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Research Article

Chemical profiles of tea tree essential oil samples available on the Bulgarian market

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Abstract

Melaleuca alternifolia Cheel (Myrtaceae), indigenous to Australia, is considered one of the tea tree essential oil (EO) sources, which have seen increased global utilisation. The EO is available in both its pure form and as an active ingredient in various commercial products. It is recognised for its safety, natural composition, and efficacy. This study aims to evaluate whether tea tree EOs available in Bulgarian markets meet established standards. Commercial EO products were randomly chosen from the Bulgarian market and analysed using gas chromatography-mass spectrometry. The analysis revealed that all samples were predominantly composed of monoterpenes and sesquiterpenes, with terpinen-4-ol as the major constituent. Other significant compounds included *γ*-terpinene, eucalyptol, *α*-terpineol, and terpinolene. All commercial products examined contained the requisite chemical compounds, indicating compliance with the necessary standards, except for one sample, which did not meet the required percentage criteria.

Keywords

commercial products, essential oil, *Melaleuca*, tea tree

Introduction

Currently, there is a significant demand for natural products across various sectors such as healthcare, cosmetics, agriculture, and the food industry (Tsitlakidou et al. 2023). Consumers strongly associate a healthy lifestyle and the prevention of many medical conditions with natural products, prompting many companies to substitute synthetic compounds with their natural counterparts.

The observed trends are closely linked to the dynamics of the essential oils (EOs) market, which holds considerable significance.

Recent reports indicate that the global EOs market is projected to exceed a revenue of 40 billion by 2030, having already surpassed 20 billion annually (Grand View Research n.d.). The European EO market represents a significant share of the global one and has generated a revenue of more than 11 billion per year over the last years. Projections indicate that this figure could surpass 20 billion annually by 2030 (Grand View Research n.d.). The European market of EOs is associated with several important key points, including various directives and regulations, the safety of EOs, lasting traditions in high-class cosmetics production, industry growth, and high levels of consumption of EOs in the agriculture, food, cosmetics, and pharmaceutical industries.

One of the most important EOs for the industry is tea tree oil. The commercially important sources for the production of tea tree EO are the leaves and the terminal branches of *Melaleuca alternifolia* Cheel, *Melaleuca linariifolia* Smith, *Melaleuca dissitiflora* F. Mueller (Table 1) and other *Melaleuca* species (Baskorowati et al. 2010; EMA 2017). These species originate from Australia and belong to the genus Melaleuca (Myrtaceae). The name "Melaleuca" originates from the Greek language and is composed of two words: "melas"—black/dark, and "leucon"—white. The genus includes about 300 species (Brophy et al. 2013). In general, species belonging to Melaleuca range from woody, multi-stemmed shrubs to high trees. However, most of the species are shrubs or small trees (high < 10 m). Forty species belonging to the genus are not more than one metre tall.

The production process of tea tree EO involves the passage of a current of steam through fresh leaves and terminal branches of the described plants. *Melaleuca alternifolia* Cheel is cultivated for the commercial extraction of tea tree EO, not only in its indigenous region of Australia but also in various other nations, including China. Australia remains the primary producer of tea tree essential oil, with an annual output ranging from 450 to 500 tonnes.

The first standard for tea tree EOs was published in 1967 by the Standards Association of Australia and was called "Oil of *Melaleuca altenifolia*". According to this standard, the cineole must be less than 15%, and terpinen-4-ol should be more than 30% (Williams and Lusunzi 1994).

Currently, several documents/standards exist about the quality of tea tree EOs: Standard International Organisation for Standardisation (ISO) 4730:2017, Technical Corrigendum 1:1997 to ISO 4730:2005, Australian Standard AS 2782-1997: Oil of Melaleuca, terpinen-4-ol type (Tea Tree oil), French Standard T75-358, the European Pharmacopoeia, the British Pharmacopoeia, Aetheroleum Melaleucae Alternifoliae (Organisation 1999; Council of Europe 2020), and others. However, the ISO Standard ISO 4730:2017 is regarded as an international reference for the quality of tea tree EOs (ISO 2017).Tea tree EO is currently incorporated into a wide range of products aimed at skin, hair, and oral hygiene. Additionally, this essential oil serves several non-cosmetic functions, such as acting as a fragrance component in household cleaning agents and providing protection for plants, among other uses.

