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The Standardization and Safety Assessment of Volatile Oils

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Abstract

In recent years, consumer preference has increasingly shifted toward natural products as alternatives to synthetic additives and pharmacologically active compounds. Essential oils have gained considerable prominence across the food, cosmetics, and pharmaceutical sectors. These oils are inherently susceptible to chemical transformation and degradation due to their complex composition of lipophilic and highly volatile compounds derived from diverse chemical families. Historical evidence from pharmacopeias, traditional medical systems, and ethnomedicine demonstrates that essential volatile oils have been utilized therapeutically for millennia, both through topical and internal administration. This review provides a comprehensive analysis of potential chemical modifications in essential oils and the factors influencing their stability. We examine the various degradation pathways that occur upon exposure to environmental conditions, with particular emphasis on how temperature, light exposure, and oxygen availability significantly compromise essential oil integrity. Furthermore, we evaluate the utility of analytical methods for assessing both authentic and adulterated essential oil profiles, focusing on their capacity to monitor chemical alterations over time. We propose that only through comprehensive evaluation using multiple analytical approaches can a thorough quality assessment be achieved that addresses consumer safety and therapeutic efficacy concerns. Such systematic evaluation is essential for maintaining standards in clinical and commercial applications of essential oils.

INTRODUCTION

Volatile secondary metabolites characterized by lipophilic properties and molecular masses under 300 Da constitute the primary components of essential oils, which are isolated from botanical sources using established extraction methodologies (Protzen 1993; Grassmann and Elstner 2003; Schmidt 2010; Sell 2010). Standardized production protocols encompass steam distillation, hydrodistillation, and mechanical expression techniques, with aqueous distillation representing the predominant commercial methodology (ISO 9235, 1997). Regulatory oversight is maintained through multiple international frameworks, including standards established by ISO and AFNOR, with usage guidelines provided by BfR, RIFM, IFRA, and SCCS organizations. Pharmacopeial

compliance enables therapeutic applications, supported by FDA safety classifications for designated uses(Smith et al 2005).The bioactive constituents demonstrate enhanced dermal bioavailability due to their exogenous nature relative to human biochemistry, facilitating topical therapeutic delivery. Compositional complexity ranges from single bioactive molecules to heterogeneous mixtures exhibiting diverse pharmacological profiles, often incorporating carrier systems and functional groups that modulate tissue penetration kinetics. Historical therapeutic applications within Ayurvedic medicine span several millennia, encompassing both monocomponent and polyherbal preparations (India Govt. 2018). Agricultural optimization studies indicate yield enhancement under specific climatic conditions, while certain source plants, particularly Jatropha and Brassica varieties, exhibit minimal heavy metal bioaccumulation [Bhattacharya, Deepak, 2010; Bhattacharya, Deepak, 2020].

Long-term agricultural monitoring spanning ten years indicates enhanced essential oil production in agro-meteorological zones characterized by annual precipitation ranging from 100-200mm, with optimal yields observed in regions combining these precipitation patterns with undulating topography and soil depths exceeding 20 feet. Preliminary data suggest that insufficient hydrostatic pressure within soil systems may negatively impact oil production efficiency. Ethnobotanical studies reveal that *Jatropha curcas* and *Brassica nigra* have maintained significant cultural importance across indigenous and traditional communities for centuries, with documented applications in ceremonial, religious, and therapeutic practices (Assad et al., 2025). *Brassica nigra* oil is commonly utilized in topical applications, such as scalp and body treatments, where it is sometimes combined with controlled solar exposure to promote volatilization of bioactive compounds. Phytoremediation analyses further suggest that this species, similar to other essential oil–producing plants, exhibits limited accumulation of heavy metals, supporting its safety and therapeutic potential. Cultural practices across Asian regions incorporate Jatropha and *Pongamia pinnata* oils extensively in ceremonial lighting during Deepavali celebrations (Rajarajan et al., 2025). Atmospheric studies suggest that combustion of these oils may influence local meteorological conditions through aerosol particle interactions, potentially affecting cloud nucleation processes and contributing to clearer atmospheric conditions that may benefit agricultural harvesting and regional weather patterns.(Bhattacharya, Deepak, 2020) agricultural optimization studies indicate yield enhancement under specific climatic conditions, while certain source plants, particularly Jatropha and Brassica varieties, exhibit minimal heavy metal bioaccumulation

Chemistry

Essential oils contain structurally diverse components, with individual formulations typically comprising over 100 distinct chemical constituents (Blitzke 2009). Minor components resulting from oxidative processes can significantly influence organoleptic characteristics when their odor threshold values reach sufficient concentrations (Grosch 2007). The aromatic impact of individual compounds depends on their specific odor thresholds, which are governed by molecular structure and volatility parameters, rather than being strictly concentration-dependent.

