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Evaluation of the quality of some widely used essential oils according to the specifications given in Turkish Pharmacopeia 2017

Burçin ERGENE [1] *, Rabia Sedanur ŞEN [2]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, Yenimahalle, Ankara, Türkiye.
[2] Faculty of Pharmacy, Ankara University, Yenimahalle, Ankara, Türkiye.
* Corresponding Author. E-mail: burcinergene@gmail.com (B.E.); Tel. +90-312-203 30 92.

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ABSTRACT: Essential oils, although they are effective due to the compounds they contain, are usually high-cost products that are obtained with low efficiency and have stability problems. Many of these oils, which can be found at various prices on the market and are claimed to be natural by manufacturers, are adulterated with other oils or synthetic substances. This study aims to provide samples of the essential oils, which are used in public, namely clove, neroli, tea tree, and cinnamon essential oils belonging to brands known to be sold widely on the market and to evaluate the compliance of these oils with the pharmacopoeia by conducting pharmacopoeia analysis. Among 25 essential oil samples evaluated with reference to the Turkish Pharmacopoeia (2017) within the scope of the study, only 2 of them were found to comply with the pharmacopoeia in terms of all tests. Investigating the compliance of essential oils with quality criteria is of great importance in preventing harm to the health of consumers and preventing them from purchasing unqualified products.

KEYWORDS: Aromatherapy; Compliance; Essential Oil; Pharmacopoeia Analysis; Turkish Pharmacopoeia.

1. INTRODUCTION

Traditional uses of essential oils have been reported, especially in the Hellenistic period and Ancient Egypt as well as in countries such as India, Iran and China. There are records showing that the Egyptians used essential oils and other extracts obtained from plants as medicine for the first time in ancient history, dating back to 2700 BC [1]. Especially in the early periods of their use, essential oils were obtained for massage applications, to obtain a perfume effect, and to add to bath water. In later periods, it began to be used for specific purposes such as protecting the skin from the sun and healing lesions such as burns and acne [2]. Studies have also yielded results supporting the traditional use of many essential oils, such as antibacterial, antiviral, antifungal, and antioxidant activities [3].

The use of essential oils has been expanded from ancient times to this century and now, it is a big market which has significant demand and use in the fields of food, health, cosmetics, and cosmeceuticals. In the field of healthcare, essential oils are used in aromatherapy applications, oral and dental care products, and veterinary medicines [4].

The World Health Organization (WHO) reported that 88% of all countries in the world prefer traditional methods of treatment [5].

Studies on the therapeutic use of essential oils in some veterinary diseases have shown that the use of essential oils can be considered as a good supplement to conventional treatments in some cases [6,7].

The components of essential oils are mainly terpenic compounds, ketones, esters, alcohols, ethers, aldehydes, and flavonoids. Terpenic compounds, which are generally found in high amounts in the essential oil composition, may belong to different groups [8], and their oxygenated derivatives are the structures exhibiting various biological activities [9]. In recent years, the use of essential oils has become widespread because of their various physiological effects. Quality controls of essential oils is important not only to provide the safe use of the essential oil, but also to prevent adulteration and counterfeiting [10].

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Adulteration is the violation of legislation or permitted specifications in the course of the production or marketing process. Adulteration is mainly carried out by adding a foreign or cheap substance to the natural content of a product. It causes some or all of the valuable components in the product to be changed, thereby reducing the product cost and thus gaining unfair profits [11].

The most common types of adulteration carried out on essential oils are; mixing the essential oil with a cheaper essential oil, mixing the essential oil with a synthetic ingredient that has a scent and aroma similar to the relevant oil, and mixing the essential oil with a fixed oil [10]. Mixing essential oils with fixed oils is a preferred method because of the low cost of fixed oils and being difficult to be detected in the content of the product even using sensitive methods for analysis. The challenging aspect of this method is that the refractive index, relative density, color and physicochemical properties of the essential oil adulterated with this method do not change [12]. Adulteration is an undesirable situation that can be detected at a significant rate in essential oil quality control analyses. In a study, in which 31 essential oil samples used in perfumery and aromatherapy were subjected to quality control analysis using gas chromatography; it was revealed that more than 45% of the essential oil samples did not even comply with ISO AFNOR standards, which have a wider acceptance range compared to other standards, and more than 19% were diluted with solvents such as propylene glycol, dipropylene glycol, triethyl and vegetable oils [13]. An estimated 80% of the commercially accessible, reported to be "natural" essential oils have some form of adulteration. The prevalence of such adulterated products in the market should be reduced by research and the creation of appropriate standards [14].