In the last two decades, many concerns about the safety of the tea tree EO were discussed. The Scientific Committee on Consumer Products (SCCP) assessed the safety of the tea tree EOs in 2004 and 2008. It was established that the EO and products containing the EO could provoke contact allergy, skin and eye irritation and other adverse reactions. However, the full safety profile was not evaluated in this period. In 2024, the European Risk Assessment Committee of ECHA classified the EO as 'Reprotoxic of Category 1B (H360Fd) (European Commission n.d.).

Tea tree oil appears as a colourless to pale yellow liquid, characterised by a distinct, sharp, camphor-like aroma and a cooling sensation reminiscent of menthol (Yadav et al. 2017). This essential oil is composed of nearly 100 different constituents, predominantly including monoterpenes, sesquiterpenes, and their alcohol derivatives. The quality of tea tree oil is largely determined by its chemical composition, with terpinen-4-ol being the primary active ingredient; however, a high concentration of 1,8-cineole is considered unfavourable due to its potential irritant effects. It is used in various commercial products like gels, soaps, and lotions (Borotová et al. 2022; Gvozdeva and Staynova 2025) but has limited administration routes due to potential toxicity and skin sensitivity. Other major chemical compounds are γ-terpinene, 1,8-cineole, and p-cymene (Borotová et al. 2022). It exhibits various biological properties and applications, as shown in Fig. 1 (Carson et al. 2006; Yadav et al. 2017).

Aim

The present study aims to determine whether tea tree EOs available in Bulgarian markets meet the required standards.

Materials and methods

Chemicals and reagents

Heptane (99%), octane (≥99%), nonane (99%), decane (≥99%), undecane (≥99%), dodecane (99%), tridecane (≥99%) and tetradecane (≥99%) were purchased from Merck KGaA (Darmstadt, Germany). Hexane (HPLC grade) was purchased from Thermo Fisher Scientific GmbH (Bremen, Germany). Toluene (HPLC grade) was purchased from Honeywell, Charlotte, North Carolina, USA.

Table 1. Important *Melaleuca* species for tea tree essential oil production.

Plant species	Description	Climate and soil type	Ref.
Melaleuca alternifolia	Small tree, 6-10 m in height. It has a distinctive white/creamy white, papery bark and a dense canopy. The leaves are arranged in alternating pairs.	Warm sub- humid climate. These species grow in different soil types, including alluvial silty loams oils, and sandy loams.	(Kasujja 2021, Brophy et al. 2013)
Melaleuca linariifolia	Shrub with a height of 1.5–4 m, with hard, grey, flaking bark. Branchlets are glabrescent and pubescent. Leaves are short-petiolate and alternate. Inflorescences are spicate and interstitial.	Grow in scrub, river flats, ridges, dry forests, rocky creeks, and summits on skeletal soil over porphyry, sandstone, and granite.	(Brophy et al. 2013)
Melaleuca dissitiflora	Shrub or tree 1–5 m in height with rough, grey bark. Branchlets rapidly glabrescent. Leaves alternate, short-petiolate. Inflorescences are spicate, pseudo terminal, or interstitial.	Grow in stony creek beds, rocky gullies, gorges, dry watercourses, sand, and alluvial soil.	(Brophy et al. 2013)

