ISSN Print: 2664-844X
ISSN Online: 2664-8458
NAAS Rating (2026): 4.97
IJAFS 2026; 8(7): 340-347
www.agriculturaljournals.com
Received: 12-04-2026
Accepted: 15-05-2026

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In vitro antifungal and sporulation-inhibitory activity of selected essential oils against *Aspergillus niger* and GC-MS characterization of selected highly active oils

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DOI: https://www.doi.org/10.33545/2664844X.2026.v8.i7e.1696

Abstract
Aspergillus niger is a filamentous ascomycete fungus and a common plant and food contaminant. Plant-pathogenic *A. niger* affects agricultural crops both in the field and after harvest. In the present study, *A. niger* was isolated from infected groundnut root, purified by sub-culturing, and identified using morphological and molecular techniques. The *in vitro* antifungal properties of 14 essential oils (EOs) were evaluated against *A. niger*, and the chemical compositions of selected highly active EOs were investigated by gas chromatography-mass spectrometry (GC-MS). *Cinnamomum zeylanicum*, *Cinnamomum cassia*, *Laurus nobilis*, and *Cymbopogon citratus* EOs exhibited higher antifungal activity than the other tested oils. The potential of these EOs as eco-friendly and economical biocontrol agents in agriculture is discussed.

Keywords: *A. niger,* Essential Oils, Plant Disease, Antifungal Activity, GC-MS

Introduction
Fungal diseases of crops are a major constraint to agricultural productivity and postharvest quality worldwide. Species of *Aspergillus*, including *A. niger*, are particularly important because they cause seedling blights, collar rot, and storage rots of oilseed and grain crops, and can reduce seed germination and marketability (Aiswarya *et al.*, 2024) [2]. In groundnut, for example, *A. niger* is associated with collar rot and storage rot, leading to significant yield and quality losses in many production systems (Kishore *et al.*, 2007) [15]. Conventional management of fungal diseases relies heavily on synthetic fungicides and, in some cases, antibiotics and copper compounds. While such chemicals are effective, their intensive and often indiscriminate use has raised environmental and human health concerns, including pesticide residues in food, non-target toxicity, and the evolution of fungicide-resistant pathogen populations (Raveau *et al.,* 2020; Samal *et al.*, 2024) [26]. Similar issues are well documented for antibiotic use in plant agriculture, where resistant bacterial strains and off-target impacts on microbiomes have been observed (McManus *et al.,* 2002) [20]. These challenges have driven a strong interest in developing eco-friendly alternatives and integrating them into disease management programs.

Plant-derived products, especially essential oils (EOs), have emerged as promising candidates for green disease management. EOs are complex mixtures of volatile secondary metabolites, primarily terpenes, terpenoids, phenylpropanoids, and related compounds, produced by aromatic plants. They are generally recognized as biodegradable, often have relatively low mammalian toxicity, and can show broad-spectrum antimicrobial activity (Nazzaro *et al.*, 2017) [21]. Over the last two decades, numerous studies and reviews have demonstrated the antifungal properties of EOs against postharvest and field fungi, including *Aspergillus, Penicillium*, *Botrytis*, *Colletotrichum*, and others, and have proposed their use as fumigants, vapours, coatings, or components of active packaging (Sivakumar & Bautista-Baños, 2014; Allagui *et al.*, 2023) [33].

The antifungal activity of EOs is closely related to their chemical composition. Phenylpropanoids such as (E)-cinnamaldehyde and eugenol, and oxygenated monoterpenes such as citral, thymol, and carvacrol, are often identified as key bioactive constituents

(Powers *et al.*, 2018) [23]. Cinnamon (*Cinnamomum* spp.) oils are typically rich in (E)-cinnamaldehyde and have shown strong inhibition of growth and spore germination in a range of fungi, including *Aspergillus spp*. (Zhang *et al.*, 2025) [37]. Likewise, lemongrass (*Cymbopogon citratus*) oil, dominated by citral (neral + geranial), and bay laurel (*Laurus nobilis*) oil, containing eugenol and monoterpene hydrocarbons, have been reported to inhibit mycelial growth and reduce mycotoxin production by *Aspergillus* species (Belasli *et al.*, 2020; Adebayo and Osuolale, 2024) [6].