Pharmacopoeias; are official sources containing monographs that provide the standards for natural or synthetic products used for therapeutic purposes or included as an auxiliary substance in a formulation. These books, which are accepted by the state authority, have been updated over the years, depending on the developing techniques and the addition of new effective and auxiliary substances to natural products. The quality, reliability, and effectiveness of medicinal plants and the products obtained from these plants can only be ensured by the evaluation of these materials in terms of accepted standards [15].

Clove bud oil is obtained from dried buds of *Syzygium aromaticum* (L.) Merr. & L.M. Perry (Syn. *Eugenia caryophyllus* (Spreng) Bullock ve S.G. Harrison) by water and steam distillation [16]. It has a wide range of uses such as the food and cosmetics industry, and especially pharmaceutical and non-pharmaceutical medical products. Clove buds, which is also used for its scent and aroma, has an important place in world trade. Clove bu oil has been stated that it has a high potential for use in the food industry to extend the shelf life of products and can be added to foods as a preservative [17].

Clove bud oil is traditionally used by dropping it directly onto the aching tooth. However, the essential oil should not come into contact with non-carious teeth and mucosa due to the risk of tooth damage or mucosal irritation in case of direct contact [18,19]. The major component of this essential oil is known to be eugenol [20]. Eugenol is widely used in dentistry for treating toothache and dental pulpitis [21]. The essential oil is used as an antiseptic, antimicrobial, analgesic, antispasmodic and carminative agent. The solution of the essential oil at the concentration of 1-5% is used as mouth wash. It is used against mild infections or wounds in the mouth after diluted in a fixed oil. The oil is mixed with fixed oil to relieve the insect bites or can also be used by mixing into an unscented cream or lotion. It is used by spraying into a room for the purpose of calming and relaxation. Clove bud oil also have a traditional use as a remedy against gastrointestinal complaints such as indigestion, bloating and diarrhea [22].

Cinnamon bark oil is obtained from the bark of young branches of *Cinnamomum cassia* Blume (Syn. *C. aromaticum* Nees) using steam distillation [16]. The major component of this essential oil is determined as cinnamaldehyde [23]. Cinnamon essential oil shows significant medicinal effects because of its healing capacity in digestive system infections [24]. The potential for use of cinnamon bark essential oil as a preservative in foods is evaluated in the food industry [25]. Cinnamon bark oil can be used as an insect repellent and has also been found to be very effective in killing mosquito larvae [26]. There are also some reports showing that this essential oil relieves mental fatigue, has an antipyretic effect, is effective against intestinal infections, and has a stimulating effect on the circulation and respiratory system [27]. The essential oil is used for the relief of gastrointestinal complaints such as colitis, diarrhea and nausea as well as against cold and menstrual pain. Diluted in fixed oil, it is used to relieve rheumatic pain by massage. Diluted oil is applied onto the skin due to its antimicrobial effect and onto the hair and scalp by massaging to revitalize and soften the hair. It is also sprayed into a room to obtain a relaxing atmosphere [22].

Tea tree oil is obtained from the leaves and small branches standing at the tips of *Melaleuca alternifolia* (Maiden & Betch) Chell, *Melaleuca linariiifolia* Smith, *Melaleuca dissitiflora* F. Mueller and/or the leaves and

small branches of other *Melaleuca* species by steam distillation [16]. The major component of the essential oil is determined as terpinen-4-ol, which is responsible for the antimicrobial activity of the oil [28]. It has been shown that the use of a solution of the essential oil in water as mouthwash improved the treatment of non-severe inflammations in the oral mucosa [29]. Clinical studies have shown that tea tree essential oil is effective in the treatment of fungal infections such as onychomycosis and oral candidiasis [30]. Tea tree oil is included in the composition of some medicinal agents in the treatment of cutaneous infections due to its antimicrobial and anti-inflammatory properties [31]. Tea tree oil, one of the frequently used essential oils, is preferred as a preservative in the food and cosmetic industry, in addition to its medical use [32, 33]. It has been observed that the use of essential oils such as tea tree, lavender and lemon together with chemical preservatives in body lotions can increase the protective effect [32]. It is used directly or after diluting in an oil to heal small superficial wounds and insect bites. It is mixed into the preparations such as lotions, gels, emulsions to apply onto the skin for the relief of skin infections, acne, eczama, dermatitis, skin rashes, psoriasis, bruises, ringworm, parasitic skin diseases and nail infections. It is used to treat itching, irritation, fungal infections of toenails, peeling, calluses and to eliminate bad odor of the feet. It is prepared in the form of gel or washing water against vaginal infections, vaginitis, cystitis, uterine and cervical wounds [22].