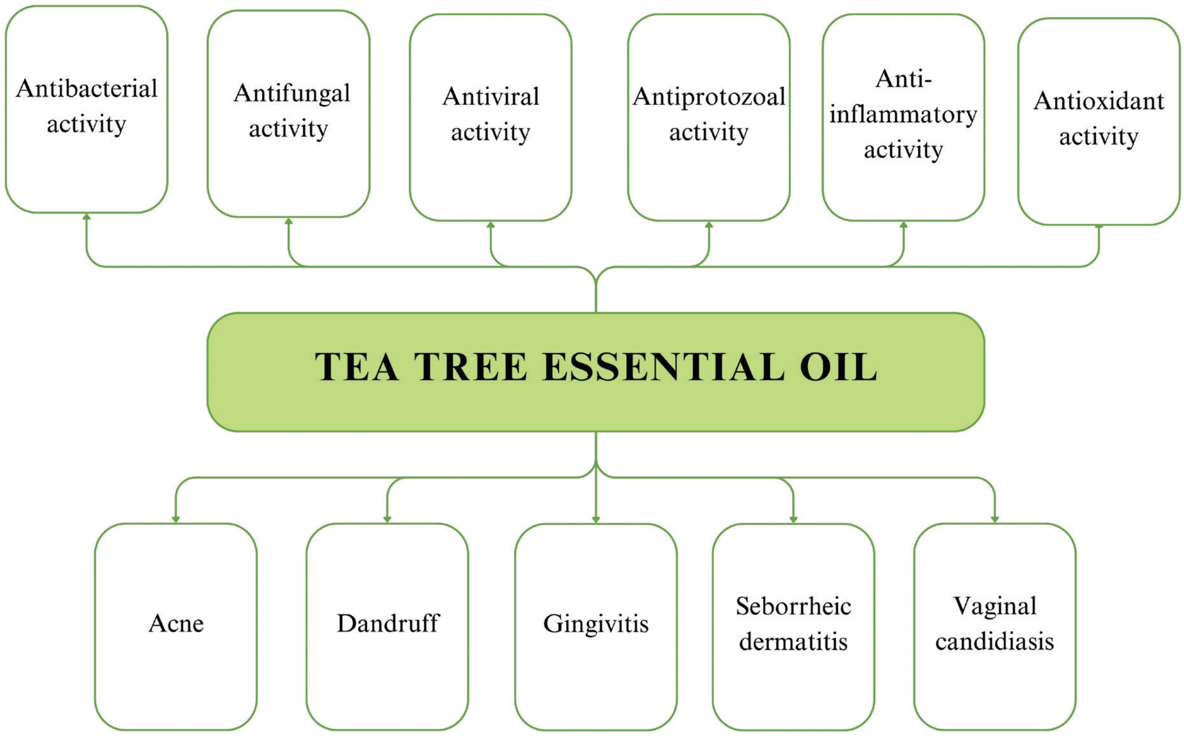

Figure 1. Biological activities and applications of tea tree EO.

Sample preparation

A total of nine commercial products labelled as containing tea tree EO were randomly purchased from pharmacies and drugstores in Bulgaria. Some of the commercial products were labelled to contain *Melaleuca alternifolia* leaf oil; the rest were only to contain tea tree EO. The commercial products are labelled as S1 to S9 through the text. Before gas chromatography-mass spectrometry analysis, the EOs were diluted in hexane to a concentration of 2%.

Physicochemical analyses

Density measurements

The density bottle was carefully cleaned and then rinsed with ethanol and acetone, followed by drying. It was thermally equilibrated and left in the weighing cabinet for 30 minutes. For the weighing of distilled water, the bottle was filled with distilled water and immersed in a water bath (20 °C) for 30 minutes. Once thermal equilibrium was achieved, the filled bottle was weighed. For the weighing of EOs, the pycnometer was emptied, washed, and dried as previously described. The procedure was performed as described for distilled water. For each sample, an average of three independent measurements was recorded.

Refractive index measurements

The refractive indices of the tested samples were determined using an Abbe refractometer (Carl Zeiss, model ORT 1RS, Jena, Switzerland), with a measurement error of n_D ± 0.0003 units. The calibration of the instrument was conducted by measuring the n_D values of distilled water (n_D = 1.3330) and toluene (n_D = 1.4969), whose refractive indices are well-known (Lechner 2008). The samples were directly introduced into the instrument's prism assembly using a syringe. For each sample, an average of three measurements was recorded.

