

## ESSENTIAL OIL AND HYDROSOL CONSTITUENTS OF *LOPHANTHUS ANISATUS*: CHEMICAL COMPOSITION AND COMPARATIVE ANALYSIS

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### ABSTRACT

*Lophanthus anisatus* (syn. *Agastache foeniculum*) produces an essential oil (EO) characterized by high concentrations of phenylpropanoids. The main constituent is estragole (methyl chavicol), which often represents 70–95 % of the oil (Charles et al., 1991; Stefan et al., 2022). Other reported components include limonene, eugenol, methyl eugenol, chavicol, and  $\beta$ -caryophyllene in smaller proportions (Nykänen et al., 1989; Bălănescu et al., 2023). The yield of EO typically ranges between 1.5 % and 2.3 % of dry aerial biomass depending on extraction method and plant organ (Stefan et al., 2022). Hydrosol, the aqueous co-product of hydrodistillation, contains trace volatiles distinct from the EO profile (Xin et al., 2023). While the EO is rich in estragole, hydrosols are enriched in more water-soluble oxygenated compounds such as alcohols and aldehydes (Monsef-Esfahani et al., 2014). This compositional difference suggests that hydrosols may have milder bioactivity compared to the corresponding EO. Nevertheless, EO studies have demonstrated antimicrobial, antioxidant, and acetylcholinesterase inhibitory effects linked to its estragole chemotype (Ivanov et al., 2019; Bălănescu et al., 2023). Due to the high estragole content, safety assessments are required because estragole is considered a potential genotoxic carcinogen (EFSA, 2011; EMA, 2023).

Overall, *L. anisatus* EO and hydrosol represent promising resources for herbal, pharmaceutical, and aromatic applications, provided that regulatory considerations are addressed.

### INTRODUCTION

*Lophanthus anisatus*, now widely accepted under the synonym *Agastache foeniculum*, is a perennial aromatic plant of the family Lamiaceae, which includes other well-known culinary and medicinal herbs such as basil, mint, and oregano. The plant is commonly referred to as anise hyssop due to its characteristic aroma reminiscent of anise (*Pimpinella anisum*), although it is not botanically related to true hyssop (*Hyssopus officinalis*). Native to North America, particularly the northern plains of the United States and southern Canada, it thrives in prairies, grasslands, and open woodlands (Kew Science, n.d.; Zielińska et al., 2014). Its square stems, serrated ovate leaves, and dense spikes of violet to blue flowers make it easily recognizable and attractive in cultivation.

For centuries, indigenous peoples of North America have valued *L. anisatus* as both a medicinal and ceremonial herb. Infusions prepared from the leaves were traditionally used to alleviate respiratory ailments such as coughs, fevers, and chest discomfort, as well as to aid digestion (Zielińska et al., 2014). Some tribes also incorporated the plant into ritual practices, burning it as an aromatic smoke during ceremonies. Its sweet, anise-like taste led to its use as a flavoring agent in teas and culinary preparations, while the flowers provided nectar for honey production. These traditional applications illustrate the long-standing integration of the plant into cultural, dietary, and medicinal contexts.

Phytochemical studies have revealed that the essential oil of *L. anisatus* is dominated by estragole (methyl chavicol), often comprising more than 85–95 % of the volatile fraction (Charles et al., 1991; Lawson et al., 2021). Minor constituents include (E)-anethole, eugenol, chavicol, and limonene, although the estragole-rich chemotype is most common. The essential oil yield typically ranges between 1.5–2.3 % of dry aerial biomass, depending on extraction method and cultivation conditions (Stefan et al., 2022). Modern pharmacological research has highlighted its antimicrobial, antioxidant, and acetylcholinesterase inhibitory activities, supporting some of its traditional medicinal uses (Ivanov et al., 2019; Bălănescu et al., 2023).

Today, *L. anisatus* is cultivated globally as an ornamental species, a pollinator-friendly garden plant, and a source of essential oils and herbal teas. Its role in supporting biodiversity, particularly bees and butterflies, adds ecological value beyond its medicinal potential. However, its high estragole content has prompted toxicological concerns, since estragole is considered a genotoxic and potentially carcinogenic compound at high doses (EFSA, 2011; EMA, 2023). As a result, while the plant remains safe in traditional culinary uses, the use of concentrated essential oils or extracts requires careful risk assessment. This dual perspective—promising bioactivity and regulatory caution—makes *L. anisatus* an intriguing subject for ongoing research in phytopharmacy, nutraceutical development, and sustainable agriculture.