Neroli oil is obtained from freshly collected flowers of *Citrus aurantium* L. subsp. *aurantium* L. (Syn. *Citrus aurantium* L. subsp. *amara* Engl.) by steam distillation [34]. Neroli essential oil is used in perfumery, cosmetics, food, and pharmaceutical industries [35]. It is used as a remedy of complementary medicine to reduce women's stress during childbirth [34]. The major components of the essential oil are determined as linalool and linalyl acetate [36]. The biological activity of neroli oil is attributed to its linalool content [37]. It has been proven in some studies that linalool is preventive against the development of fungal diseases and has insecticidal activity [38]. Nerolidol, another component found in neroli oil, provides a characteristic odour to the oil. In particular, nerolidol was shown to provide strong antioxidant activity by scavenging free radicals, preventing lipid peroxidation, and increasing the production of antioxidant enzymes [39]. Due to its effect on central nervous system, it is used for calming and relaxation in case of stress, anxiety, uneasiness, fear and depression. It is commonly used by inhalation with the aid of a diffuser. Mixing into a carrier oil, it is used to heal the scars of acnes and as an emollient [22]. It is known that the use of neroli oil by inhalation provides positive effects on sleep duration and anxiety-related electrophysiological properties in the heart, such that cocaine users have been reported to have a sedative effect during withdrawal. Clinical studies have proven that neroli essential oil exhibits relieving effect on anxiety before surgery, during birth, or during hemodialysis [40]. Due to its relaxing effect on smooth muscles, it is used agains cardiac spasms, tachycardia, colitis-type pains in the intestine, irritable bowel syndrome and diarrhea induced by stress [22].

Although essential oils are effective due to various compounds they contain, they are generally high-cost products that are obtained with low yield and have stability problems. Many of these oils, which can be found in the market at various prices and some of which are claimed to be "completely natural" by some suppliers, are adulterated with other oils or synthetic substances. In the content of this study, four essential oils known to be used for aromatherapy applications, namely clove bud oil, cinnamon bark oil, tea tree oil, and neroli oil, were obtained from the market. The compliance of these oils with Turkish Pharmacopoeia 2017 (TF 2017) was tested with the purpose of evaluating their safety and quality.

2. RESULTS

2.1. Cinnamon Bark Oil

2.1.1. Appearance

The clarity and viscosity of all samples were compatible; the color of two samples was yellow, one sample was light yellow, and three samples were pale yellow.

2.1.2. Thin layer chromatography

The blue fluorescent zones, which were referred to as coumarin, were observed in the chromatograms obtained using the samples. The zones of eugenol and *trans*-cinnamaldehyde, which have the same retention factor (Rf) values as the references, were observed in the chromatograms of all samples. Other pale spots were also detected as mentioned in the pharmacopeia (Figure 1) [16].

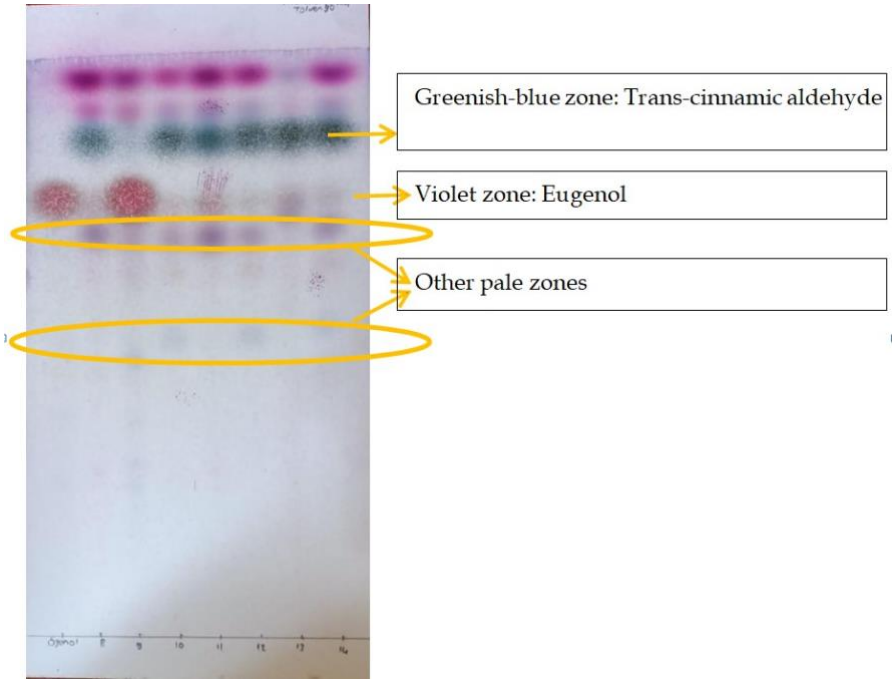

Figure 1. TLC chromatogram of cinnamon bark oil samples

2.1.3. Relative density

The reference range given in the monograph of cinnamon oil is 1.052–1.070, and only one sample was found to be compatible with the given range [16].

