Journal of Research in Pharmacy

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Evaluation of commercial *Syzygium aromaticum* L. (clove) essential oil samples from market in accordance with the European Pharmacopoeia 10.0 criteria

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Received: 3 October 2023 / Revised: 7 November 2023 / Accepted: 7 November 2023

ABSTRACT: Clove (*Syzygium aromaticum* L.) oil is widely used in cosmetics, medicine and food industries. Previous studies have revealed many pharmacological effects of clove oil, including anti-infective, antioxidant, anesthetic and anti-inflammatory. In this study, clove essential oil (CEO) samples obtained from different places in Türkiye, have been extensively evaluated in terms of their compliance with European Pharmacopoeia (EP) standards, which is the official guide that includes international rules and methods. In this study, 13 CEO obtained from Turkish market (Pharmacy and other) were analyzed according to the criteria specified in the "Clove Oil" monograph in European Pharmacopoeia 10.0. Appearance, solubility, thin layer chromatography, relative density, refractive index, optical rotation analysis was conducted for all samples. In addition, GC-MS was used to elucidate detailed phytochemical profiles of the samples. Results revealed that none of the samples from Turkish Market fully met the EP criteria. When the difference between the sources of purchase evaluated, results revealed that there is a significant difference between the sources from which the oils are taken in terms of standards. The deficiency of the CEO samples to fulfil standards of the EP indicates the requirements of performing the necessary effort and auditing to increase the quality of the products on the market.

KEYWORDS: Clove oil; European pharmacopoeia; GC-MS; essential oil; *Syzygium aromaticum* L..

1. INTRODUCTION

Essential oils are valuable aromatic and volatile extracts obtained from parts of plants such as seeds, flowers, peel, stem, bark and whole plants [1]. They have been used in traditional medicine for centuries and now their popularity as a complementary and alternative therapy continues to increase. They have been marketed as antibacterial, anti-fungal, antiviral, reducing stress, increasing attention, improving mood, improving sleep in pharmaceutical, cosmetic, agricultural and food industries [2]. Myrtaceae is an important family with a wide distribution in temperate, subtropical, and tropical regions, with a particular concentration in Australia and tropical America. It comprises around 142 genera and 5500 species [3]. *Szygium aromaticum* L. (clove) belongs to Myrtaceae family and clove essential oil (CEO) is derived from the flower buds of this plant by steam distillation [4]. CEO has broad usage for various purposes. Especially it is traditionally used for toothache pain relief. Beside the folk medicine, previous studies have revealed many pharmacological effects of clove oil, including antibacterial, anti-infective, insecticidal, antioxidant, anesthetic and anti-inflammatory [5].

Due to increasing public demand for natural based products, essential oil market expanding rapidly. The total market for EOs in 2010 was estimated to be at US $22 billion. Additionally, the demand for these EOs has been rising over time and is projected to reach 247.08 kilotons in 2020. By 2027, the market is expected to rise by 7.5 percent [6]. When the area of their usage investigated, 29% of the essential oils market is covered by cosmetics and aromatherapy sector while 15% is used for pharmaceutical industry [7]. Cosmetics, aromatherapy and pharmaceuticals are sectors which directly connected to public healthcare thus, ingredients and end products used for these purposes must meet the international standards. Pharmacopoeias are recognized governmental documents that specify the necessary standards of quality for

How to cite this article: Barak TH, Gültekin E, Şentürk TB, Özdemir K. Evaluation of commercial Syzygium aromaticum L. (clove) essential oil samples from market in accordance with the European Pharmacopoeia 10.0 criteria. J Res Pharm. 2024; 28(4): 1088-1098.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.791