Chromatographic conditions

Gas chromatography-mass spectrometry (GC-MS) was employed for the analysis, utilising a Bruker Scion 436-GC SQ MS system (Bremen, Germany). The separation was performed on a Bruker BR-5 ms fused silica capillary column with dimensions of 30 m × 0.25 mm internal diameter and a film thickness of 0.25 µm. The oven temperature was initially held at 70 °C for 1 min and then increased to 80 °C at 1.5 °C/min, subsequently increased to 160 °C at 15 °C/min, then increased to 175 °C at 3 °C/min, and after that increased to 220 °C at 30 °C/min and held for 1 min. Helium was the carrier gas with a flow rate of 1 mL/min. The injector split ratio was 1:60 with the injection volume – 1 µL. The range of *m*/*z* was 50–450 in the full-scan mode. To compare the spectral data and retention indices of compounds, the Wiley NIST11 Mass Spectral Library (NIST11/2011/EPA/NIH, Adams' book for retention indices and the literature data were used. Retention index values were calculated and compared with reported values for a C_7–C_{20} series of *n*-alkane standards (Linstrom 1997; Adams 2007).

Results and discussion

The first step in the study was to determine whether the examined EOs met the physicochemical requirements (appearance, colour, relative density and refractive index) according to the ISO standard and the European Pharmacopoeia (Ph. Eur.), shown in Table 2.

Table 2. Characters and physical-chemical requirements for the Melaleuca EO, terpinen-4-ol type, where ✓ means meets the requirements and × means does not meet the requirements.

Characteristics	S1	S2	S3	S4	S5	S6	S7	S8	S9
Appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓
Colour	✓	✓	✓	✓	✓	✓	✓	✓	✓
Relative density	✓	✓	✓	✓	✓	✓	✓	✓	×
Refractive index	✓	✓	✓	✓	✓	✓	✓	✓	×

The obtained results provide an initial confirmation for establishing the physicochemical parameters of applicable products. The mean value for each of the samples was calculated with the relative standard deviation (RSD) remaining below 2%. Additionally, the physicochemical characteristics of the analysed S1–S8 comply with the requirements of ISO standards (ISO 2017) as well as those of the European Pharmacopoeia (Council of Europe 2020), while S9 did not meet the criteria about relative density and refractive index.

The subsequent step involved comparing the chemical profile of the analysed products with the standards outlined by ISO and the European Pharmacopoeia (Council of Europe 2020; ISO 2017). The chromatograms obtained from the analysis of each EO sample are presented in Fig. 2, while the identified chemical constituents are detailed in Table 3.

The GC-MS analysis revealed that the total concentration of identified components ranged from 98.23% to 99.98%. Among the various terpenes, monoterpene hydrocarbons constituted the largest proportion across all samples. Furthermore, all commercial products were classified under the terpinen-4-ol chemotype, as this compound was found in the highest concentration.

Upon reviewing the chemical profiles of the tested tea tree EO samples and comparing them to the ISO 4730:2004 standards for tea tree EO, the results show a generally high degree of compliance, although some deviations were noted (Council of Europe 2020; ISO 2017). *α*-Pinene content was within the ISO range in all samples, ensuring conformity and confirming that this compound is present at acceptable levels in the commercial products. Sabinene was not detected in S3, S7 and S9. *α*-Terpinene was present within the ISO range in all products, indicating that the samples meet the ISO requirement for this important compound. Limonene levels were observed to exceed the ISO limit in some samples (S4 and S5), where concentrations were 2.18% and 2.21%, respectively. However, the observed levels slightly exceed the ISO requirement, suggesting a need for careful monitoring of this compound in future formulations. 1,8-cineole (eucalyptol) exceeded the ISO limit, particularly in sample S9, which had 19.34%. Moreover, eucalyptol is considered a skin sensitiser, and its levels in cosmetics should be monitored (Api et al. 2022).

