PROSPECTS
IN PHARMACEUTICAL SCIENCES

Prospects in Pharmaceutical Sciences, 24(3), 45-53
https://prospects.wum.edu.pl/

Review Article

PROBLEMS AND CHALLENGES IN THE DETECTION OF ESSENTIAL OIL ADULTERATION – A REVIEW OF CURRENT ANALYTICAL METHODS

Oliwia Szambora[1], Katarzyna Paradowska[*1]

[1] *Department of Organic and Physical Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland.*

* Correspondence, e-mail: katarzyna.paradowska@wum.edu.pl

Received: 01.01.2026 / Revised: 15.03.2026 / Accepted: 06.04.2026 / Published: 23.08.2026

ABSTRACT

Essential oils are volatile, aromatic plant metabolites with a complex chemical composition and a wide range of applications in the pharmaceutical, cosmetic, and food industries. Due to their high market value and the limited availability of certain raw materials, essential oils are susceptible to numerous forms of adulteration, such as dilution with cheaper substances, the addition of synthetic compounds, or substitution of the botanical source. Quality control and authenticity assessment of essential oils require advanced analytical methods. The European Pharmacopoeia recommends basic physicochemical determinations, including relative density and refractive index. Gas chromatography coupled with mass spectrometry (GC–MS) represents the gold standard for the analysis of volatile constituents of essential oils, enabling both identification and quantitative evaluation of terpene compounds. High-performance liquid chromatography (HPLC) allows for the determination of non-volatile and thermolabile compounds, which is particularly important in citrus oils. Nuclear magnetic resonance (NMR) spectroscopy offers a rapid, non-destructive method for authenticity verification and impurity detection, despite its lower sensitivity compared to chromatographic techniques. Contemporary approaches to essential oil quality control are based on the combination of complementary analytical techniques, enabling comprehensive chemical characterization and effective detection of adulteration.

KEYWORDS: essential oils, quality control, GC–MS, HPLC, NMR

1. Introduction

The European Pharmacopoeia defines an essential oil as a “fragrant product, usually of complex composition, obtained from a botanically defined plant raw material by steam distillation, dry distillation, or an appropriate mechanical process without heating” [1]. Chemically, essential oils (EOs) constitute a group of volatile, aromatic secondary plant metabolites, i.e., mixtures of terpenoid, phenolic, and phenylpropanoid-derived compounds. They differ in chemical composition and biological properties; however, common features of all essential oils include volatility, lipophilicity, and a characteristic odor specific to each oil. The biological properties exhibited by a given essential oil depend directly on its chemical composition. Among the most frequently reported and most significant activities are antimicrobial, anti-inflammatory, sedative, skin-irritating, and expectorant effects [2-4].

Many plant species synthesize essential oils, however, only some of them constitute essential-oil-bearing raw materials used for their industrial extraction. A typical example of an essential-oil plant is *Lavandula angustifolia*.

Essential oils are used in many industrial sectors, including the cosmetic, pharmaceutical, and food industries. For example, peppermint oil is used for gastrointestinal disorders, such as irritable bowel syndrome, and exhibits antispasmodic and analgesic effects [5]. Lavender oil, due to its calming, anxiolytic, and sleep-improving properties, is used in the treatment of anxiety and insomnia [6]. Tea tree oil, because of its antiseptic and antibacterial activity, is applied in dermatological formulations such as creams and gels [7]. For the treatment of upper respiratory tract diseases, eucalyptus oil is used, offering expectorant, anti-inflammatory, and breathing-facilitating effects [8]. Bitter orange oil, due to its sedative properties, is used in the treatment of insomnia, while citrus oils are commonly employed as flavoring agents in the food industry [9]. Bergamot oil has found application as a preservative in food and cosmetics, a flavoring component of teas, and an ingredient in perfume production [10].

Due to their wide range of applications and natural origin, essential oils attract considerable scientific and

commercial interest. Nevertheless, the limited availability of certain plant raw materials, such as agarwood, combined with variability in chemical composition resulting from differences in extraction efficiency, environmental conditions, and geographical origin, often leads to high market prices. As a consequence, adulteration has become a common problem in this sector [11, 12].

Owing to the various adulteration techniques and the inherent variability in the quantitative composition of essential oils of a given species – resulting from the plant part used (e.g., flowers, leaves), harvest time, geographical location, genetic factors, and vegetation phase, an oil may fail to meet the requirements imposed by legal regulations or standards recognized by pharmacopoeias [12, 13]. Therefore, quality control of essential oils is crucial, both in terms of ensuring safe use and confirming authenticity. Authenticity can be understood as an essential oil free from foreign bodies and substances, as well as the absence of contamination in the raw material from which it is obtained [11, 12]. This article presents a review of analytical techniques used in the quality control of essential oils.

2. Methods of Essential Oil Adulteration

Essential oils are widely used in many branches of industry; therefore, analysis of their composition is crucial to ensure appropriate quality. The composition of an essential oil is influenced by both internal factors, as described above, and external factors associated with adulteration. In recent years, the problem of essential oil adulteration has attracted considerable research attention, due both to safety concerns and the growing challenge of detecting adulteration. Such practices not only negatively affect economic aspects but also pose risks to consumers. A composition different from that declared may result in allergic or toxic reactions. Therefore, the possibility of rapid and accurate qualitative verification of essential oils is of great importance [11, 12, 14].