Despite this growing body of work, there is still value in comparative screening of multiple commercial EOs against important storage fungi, especially when combined with chemical profiling that allows a preliminary structure–activity interpretation. Such data can help to prioritize candidate oils for formulation development (e.g., coatings, nanoemulsions, or EO-fungicide combinations) and to identify oils that reduce sporulation, which is especially critical for lowering inoculum pressure and airborne spread (Rabari *et al.,* 2018) [25].

In this study, we evaluated the *in vitro* antifungal activity of 14 commercial EOs against *Aspergillus niger* using a disc diffusion assay. We recorded both inhibition of mycelial growth (zone of inhibition) and the qualitative effect on sporulation. Two selected highly active oils, *Cinnamomum cassia* and *Laurus nobilis*, were further subjected to GC-MS analysis to determine their major chemical constituents and to relate composition to bioactivity. The overarching aim was to identify promising EO candidates for the development of plant-based fungicidal or fungistatic products against *A. niger* and similar storage pathogens.

Materials and Methods

Isolation of Fungi

Infected *Arachis hypogaea* roots were collected from a farm in Jamvadi village, Gondal, Rajkot, Gujarat. The infected material was washed under running tap water for 3 h and surface sterilized with 0.1% HgCl2 for 15 min. It was then washed three times with sterile distilled water. After surface sterilization, infected root pieces were cut using sterile forceps and a scalpel. The cut infected pieces were inoculated on PDA medium under aseptic conditions and incubated at 37 ºC. After 48 h of incubation, mycelial growth was observed around the leaf. Morphological identification was carried out by observing the growth pattern and colour of the hyphae.

Essential oils

Fourteen EOs were obtained from Vimal Research Society for Agro-Biotech and Cosmic Powers (VIRSACO). The oils evaluated is listed in Fig. 1 together with their antifungal activity data.

Disc diffusion assay for antifungal activity

Antifungal activity of the 14 EOs against *A. niger* was evaluated using a disc diffusion method adapted from Rabari *et al.* (2018) [25]. *A. niger* was grown on PDA plates for 96 h at 37 °C to obtain actively growing mycelium and conidia. After 96 h, fungal mycelium and conidia were suspended in 10 mL sterile distilled water (D/W). One hundred microlitres (100 µL) of fungal suspension was spread over PDA plates using a glass spreader to obtain uniform fungal growth on test plates. For preparing the test discs, 5 µL of EO was pipetted onto a 5-mm Whatman filter paper disc and placed carefully on the PDA medium. All Petri plates were sealed with sterile laboratory parafilm to avoid evaporation of the test samples. The plates were left for 30 min at room temperature to allow diffusion of the oil and then incubated at 37 °C for 96 h. After incubation, the zone of inhibition was measured for all 14 oils in millimetres. The experiment was performed in triplicate, and the mean value was calculated. The antifungal activity of the oils is summarized in Figs. 1 and 2. In addition to mycelial growth inhibition, the effect of each EO on sporulation was qualitatively recorded as complete inhibition of growth (no visible mycelium), sporulation inhibition (mycelial growth present but with markedly reduced or absent conidiation around the disc), or no apparent effect on sporulation relative to the control.

GC–MS analysis of selected essential oils

The two selected highly active oils, *C. cassia* and *L. nobilis*, were analyzed using gas chromatography-mass spectrometry (GC-MS) to identify major constituents. Analyses were carried out on a Perkin Elmer AutoSystem XL gas chromatograph coupled to a Perkin Elmer TurboMass mass spectrometer. Separation was achieved on a DB-1 capillary column (30 m x 0.25 mm, 0.25 µm film thickness) with helium as carrier gas at a flow rate of 1 mL min-1. The injector and detector temperatures were set at 250 °C and 280 °C, respectively. The oven temperature was programmed from 60 to 250 °C at 5 °C/min. A 2 µL sample of each EO diluted in an appropriate solvent was injected in split mode. The transfer line temperature was maintained at 280 °C. Mass spectra were acquired over an appropriate mass range, and EO components were identified by comparing their retention times and mass spectra with those in the NIST library and, when possible, with published data. Relative percentages of components were calculated from peak area normalization (Table 1).