This significant deviation raises concerns and suggests that further analysis or adjustments to product formulations may be necessary to ensure consistency with ISO specifications. *γ*-Terpinene content was within the ISO range in all samples, confirming that the product formulations meet the expected levels of this major component. *p*-Cymene was found within the accepted ISO range, ensuring that all samples comply with this requirement. Terpinolene levels were consistent with the ISO range, except for sample S5, where it fell below the required level (1.35%). This deviation suggests that product formulation in S5 may require adjustments to maintain consistency with ISO standards. Terpinen-4-ol, which is a critical marker for tea tree oil, was above the required minimum in all samples, except in S9, where it was recorded at 27.31%. This result is below the acceptable ISO minimum, highlighting the need for attention to the terpinen-4-ol concentration in sample S9. Aromadendrene, ledene, and other sesquiterpenes such as *δ*-cadinene and globulol were all within the ISO specifications, with only slight variations observed in certain samples. These minor deviations are within acceptable limits and do not significantly affect overall compliance. Additionally, some of the products were labelled as containing linalool,

Table 3. Percentage chemical composition of tested samples from commercial products according to requirements.

№	Compound	RI*	Terpene classes	Molecular formula	S1 (%)	S2 (%)	S3 (%)	S4 (%)	S5 (%)	S6 (%)	S7 (%)	S8 (%)	S9 (%)
1	*α*-Pinene	920	MH	$C_{10}H_{16}$	2.810	2.560	3.105	3.547	3.424	4.120	4.450	2.957	4.076
2	Sabinene	940	MH	$C_{10}H_{16}$	0.158	0.150	ND	0.198	0.145	0.143	ND	0.149	ND
3	*α*-Terpinene	1011	MH	$C_{10}H_{16}$	11.263	11.850	12.251	11.468	11.478	12.065	10.005	12.255	9.793
4	*p*-Cymene	1029	MH	$C_{10}H_{14}$	3.985	3.311	4.848	4.978	4.494	6.255	8.260	3.522	5.262
5	Limonene	1039	MH	$C_{10}H_{16}$	0.871	0.837	1.297	2.184	2.213	2.952	2.956	0.979	3.926
6	1,8-Cineole (eucalyptol)	1044	MO	$C_{10}H_{18}O$	2.740	3.395	4.229	3.120	2.774	3.218	2.422	2.421	19.339
7	*γ*-Terpinene	1065	MH	$C_{10}H_{18}O$	18.952	18.462	21.163	20.104	22.109	20.494	19.241	20.920	17.553
8	Terpinolene	1095	MH	$C_{10}H_{16}$	3.964	4.058	4.453	3.567	1.348	3.679	3.077	4.237	2.163
9	Terpinen-4-ol	1175	MO	$C_{10}H_{18}O$	34.616	32.524	33.919	33.735	33.506	33.344	35.138	33.944	27.309
10	*α*-Terpineol	1186	MO	$C_{10}H_{18}O$	4.003	3.475	3.616	5.460	5.578	7.416	6.719	3.771	6.027
11	Aromadendrene	1428	SH	$C_{15}H_{24}$	0.171	0.156	0.282	0.219	2.619	2.852	2.548	1.257	2.149
12	Ledene (syn. Viridiflorene)	1440	SH	$C_{15}H_{24}$	1.493	1.869	1.179	1.297	1.366	0.624	0.917	1.079	0.430
13	*δ*-Cadinene	1509	SH	$C_{15}H_{24}$	1.642	1.920	0.213	0.996	1.074	ND	ND	0.263	ND
14	Globulol	1560	SO	$C_{15}H_{26}O$	0.635	0.804	0.205	0.435	0.468	0.411	0.368	0.292	0.277
15	Viridiflorol	1579	SO	$C_{15}H_{26}O$	0.656	0.737	ND	0.315	0.331	ND	ND	ND	ND

*RI – obtained retention indices; the percent of total is presented as mean values from three independent measurements; the RSD does not exceed 2% for each sample.