Currently, four main types of adulteration are encountered on the market. One involves dilution of the obtained essential oil with vegetable oils (VO). This type of adulteration is particularly difficult to detect due to the lack of changes in physicochemical properties of essential oils, such as color, refractive index or density; however, the aroma of the original oil may be diminished. Due to similar chemical compositions, especially with respect to volatile compounds, gas chromatography (GC) does not always allow detection of vegetable oils addition. Dilution with vegetable oils is a common practice because of its low cost, availability, and the difficulty of detection using standard analytical methods [14, 15]. Therefore, in the case of such adulterations, infrared (IR) spectroscopy combined with multivariate analysis is most commonly employed. This approach allows the detection and quantitative determination of contaminating substances, but it does not enable their precise identification. This limitation arises primarily from the fact that vegetable oils consist mainly of triglycerides, which differ only in the relative composition of individual fatty acids. However, their absorbance in IR spectra is almost identical, resulting in overlapping signals. Consequently, it is possible to confirm the botanical origin of the oil, but not to identify the individual components. This challenge can be addressed by nuclear magnetic resonance (NMR) spectroscopy, which is based on the detection of the electron cloud surrounding atomic nuclei, which differs among specific atoms and molecules. NMR allows the bypassing of complex multivariate analyses: if the target signals do not overlap and are not directly assigned, integration of the relevant peaks enables rapid and precise qualitative and quantitative analysis of the sample, without the need for elaborate data manipulation [15].

Another common method involves the addition of a cheaper oil from the same species or co-extraction with oil obtained from a different part of the plant or from a different geographical origin. Due to very similar qualitative compositions and physicochemical properties, this type of adulteration is again difficult to detect. Particularly important, however, are differences in quantitative composition, which determine the biological activity of a given oil. Similar problems arise with the addition of cheaper essential oils from closely related species, which often represent more affordable and readily available plant raw materials, such as the addition of lavandin oil to lavender oil. The last of the most common adulteration methods is the addition of synthetic or natural compounds aimed at obtaining a desired - often superior to the original - terpene composition, thereby increasing the market value of the essential oil [11, 12, 14, 15].

One of the essential oils most frequently subjected to adulteration — either by dilution with essential oils from other species or by the addition of synthetic compounds — is lavender essential oil, *Lavandula angustifolia* Mill. (LEO). Non-volatile compounds, such as glycols, benzyl alcohol, or vitamin E, are often added, which are undetectable by GC methods. In such cases, high-performance liquid chromatography (HPLC) is recommended, and its application in these scenarios is discussed in more detail in the following chapter. LEO is also commonly adulterated with racemic, synthetic linalool and linalyl acetate, which are among its major constituents. In these instances, enantioselective GC can be employed. Beale et al. described a method for the rapid quality assessment of LEO based on chemometric analysis of both targeted and untargeted GC-MS data [16, 17].

Adulteration of essential oils represents a complex and challenging issue in the context of qualitative analysis. Such analyses often require the combination of multiple analytical techniques, and even then, they do not always allow for precise identification of contaminating substances. Therefore, in terms of ensuring the safe use of essential oils, it is essential to conduct comprehensive, multi-step analyses employing a variety of, often sophisticated, analytical methods.

3. Qualitative Analysis of Essential Oils

Quality control of essential oils aims to confirm their compliance with pharmacopeial monographs or legal standards. However, not only the aforementioned adulterations may negatively affect analytical evaluation. Deviations from standard values may also result from improper storage, temperature fluctuations, packaging type, or aging processes of the oil [11, 18,19].

Although the Pharmacopeia has fundamental regulatory and standardization significance in the context of pharmacy, essential oils are also subject to other regulations. One of the most important quality and standardization benchmarks are the standards for essential oils set by the International Organization for Standardization. The ISO 9235 document defines essential oils as "product obtained from a natural raw material of plant origin, by steam distillation, by mechanical processes from the epicarp of citrus fruits or by dry distillation, after separation of the aqueous phase - if any - by physical processes." The ISO 11024 standard designates gas chromatography as the flagship method for identifying the composition of essential oils. Additionally, ISO standards specify the identification of synthetic additives, maximum concentrations of individual components, chromatographic profiles of specific essential oils, and guidelines for them [20, 21].

The use of essential oils in cosmetics is regulated by Regulation of the European Parliament and of the Council of 30 November 2009 on cosmetic products. This regulation requires the preparation of a cosmetic product safety report, adherence to Good Manufacturing Practice (GMP), notification of the product through the EU Cosmetic Products Notification Portal (CPNP), and prohibits testing cosmetics on animals [22].

Legal acts and the Pharmacopeia also require the control of contaminants in essential oils, including, among others, heavy metal content (Pb, Cd, Hg, As), solvent residues, and pesticides. The table below summarizes the most important legal acts in force within the European Union that specify limits for these contaminants depending on the industrial sector (Table 1) [23-34].

The Pharmacopeia contains both general monographs on essential oils as well as specific monographs for particular EOs. These include numerous identification tests and purity studies. The European Pharmacopoeia recommends determination of relative density, optical rotation, refractive index, acid value, and acid value after acetylation for the identification and quality assessment of essential oils [1, 35]. The Pharmacopeia also describes tests for detecting the presence of contaminants or adulterations, such as foreign esters, fatty oils, or resinous fractions of essential oils. In addition, quality assessment also includes organoleptic evaluation (odor and taste) and determination of solvent residues. For identification and composition profiling, gas chromatography, thin-layer chromatography, and high-performance thin-layer chromatography (HPTLC) are recommended [1, 36].