pharmaceuticals with natural and synthetic bases that aim to advance and safeguard public health. The Republic of Türkiye is legally bound by the European Pharmacopoeia (EP), which has more than 200 herbal product monographies, and must follow its regulations[8]. Due to these factors, for any marketed product in cosmetic and pharmaceutical purposes must meet the pharmacopoeia criteria in order to maintain minimum risks for public health. In the light of these information for this study, 13 pure CEO containing products from Turkish market were investigated for their compliance with standards which stated in the latest version of EP. In order to comprehend differences between sources of purchase, 5 of the samples were selected from pharmacies and 8 of the samples were taken from other sources such as akhtars and online markets. Various physical and chemical analysis were conducted as stated in EP 10.0. Characters, solubility tests, fatty oils and resinified essential oils tests were done as stated in EP 10.0 in order to primarily evaluate possible adulterations. Relative density, refractive index and optical rotation assays were conducted as given in pharmacopoeia for higher understanding of the quality issues of the products. In addition to physical assays, chromatographic analysis was applied to all samples for deeper evaluation of quality. Thin Layer Chromatography method was used with two main ingredients as reference substances. Gas-Chromatography-Mass Spectrometry (GC-MS) is most widespread used method for investigating chemical profiles of essential oils. Therefore, GC-MS analysis were applied to all 13 samples and to compare the results with the necessities given in EP 10.0. In the present study, it was intended to estimate current quality status of the CEO 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, Methylene Chloride, Toluene and Fatty Oils and Resinified Essential Oils

According to EP standards, clove oil should be clear, yellow liquid, which becomes brown when exposed to air. Analysis showed that all samples were compatible with EP 10.0 standards. Clove oil must be soluble in 5 volumes of 70% ethanol, methylene chloride, toluene and fatty oil. After the analysis it was seen that P5, A1, A2, A5, A7 and A8 were not soluble in alcohol while A4 was not soluble in toluene. In contrary, all samples were soluble in methylene chloride and fatty oils. Fatty oils and resinified essential oils assay is a simple method for observing possible undesirable presence of non-volatile materials which might be the apparent sign of adulteration. To perform fatty oils and resinified essential oils test, 1 drop of each sample was dropped onto filter paper and kept for sufficient time for complete evaporation. A remaining stain were observed for A1, A2, A5, A6 and A7 samples which is not compatible with standards.

2.2. Relative density, Refractive index and Optical Rotation

Table 1 presents the results of the relative density, refractive index, and optical rotation tests conducted on 13 essential oil samples. According to the EP 10.0 standards, the relative density value for clove oil should be within the range of 1.030 to 1.063, the refractive index value should be between 1.528 to 1.537, and the optical rotation value should be between − 2° to 0°. The results of the relative density, refractive index, and optical rotation tests were obtained through triplicate measurements and are presented as averages ± standard deviation. Results demonstrated that all samples were found in the standard range given in EP 10.0 in optical rotation test. Dissimilar to optical rotation test, several samples were found out of the range in refractive index and relative density. A1, A2, A4, A5 and A7 samples were failed to meet the criteria of EP for both refractive index and relative density while P1 and P3 were only failed with relative density assay. All results were compared with standards in Figure 1.

2.3. TLC Analysis

According to EP 10.0, main component of CEO is eugenol, and it must seen at the middle part a quenching zone, while acetyl eugenol seen at weak faint violet-blue quenching zone just below in TLC plate. β-caryophyllene should seen at reddish-violet zone in the upper part of TLC plate. These characteristic zones serve as markers for the identification of specific compounds and enable the qualitative analysis of complex mixtures. TLC plaques were given in Figure 2 and results of the evaluation is stated in Figure 1. P3, P5, A1,

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A4 and A8 samples were found to be inconsistent with standards. Eugenol, the main ingredient of CEO was spotted in all samples however, acetyl eugenol zone was was not seen in 5 mentioned products.

Table 1. The results of the relative density, refractive index, and optical rotation tests were obtained through triplicate measurements and are presented as averages ± standard deviation.