2.1.4. Refractive index

The reference range given in the monograph of cinnamon oil is 1.600–1.614. Four of the seven samples were found to comply with the standards in terms of refractive index [16].

2.1.5. Optical rotation

The reference range given in the monograph of cinnamon oil was (-1°)-(+1°). The values determined for the five samples were found to be in this range [16].

2.1.6. Gas chromatography

The results of the gas chromatography analysis showed that the sample was incompatible with the pharmacopeia standards (Table 1).

Table 1. The content of the cinnamon bark oil sample determined by GC-MS analysis.

Sample code	Component	Relative content (%)	Values given in TF 2017 (%) [16]
1	Linalool	1.80	
2	β-Caryophyllene	0.80	
3	Safrole	0.60	
4	(E)-Cinnamaldehyde	9.20	70.00–90.00
5	Cinnamyl acetate	1.10	1.00–6.00
6	Eugenol	79.30	Max 0.50
7	Eugenyl acetate	1.50	
8	o-Metoxy-cinnamaldehyde	0.70	3.00–15.00
9	Benzyl benzoate	2.40	
	Total	97.40	

2.2. Clove Bud Oil

2.2.1. Appearance

The clarity and viscosity of all samples were compatible; the color of two samples was yellow, one sample was light yellow, two samples were pale yellow, and one sample was reddish brown.

2.2.2. Thin layer chromatography

Pale zones at the wavelength of 254 nm and brownish purple zones at visible light were observed in the chromatograms of all samples. These zones were referred to as eugenol in the monograph. Dark reddish-purple zones belonging to β-caryophyllene were detected in the chromatograms of all tested samples (Figure 2) [16].

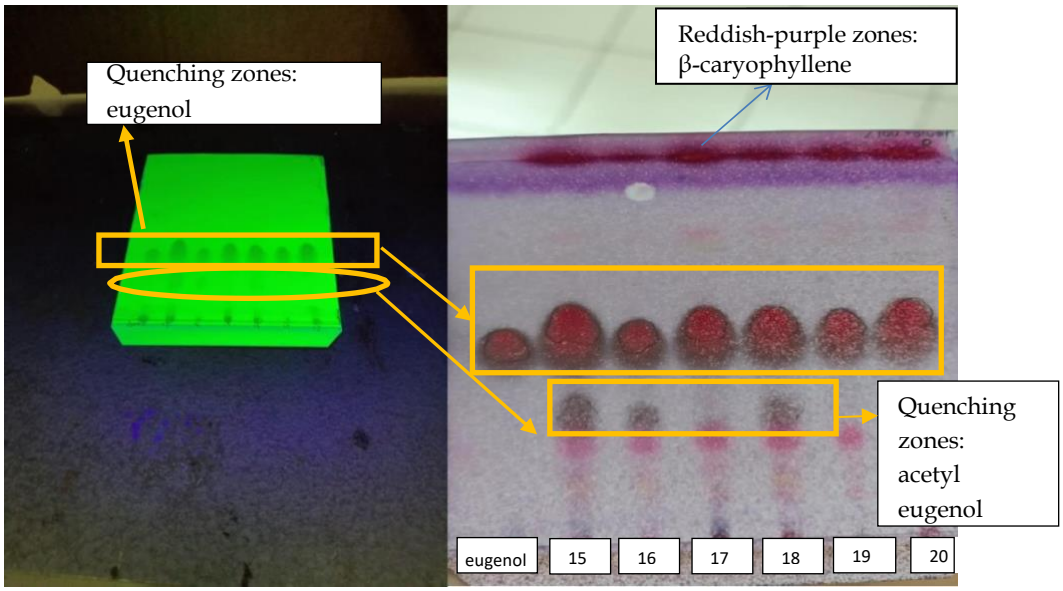

Figure 2. TLC chromatogram of clove bud oil samples

2.2.3. Solubility

All of the samples were mixed with fixed oil, methylene chloride, and toluene, demonstrating that the samples complied with the pharmacopoeia in terms of solubility [16].

2.2.4. Relative density

The reference range given in the monograph of cinnamon oil is 1.030–1.063, and none of the samples were found to be compatible [16].