Specific gravity compares the density of the sample with that of a reference, verifying oil identity and purity, with adulteration causing unacceptable deviations. Determination of the refractive index involves passing light through a thin layer of the sample between agate prisms. Each essential oil refracts light in a characteristic manner due to its unique chemical composition, and comparison with reference values allows assessment of identity and purity — dilution results in unacceptable discrepancies. In IR spectroscopy, infrared radiation excites chemical bonds in the sample, inducing vibrations with

Table 1. Regulatory limits and analytical standards for selected contaminants (heavy metals, pesticides, residual solvents, phthalates and PAHs)

Type of Contaminant	Standard/ Regulation	Permissible Limit (example)	Notes
Heavy metals (Pb, Cd, Hg, As)	European Pharmacopoeia 11.0 - Heavy metals test	Pb ≤ 10 ppm, Cd ≤ 1 ppm, Hg ≤ 0.1 ppm, As ≤ 1 ppm	Applies to pharmaceutical raw materials; analytical methods: ICP-MS, AAS
	Regulation (EC) No 1881/2006	Depends on food product	Applies to food flavorings
	REACH Regulation	No global limit, registration required	Applies to chemicals in the EU
Pesticides	Regulation (EC) No 396/2005	Depends on pesticide (e.g., 0.01-0.1 mg/kg)	Applies to plant raw materials and food flavorings
	ISO 22000 / HACCP	No numerical limit, monitoring required	Good manufacturing practices
Residual solvents	European Pharmacopoeia 11.0 - Residual solvents	Class 1: prohibited; Class 2: e.g., methanol ≤ 3000 ppm, acetone ≤ 5000 ppm	Applies to pharmaceutical raw materials
	ICH Q3C	Aligned with Ph. Eur.; limits for volatile solvents	International standard for medicinal products
Phthalates (from packaging/ migration)	Directive 2005/84/EC	DEHP, DBP, BBP ≤ 0.1% in children's articles	Relevant for contact with cosmetics and food
	REACH - Substances of Very High Concern	Restricted or banned substances	Monitoring required in raw materials
PAHs (polycyclic aromatic hydrocarbons)	European Pharmacopoeia 11.0 - Vegetable fatty oils	8 PAHs ≤ 10 µg/kg in vegetable oils	Applies to pharmaceutical and cosmetic raw materials
	ISO 21461	No numerical limit, defined analytical methods	Detection by GC-MS / HPLC
	CLP Regulation (EC 1272/2008)	CMR classification - labeling required	Carcinogenic, mutagenic, toxic substances

a unique frequency distribution that constitutes a material "fingerprint." Comparison of a spectrum of the sample with a standard enables identity verification and detection of adulteration [35]. Analytical methods used to study the composition of essential oils include gas chromatography (GC), high-performance liquid chromatography (HPLC), and nuclear magnetic resonance (NMR).

3.1. Gas Chromatography (GC)

Gas chromatography (GC) is a highly sensitive, accurate, and selective analytical method used for the analysis of complex volatile organic compounds [37, 38, 39]. When coupled with MS or FID detectors, it is the most commonly chosen technique for studying chemical composition, assessing quality, authenticity, and originality of EOs [38, 39, 40]. The use of conventional GC columns provides high separation efficiency for volatile compounds and allows their identification based on mass spectra. Essential oils often contain analogs, isomers, or homologs of compounds belonging to different chemical classes. These compounds have similar structures and physical properties, which can lead to co-elution and complicate the separation of mixtures using conventional columns. For this reason, columns with orthogonal stationary phase selectivity are frequently employed in essential oil analysis, enabling the examination of the same sample on columns of different polarity, thereby improving the reliability of compound identification. In more complex cases, techniques with higher separation capacity, such as two-dimensional gas chromatography (GC×GC) or chiral columns, are also used, allowing the separation of terpene enantiomers and a more detailed characterization of essential oil composition [38, 41, 42].

Many studies also focus on reducing analysis time by using shorter columns, lowering outlet pressure, faster oven heating, etc. For example, studies conducted by Tranchida et al. employed GC-MS with a triple quadrupole, enabling shortened analysis of citrus EOs [43]. However, there is still an insufficient number of samples, particularly for more complex analytes, to fully validate and assess improvements in GC-MS techniques aimed at reducing analysis time [39]. Nevertheless, GC-MS remains the "gold standard" for essential oil composition analysis.

Shi et al., based on GC and GC-MS analyses, reported for the first time the chemical composition of essential oil from the roots of *Seseli mairei* H. Wolff, identifying 37 components accounting for 97.84% of the total oil [44]. Thabet et al., by comparing retention indices and data with the NIST Mass Spectral Library, determined and compared the composition of oils obtained from different vegetative parts of *Bontia daphnoides* L. They identified dehydroepingaione as the dominant volatile constituent and demonstrated compositional differences among oils from flowers, leaves, stems, and fruits of the plant [45].

GC-MS analysis is also useful for authenticity verification of essential oils. Dubnicka et al. examined commercially available oils of tea tree, lavender, sandalwood, rose, eucalyptus, and lemongrass. In four samples, they detected the addition of diethyl phthalate (Fig. 1a), and in all samples they identified carbitol – ethoxydiglycol (Fig. 1b) [46].

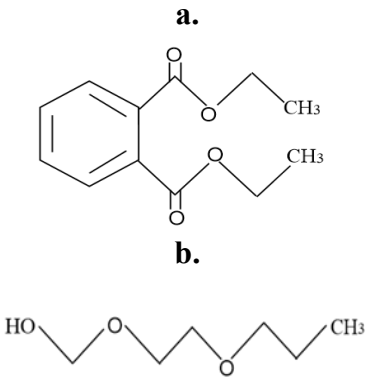

Fig. 1. Structures of: (a) diethyl phthalate and (b) ethoxydiglycol

The presence of these toxic additives is particularly concerning given the intended use of the oils for aromatherapy. Brett et al. focused on the analysis of fennel and star anise oils. In addition to qualitative profiling, they compared stable isotope ratios of E-anethole. This approach allowed them to demonstrate that as many as 27% of the commercially available samples analyzed were adulterated [47].