	Relative Density 1.030 to 1.063	**Refractive Index** 1.528 to 1.537	**Optical Rotation** − 2° to 0°
P1	1.074	1.534 ± 0,0006	-0,06 ± 0,00
P2	1.049	1.534 ± 0,0001	-0,91 ± 0,031
P3	1.020	1.535 ± 0,0001	-0,81 ± 0,012
P4	1.046	1.533 ± 0,0002	-0,35 ± 0,012
P5	1.043	1.535 ± 0,0000	-0,61 ± 0,525
A1	1.003	1.526 ± 0,0001	-0,54 ± 0,035
A2	0.951	1.490 ± 0,0030	-0,66 ± 0,020
A3	1.034	1.534 ± 0,0001	-1,56 ± 0,035
A4	1.007	1.469 ± 0,0009	-0,65 ± 0,042
A5	1.079	1.490 ± 0,0001	-0,44 ± 0,020
A6	1.041	1.534 ± 0,0001	-0,77 ± 0,012
A7	1.013	1.489 ± 0,0001	-0,11 ± 0,046
A8	1.043	1.533 ± 0,0001	-1,05 ± 0,031

Experiment	Reference Interval	P1	P2	P3	P4	P5	A1	A2	A3	A4	A5	A6	A7	A8
Appearance		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Methylene Chloride Solubility		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Toluene Solubility		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓
Fatty Oils Solubility		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Relative Density	1.030-1.063	1.074	1.049	1.020	1.046	1.043	1.003	951	1.034	1.007	1.079	1.041	1.013	1.043
Refractive Index	1.528-1.537	1.534	1.534	1.535	1.533	1.535	1.526	1.490	1.534	1.469	1.490	1.534	1.489	1.533
Optical Rotation	− 2° to 0°	-0,06	-0,91	-0,81	-0,35	-0,61	-0,54	-0,66	-1,56	-0,65	-0,44	-0,77	-0,11	-1,05
Fatty Oils and Resinified Essential Oils		✓	✓	✓	✓	✓	X	X	✓	✓	X	X	X	✓
Alcohol Solubility		✓	✓	✓	✓	X	X	X	✓	✓	X	✓	X	X
TLC		✓	✓	X	✓	X	X	✓	✓	X	✓	✓	✓	X
GC ASSESMENT														
β-caryophyllene	5.0 to 14.0 per cent	7,58	3,59	11,09	8,50	10,72	16,52	8,67	11,47	7,80	9,22	7,51	10,86	12,02
Eugenol	75.0 to 88.0 per cent	68,59	89.0	77,50	72,67	85,83	79,93	79,63	83,07	90,77	77,67	72,94	77,34	58,10
Acetyl eugenol	4.0 to 15.0 per cent	18,64	7,41	X	10,96	X	1,05	8,06	0,35	0,35	8,58	15,97	8,68	X

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

http://dx.doi.org/10.29228/jrp.791
J Res Pharm 2024; 28(4): 1088-1098

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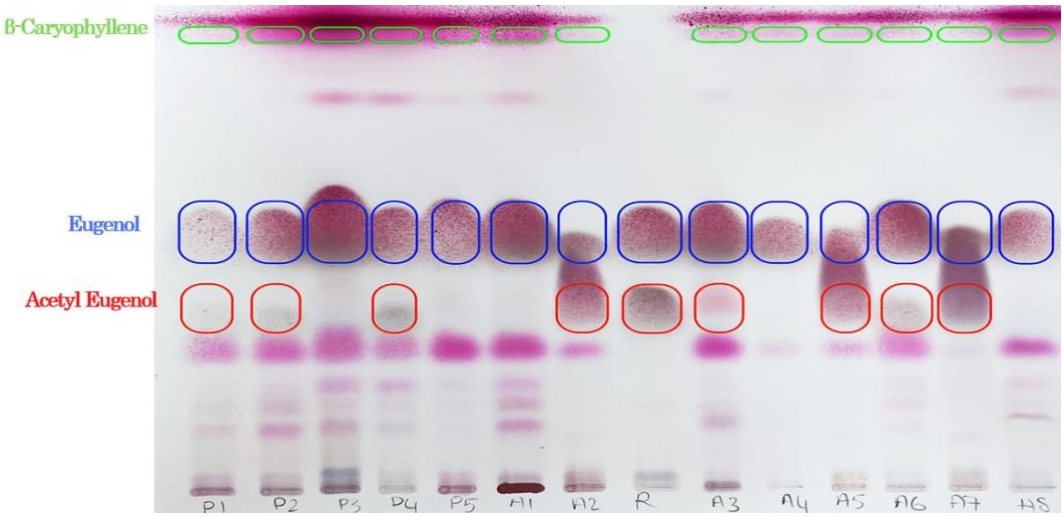

Figure 2. TLC chromatogram of 13 samples. R: Reference mixture; eugenol and acetyl eugenol from top to bottom. Mobile phase; toluene R; Stationary phase: Paper silica gel Detection: sprayed with anisaldehyde solution R and heated at 100-105 °C for 5-10 min; examined in daylight.