2.2.5. Refractive index

The reference range given in the monograph of cinnamon oil is 1.528–1.537. All the samples were found to comply with the standards in terms of refractive index [16].

2.2.6. Optical rotation

The reference range given in the monograph of cinnamon oil was (-2°)-(0°). The values determined for all samples were found to be in this range [16].

2.2.7. Solubility in alcohol

All of the samples were found to be soluble in 70% (*v/v*) ethanol, as indicated in the monograph [16].

2.2.8. Fixed Oil and Resinous Essential Oil

After 24 h, it was observed that there was no residue on the filter papers on which the essential oil samples were dripped separately.

2.2.9. Gas chromatography

The results of the gas chromatography analysis showed that the sample was compatible with the pharmacopeia standards (Table 2).

Table 2. The content of the clove bud oil sample determined by GC-MS analysis

Sample code	Component	Relative content (%)	Values given in TF 2017 (%) [16]
1	β-Caryophyllene	5.50	5.00–14.00
2	α-Humulen	0.70	
3	Eugenol	83.10	75.00–88.00
4	Eugenyl acetate	98.50	4.00–15.00
	Total	97.80	

2.3. Tea Tree Oil

2.3.1. Appearance

The clarity and viscosity of all samples were compatible; six of seven samples were colorless, whereas one sample was pale yellow. According to this examination, the samples' appearances were compatible with the pharmacopoeia [16].

2.3.2. Thin layer chromatography

In the chromatogram of all samples, brownish pink zones, which were considered to be cineol, were observed at the upper part of the plate, along with brownish purple and pale brown zones belonging to terpinen-4-ol and α-terpineol, respectively (Figure 3) [16].

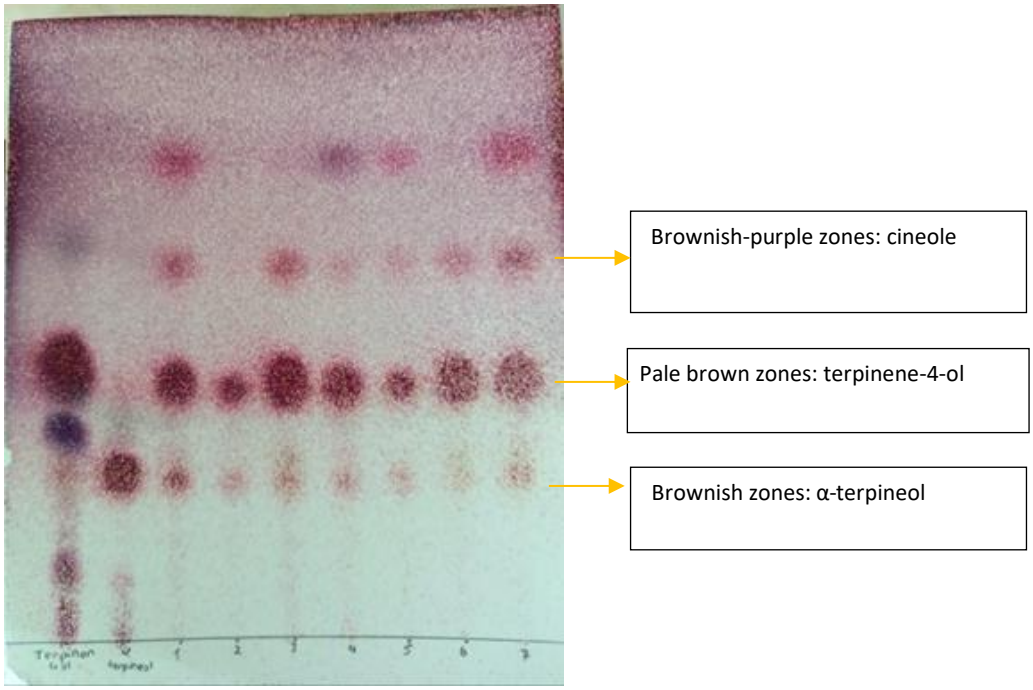

Figure 3. TLC chromatogram of tea tree oil samples

2.3.3. Relative density

The reference range given in the monograph of cinnamon oil is 0.885–0.906, but only one of the samples was found to be in the given range [16].

2.3.4. Refractive index

The reference range given in the monograph of cinnamon oil is 1.475–1.482. This value was out of the given range for one of the samples [16].

2.3.5. Optical rotation

The reference range given in the monograph of cinnamon oil was (+5°)-(+15 °). The values calculated for all samples were found to be in this range [16].