Data handling and R-based visualization

All data handling and visualization were carried out in R (version 4.5.0; R Core Team, Vienna, Austria). The dataset containing botanical name, family, common name, zone of inhibition, and qualitative sporulation response for each EO was entered into a comma-separated values (CSV) file and imported into R using the readr package. Data wrangling and variable derivation were performed using functions from dplyr and tidyr within the tidyverse ecosystem.

For each EO, an efficacy class was assigned based on the measured zone of inhibition: very low (<10 mm), low (10-30 mm), moderate (30-60 mm), and high (>60 mm). These classes were defined a priori to facilitate visualization and are not intended as formal breakpoints. A numerical rank variable (1 = highest activity) was generated by ordering oils in descending order of zone diameter and resolving ties by assigning the same rank. These derived variables are included in Fig. 1. Graphical representation of the antifungal activity was produced using ggplot2. A Cleveland dot (lollipop) plot was constructed with zone of inhibition (mm) on the x-axis and EO name on the y-axis. Horizontal segments were drawn from zero to the observed zone value for each oil, with points at the terminal zone value. Panels were faceted according to the qualitative sporulation response category (no apparent effect, sporulation inhibited, complete inhibition of growth and sporulation), and points

were coloured by efficacy class to emphasize differences among oils.

Results and Discussion

Antifungal and sporulation-inhibitory activity of essential oils

Thirteen of the 14 EOs exhibited measurable antagonistic activity against *A. niger*in vitro, although the magnitude of inhibition varied markedly among oils (Figs. 1 and 2). *Vetiveria zizanioides* oil showed no detectable inhibition under the conditions tested. The largest inhibition zones were observed for *Laurus nobilis* and *Cymbopogon citratus*, each with zones of 85 mm, followed by *Cinnamomum cassia* (81 mm) and *C. zeylanicum* (73 mm). Oils of *Syzygium aromaticum* (58 mm), *Geranium macrorhizum* (55 mm), and *Apium graveolens* (52 mm) showed strong activity, whereas *Melaleuca alternifolia* (48 mm), *Eucalyptus globulus* (29 mm), *Jasminum grandiflorum* (15 mm), *Lavandula officinalis* (12 mm), *Rosmarinus officinalis* (12 mm), and *Santalum album* (9 mm) produced smaller but still discernible zones.

An interesting qualitative feature of the dataset is that several oils not only inhibited mycelial expansion but also suppressed conidial production. *M. alternifolia, L. officinalis, G. macrorhizum, S. album, C. cassia, and A. graveolens* all showed "sporulation inhibition" in the inhibition zone, while *L. nobilis* and *C. citratus* completely prevented both mycelial growth and sporulation in the region around the disc. From a plant disease management perspective, suppression of sporulation is extremely valuable because it lowers inoculum potential, limits airborne spread, and may reduce the risk of mycotoxin contamination in storage environments.

Overall, the ranking of oils in our disc diffusion assay is broadly consistent with previous reports that identify cinnamon, lemongrass, clove, and bay laurel oils as among the most potent antifungal EOs. *Cinnamon* bark and *cassia* oils, rich in (E)-cinnamaldehyde and eugenol, have repeatedly shown strong inhibition of *Aspergillus* spp. and other plant pathogens in vitro (Kishore *et al.*, 2007) [15]. Similarly, lemongrass oil, dominated by citral, has been documented to completely inhibit mycelial growth of several *Aspergillus* species and other fungi at relatively low concentrations (Matasyoh *et al.,* 2011) [19]. Recent studies on *L. nobilis* EO also highlight its strong antifungal and antitoxigenic effects against *Aspergillus* and yeast pathogens, supporting the high activity observed in our work (Belasli *et al.*, 2020) [6].