2.4. GC-MS analysis

To determine the detailed phytochemical component in all 13 samples, GC-MS analysis was conducted, and the results were presented in Table 2 with percentages. The analysis was able to determine 95.22% to 100% of the components, which are detailed in Table 2. Moreover, Figure 3 was included to provide a representing chromatogram, demonstrating the occurrence of the phytochemicals listed in the EP 10.0. Even though eugenol was determined as the major ingredient for all samples, 6 of them was not detected in the reference interval of EP 10.0. 2 of them were found higher 88% which is the highest suitable limit and 4 of them were found to be lower than 75% which is the lowest limit. β–caryophyllene was detected in all samples nonetheless, in A2 sample the amount was lower than the interval and in A1 sample it was higher. Acetyleugenol content was found to be compatible in only 5 samples while 8 of them were not suitable with the standards. Acetyleugenol was not found to be present in 3 samples. In Table 2, general results of GC-MS analysis were given and total 14 compounds were identified from 13 samples which some of them are clear signs of adulteration.

3. DISCUSSION

Syzygium aromaticum L. is one of the most valuable medicinal plants in the world. Plant and its essential oil are used widely in folk medicine, food industry, and cosmetics due to extensive spectrum of its pharmacological activity. Various studies found that clove essential oil has anti-inflammatory, antibacterial, antifungal, antimicrobial, anesthetic, analgesic, anticancer activity. For those reasons, public demand for products containing pure CEO for medical purposes are increasing [4,5].

Any product that is used for medicinal purposes must be meeting the international standards to avoid possible health hazards and also to apply evidence-based medicine with reproducible physiological outcome. Pharmacopoeias are the primary scientific publications for pharmaceutically utilized products including active pharmaceutical ingredients, excipients and herbal drugs. They are confirmed by scientific committees and excepted by nearly all around the world. Pharmacopoeias includes standardization methods for pharmaceutical products including herbal drugs [9]. Türkiye is legally bound to EP thus it should be expected for pure CEO containing products to meet the criteria given in "Clove Oil" monograph of EP 10.0. All of the samples evaluated in this study was licensed as cosmetics via Ministry of Health of Republic of Türkiye. 5 of the samples were purchased from pharmacies and 8 of them from other sources.

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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 A: Mono-terpene B: Oxygenated monoterpene C: Sesquiterpene D: Sesquiterpenoid E: Aromatic F: Other

Components	RT	RI^{Exp}	RI^{Lit}	Id	P1	P2	P3	P4	P5	A1	A2	A3	A4	A5	A6	A7	A8
α-Pinene[a]	6.17	928	936	a,b				0.7									
D-Limonene[a]	9.44	1030	1037	a,b			0.1	0.4									
Benzyl Alcohol[e]	10.03	1046	1045	a,b			0.1										24
Methyl salicylate[e]	16.21	1198	1990	a,b	0.5										0.2		
Estragole[b]	16.39	1202	1200	a,b				0.9									
Chavicol[e]	19.05	1263	1265	a,b	0.2		1.5										0.8
(E)-Cinnamaldehyde[e]	19.53	1275	1277	a,b				1.2									
Eugenol[b]	23.75	1374	1373	a,b,c	69	89	78	72	86	80	80	83	91	78	73	77	58
β-Caryophyllene[c]	25.87	1425	1424	a,b	7.6	3.6	11	8.5	11	17	8.5	12	7.6	9.2	7.5	11	12
Humulene[c]	27.20	1458	1456	a,b	1.3		4.1	3.2	2	1	2	2	1.4	2.3	1.1	2.7	2.3
Calemenene[c]	29.97	1527	1527	a,b				0.5						0.5	0.9	0.4	0.4
Acetyl eugenol[e]	30.17	1533	1525	a,b,c	19	7.4		11		1	8	0.5		8.6	16	8.7	
Caryophyllene oxide[d]	32.27	1587	1588	a,b	1.4		1	0.6		1	1.5	1.5		1.8	0.6		0.9
Diisooctyl phthalate[f]	62.06	2576	2545	a,b													1.6
			Total (%)		99	100	95.8	99	99	100	100	99	100	100	99.3	100	100