Chemical classification of essential oil constituents encompasses three primary categories: lipophilic terpenoids, phenylpropanoids, and short-chain aliphatic hydrocarbon derivatives, representing the most prevalent low molecular weight components (Kubeczka 1979). Terpenoid compounds, including tricyclic monoterpenes, allylics, monoterpenes, and sesquiterpenes, constitute the predominant fraction of essential oils and exist in various functional forms, including alcohols, oxides, ketones, phenols, hydrocarbons, and esters (Masyita et al., 2022). Biosynthetic pathways govern the formation of essential oil constituents through well-characterized metabolic routes. Terpenoids are derived from the five-carbon precursor's isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate, while phenylpropanoids originate via the shikimic acid pathway leading to phenylalanine biosynthesis (Pichersky and others 2006; Colquhoun and others 2010). The structural organization of terpenoid molecules follows the isoprene rule, initially proposed by Otto Wallach in 1887 and subsequently refined by Sir Robert Robinson and Leopold Ruzicka, which states that terpenoid structures are constructed from isoprene units (C5H8) linked in head-to-tail configurations through direct condensation or cyclization mechanisms (Ruzicka 1953; Banthorp et al. 1972; Hanson 2003).

Analysis of Volatile Oils

Comprehensive analytical verification is essential to establish the therapeutic value and authenticate the chemical identity of essential oils, given the extensive compositional diversity these complex mixtures exhibit. Multiple characterization methodologies have been developed for volatile oil assessment, incorporating both physicochemical parameters and stability evaluation during storage conditions. The European Pharmacopoeia establishes quality control protocols encompassing optical rotation measurements, freezing point determination, specific gravity assessment, and compound-specific analytical procedures to ensure pharmaceutical grade standards (Herve et al. 2025). Sensory evaluation techniques provide complementary authentication methods, particularly valuable for confirming characteristic aromatic

profiles that serve as preliminary identification markers for specific essential oil varieties (Pudilet al. 1998).

Chromatography

Multiple analytical detection methods are employed for comprehensive screening of essential oil composition, providing critical information regarding adulteration, potential contaminants, and chemical identity verification (Scott 2005; Acampora Zellner et al. 2010). Thin-layer chromatography has maintained a fundamental role in essential oil analysis, serving as a reliable technique for compositional assessment and quality control applications (Fischer and Still 1967; Hefendehl 1993). Contemporary separation methodologies have demonstrated significant advancement in aromatic compound analysis, offering enhanced resolution and sensitivity for complex essential oil characterization.

- **Gas Chromatography (GC)**

Gas chromatography (GC) represents the analytical method of choice for complex essential oil mixture analysis due to its exceptional separation capabilities (Cserhati 2010). The high theoretical plate count characteristic of GC systems provides outstanding resolution for volatile compound analysis, making it particularly suitable for aromatic substance investigation. Advanced methodologies, including enantioselective gas chromatography and multidimensional techniques, achieve enhanced resolving power, while fast chromatography approaches improve analytical throughput and efficiency.

Comprehensive reviews of essential oil analytical methodologies have been published by Mariotte et al. (2001), Rubiolo et al. (2010), and Tranchida et al. (2012). Gas chromatographic olfactometry combines chromatographic separation with sensory detection systems, enabling identification of off-flavours and comprehensive organoleptic characterization (Bonnefille 2011; Mahattanatawee and Rouseff 2011). For terpenoid quantification, GC coupled with mass spectrometry detection or flame ionization detection provides optimal analytical capabilities (Cicchetti et al. 2008). Electron ionization techniques have been extensively documented for volatile oil and fragrance product analysis (Rastogi 1995; Masotti et al. 2003; Radulescu et al. 2004).