At the same time, some oils with modest zones (e.g., *M. alternifolia* and *L. officinalis*) nonetheless markedly suppressed sporulation. This duality, moderate growth inhibition but strong impact on conidiation, suggests that different EO components or concentration thresholds may differentially affect hyphal extension and spore differentiation. Such effects have been noted in mechanistic studies showing that EOs can alter membrane integrity, interfere with ergosterol biosynthesis, and disrupt signaling pathways involved in sporulation and secondary metabolism (Leiva-Mora *et al.*, 2025) [16]. For storage disease management, oils that primarily reduce sporulation could be combined with other agents (e.g., biological control organisms or reduced-dose fungicides) to achieve both growth suppression and inoculum reduction.

GC–MS profile of *C. cassia* and *L. nobilis* essential oils

GC-MS analysis of *C. cassia* EO revealed (E)-cinnamaldehyde as the dominant component (66.36% of total peak area), accompanied by benzyl benzoate (10.24%), beta-linalool (9.16%), eugenol (7.74%), cinnamyl acetate (3.97%), eugenyl acetate (0.52%), benzaldehyde (0.15%), benzyl alcohol (0.23%), and minor unidentified constituents (1.63%) (Table 1). This composition is in good agreement with published data for *C. cassia* and *C. zeylanicum*, where (E)-cinnamaldehyde typically represents 60-90% of the oil, with benzaldehyde and cinnamyl acetate as additional major components (Singh *et al.*, 2007) [32].

For *L. nobilis* EO, eugenol (50.40%) was the predominant peak, followed by benzaldehyde (10.96%), caryophyllene (10.23%), beta-linalool (9.90%), beta-pinene (3.17%), limonene (3.71%), 1,8-cineole (2.63%), 1-terpinene (2.10%), and other minor constituents (7.99%) (Table 1). This eugenol-rich chemotype is consistent with earlier reports in which *L. nobilis* leaf and flower oils contain large proportions of eugenol, 1,8-cineole, and monoterpene hydrocarbons, and exhibit notable antifungal activity (Belasli*et al.*, 2020) [6].

The strong antifungal effects of *C. cassia* and *L. nobilis* against *A. niger* in our assay therefore likely stem from the high abundance of phenylpropanoids such as (E)-cinnamaldehyde and eugenol. Both compounds have been shown to disrupt fungal membranes, increase cell permeability, and cause leakage of intracellular constituents, ultimately leading to growth inhibition and cell death (Powers *et al.,* 2018) [23]. Aldehydes like cinnamaldehyde are particularly reactive toward nucleophiles in proteins and may interfere with key metabolic enzymes, while eugenol can insert into lipid bilayers and disturb membrane structure (Al-Harrasi *et al.,* 2022) [3].

Sporulation-targeted EO activity and formulation potential

A notable feature of this study is the differentiated response of *A. niger* in terms of hyphal growth versus sporulation. Oils such as *C. citratus* and *L. nobilis* completely inhibited both mycelial growth and sporulation, whereas *M. alternifolia, G. macrorhizum, A. graveolens*, and *C. cassia* strongly reduced sporulation despite moderate growth zones. This pattern suggests that some EO components may preferentially target developmental processes leading to conidiation rather than primary vegetative growth. From a disease management standpoint, this is an under-exploited angle: many fungicide screening assays focus only on radial growth, ignoring sporulation behaviour.

Recent reviews on EO mechanisms emphasize the need to move beyond simplistic MIC values and to examine multiple endpoints, including membrane integrity, oxidative stress, sporulation, biofilm formation, and mycotoxin production (Mani-López *et al.,* 2021) [18]. In the context of *A. niger*, EOs that primarily suppress sporulation could help maintain low inoculum loads on seeds and in storage environments even when some mycelial growth persists, thereby reducing spread and potentially lowering mycotoxin risk. Future work could quantify conidial counts microscopically and evaluate effects on spore viability and germination.