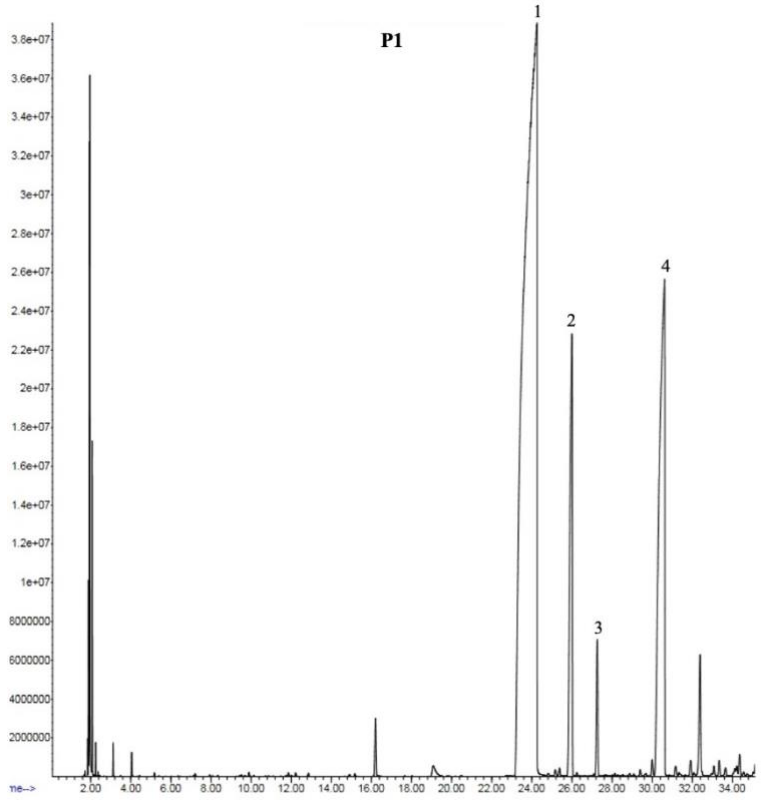

Figure 3. GC-MS chromatogram of P1 sample showing the chemical components given in pharmacopoeia 1: Eugenol, 2: Caryophyllene. 3: Humulene, 4: Acetyl Eugenol