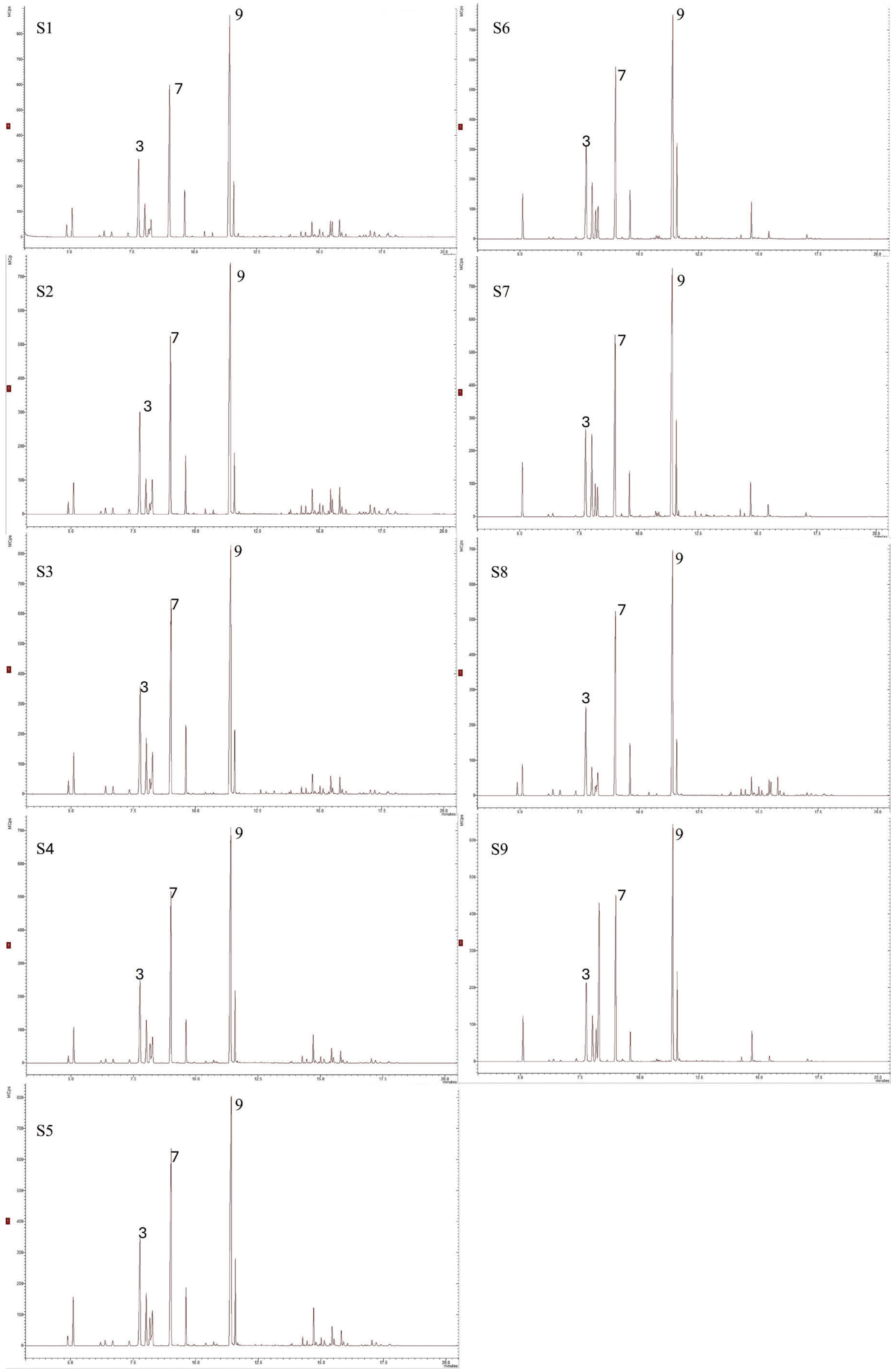

Figure 2. Chromatograms of analysed commercial products: 3 – α-terpinene, 7 – γ-terpinene and 9 – terpinen-4-ol.

limonene, and geraniol as components of the essential oil; they were detected and confirmed in the respective samples (ISO 2017).

Most of the tested commercial products met the ISO standards for tea tree EO; several samples displayed deviations in certain components like limonene, 1,8-cineole, and terpinen-4-ol, which require attention. These findings highlight the importance of quality control and the need for regular monitoring of tea tree essential oil compositions to ensure ongoing compliance with ISO standards (ISO 2017).