Nevertheless, gas chromatography has its limitations. In the case of non-volatile substances or those with high boiling points – such as castor oil – this method is unable to identify them, and other techniques, such as HPLC, must be employed [9, 12 , 48].

3.2. High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is a widely used analytical technique applied for the separation and identification of mixture components, the creation of chemical profiles and chemical "fingerprints", as well as for quantitative analysis. This method is particularly useful for the analysis of complex matrices, such as plant extracts and essential oils, as it enables the identification of thermolabile or non-volatile compounds that cannot be analyzed by gas chromatography. In the study of herbal products, HPLC plays an important role in quality control by enabling the identification of chemical markers and the creation of chemical fingerprints supported by chemometric methods. The most commonly used detection techniques are HPLC-UV and HPLC-DAD, while HPLC-MS is increasingly applied. The development of UHPLC and hyphenated techniques, such as HPLC-DAD-MS and HPLC-NMR, has significantly expanded the possibilities for chemical profiling of natural products; however, the development of fully universal detection methods remains a challenge [49-51].

In the context of essential oil analysis, high-performance liquid chromatography enables the detection of terpenes, alkaloids, flavonoids, and phenolic compounds. Non-volatile constituents of essential oils are especially important for the identification of essential oils (EOs) derived from different citrus species, as they are more specific than volatile components. HPLC allows both the identification and quantitative determination of compounds such as coumarins and oxygenated heterocyclic compounds (OHCs) present in cold-pressed citrus essential oils [52-54].

Arigò et al. developed an HPLC-PDA method combined with a linear retention index (LRI) approach and a UV-Vis library, which enabled the characterization of oxygenated heterocyclic compounds (OHCs) in citrus essential oils. This approach significantly facilitated the determination of OHCs, even when they possessed very similar structural features. In such cases, coupling HPLC with a mass spectrometric detector also represents a considerable advantage [55, 56]. Dosoky et al. developed a UPLC-MS/MS method that enabled the detection and quantification of 14 coumarins and furanocoumarins in essential oils obtained from 12 citrus species [54].

Puthenvitil et al. scaled up an HPLC method and validated it at the preparative HPLC level for the isolation and purification of thymol from the essential oil of *Trachyspermum ammi*. Using this approach, they obtained thymol with a purity of 98.87%, which was further confirmed by gas chromatography–mass spectrometry (GC-MS), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and nuclear magnetic resonance (NMR) analyses. Their results confirm the effectiveness and efficiency of preparative HPLC in the isolation of chemical compounds from complex plant matrices [57].

Although high-performance liquid chromatography is highly effective in the identification of non-volatile compounds and, when combined with GC-MS, provides complementary information on the phytochemical composition of essential oils, a major drawback – especially in routine analyses – is the long analysis time, which is approximately 45 minutes. Russo et al. developed two LC methods: a 10-minute method providing an accurate chemical profile and a 3-minute method ideal for preliminary analyses. The chemical profiles obtained with both methods focused on OHCs characteristic of the respective citrus essential oils and were consistent with chromatograms obtained using conventional HPLC methods. This demonstrates the feasibility of applying the developed methods in routine analyses [58].

3.3. Nuclear Magnetic Resonance (NMR)

NMR spectroscopy is based on the magnetic properties of nuclei (^{1}H, ^{13}C, or ^{15}N), enabling highly accurate analysis and identification of compounds. A major advantage of this technique is its high experimental reproducibility, relative ease of measurement, and simple sample preparation, as it does not require derivatization or sample destruction. Moreover, NMR analysis does not require prior separation of mixture components, which greatly facilitates work with complex matrices such as essential oils. In addition, nuclear magnetic resonance enables the analysis and identification of compounds such as sugars, organic acids, alcohols, and other highly polar compounds, which are much more difficult to analyze using chromatographic techniques. The main limitation of NMR spectroscopy is its low sensitivity - approximately 10 to 100 times lower than that of GC-MS. Nevertheless, it remains a very good choice for routine, rapid quality control analyses [58, 59].

Schripsema et al. investigated the composition of vetiver essential oil using GC-MS and NMR. Their study showed that out of eight commercially available oils, as many as four contained added vegetable oils, which was revealed only by NMR spectroscopy. They demonstrated that ^{13}C NMR spectra are the most effective method for the unequivocal identification of contaminating substances. An additional advantage of NMR is the lack of necessity for simultaneous measurement of a reference standard. The use of differential spectroscopy enabled the detection of signals originating from adulterants [60].

Studies conducted by Fabry et al. focused on the analysis of isoeugenol and other structurally related phenylpropanoids. They developed and validated a rapid ^{1}H NMR method for the simultaneous analysis of phenylpropanoids in essential oils [61]. Mahanta et al. focused on the essential oil from black turmeric rhizomes, in which they identified four thermolabile sesquiterpene markers whose determination had been impossible in previous GC-MS analyses. Once again, the ^{1}H NMR technique was used to validate a method applied to the analysis of variability in the oleochemical profile of samples originating from different harvest batches and subjected to different storage or drying conditions. This approach enables rapid routine analysis of black turmeric rhizome essential oil [62].

Zhao et al. focused on the characterization and differentiation of peppermint essential oil. Analysis of 1D and 2D NMR spectra enabled the assignment of ^{1}H NMR signals for the main constituents of this oil and additionally revealed differences in chemical composition between oils originating from different geographical regions. Based on these data, they developed a quotient-difference method allowing the determination of whether a given oil originated from the USA or India - the major suppliers of peppermint essential oil. At the same time, they subjected approximately 50 oil samples to detailed NMR analysis, which revealed that 42% of them had been adulterated [6].