Authentication and standardization are crucial for medicinal utilization of essential oils. One of the main issues with industrial essential oil productions on a wide scale is the relatively low yield per mass of raw materials, which emerges to be the source of the high prices on the global market. Combination of high prices, low yield and high demand may be the main driving forces of adulteration. Essential oils adulteration can be applied with different methods such as addition of cheaper EO or vegetable oil or via addition of cheap synthetic chemicals. Even though the safety profiles of various essential oils were studied in a detailed manner, undeclared adulterants may lead unanticipated adverse effects[7,10]. Herbal products are advertised and used around the world for their claimed or expected health benefits. However, with this increased demand derives potential hazards such as intentional and accidental adulterations. A review study found that 41% of 508 microscopically examined plant products from 13 countries were adulterated which indicates that there is an international problem with standardization and quality status of herbal based products[11]. Numerous studies in the literature demonstrated essential oil adulteration in diverse countries all around the world [12-14]. Given the growing public demand for aromatherapy products and the deficiency of scientific reporting on the subject, the importance of such research appear evident. EP's main goal is to maintain product standards so that customers can purchase any product without abstaining on quality. In this regard, 13 products that purport to contain pure CEO were obtained for this study (5 from pharmacies and 8 from other sources), and they were assessed in accordance with the criteria outlined in the EP 10.0 "Clove oil" monograph. Addition of carrier oils and other non-volatile contaminants can be considered as the lowest degree of adulteration. Fatty oils and resinified essential oils test were given in the monograph for simple detection of such contaminants. All the labels of products were stated that clove oil is obtained with steam distillation thus it is expected for them to fully evaporate, and it is inappropriate to observe a significant mark following the dropping on filter paper. Figure 1 shows the results of the fatty oils and resinified essential oils assay. Five of the samples from non-pharmacy (A1, A2, A5, A6 and A7) left permanent stains on the filter paper, indicating the presence of non-volatile principles in the products and indicate lowest degree of adulteration [3]. In the monograph, appearance section indicates that CEO is clear, yellow liquid which turns to brownish color when exposed to air. It was found that all the samples tested in this study complied with the specified criteria. 4 different solubility tests were stated in EP standards for CEO. These are methylene chloride, toluene and fatty oils solubility. All samples were soluble in methylene chloride and fatty oil however, A4 is not soluble in toluene. A significant layer was observed on the toluene phase which indicated insolubility. Alcohol solubility test were stated as a separate assay and results showed that 6 of the samples were failed on alcohol solubility test and 4 of the samples also failed in fatty oils and resinified essential oils test which may be an indication of adulteration. 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 clove oil, three decimal places are mandatory. Reference interval of refractive index of CEO is stated as 1.528 - 1.537 in the monograph and result showed that all the samples from pharmacies was compatible with the monograph. In contrary, 5 of the 8 samples from other sources had failed to be consistent with monograph. Optical rotation is a characteristic of chiral substances that rotates linearly polarized light. The monograph stated that the optical rotation value of CEO should be between 0° and -2°. Even though the interval is very narrow, all the samples were found to be compatible with the monograph. 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 monograph, CEO has a specific density range which is between 1.030 and 1.063. 7 of 13 samples (P1, P3, A1, A2, A4, A5 and A7) were not in the specified range. The averages and standard deviations of results were given in Table 1.

Chemical methods are the most important and widely used plant identification techniques recommended by national and international pharmacopoeias. In a systematic review, studies involving 2,386 commercial herbal products sold in 37 countries on six continents were investigated. The majority of the products analyzed were reported to be genuine (73%), but more than a quarter (27%) turned out to be adulterated. Additionally, it was reported that 57% of the products from Türkiye are adulterated which is more than two-fold when compared to the world average [15]. In the light of these information, it is crucial to determine the status of the herbal products in Turkish market, including pure CEO containing ones. Chromatographic profile can be considered the most central characteristic of an essential oil. Since, biological activity of the volatile components, consequently phytochemical profile, regulates the biological activity [16]. TLC is widely used and simple method to internationally used for authentication of essential oils [17]. For

this study, TLC analysis were conducted to all samples and image of the plate were given in Figure 2. Eugenol and acetyleugenol were used as reference substances as stated in the monograph and results demonstrate that β-caryophyllene and eugenol zones were observed in all samples. However, acetyleugneol zones were not observed in 6 samples (P3, P5, A1, A4 and A8) consequently they failed to comply with EP 10.0 requirements.