Moreover, the analysis revealed that most of the tested products meet the requirements set by the European Pharmacopoeia for their chemical composition, with a few exceptions. The individual compounds in the tea tree EOs were compared against the stipulated ranges provided by the Ph. Eur. The levels of α-pinene in all samples (ranging from 2.56% to 4.45%) were within the required range of 1.0–6.0%. Thus, all products comply with the European Pharmacopoeia standards for this compound. The concentrations of α-terpinene in all samples (ranging from 9.79% to 12.26%) fall within the required 5.0–13.0% range. As such, the results follow the specified limits. The levels of limonene in some of the samples exceeded the upper limit of 4.0% set by the pharmacopoeia. Specifically, sample S6 (2.95%) and S9 (3.93%) were within the permissible range, but other samples exceeded the upper threshold. Consequently, there is partial non-compliance regarding limonene. Regarding 1,8-cineole, an exceptional variation is demonstrated in the samples, with S9 exceeding the upper limit of 15.0% (19.34%). Other samples, however, maintained concentrations within the acceptable range (2.42% to 4.23%). The significant deviation in S9 is a cause for concern regarding its compliance. The levels of γ-terpinene in all the tested samples (ranging from 17.55% to 22.11%) are well within the required range of 10.0–28.0%. Thus, the samples meet the pharmacopoeia standard for this compound. The concentration of p-cymene in all the samples (ranging from 3.31% to 8.26%) followed the European Pharmacopoeia's range of 0.5–12.0%, confirming conformity. While most samples (S1 to S4, S6 to S9) had concentrations within the required range (1.5–5.0%) for terpinolene, sample S5 (1.35%) was below the minimum threshold. Therefore, some samples do not fully comply with the prescribed limits for this compound. All samples exceeded the minimum required concentration of 30.0% for terpinen-4-ol, with values ranging from 27.31% to 35.14%. Despite sample S9 being slightly below the minimum limit (27.31%), most samples complied, thereby ensuring that the terpinen-4-ol content in most products meets the standard. The α-terpineol content in all samples (ranging from 3.47% to 7.42%) was in line with the pharmacopoeia's required range of 1.5–8.0%. As a result, the samples comply with the standards for this compound (Council of Europe 2020).

Most tested samples comply with both Ph. Eur. and ISO standards. However, sample S9 shows deviations, particularly in 1,8-cineole (exceeding the ISO limit) and terpinen-4-ol (falling below the Ph. Eur. minimum). Additionally, some samples slightly exceed the ISO limits for limonene, α-terpineol, and aromadendrene, but they generally conform to Ph. Eur. standards. Furthermore, not all the tested samples are described as meeting the ISO standards, but despite this, the majority comply with these requirements (Council of Europe 2020; ISO 2017). Further studies could explore additional factors such as storage conditions, shelf life, and potential adulteration to provide a more comprehensive assessment of tea tree EO quality in commercial markets.

Although EOs may comply with ISO standards and pharmacopoeia requirements and possess diverse applications, they have the potential to induce adverse effects, including skin irritation, allergic reactions, inflammation, and dermatitis (Sanajou et al. 2024). Given the increasing interest in natural remedies, ongoing research is imperative to facilitate informed and safe incorporation into routine health and skincare practices.

Conclusion

The GC-MS profiling of tea tree EOs available on the Bulgarian market revealed that most of the tested commercial products meet established international quality standards, including ISO 4730:2017 and the European Pharmacopoeia. All analysed samples predominantly contained monoterpenes and sesquiterpenes, with terpinen-4-ol as the major constituent—aligning with standard requirements for therapeutic-grade tea tree EO. However, one sample exhibited deviations in both physicochemical and chemical composition parameters, indicating potential quality inconsistencies in certain market offerings. These findings highlight the overall compliance of tea tree EOs with regulatory standards but also underscore the importance of continuous quality control to ensure product authenticity and consumer safety.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

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Author contributions

Conceptualization, K.I., V.T., N.K., D. G-K. and S.I.; methodology, K.I., V.T. and S.I.; software, K.I.; investigation, K.I., N.K. and D. G-K.; writing –original draft preparation, K.I., V.T. and S.I.;

writing – review and editing K.I., V.T. and S.I.; visualization K.I.; supervision, K.I. and S.I.

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Data availability

All of the data that support the findings of this study are available in the main text.

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