The above studies demonstrate the potential of nuclear magnetic resonance in chemical and qualitative analyses as well as in the authentication of essential oils, while also constituting a rapid, simple, non-destructive, and highly reproducible analytical method (Table 2).

Table 2. Comparison of analytical techniques used for essential oil analysis

Method	Types of compounds analyzed	Main advantages	Main limitations
GC-MS	Volatile compounds	High sensitivity and selectivity; qualitative and quantitative profiling	Not suitable for non-volatile or thermolabile compounds
HPLC	Non-volatile or polar compounds	Excellent resolution; suitable for thermolabile compounds; quantitative determination	Requires solubility in the mobile phase; longer analysis time
NMR	Broad range of compounds	Provides structural and quantitative information; non-destructive; reliable for authenticity assessment	Lower sensitivity

4. Conclusions

Verification of the quality and authenticity of essential oils constitutes a key step in ensuring the safety of their use and compliance with pharmacopoeial requirements and relevant standards. Due to the complex chemical nature of essential oils and the natural variability of their composition, the application of advanced analytical methods with high sensitivity and selectivity is essential. Gas chromatography coupled with mass spectrometry (GC-MS) remains the primary technique for the analysis of the volatile fraction of essential oils, enabling detailed identification and quantitative assessment of terpene components.

High-performance liquid chromatography (HPLC, UHPLC), in turn, serves as a complementary method, allowing for the determination of non-volatile and thermolabile compounds that are of significant importance for biological activity and oil authenticity, particularly in the case of essential oils derived from citrus plants. Increasingly, hybrid approaches combining GC-MS and LC-MS are being employed, enabling the acquisition of a comprehensive chemical profile of the sample and the detection of adulteration, such as the addition of cheaper oils, solvents, or synthetic components.

NMR spectroscopy, despite its lower sensitivity compared to chromatographic methods, is gaining importance in rapid, non-destructive sample analysis and in the confirmation of essential oil authenticity. Contemporary studies indicate that only the integration of multidimensional analytical techniques (Table 2) enables a complete and reliable chemical characterization of essential oils, thereby forming the basis for quality control and the detection of adulteration.

In laboratory practice, the identification of essential oil constituents requires the strategic application of analytical techniques. The separation of mixture components is usually performed using GC-MS or GC-FID, which enables both the analysis of the chemical profile and the comparison of retention times with reference data [11]. Mass spectra are compared with spectral libraries, while the presence of individual compounds can be confirmed using reference standards or certified reference materials by recording NMR or MS spectra [63]. In cases where reference standards are not available, hyphenated analytical techniques are applied, such as the combination of gas chromatography, mass spectrometry and ^{1}H NMR, as well as FT-IR coupled with chemometric analysis. These approaches allow the detection of subtle changes in chemical composition and the identification of potential adulterants [64]. Such strategies enable reliable quality assessment of essential oils, the detection of non-authentic additives, and the monitoring of the degradation of their components.

Authors' contributions: Concept: O.S. and K.P.; resources: O.S. and K.P.; writing — preparation of the original draft, O.S.; writing — review and editing, O.S. and K.P.; visualisation, O.S. and K.P.; supervision, K.P.; project administration, O.S. and K.P. All authors have read and approved the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. European Pharmacopoeia. 11th ed. Strasbourg: European Directorate for the Quality of Medicines & HealthCare; **2022**. Essential oils; p. 1077-1078
2. Masyita, A.; Mustika Sari, R.; Dwi Astuti, A.; et al. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. *Food Chem. X* **2022**, *13*, Art. No: 100217. DOI: 10.1016/j.fochx.2022.100217
3. de Sousa, D.P.; Damasceno, R.O.S.; Amorati, R.; Elshabrawy, H.A.; de Castro, R.D.; Bezerra, D.P.; Nunes, V.R.V.; Gomes, R.C.; Lima, T.C. Essential Oils: Chemistry and Pharmacological Activities. *Biomolecules* **2023**, *13*(7), Art. No: 1144. DOI: 10.3390/biom13071144
4. Prakash, B.; Kedia, A.; Mishra, P.K.; Dubey, N.K. Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri-food commodities – Potentials and challenges. *Food Control* **2015**, *47*, 381–391. DOI: 10.1016/j.foodcont.2014.07.023
5. Koulivand, P.H.; Khaleghi Ghadiri, M.; Gorji, A. Lavender and the nervous system. *Evid. Based Complement. Alternat. Med.* **2013**, *2013*, Art. No: 681304. DOI: 10.1155/2013/681304
6. Zhao, J.; Wang, M.; Lee, J.; Ali, Z.; Khan, I.A. Characterization, differentiation, and adulteration detection of peppermint essential oil: An NMR approach. *J. Pharm. Biomed. Anal.* **2025**, *263*, Art. No: 116941. DOI: 10.1016/j.jpba.2025.116941
7. Imam, M.W.; Luqman, S. Unveiling the mechanism of essential oil action against skin pathogens: from ancient wisdom to modern science. *Arch. Microbiol.* **2024**, *206*, Art. No: 347. DOI: 10.1007/s00203-024-03986-6
8. Hoch, C.C.; Petry, J.; Griesbaum, L.; Weiser, T.; Werner, K.; Ploch, M.; Verschoor, A.; Multhoff, G.; Dezfouli, A.B.; Wollenberg, B. 1,8-cineole (eucalyptol): A versatile phytochemical with therapeutic applications across multiple diseases. *Biomed. Pharmacother.* **2023**, *167*, Art. No: 115467. DOI: 10.1016/j.biopha.2023.115467
9. Fan, H.; Wu, Q.; Simon, J.E.; Lou, S.N.; Ho, Ch.T. Authenticity analysis of citrus essential oils by HPLC-UV-MS on oxygenated heterocyclic components. *J. Food Drug Anal.* **2015**, *23*, 30–39. DOI: 10.1016/j.jfda.2014.05.008
10. Ma, J.; Wang, Y.; Liu, Y.; Gao, S.; Ding, L.; Zhao, F.; Chen, L.; Qiu, F. Cadinane sesquiterpenes from Curcuma phaeocaulis with their inhibitory activities on nitric oxide production in RAW 264.7 cells. *Fitoterapia* **2015**, *103*, 90–96. DOI: 10.1016/j.fitote.2015.03.020
11. Do, T.K.T.; Hadji-Minaglou, F.; Antoniotti, S.; Fernandez, X. Authenticity of essential oils. *Trends Anal. Chem.* **2015**, *66*, 146–157. DOI: 10.1016/j.trac.2014.10.007