GC-MS is the most typical and notable method used to examine the components of essential oils [7]. Thus, EP requires GC analysis for quality control analysis of pure essential oils. For these reasons GC-MS analysis were conducted to all 13 samples from Turkish market in order to detailed evaluation of their chemical components. 14 different chemicals were detected and 95.22 to 100% of ingredients from all samples were disclosed Table 2. 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. Thus, previous studies showed extreme diversities on phytochemical components of clove essential oils all around the world. Eugenol is dominantly major ingredient of clove essential oil. In a previous study, Fadel et al. (2020) observed that 89.9% of the clove essential oil from Egypt is eugenol [18]. In contrary, Chen et al. (2022) reported 32.6% of the hydrodistilled clove essential oil from China is Eugenol [19]. Correspondingly, 13 samples from this study demonstrated significant variation for their eugenol content. Results varied from 58.2% to 90.77% of eugenol content while interval for eugenol content in the monograph is 75 to 88%. Two of the samples P2 and A4 were found to be higher than the limit value of monograph while 4 of the samples (P1, P4, A6 and A8) were detected lower than the minimum required level of EP 10.0. As a result, only 7 of the 13 samples are corresponding with the limit given in the monograph (Figure 1). Acetyleugenol is one of the major ingredients of CEO and in the monograph it was stated that the amount must be between 4 to 15%. Previous studies showed notable variations. In a previous study, researchers declared that 14.24% of the clove oil from Iran is acetyleugneol while other studies found that acetyleugneol was undetected [20,21]. In this study, acetyleugneol content of the samples showed fluctuations and only 5 of the samples were in the specified range. In P3, P5 and A8 samples acetyleugneol was not detected while in P1 and A6 samples it was found higher than the maximum limit. β-caryophyllene is the third major ingredient of CEO and the monograph states that content of it must be between 5 to 14%. In our study two of the sample were found to be unsuitable with the monograph, P2 sample have lower amount than stated while A1 has higher. Likewise previous studies showed significant variations such as Chen et al. (2022) reported 50.75% β-caryophyllene content while Laaziz et al. (2022) reported absence of it [19,22].

Given the high demand and valuable properties of some essential oils, it is no wonder that counterfeit and adulterated essential oils are on the market. Formulating medicines with counterfeit and adulterated essential oils is particularly threatening and can pose serious health risks to consumers [23]. Previous studies showed various examples of poor-quality status of essential oil markets for different countries. Kucharska et al. (2021) investigated 6 different products of sandalwood essential oil from Poland and reported that none of them were met the ISO criteria [12]. Schripsema et al. (2022) investigated 8 vetiver essential oils samples obtained from online channels of Brazil and reported that four were found to have been adulterated by solvents or semisynthetic compounds [14]. In addition, in our previous study it was observed that none of the rosemary oil samples from Turkish market have accomplish to fully met the EP 10.0 criteria [8]. Adulteration is one of the most important challenges in the essential oil market. There are various novel methods suggested for detecting adulteration in essential oils such as chiral gas chromatography [13], Mid-infrared spectroscopy [24], isotope ratio mass spectrometry [25], Raman spectrometer [23] etc. The reason is there may be different degrees of sophistication for adulteration of essential oils which requires novel methods to overcome. However, in this study none of the samples were fully comply with the EP 10.0 standards thus further investigation was not required to disclose their quality status. Previous studies were investigated adulteration problem of essential oils with particularly benzyl alcohol which is a very common chemical used for that purpose [26]. A previous study investigated 18 CEO samples from Germany for possible adulteration with benzyl alcohol with a novel method and it was shown that two of the sample were adulterated with benzyl alcohol [23]. In addition, researchers from Türkiye developed a novel method for quick determination of ethyl acetate and benzyl alcohol adulteration of CEO. 51 samples were studied with Fourier transform infrared (FTIR) spectroscopy and results showed that 6 of the samples were adulterated with benzyl alcohol and 7 of the samples with ethyl acetate [27]. Correspondingly in our study it was observed that A8 sample were severely adulterated with benzyl alcohol

[23], 24.14% of the sample were found to be consist of benzyl alcohol. Previous studies investigated CEO adulteration with specific ingredients, however this is the first study which thoroughly investigated quality status of clove oil samples from Türkiye depending on EP 10.0 standards, to our knowledge. When difference between sources of purchases investigated, fulfillment rate of samples from pharmacies were calculated as 81.5% while it was 68.27% in other sources. Results indicated that none of the samples were fully met the criteria. Reason for poor quality status may be due to poor storage conditions [28] or intentional-unintentional adulteration. Whatever the reason, the results revealed that there are significant problems with the quality of clove oil on the Turkish market. Results are consistent with the previous studies indicating similar problems with other essential oils in the Turkish market [29].