*Lophanthus anisatus* (syn. *Agastache foeniculum*, anise hyssop) produces a phenylpropanoid-rich essential oil (EO) dominated by estragole (methyl chavicol), with notable contributions from limonene, methyl eugenol/eugenol, and  $\beta$ -caryophyllene; proportions vary with organ, genotype, and environment. Across reports, estragole typically constitutes the primary constituent—often >70% and up to ~90% in flower oils—while leaves may show comparatively higher eugenol/methyl eugenol and chavicol alongside limonene ( $\approx$ 3–9%) (Bălănescu et al., 2023; Badea et al., 2022; Nykänen, 1989; Stefan et al., 2022). Recent profiling within *Agastache* confirms chemotypic diversity (e.g., estragole–limonene or menthone/pulegone types), but *A. foeniculum* commonly expresses the estragole chemotype (Nechita et al., 2024; Ivanov et al., 2019).

Hydrosol (hydrolate), the aqueous co-product of hydrodistillation, contains trace volatiles enriched in more water-soluble oxygenated compounds relative to EO (e.g., monoterpene alcohols and aldehydes), while lipophilic hydrocarbons are strongly depleted. Although species-specific hydrosol GC-MS data for *L. anisatus* remain scarce, comparative studies across Lamiaceae hydrosols demonstrate consistent enrichment of alcohols (e.g., linalool/ $\alpha$ -terpineol/geraniol in model systems) and an overall distinct profile from the corresponding EO (Xin et al., 2023; Monsef-Esfahani et al., 2014). Accordingly, the *L. anisatus* hydrosol is expected to contain markedly lower estragole than its EO and relatively higher proportions of polar oxygenated volatiles extracted into the distillate water. This compositional

divergence has practical implications: EO-driven bioactivities in *L. anisatus* (e.g., antimicrobial effects mainly against *Staphylococcus aureus*) are linked to estragole and allied phenylpropanoids, whereas hydrosol activities likely reflect its oxygenated monoterpene fraction at much lower total volatile levels (Stefan et al., 2022; Nechita et al., 2024). Targeted, species-specific hydrosol analyses for *L. anisatus* are warranted to quantify these expectations and guide applications.

## MATERIAL AND METHODS

Essential oils (EOs) were extracted primarily by hydrodistillation, a method in which plant material is submerged in water and heated to generate steam, carrying volatile compounds into a condenser where the oil separates from water (Almeida et al., 2024). Steam distillation is another common approach, in which steam passes through plant tissues, vaporizing volatiles that are then condensed, allowing EO collection separate from water. For sensitive compounds, supercritical fluid extraction (SFE) using CO<sub>2</sub> is employed, offering efficient extraction while preserving thermolabile constituents. Additionally, solvent extraction with organic solvents such as hexane may be used, particularly for delicate plant parts like flowers, dissolving volatile compounds that are subsequently concentrated.

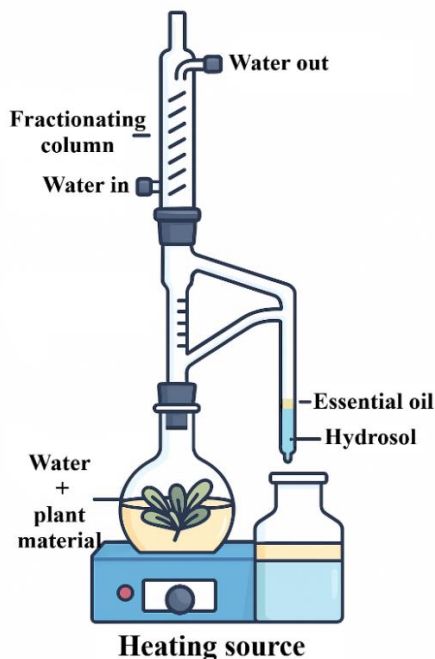


Figure 1. Illustrative representation of hydrodistillation.

Hydrosols, or floral waters, are obtained simultaneously during hydrodistillation or steam distillation; these aqueous distillates contain water-soluble volatiles and traces of EO, and are collected separately for further use (Almeida et al., 2024) (Figure 1). The choice of extraction method influences the yield, chemical composition, and bioactivity of both the EO and hydrosol, making method selection critical depending on the intended application in pharmacology, aromatherapy, or food products.

Plant material of *Lophanthus anisatus* was collected in August 2024 from a cultivated garden in Constanța, Romania (44°10'24"N, 28°38'18"E). Aerial parts of the plant (stems, leaves, and inflorescences) were dried, and 100 g of material was subjected to hydrodistillation with 700 mL of distilled water.