12. Capetti, F.; Marengo, A.; Cagliero, C.; Sgorbini, B.; Liberto, E.; Bicchi, C.; Rubiolo, P. Adulteration of Essential Oils: A Multitask Issue for Quality Control. Three Case Studies: *Lavandula angustifolia* Mill., *Citrus limon* (L.) Osbeck and *Melaleuca alternifolia* (Maiden & Betche) Cheel. *Molecules* **2021**, *26*, Art. No: 5610. DOI: 10.3390/molecules26185610

13. Rathore, S.; Mukhia, S.; Kumar, R.; et al. Essential oil composition and antimicrobial potential of aromatic plants grown in the mid-hill conditions of the Western Himalayas. *Sci. Rep.* **2023**, *13*, Art. No: 4878. DOI: 10.1038/s41598-023-31875-3

14. Yang, H.; Huang, X.; Yang, M.; Zhang, X.; Tang, F.; Gao, B.; Gong, M.; Liang, Y.; Liu, Y.; Qian, X.; Li, H. Advanced analytical techniques for authenticity identification and quality evaluation in Essential oils: A review. *Food Chem.* **2024**, *451*, Art. No: 139340. DOI: 10.1016/j.foodchem.2024.139340

15. Truzzi, E.; Marchetti, L.; Benvenuti, S.; Ferroni, A.; Rossi, M.C.; Bertelli, D. Novel Strategy for the Recognition of Adulterant Vegetable Oils in Essential Oils Commonly Used in Food Industries by Applying 13C NMR Spectroscopy. *J. Agric. Food Chem.* **2021**, *69(29)*, 8276–8286. DOI: 10.1021/acs.jafc.1c02279

16. Wang, M.; Zhao, J.; Ali, Z.; Avonto, C.; Khan, I.A. A novel approach for lavender essential oil authentication and quality assessment. *J. Pharm. Biomed. Anal.* **2021**, *199*, Art. No: 114050. DOI: 10.1016/j.jpba.2021.114050

17. Beale, D.J.; Morrison, P.D.; Karpe, A.V.; Dunn, M.S. Chemometric Analysis of Lavender Essential Oils Using Targeted and Untargeted GC-MS Acquired Data for the Rapid Identification and Characterization of Oil Quality. *Molecules* **2017**, *22*(8), Art. No: 1339. DOI: 10.3390/molecules22081339

18. Turek, C.; Stintzing, F. Impact of different storage conditions on the quality of selected essential oils. *Food Res. Int.* **2012**, *46*(1), 341–353. DOI: 10.1016/j.foodres.2011.12.028

19. Ganosi, E.; Barda, Ch.; Grafakou, M.E.; Rallis, M.Ch.; Skaltsa, H. An In-Depth Stability Study of the Essential Oils from *Mentha × piperita, Mentha spicata, Origanum vulgare*, and *Thymus vulgaris*: The Impact of Thermal and Storage Conditions. *Separations* **2023**, *10*(9), Art. No: 488. DOI: 10.3390/separations10090488

20. International Organization for Standardization. *ISO 9235:2021: Aromatic natural raw materials — Vocabulary*. Available online: https://www.iso.org/standard/78908.html Edition 3, **2021**

21. International Organization for Standardization. *ISO 11024-1:1998: Essential oils — General guidance on chromatographic profiles*. **2020** Available online: https://www.iso.org/standard/19008.html

22. Regulation (EC) No 1223/2009 of the European Parliament and of the Council. Available online: https://eur-lex.europa.eu/eli/reg/2009/1223/oj/eng (accessed on: 01.05.2026)

23. Heavy metals test. In: *European Pharmacopoeia. 11th ed*. European Directorate for the Quality of Medicines & HealthCare, Strasbourg, France, **2022,** pp. 244-247

24. Commission Regulation (EU) 2023/915. Available online: https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32023R0915 (accessed on 08.10.2025)

25. Regulation (EC) No 1272/2008. **14.03.2024** Available online: https://osha.europa.eu/en/legislation/directives/regulation-ec-no-1272-2008-classification-labelling-and-packaging-of-substances-and-mixtures

26. Regulation (EC) No 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin. Available online: https://eur-lex.europa.eu/eli/reg/2005/396/oj (accessed on 12.05.2026)

27. International Organization for Standardization. *ISO 22000:2018: Food safety management systems — Requirements for any organization in the food chain*. Available online: https://www.iso.org/standard/65464.html

28. Residual solvents. In: *European Pharmacopoeia. 11th ed*. European Directorate for the Quality of Medicines & HealthCare, Strasbourg, France, **2022**, pp. 891-902