2.3.6. Gas chromatography

The results of the gas chromatography analysis are shown in Table 3.

Table 3. The content of the tea tree oil sample determined by GC-MS analysis.

Sample code	Component	Relative content (%)	Values given in TF 2017 (%) [16]
1	β-Pinene	4.40	
2	α-Terpinen	8.30	5.00–13.00
3	Limonene	3.60	0.50–4.00
4	1,8-Cineol	7.00	Max 15.00
5	γ-Terpinen	14.60	10.00–28.00
6	p-Cymen	5.80	0.50–12.00
7	α-Terpinolen	2.30	1.50–5.00
8	Terpinen-4-ol	40.20	Min 30.00
9	β-Terpineol	0.50	
10	α-Terpineol	5.50	1.50–0.80
11	γ-terpineol	1.10	
12	Viridifloren	0.50	
13	δ-Cadinen	0.60	
	Total	94.40	

2.4. Neroli Oil

2.4.1. Appearance

The clarity and viscosity of all samples were compatible; four of the five samples were colorless, while one sample was pale yellow. According to the monograph, the color of the oil was yellow or pale yellow. In this respect, only one sample was compatible in respect to this specification [16].

2.4.2. Thin layer chromatography

Pale blue fluorescent zones referring to methyl anthranilate zones below the zones of brownish red linalyl acetate were observed in the chromatograms of all samples. According to the pharmacopoeia criteria, neroli oil contains no bergapten; therefore, all of the samples were compatible. Reddish brown zones referring to linalool were also detected in the chromatogram of all samples. The intensity of the linalool zone was required to be lower than that of linalyl acetate. Four of the five samples were found to meet this criterion. Likewise, as indicated in the monograph, other unidentified, reddish brown zones were observed in the chromatograms of the samples (Figure 4) [16].