Essential oil and hydrolate samples were analyzed without prior preparation or dilution. One microliter of each sample was injected into a gas chromatograph–mass spectrometer (GC-MS, Perkin Elmer Clarus 680/SQ 8T, Perkin Elmer, CT, USA) equipped with an Elite-5MS capillary column (30 m × 0.25 mm × 0.25 μm; Perkin Elmer, CT, USA). Helium served as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature program was: initial temperature 40 °C (2 min hold), ramped at 10 °C/min to 300 °C, followed by a 2 min hold. Injections were performed in split mode (split ratio 50:1 for hydrolate, 200:1 for essential oil), with the injector temperature set at 200 °C.

MS conditions were as follows: ion source temperature 210 °C, transfer line temperature 310 °C, electron impact ionization (EI+) at 70 eV, and solvent delay of 3 min. Compound identification was achieved by comparison with the NIST 2017 and Wiley 9.0 mass spectral libraries. For each sample, the 30 most intense peaks were considered in the library search, with a minimum match threshold of 700 (70 % probability) applied for compound confirmation.

The pH of *Lophanthus anisatus* hydrosol was determined in an aqueous suspension (sample:water = 1:2.5), according to SR 7184-13:2001, PTL 04, ed. 3, rev. 0.

## RESULTS AND DISCUSSIONS

In the essential oil of *Lophanthus anisatus*, the analysis reveals that estragole is the predominant compound (Table 1, Figure 2). Estragole is a phenylpropanoid (allylbenzene) occurring in several aromatic plants—especially tarragon (*Artemisia dracunculus*) and estragole-chemotype basil (*Ocimum basilicum*), as well as anise and star anise. Typical presence in these natural complex substances is documented by IFRA (the fragrance industry safety body) (IFRA, 2020).

Botanical insecticides offer promising alternatives for crop pest management. Pure methyl chavicol (estragole) exhibited potent toxicity against the corn pest *Spodoptera frugiperda*, causing 100 % larval mortality within 24 hours at a concentration of 0.92 mg mL<sup>-1</sup> in a diet-incorporation assay, underscoring its potential as an effective biocontrol agent (Menezes CWG et al., 2019).

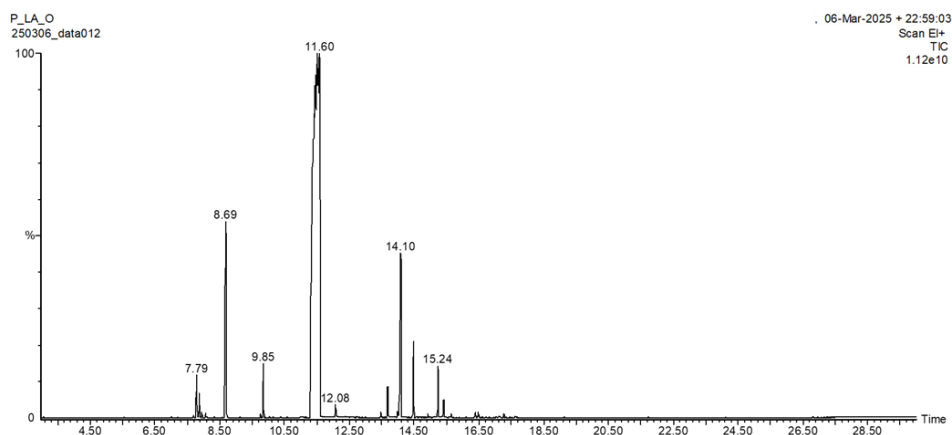
For antifungal activity against plant pathogens, an estragole-rich essential oil from *Ocimum selloi* and its major constituent, methyl chavicol, demonstrated inhibitory effects on phytopathogenic fungi in vitro. Against *Moniliophthora perniciosa* (causal agent of witches' broom disease in cacao), methyl chavicol at approximately 1000 ppm exhibited activity comparable to the whole oil. Similarly, estragole-type basil oil has been reported to suppress *Botrytis fabae* (broad bean pathogen) (Oxenham, S. K. et al. 2005). These findings highlight the potential of estragole-based formulations for managing certain fungal diseases (Costa, L. C. B., et al. 2015, Ajmal, M. et al. 2025 ).

Antibacterial activity against plant-associated bacteria. An estragole-rich essential oil from *Ocimum ciliatum* demonstrated in vitro antibacterial effects on plant-pathogenic bacteria, suggesting potential applications in pre- or post-harvest hygiene, depending on the formulation ( Mohammad M. et al. 2014).