29. International Council for Harmonisation. *ICH Guideline Q3C (R9) on Impurities: Guideline for Residual Solvents*. Available online: https://www.ema.europa.eu/en/ich-q3c-r9-residual-solvents-scientific-guideline Published: **29.04.2024**

30. Directive 2005/84/EC of the European Parliament and of the Council. Available online: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:344:0040:0043:en:PDF Published: **14.12.2005**

31. Regulation (EC) No 1907/2006 of the European Parliament and of the Council. Available online: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32006R1907 Published: **18.12.2006**

32. Vegetable fatty oils. In: *European Pharmacopoeia. 11th ed*. European Directorate for the Quality of Medicines & HealthCare, Strasbourg, France, **2022,** pp. 1090-1091

33. International Organization for Standardization. *ISO 21461: Rubber — Determination of the aromaticity of oil in vulcanized rubber compounds*. Available online: https://www.iso.org/obp/ui/en/#iso:std:iso:21461:ed-3:v1:en Published: **2012**

34. Regulation (EC) No 1272/2008 of the European Parliament and of the Council https://eur-lex.europa.eu/eli/reg/2008/1272/oj/eng **01.07.2026**

35. Barak, T.H.; Bölükbaş, E.; Ardakci, H. Evaluation of Marketed Rosemary Essential Oils (*Rosmarinus officinalis* L.) in Terms of European Pharmacopoeia 10.0 Criteria. *Turk. J. Pharm. Sci.* **2023**, *20*(4), 253-260. DOI: 10.4274/tjps.galenos.2022.78010

36. 2.2.27. Thin-layer chromatography - 2.2.28. Gas chromatography; In: *European Pharmacopoeia. 11th ed*. European Directorate for the Quality of Medicines & HealthCare, Strasbourg, France, **2022**, pp. 146-148

37. Michalski, R. Podstawy chromatografii, czyli co było. *LAB Laboratoria, Aparatura, Badania* **2013**, *4*, 11-13.

38. Cagliero, C.; Sgorbini, B.; Bicchi, C.; Marengo, A.; Rubiolo, P. Gas chromatography of essential oil: State-of-the-art, recent advances, and perspectives.