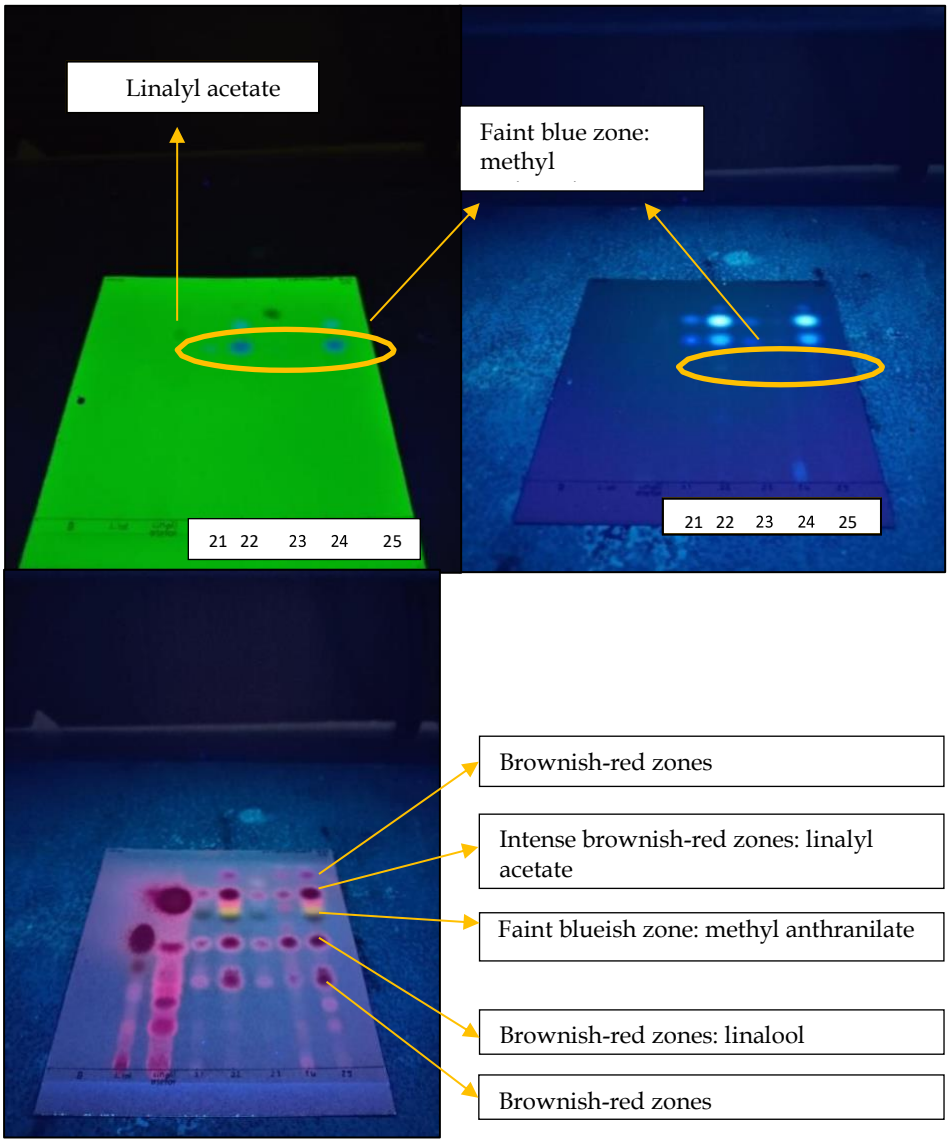

Figure 4. TLC chromatogram of neroli oil samples

2.4.3. Relative density

The reference range given in the monograph of cinnamon oil is 0.863–0.880, but none of the samples were found to be in the given range [16].

2.4.4. Refractive index

The reference range given in the monograph of cinnamon oil is 1.464–1.474. The refractive index values of four samples were found to be in the given range [16].

2.4.5. Optical rotation

The reference range given in the monograph of cinnamon oil was (1.5 °)-(+11.5 °). The specific optical rotation value of one sample was found to be out of the given range [16].

2.4.6. Acide Value

The maximum value given for the acid value in TF 2017 is 2.0. The acid values calculated for all samples were lower than this limit [16].

2.4.7. Gas chromatography

The results of the gas chromatography analysis showed that the terpinen-4-ol amount in the sample was higher than the maximum content given in the monograph (Table 4).

Table 4. The content of the neroli oil sample determined by GC-MS analysis.

Sample code	Component	Relative content (%)	Values given in TF 2017 (%) [16]
1	β-Pinene	9.40	7.00–17.00
2	δ-3-Carene	1.30	
3	Limonene	12.30	9.00–18.00
4	Linalool	36.40	28.00–44.00
5	Linalyl acetate	7.90	2.00–15.00
6	β-Caryophyllen	1.20	
7	α-Terpineol	7.60	2.00–5.50
8	α-Terpinyl acetate	3.20	
9	Neryl acetate	1.50	Max 2.50
10	Geranyl acetate	2.60	
11	Nerol	1.10	
12	Geraniol	1.60	
13	Phenylethyl alcohol	2.50	
14	Unidentified	1.00	
15	Caryophyllene oxide	0.70	
16	Nerolidol	1.60	1.00–5.00
	Total	91.90	

3. DISCUSSION

Herbal products are defined as " unprocessed or simply processed products of plant origin, that do not fall within the definition of plant by Turkish Republic Ministry of Health. The rising popularity of use of these products among the public increases the importance of ensuring the quality and standardization. In this context; collaboration between the Ministry of Agriculture and Forestry, which is responsible for the authorization and control of herbal products that are not included in the scope of medicine, and the Ministry of Health, which is responsible for the licensing of herbal medicines and traditional herbal medicinal products, ensures safe and controlled use by keeping the monographs and plant lists prepared for medicinal plants and up to date. The importance of ensuring proper use is also stated in the report of 2013-2023 Multi-Stakeholder Health Responsibility Development Program prepared by the Ministry of Health [41]. An important service for this goal will be provided by conducting analyses to assess the content of herbal products and raising public awareness through a variety of media.

In the content of this study, 25 essential oil samples were tested and the results were evaluated according to the criteria given in TF 2017 [16]. Among the seven samples of cinnamon bark oil, all the samples complied with the pharmacopoeia standards in terms of appearance and TLC analysis. The relative density of only one sample was found to be within the range given in the monograph. The refractive index of four samples and specific optical rotation of five samples were compatible. After the evaluation of these tests, one sample was selected for analysis by GC-MS, but the content of the sample did not meet the specifications.

Six clove bud oil samples were analyzed in the content of the study. In the monograph of the oil, it is stated that the oil is clear but brown when exposed to air [16]. According to this statement, the oil, which was found to be brown, was not kept in convenient conditions. All the zones that were required to be observed in the chromatograms of the samples were detected in TLC analyses. The solubility of the samples was as expected, but none of the samples met the qualifications in terms of relative density. The refractive index and specific optical rotation values were within the given ranges, and all of the samples were compatible in terms of fixed oil and resinous essential oil content. GC-MS analysis of the selected sample revealed that the content of the sample complied with the pharmacopoeia standards.