Table 1

*Lophanthus anisatus* – essential oil main compounds

	Compound name	Retention time (min)	Probability (%)	CAS #	Area	% from the sum of areas
1	1-OCTEN-3-OL	7,79	93,9	53907-72-5	32642264	1,23
2	3-Octanone	7,87	92,7	106-68-3	16803854	0,64
3	D-Limonene	8,69	85,7	5989-27-5	23353617 6	8,83
4	1-OCTEN-3-YL ACETATE	9,85	93,1	2442-10-6	37872860	1,43
5	Estragole	11,60	74,4	140-67-0	26448542 72	100,00
6	Methyleugenol	14,10	88,5	93-15-2	19630296 0	7,42
7	Caryophyllene	14,49	94,1	87-44-5	61962592	2,34
8	GERMACRENE-D	15,24	92,3	23986-74-5	39347140	1,49
9	ç-Elemene	15,42	87,4	29873-99-2	13998462	0,53
10	(+) spathulenol	16,40	78,8	77171-55-2	5330995	0,20
11	(-)-Caryophyllene oxide	16,49	87,6	1139-30-6	4605159	0,17
12	Phytol	21,74	87,3	150-86-7	987471	0,04

Figure 2. Total ion chromatogram recorded (TOC) – for *Lophanthus anisatus* essential oil.

Analysis of the *Lophanthus anisatus* hydrosol showed estragole as the principal component, with methyleugenol detected as the secondary compound (Table 2, Figure 3).

The pH of *Lophanthus anisatus* hydrosol was measured at 5.04, indicating a slightly acidic character typical of plant-derived hydrosols.

Methyleugenol (often referred to as methyl eugenol) is a powerful male fruit fly attractant widely utilized in integrated pest management (IPM) for horticultural crops. It specifically attracts males of several *Bactrocera* species, such as *B. dorsalis*, *B. zonata*, and *B. correcta*, leading to substantial reductions in fruit fly

populations in orchards of mango, guava, papaya, and citrus (Tan & Nishida, 2012; Singh et al., 2018).

This compound is typically deployed in pheromone traps or lethal bait traps, where it selectively lures pest insects while limiting effects on beneficial insect populations (Shelly et al., 2014). Because of this specificity, methyleugenol plays an essential role in mass trapping programs and IPM, significantly lowering the need for broad-spectrum pesticide applications (Tan & Nishida, 2012; Singh et al., 2018).

In addition, methyleugenol has been incorporated into Sterile Insect Technique (SIT) programs, where brief exposure—sometimes termed “aromatherapy”—enhances the mating competitiveness of sterile males, thereby improving the overall efficiency of SIT-based fruit fly suppression (Shelly et al., 2014). Interestingly, while attractive at lower concentrations, methyleugenol can act as a repellent against certain insects such as the cigarette beetle (*Lasioderma serricorne*) when applied at higher doses, suggesting a dose-dependent dual role in pest management (Zhang et al., 2018).

Furthermore, methyleugenol has been classified in regulatory frameworks as a biopesticide or metabolite insecticide, with high-purity formulations (>98 %) commonly used for labeled agricultural applications (Tan & Nishida, 2012).

Methyleugenol is frequently a major constituent of essential oils, where it contributes to antifungal efficacy. For instance, in *Ocimum campechianum* oil, methyleugenol accounts for 75–87% of the total composition, and both the crude oil and pure compound demonstrate potent fungicidal effects against plant pathogens such as *Fusarium oxysporum* and *Colletotrichum gossypii* (Figueiredo et al., 2018). Although its antifungal performance is notable, the associated antioxidant activity tends to be moderate (<40 %) (Figueiredo et al., 2018).

Beyond antifungal uses, methyleugenol also exhibits antibacterial and antibiofilm activities. A recent study showed that it can interfere with bacterial quorum sensing—thereby inhibiting biofilm formation—and also disrupt bacterial cell membranes by causing depolarization. These properties make it a promising candidate for eco-friendly antimicrobial surface applications (Zhou et al., 2024).

Moreover, essential oils containing methyleugenol, such as those derived from *Gardenia jasminoides*, have demonstrated moderate antibacterial activity. Molecular docking analyses suggest that methyleugenol may interact with key bacterial enzymes, including topoisomerase II, which could help explain its antimicrobial activity (Lopes et al., 2023).