However, analytical limitations exist for compounds with vapor pressures below 350-400°C, as thermal stability during separation and vaporization becomes problematic (Cserhati 2010). Thermally induced structural modifications of less volatile substances during GC analysis have been documented, potentially compromising analytical accuracy (Mateus et al. 2010). Catalytically active column surfaces and heated injection port liners may promote unintended decomposition and molecular rearrangement

reactions during chromatographic separation, as noted by Hinshaw (2002) and Klee (2010). Additionally, GC analysis of aqueous-based products including foods, detergents, toiletries, and cosmetic formulations presents significant analytical challenges and extended analysis times (Radulescu et al. 2004; Tschiggerl and Bucar 2010). Recent developments have introduced transformative approaches to essential oil analysis, including the integration of artificial intelligence and machine learning algorithms for automated pattern recognition and quality assessment (Srati et al., 2024). Advanced chromatographic data processing systems now utilize AI-driven methodologies to enhance separation optimization and reduce analysis time, while modern innovations in essential oil recovery and analysis incorporate AI integration for improved authenticity verification (Nayak et al., 2025). Machine learning applications, particularly Random Forest algorithms, have demonstrated exceptional capability in GC-MS data classification and essential oil characterization, enabling rapid identification of botanical origins and detection of adulteration with unprecedented accuracy (Ratnasekhar, C. H., et al., 2025). Contemporary analytical workflows leverage deep learning neural networks for real-time chromatographic peak identification and quantitative analysis, representing a paradigm shift toward fully automated, high-throughput essential oil authentication systems that significantly enhance both analytical precision and laboratory efficiency

- **High Performance Liquid Chromatography (HPLC)**

  High-performance liquid chromatography (HPLC) has been established as a sensitive analytical technique for detecting volatile oils (Rauber et al., 2005; Turek &Stintzing, 2011a). Hajimehdipoor et al. (2010) developed a validated HPLC-based method as an alternative to gas chromatography (GC) for quantifying carvacrol and thymol in thyme oil and citral in lemongrass oil. However, the application of HPLC to volatile oil analysis remains relatively limited, with most studies employing it for the characterization of non-volatile fractions, such as those found in cold-pressed citrus oils (Lockwood, 2001; d'Acampora Zellner et al., 2010; Tranchida et al., 2012). Historically, HPLC was primarily used for sample cleanup prior to GC analysis, although in rare cases it has enabled the separation of volatile terpenoids and hydrocarbons from complex mixtures. Turek and Stintzing (2011a) later introduced a universal HPLC method for characterizing volatile oils and assessing individual constituents. By employing mass spectrometric (MS) detection at low eluent flow rates, along with optimized gradient programs and end-capped reverse-phase columns, high-resolution chromatographic fingerprints could be obtained. For detection, UV-Vis and diode array detectors (DAD) have been widely applied due to their flexibility and selectivity, with DAD providing rapid spectral data for each chromatographic peak. Typical detection wavelengths range from 195–275 nm,

depending on the analyte (Ogawa et al., 2002). Since volatile oils contain compounds from diverse chemical classes, simultaneous monitoring at multiple characteristic wavelengths is often required. For example, α- and γ-terpinene exhibit highly similar chromatographic and spectral properties, necessitating careful method optimization (Turek &Stintzing, 2011a).

- **Liquid Chromatography (LC)**

For compound identification, the combination of LC (liquid chromatography) with mass spectrometry represents a powerful analytical tool. To comprehensively assess compounds in numerous volatile oils alongside GC (gas chromatography), a novel approach was established by Turek and Stintzing (2011a), utilizing HPLC/DAD (high-performance liquid chromatography with diode array detection) in sequence with cycle MS (multiple reaction monitoring mass spectrometry) and employing specific tension substance ionization in the positive mode. For the guidance of multiple finding modes, such as UV absorption coupled with MS and the possibility of rapid screening at various frequencies when using a diode array detector, the authors concluded that the moderate separation limit of HPLC compared to GC due to a lower number of theoretical plates could be compensated to a significant extent (Turek and Stintzing 2011a; Turek and Stintzing 2012).Researchers have identified novel techniques for monitoring oxidative changes and degradation reactions in essential oils, providing more accurate assessments of their physicochemical reliability. The adoption of advanced analytical platforms, including HPLC-UV-MS and cycleMS (Vadivel et al., 2024), has substantially improved the characterization of volatile oil constituents. Innovations such as novel ionization techniques and the integration of diode array detectors have further enhanced analytical sensitivity and specificity. Collectively, these developments support more rigorous quality control across the food, cosmetic, and therapeutic sectors, thereby ensuring both the safety and efficacy of essential oil-based formulations.