Another forward-looking avenue is the incorporation of these highly active oils into delivery systems such as nano emulsions, edible coatings, and EO-loaded films, which can

stabilize volatile compounds and provide controlled release. Recent studies have demonstrated the success of EO nano emulsions (including *L. nobilis* EO) and EO-incorporated biopolymer films in inhibiting *Aspergillus* spp. on food substrates while improving barrier properties (Singh*et al.,* 2023) [30]. The strong activity of *C. cassia, L. nobilis, C. citratus,* and *S. aromaticum* in our in vitro assay suggests that these oils would be promising candidates for such formulations aimed at protecting stored seeds or grains.

Comparison with previous EO screenings against *Aspergillus*

Large screening studies of EOs have shown that a relatively small subset of oils display consistently strong antifungal activity across multiple fungi. For example, Rabari *et al.* (2018) [25] screened 75 EOs and identified cinnamon, clove, lemongrass, and thyme among the most broadly active oils. Similar conclusions emerge from other surveys and meta-analyses of EOs against *Aspergillus* and *Penicillium spp.*, where phenylpropanoid-rich and citral-rich oils typically rank high in potency. Our results align with these patterns and reinforce the idea that these chemotypes could serve as backbone components in botanical fungicide formulations (Powers *et al.*, 2018) [23].

However, we also observed that some oils traditionally considered weaker fungicides, such as sandalwood and jasmine, still produced small but measurable inhibition zones. While these effects may not be sufficient on their own, they could potentially contribute to synergistic or additive interactions in multi-component blends, for instance, cinnamon–clove or cinnamon–lemongrass mixtures, which have shown synergistic effects against *A. niger* and other microbes in previous work (Purkait *et al.,* 2020) [24]. Exploring such combinations could allow dose reduction and help address cost and sensory constraints (e.g., strong odour or flavour) in practical applications.

Limitations and future directions

This work was designed as an exploratory *in vitro* screening rather than an exhaustive evaluation of all parameters relevant to product development. The disc diffusion assay provides a rapid and widely used first indication of antifungal activity, but zone diameters can be influenced by factors such as volatility and diffusion of the oils in agar and therefore do not directly correspond to MIC values or detailed dose-response relationships (Wilson *et al.*, 1997) [35]. Follow-up studies using broth microdilution and vapor-phase assays, together with quantification of spore germination and mycelial growth rates at different concentrations, would allow a more precise determination of fungistatic and fungicidal thresholds for the most promising oils.

Finally, the current experiments were performed under controlled *in vitro* conditions. For practical application in crop or storage protection, the most active oils, particularly *C. cassia*, *L. nobilis*, *C. citratus*, and *S. aromaticum*, should be evaluated in more realistic systems, such as EO-based coatings, nanoemulsions, or active packaging applied to seeds or stored commodities. Such studies could be extended to examine seed germination, phytotoxicity, residue behaviour, and safety for mammalian cells. Despite these natural limitations of a first screening, the present results provide a clear and useful baseline for prioritizing candidate oils and guiding the design of more detailed mechanistic and formulation studies.

Conclusion

The present study demonstrates that selected plant essential oils can strongly inhibit the growth and sporulation of *Aspergillus niger* under *in vitro* conditions. Among the 14 essential oils tested, *Laurus nobilis*, *Cymbopogon citratus*, *Cinnamomum cassia*, and *Cinnamomum zeylanicum* showed the highest antifungal activity, while several oils also reduced conidial production. This sporulation-inhibitory effect is important because suppression of spores can reduce inoculum load and may help limit the spread of *A. niger* in crop and storage environments.

GC-MS analysis showed that the strong activity of *C. cassia* and *L. nobilis* was associated with major bioactive constituents such as (E)-cinnamaldehyde and eugenol, respectively. These compounds are known to affect fungal membrane integrity and may contribute substantially to the observed inhibition. Although the work is an initial laboratory screening, the results identify these essential oils as promising candidates for further development as botanical fungistatic or fungicidal agents. Future studies should evaluate dose-response relationships, vapor-phase activity, effects on spore germination and mycotoxin production, and performance in seed coating, nano emulsion, or storage-protection systems. Overall, this study supports the potential use of essential oils as eco-friendly, residue-conscious tools for integrated management of *A. niger* and related storage pathogens.