4. CONCLUSION

Essential oils are marketed with notorious health claims. Among other things, clove oil is assumed to have health benefits and is sold through multiple channels such as akhtars, websites and pharmacies without sufficient 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 clove oil on the market to determine the current status and quality level of products on the market. In this study, 13 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. Com-paring compliance rates with place of purchase, it is found that pharmacy products per-formed higher than other channels (81.5% and 68.27%, respectively). Eventually, it is obvious that there is an urgent need to raise quality and auditing standards for clove oil in the Turkish market.

5. MATERIALS AND METHODS

5.1. Materials

Thirteen samples of pure clove essential oil (CEO) were procured from different sources in the Turkish market, including pharmacies, herbalists, and website-based markets. Registration of all samples were checked and determined that all of them are licensed as cosmetic products via Ministry of Health of Turkish Republic. To preserve the quality and integrity of each sample, they were stored according to the instructions mentioned on their labels. To maintain accuracy during testing and analysis, each sample was labeled and coded based on its purchase location, allowing for easy tracking of their origin. P codes are given to samples obtained from pharmacies and A code is given to products from other sources. All standard compounds and solvents were purchased from Sigma-Aldrich.

5.2. Characters, Solubility in Alcohol, Methylene Chloride, Toluene and Fatty Oils and Resinified Essential Oils

All tests conducted in this study were carried out in accordance with the 10th edition of the European Pharmacopoeia, with minor adjustments made to suit the specific requirements of the research. To perform resinified essential oil test, 1 drop of each sample were dripped on the coded filter paper then kept in an 80 °C oven for 30 minutes. For solubility test, 4 ml solvent and 1 ml of sample poured into test tube separately for each solvent (96% ethanol, methylene chloride toluene and fatty oil) and each sample with the help Pasteur Pipette. According to EP, CEO should soluble in 96% ethanol, methylene chloride toluene and fatty oil[30].

5.3. Relative Density, Refractive Index, Optical Rotation

To determine the relative density, refractive index, and optical rotation of the clove essential oil samples, the procedures detailed in EP 10.0 were followed. The volume of the oil samples was measured using a pycnometer and was compared to an equivalent volume of water at 20 °C to obtain the relative density results. The refractive index assay was conducted using an Anton Paar - Abbemat 3100 refractometer

device, while the optical rotation analysis was carried out using an Anton Paar – MCP 150 instrument. The results were presented as an average and standard deviation[30].

5.4. TLC Analysis

The standard solution used in this assay is composed of a mixture of eugenol and acetyl eugenol substances. A Silica gel F254 plate was used as the stationary phase, and 100% Toluene was used as the mobile phase. After completing the solvent mobilization process, the TLC plate was dried and sprayed with anisaldehyde solution and examined in daylight while heating at 100-105 °C for 5-10 minutes[30].

5.5. GC-MS Analysis

The GC-MS method that is used in this experiment was method of Servi et al. (2023). A HP-5MS column comprising 5% diphenyl and 95% dimethyl polysiloxane was employed, with dimensions of 30 m × 0.25 mm and 0.25 m film thickness. The oven temperature was programmed to maintain isothermal conditions at 60 °C for one minute, followed by an increase up to 246 °C at a rate of 3 °C per minute, and a subsequent isothermal hold for 30 minutes. Helium was used as the carrier gas at a flow rate of 0.9 mL per minute. The identification of the components in the essential oil was accomplished by comparing their relative retention indices (RRI) obtained from a series of n-alkanes (C5 to C30) with those found in the literature, and by comparison with mass spectra. The comparison of the mass spectra was achieved using computer matching with the NIST14 and Wiley7 mass spectra libraries commercially available[31].

Acknowledgements: This study is supported by a grant (2209-A) from Scientific and Technological Research Council of Turkey (TÜBİTAK)

Author contributions: Conceptualization; T.H.B. Methodology; T.H.B., K.Ö. Formal analysis; T.H.B., T.B.Ş. Investigation; T.H.B., E.G., T.B.Ş, K.Ö. Resources; T.H.B., E.G. Writing - Original Draft; T.H.B. Visualization; T.B.Ş, K.Ö. Funding acquisition; T.H.B., E.G.

Conflict of interest statement: "The authors declared no conflict of interest"

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