Standardization of volatile oil

Safety Evaluation of Volatile Oil

The regulation and safety assessment of volatile oils presents unique challenges due to their complex relationship with food flavouring compounds. Under the Federal Food Drug and Cosmetic Act, ingredients naturally occurring in food are subject to different regulatory standards than those deliberately added, with naturally occurring substances required only to meet the general safety standard that they do not "ordinarily render [food] injurious to health" (21 CFR 172.30), while additives face more stringent requirements.

Given their extensive historical use in food applications, volatile oils benefit from an established safety profile based on recognized patterns of consumption without documented adverse effects. In the United States, comprehensive annual surveys have documented the safety history of numerous volatile oils used as flavouring agents, providing a foundation for safety assessments that begin with the presumption of safety for substances with demonstrated histories of safe use.

However, several factors have introduced complexity into contemporary safety evaluations. Modern dietary patterns have altered exposure levels to volatile oils in various ways - for instance, the incorporation of cinnamon oil in low-fat baked goods may modify population exposure profiles compared to traditional consumption patterns. Additionally, increased global trade has facilitated the introduction of plant extracts from previously geographically distant regions, with the Flavour and Extract Manufacturers Association (FEMA) GRAS list recently including compounds derived from non-native plants such as Osmanthus absolute (FEMA No. 3750) and Jambu oleoresin (FEMA No. 3783).

Contemporary safety assessment must also account for the fact that natural flavour compounds may be consumed not only as flavouring substances but also as dietary supplements marketed for purported health benefits. These evolving consumption patterns have renewed interest in comprehensive safety evaluations of natural flavour compounds, including essential oils (Ivanovo et al., 2025). While safety assessment of these compounds should continue to rely substantially on historical usage data, a flexible, science-based approach is necessary to address the challenges of evaluating multiple applications for identical volatile oils across different context.

Pharmacology of Volatile Oil

The high monoterpene content of volatile oils confers substantial lipophilicity, enabling these compounds to readily traverse biological barriers. Systemic absorption of specific chemical constituents following pulmonary or dermal administration of volatile oils presents both toxicological concerns and potential therapeutic benefits (Kohlert et al., 2000).

Oral administration demonstrates significant systemic absorption of many volatile oil constituents. Investigations employing radiolabelled trans-anethole in rats demonstrated that nearly 90% of the administered compound is absorbed through the gastrointestinal tract, enters systemic circulation, and undergoes subsequent metabolism before being excreted via both faecal and urinary pathways (Bounds and Caldwell, 1996). Similarly, oral administration of geraniol to Sprague-Dawley rats demonstrated complete bioavailability of 92%, indicating substantial systemic

absorption (Pavan et al., 2018). Human studies using enteric-coated capsules contain three terpenoids (1, 8-cineole, α-pinene, and limonene) confirmed significant gastrointestinal absorption of 1, 8-cineole (Zimmerman et al., 1995).

Dermal application of volatile oils, commonly employed in aromatherapy massage, can result in systemic absorption of chemical constituents. The extent of absorption depends on factors including the surface area of exposed skin, contact duration, and concentration of the applied compound. Volatile oil components can penetrate the skin barrier by enhancing drug distribution and inducing conformational changes in the stratum corneum, potentially affecting the absorption of other topically applied substances (Herman and Herman, 2015).

Transdermal absorption has been documented for numerous monoterpenes, including α-pinene, limonene, and camphor, which can produce both local cutaneous effects and systemic distribution. Other volatile oil constituents have similarly demonstrated the capacity to traverse the skin barrier (Kohlert et al., 2000). The volatility of essential oils enables pulmonary administration for respiratory therapeutic applications. Pulmonary absorption rates depend on physiological factors such as respiratory mechanics and the physicochemical properties of the inhaled volatile compounds. Confirmed pulmonary absorption has been demonstrated for camphor, menthol, and α-pinene (Kohlert et al., 2000). Studies have explored the therapeutic potential of essential oils in treating various ailments, including pain, inflammation, and anxiety (Qneibi et al., 2024). The development of new formulations and delivery systems has enhanced the bioavailability and efficacy of these oils, leading to improved patient outcomes (Kohlert C2000).