J. Sep. Sci. **2022**, *45*, 94–112. DOI: 10.1002/jssc.202100681

39. Khatri, P.K.; Paolini, M.; Larcher, R.; Ziller, L.; Magdas, D.A.; Marincas, O.; Roncone, A.; Bontempo, L. Validation of gas chromatographic methods for lavender essential oil authentication based on volatile organic compounds and stable isotope ratios. *Microchem. J.* **2023**, *186*, 94-112. DOI: 10.1016/j.microc.2022.108343
40. Kivrak, S.; Göktürk, T.; Kivrak, İ. Assessment of Volatile Oil Composition, Phenolics and Antioxidant Activity of Bay (Laurus nobilis) Leaf and Usage in Cosmetic Applications. *Int. J. Sec. Metabolite* **2017**, *4*(2), 148–161. DOI: 10.21448/ijsm.323800
41. Omar, J.; Alonso, I.; Olivares, M.; Vallejo, A.; Etxebarria, N. Optimization of comprehensive two-dimensional gas-chromatography (GC×GC) mass spectrometry for the determination of essential oils. *Talanta* **2012**, *88*, 145–151. DOI: 10.1016/j.talanta.2011.10.023
42. Koljančić, N.; Vyviurska, O.; Špánik, I. Aroma Compounds in Essential Oils: Analyzing Chemical Composition Using Two-Dimensional Gas Chromatography–High Resolution Time-of-Flight Mass Spectrometry Combined with Chemometrics. *Plants* **2023**, *12*(12), Art. No: 2362. DOI: 10.3390/plants12122362
43. Tranchida, P.Q.; Zoccali, M.; Franchina, F.A.; Bonaccorsi, I.; Dugo, P.; Mondello, L. Fast gas chromatography combined with a high-speed triple quadrupole mass spectrometer for the analysis of unknown and target citrus essential oil volatiles. *J. Sep. Sci.* **2013**, *36(3)*, 511-516. DOI: 10.1002/jssc.201200742
44. Shi, S.; Zhang, X.; Liu, X.; Chen, Z.; Tang, H.; Hu, D.; Li, H. GC-MS Analysis of the Essential Oil from *Seseli mairei* H. Wolff (Apiaceae) Roots and Their Nematicidal Activity. *Molecules* **2023**, *28(5)*, Art. No: 2205. DOI: 10.3390/molecules28052205
45. Thabet, A.A.; Moghannem, S.; Ayoub, I.M.; Youssef, F.S.; Al Sayed, E.; Singab, A.N.B. GC/MS profiling of essential oils from *Bontia daphnoides* L., chemometric discrimination, isolation of dehydroepingaione and evaluation of antiviral activity. *Sci. Rep.* **2022**, *12*, Art. No: 17707. DOI: 10.1038/s41598-022-22174-4
46. Dubnicka, M.; Cromwell, B.; Levine, M. Investigation of the Adulteration of Essential Oils by GC-MS. *Curr. Anal. Chem.* **2019**, *16*(8), 965–969. DOI: 10.2174/1573411015666191127093710
47. Murphy, B.J.; Wilson, T.M.; Ziebarth, E.A.; Bowerbank, C.R.; Carlson, R.E. Authentication of Fennel, Star Anise, and Anise Essential Oils by Gas Chromatography (GC/MS) and Stable Isotope Ratio (GC/IRMS) Analyses. *Plants* **2024**, *13*, Art. No: 214. DOI: 10.3390/plants13020214
48. Ali, S.; Ekbbal, R.; Salar, S.; Yasheshwar; Ali, S.A.; Jaiswal, A.K.; Singh, M.; Yadav, D.K.; Kumar, S.; Gaurav. Quality Standards and Pharmacological Interventions of Natural Oils: Current Scenario and Future Perspectives. *ACS Omega* **2023**, *8*, 39945−39963. DOI: 10.1021/acsomega.3c05241
49. Sarker, S.D.; Nahar, L. Applications of High Performance Liquid Chromatography in the Analysis of Herbal Products. In *Evidence-Based Validation of Herbal Medicine*; Elsevier, **2015**, 405-425. DOI: 10.1016/B978-0-12-800874-4.00019-2
50. Wolfender, J.L. HPLC in Natural Product Analysis: The Detection Issue. *Planta Med.* **2009**, *75*, 719–734. DOI: 10.1055/s-0028-1088393
51. Belanger, J.M.R.; Pare, J.R.J.; Sigouin, M. Chapter 2: *High Performance Liquid Chromatography (HPLC): Principles and Applications*; Instrumental Methods in Food Analysis; Elsevier, Volume 18, **1997**, pp. 37-59
52. Zhang, M.; Zhao, J.; Dai, X.; Li, X. Extraction and Analysis of Chemical Compositions of Natural Products and Plants. *Separations* **2023**, *10*(12), Art. No: 598. DOI: 10.3390/separations10120598
53. Russo, M.; Bonaccorsi, I.; Costa, R.; Trozzi, A.; Dugo, P.; Mondello, L. Reduced time HPLC analyses for fast quality control of citrus essential oils. *J. Essent. Oil Res.* **2015**, 27(4), 307–315 DOI: 10.1080/10412905.2015.1027419
54. Dosoky, N.S.; Satyal, P.; Setzer, W.N. Authentication of *Citrus* spp. Cold-Pressed Essential Oils by Their Oxygenated Heterocyclic Components. *Molecules* **2022**, *27*(19), Art. No: 6277. DOI: 10.3390/molecules27196277
55. Arigò, A.; Rigano, F.; Micalizzi, G.; Dugo, P.; Mondello, L. Oxygen heterocyclic compound screening in *Citrus* essential oils by linear retention index approach applied to liquid chromatography coupled to photodiode array detector. *Flavour Fragr. J.* **2019**, *34*, 349–364. DOI: 10.1002/ffj.3515
56. Cafeo, G.; Irrera, E.; Russo, M.; Dugo, P. Extraction and Chromatographic Approaches for Coumarin, Furocoumarin, and Polymethoxyflavone Characterization in Foods. *Foods* **2024**, *13*(16), Art. No: 2517. DOI: 10.3390/foods13162517
57. Puthenvitil, S.K.; Perumal, P. Optimization of preparative HPLC method for the purification of thymol from the essential oil of *Trachyspermum ammi* (L.). *J. Chromatogr. B* **2025**, *1263*, Art. No: 124707. DOI: 10.1016/j.jchromb.2025.124707
58. Pătruică, M.R.; Gădălean, F.; Vlad, A.; Sturza, A.; Jianu, D.C.; Balint, L.; Ienciu, S.; Petrica, L.; Muntean, D.M.; Socaciu, C. Metabolomics in Chronic Kidney Diseases: Here to Stay. *Timisoara Med.* **2020**, *2020*(2), Art. No: 8. DOI: 10.35995/tmj20200208
59. Emwas, A.H.; Roy, R.; McKay, R.T.; Tenori, L.; Saccenti, L.; Nagana Gowda, G.A.; Raftery, D.; Alahmari, F.; Jaremko, Ł.; Jaremko, M. NMR Spectroscopy for Metabolomics Research. *Metabolites* **2019**, *9*(7), Art. No: 123. DOI: 10.3390/metabo9070123
60. Schripsema, J.; da Silva, S.M.; Dagnino, D. Differential NMR and chromatography for the detection and analysis of adulteration of vetiver essential oils. *Talanta* **2022**, *237*, Art. No: 122928. DOI: 10.1016/j.talanta.2021.122928
61. Fabry, P.; Weber, S.; Teipel, J.; Richling, E.; Walch, S.G.; Lachenmeier, D.W. Quantitative NMR Spectrometry

of Phenylpropanoids, including Isoeugenol in Herbs, Spices, and Essential Oils. *Foods* **2024**, *13*, Art. No: 720. DOI: 10.3390/foods13050720

62. Mahanta, B.P.; Sut, D.; Kemprai, P.; Paw, M.; Lal, M.; Haldar, S. A ^{1}H-NMR spectroscopic method for the analysis of thermolabile chemical markers from the essential oil of black turmeric (*Curcuma caesia*) rhizome: application in post-harvest analysis. *Phytochem. Anal.* **2020**, *31*(1), 28–36. DOI: 10.1002/pca.2863
63. Motta, E.V.S.; Sampaio, B.L.; Costa, J.C.; Teixeira, S.P.; Bastos, J.K. Quantitative analysis of phenolic metabolites in *Copaifera langsdorffii* leaves from plants of different geographic origins cultivated under the same environmental conditions. *Phytochem. Anal.* **2019**, *30*(3), 364–372. DOI: 10.1002/pca.2819
64. Oliveira, M.F.L.de; Pinheiro, J.F.; Freitas, A.S.; Mendonça, C.de J.S.; Silva, G.S.da; Sousa, E.R.de. Exploratory analysis of adulterant detection in essential oils using infrared spectroscopy and chemometric tools. *J. Essent. Oil Res.* **2025**, *37*(5), 357–367. DOI: 10.1080/10412905.2025.2532375