Table 2

*Lophantus anisatus* – hydrosol main compounds

	Compound name	Retention time (min)	Probability (%)	CAS #	Area	% from the sum of areas
1	1 OCTEN 3 OL	7,80	87	3391-86-4	807129	21,46
2	Estragole	11,32	94,3	140-67-0	3760226	100,00
3	Eugenol	13,70	89	97-53-0	765733	20,36
4	Methyleugenol	14,06	91,9	93-15-2	2444517	65,01

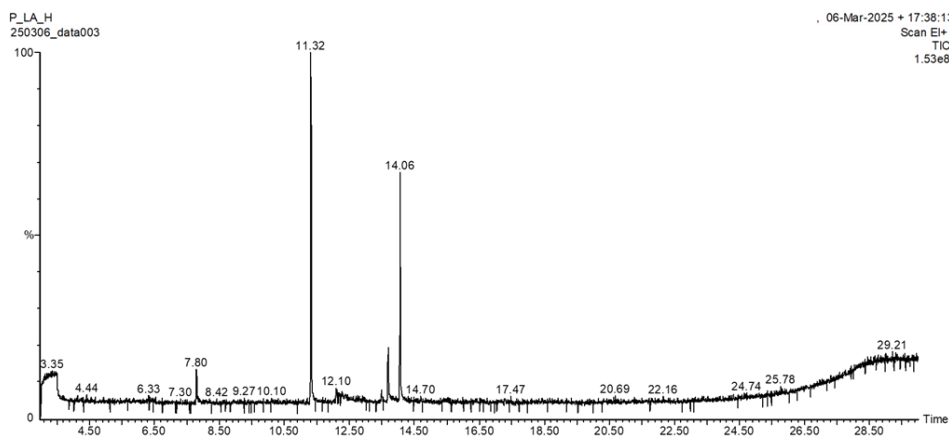


Figure 3. Total ion chromatogram recorded (TOC) – for *Lophanthus anisatus* hydrosol.

### CONCLUSIONS

The chemical profiles of *Lophanthus anisatus* essential oil and hydrosol demonstrate clear differences, despite sharing estragole as the predominant compound. In the essential oil, estragole accounted for nearly the entire composition, with smaller proportions of methyleugenol, D-limonene, and caryophyllene. Conversely, the hydrosol, while still rich in estragole, was characterized by comparatively higher levels of methyleugenol, eugenol, and 1-octen-3-ol, indicating a broader volatile spectrum. These findings align with previous reports that hydrosols often retain a more diverse set of water-soluble compounds compared to essential oils (Di Vito et al., 2021).

The high content of estragole in both preparations is notable. While estragole contributes to the aromatic character and potential bioactivity of *L. anisatus*, it has also been classified as a genotoxic and potentially carcinogenic compound in animal studies, which has prompted regulatory bodies such as the European Medicines Agency (EMA, 2023) to recommend restrictions on its use. The comparatively diluted concentration of estragole in hydrosols could mitigate some of these safety concerns, making them more suitable for direct applications such as aromatherapy, topical cosmetics, or food flavoring.

Beyond estragole, the presence of methyleugenol and eugenol in the hydrosol is of particular interest. Both compounds are known for their antimicrobial, antioxidant, and anti-inflammatory activities (Sharifi-Rad et al., 2017; Tangpao, 2018). Their relatively high abundance in the hydrosol suggests that this preparation may offer greater therapeutic potential compared to the essential oil, particularly in applications where phenylpropanoid activity is desirable. Similarly, the detection of 1-octen-3-ol, a compound often linked to antifungal activity and characteristic mushroom-like aroma, may further enhance the biofunctional versatility of the hydrosol.

Altogether, the findings suggest that while estragole defines the core chemical identity of *Lophanthus anisatus*, the hydrosol composition provides a safer and potentially more functionally diverse product than the essential oil. This supports the growing view that hydrosols, often overlooked in favor of essential oils, should be considered valuable botanical preparations in their own right (Di Vito et al., 2021).

Further pharmacological and toxicological studies are recommended to clarify the biological significance of methyleugenol and eugenol in *L. anisatus* hydrosols and to assess their safe application ranges.

Beyond their chemical merits, hydrosols also present significant **economic advantages**. As byproducts of essential oil distillation, hydrosols are often undervalued or discarded, despite their considerable bioactive potential (Di Vito et al., 2021). Utilizing hydrosols not only adds value to the distillation process but also contributes to a **circular bioeconomy** by reducing waste and diversifying product portfolios for small-scale producers and the fragrance, cosmetic, and food industries. Their lower production costs, ease of formulation in aqueous systems, and increasing consumer demand for natural, mild, and multifunctional products further strengthen the economic case for their broader use.

In conclusion, hydrosols—exemplified by *Lophanthus anisatus*—should be regarded not merely as secondary products but as valuable botanical resources with both functional and economic significance. Future efforts to systematically characterize hydrosols and explore their applications may enhance their role as sustainable, marketable, and health-promoting alternatives to essential oils.

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