Among the five samples of neroli oil, only one sample was compatible in terms of appearance. TLC analyses revealed that none of the samples contained bergapten, which was the expected result for neroli oil. The other zones observed in the chromatograms were as described in the monograph. None of the samples was found to possess the relative density value given in the monograph, whereas five samples were suitable

in terms of refractive index. The acid values of the samples were found to be within the given range, but only a suitable value for optical rotation was calculated for only one sample. The GC-MS results of the tested sample showed a higher terpinen-4-ol content than expected.

The appearance of the seven tea tree oil samples tested were in accordance with the identification given in the monograph. All samples complied with the given specifications in terms of TLC analysis and optical rotation. The relative density of one sample was calculated as it was given in the monograph, whereas only one sample's refractive index was found to be out of the range. GC-MS results of the tested sample showed that the amount of terpinen-4-ol in the sample was higher than the maximum limit given in the monograph.

4. CONCLUSION

Herbal products to be used for medical purposes must meet certain specifications, especially for safety and effectiveness. In this respect, quality control of the finished product, control methods, and accepted limits determined by the necessary authorities are of great importance [42].

Turkish Pharmacopoeia 2017 is an important source for the determination of the quality of medicinal plants and herbal products. For the essential oil to be used for pharmaceutical purposes, it must be of pharmaceutical and therapeutic quality [29].

For this study, clove bud oil, cinnamon bark oil, tea tree oil, and neroli oil were selected as the subjects, and 25 samples were purchased from the market to be analyzed with reference to the Turkish Pharmacopoeia (2017). Among these samples, only two were found to comply with the pharmacopoeia in terms of all tests.

The results of the study showed that essential oils sold on the market claiming to be pure or completely natural do not actually meet the necessary criteria. This study, which shows the reality of abuse in this field, also reveals the importance of choosing reliable sources for the supply of essential oils and conducting the necessary analyses on the oils on the market. Universities and research laboratories also have a responsibility to carry out the necessary analyses on these products.

5. MATERIALS AND METHODS

5.1. Materials

For pharmacopoeia analyses, 7 cinnamon bark oil, 6 clove bud oil, 5 neroli oil, and 7 tea tree oil samples were purchased. The study involved 25 essential oil samples from nine different brands. In order not to affect the study results, brands and essential oil samples were coded before the analysis, and the analysis results were interpreted regardless of the brands. The essential oils selected for analysis within the scope of the study are given in Table 5.

Table 5. Essential oils and the number of samples selected for analysis.

Essential oil	Source of essential oil	Number of samples
Tea tree oil	*Melaleuca* sp.	7
Cinnamon bark oil	*Cinnamomum cassia* Blume (Syn. *C. aromaticum* Nees)	7
Clove bud oil	*Syzygium aromaticum* (L.) Merr. & L.M.Perry	6
Neroli oil	*Citrus aurantium* L. subsp. *aurantium* L.	5

5.2. Methods

Pharmacopoeia analyses of essential oil samples were conducted according to the monographs of the essential oils given in Turkish Pharmacopoeia 2017. The tests required to be performed for each essential oil group in the pharmacopoeia are listed in Table 6. In the analyses, a sample from each essential oil group that was found to be compatible with the pharmacopoeia standards was selected, and GC analysis of the sample was performed.

Table 6. Tests required to be conducted for each essential oil sample according to TF 2017

Tests	Tea tree oil	Cinnamon bark oil	Clove bud oil	Neroli oil
Volume and Page of the monograph in TF 2017	3/1887-1888	5/4199-4200	4/2803-2804	4/3406-3408
Appearance	+	+	+	+
Solubility	+	+	+	
Thin layer chromatography (TLC)	+	+	+	+
Gas chromatography (GC)	+	+	+	+
Relative Density	+	+	+	+
Refractive Index	+	+	+	+
Optical Rotation	+	+	+	+
Acid Value	+		+	+
Fatty Oils And Resinified Essential Oils			+	+
Solubility in Alcohol			+	

5.2.1. GC-MS Analysis

Device: Agilent 7890B GC 5977B Mass Selective Detector System
Stationary phase: HP Innowax column (60 m, 0.25 mm, 0.25 µm)
Temperature of the injection port: 250°C
Carrier gas: Helium
Carrier gas flow rate: 0.7 mL/min
Detector: Flame ionization detector (FID)
Electron ionization system: 70 eV
Ion source: 230°C, 35–450 m/z
Temperature of the detector: 250°C
Database: Wiley 9-Nist 11 Mass Spectral Database
The temperature program in the column is given in Table 7.

Table 7. Temperature program of the column for GC– MS analyzes.

Temperature (˚C)	Rate of Increase (˚C)	Time (per min)	Total time (min)
60	-	-	10
220	4	1	50
240	1	1	70
240	-	-	80

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