Volatile Oil Toxicity

Volatile oils are widely used by the general public and can be found in supermarkets or online. A recent study indicated that 11% of Australians self-prescribed volatile oils for medicinal purposes in 2016 (Harnett, JE, 2019). Specific constituents of these oils can lead to unwanted effects, and the chemical complexity of volatile oils complicates their investigation. Toxicity studies conducted on laboratory animals have assessed the potential harmful effects of volatile oils and their components. In rats, acute toxicity was evaluated using the LD50 test, revealing low toxicity levels, with an LD50 range of 1 to 20 g/kg. For instance, lemon oils have an LD50 greater than 5 g/kg in humans, making it challenging to reach a fatal dose, which would be approximately 350 grams for a 70-kilogram adult. Notable exceptions include volatile oils from Chenopodium, Boldo leaf, *Mentha pulegium*, savory (*Satureja hortensis*), and Thuja, which exhibit an LD50 between 0.1 and 1 g/kg in mice, indicating significant toxicity and underscoring the need for safety measures in their use (Lis-Balchin, M., 2005). Additionally, certain molecules, such

as oxidation products of limonene, can act as skin sensitizers, and volatile oils are susceptible to degradation through oxidation (Karlberg, AT, 1992). To minimize the risk of adverse effects, proper storage of volatile oils is crucial to maintain their efficacy. They should be kept in tightly sealed containers, stored in a cool, dark place. The Flavor and Extract Manufacturers Association (FEMA) has granted GRAS status to many volatile oils, although this assessment was based on their use as flavoring agents. It is important to note that concentrated volatile oils can potentially exhibit local or systemic toxic effects under specific circumstances(Lis-Balchin M., 2005). Novel research highlighted the potential for volatile oils to cause severe dermal damage and toxicity in purified fractions, raising concerns about their safety in food processing (Manful et al., 2024). Recent studies have also emphasized the need for rigorous quality control measures to mitigate these risks and ensure the safety of essential oil-based products. Novel air techniques like computer vision (CV), machine learning (ML), natural language processing (NLP), and reinforcement learning (RL) are supporting to lower such risks (Dhal, S. B., & Kar, D. (2025).

Stability

Essential oils are prone to conversion and degradation reactions at room temperature, particularly due to the diverse chemical classes that constitute the entire oil (Turek et al. 2012). Additionally, these oils undergo degassing, which can lead to failure, degeneration, and the formation of new compounds upon contact with polymer containers. Furthermore, they react with tin during long-term storage. The processes of fractionation and purification are challenging, costly, and inefficient for yield extraction, use, and handling. Purified fractions are highly toxic and can cause severe dermal damage, rendering them unsuitable for use in the food processing industry. Historically, the use of whole oil has likely served as the foundation of all medical practices. Whole oils exhibit relatively low volatility, making them attractive for applications in the medicinal rubrics industry.

Anti-Viral Property

Numerous essential oils demonstrate documented antiviral activities across a broad spectrum of viral pathogens (da Silvaet al. 2020)]. Of particular interest is *Artemisia vulgaris* (Sanskrit: Dayana/durlavaa), which has been systematically cultivated within Hindu temple and monastic complexes since approximately 900-1000 CE for its recognized therapeutic properties. Traditional ceremonial applications involve the ritualistic use of this species within the inner sanctuaries of major Hindu temples, specifically in enclosed spaces protected from natural light, following seasonal and liturgical protocols. Contemporary research has validated the broad-spectrum antiviral

efficacy of *A. vulgaris*, including demonstrated activity against various viral strains (Behera Jagdish, 2020a; Behera Jagdish 2020b). Recent investigations have extended these findings to include potential efficacy against SARS-CoV-2, suggesting therapeutic relevance for contemporary viral challenges (Bhattacharya, et al, 2020a; Bhattacharya, et al, 2020b; Bhattacharya Deepak, et al. 2021).

Conclusion

A review of the existing literature on essential oil stability revealed that oxidative changes and degradation reactions, which can lead to significant tactile and pharmacological alterations, have generally been overlooked. The impact of external storage conditions on the physicochemical reliability of essential oils demonstrated that this topic warrant further investigation. HPLC (high-performance liquid chromatography) appears to be a reliable method for profiling both authentic and oxidized essential oils, offering valuable insights for quality control purposes. Additionally, HPLC may be appropriate for assessing essential oil components in food products, beauty care items, and restorative formulations. Conductivity and pH measurements, alongside POV (photostability) evaluation, should be included in the analytical toolkit to gain a more comprehensive understanding of the oxidative events that a single essential oil may have undergone.

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