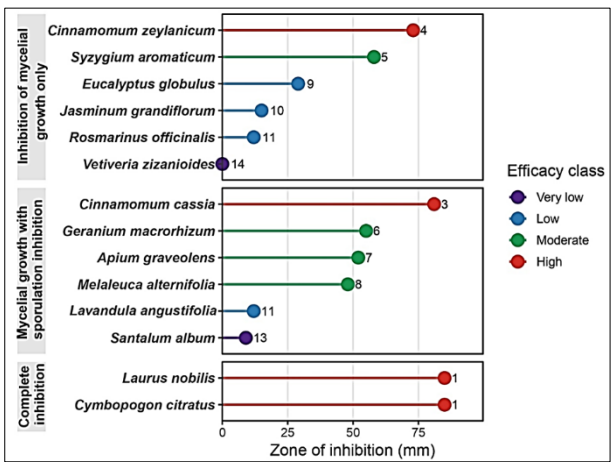

Fig 1: *In vitro* antifungal efficacy of selected essential oils based on zone of inhibition and sporulation response

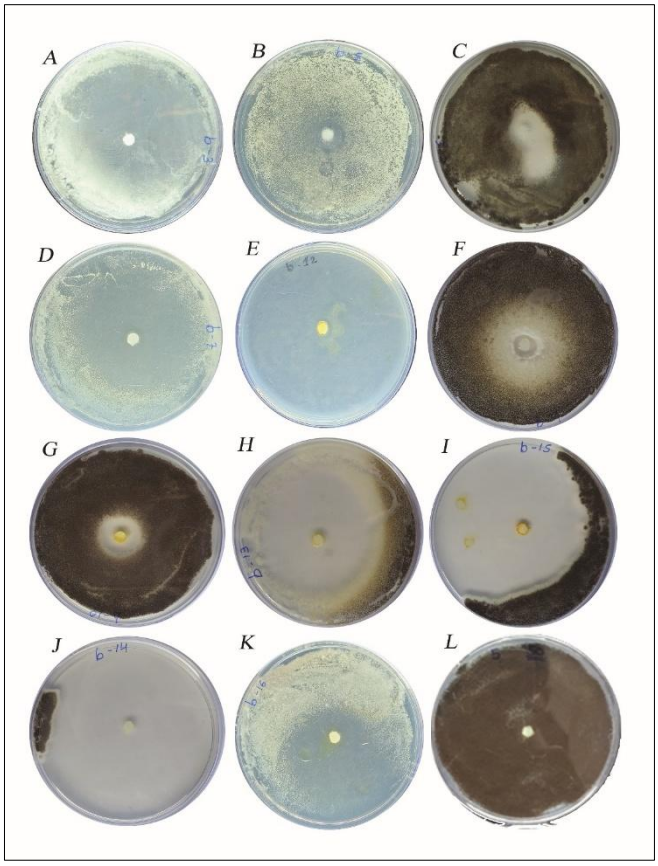

Fig 2: Zone of inhibition of *A. niger* against (A) *M. alternifolia*, (B) *L. officinalis*, (C) *E. globulus*, (D) *G. macrorhizum*, (E) *L. nobilis*, (F) *S. album*, (G) *J. grandiflorum*, (H) *S. aromaticum*, (I) *C. zeylanicum*, (J) *C. cassia*, (K) *A. graveolens*, and (L) *V. zizanioides* essential oils

Table 1: Chemical composition of selected essential oils

No.	Essential oil	Compound	RT (min)	Area (%)	Chemical class	Major reported bioactivities*	Antifungal / anti-*Aspergillus* evidence
1	*C. cassia*	β-Linalool	11.666	9.16	Monoterpene alcohol	Antimicrobial, antioxidant, anti-inflammatory, analgesic, anxiolytic, neuroprotective (Kamatou and Viljoen, 2008) [13]	Inhibits *Aspergillus flavus* and other fungi; fungicidal against various yeasts and molds (Li *et al.,* 2022) [17]
		(E)-Cinnamaldehyde	15.369	66.36	Phenylpropanoid aldehyde	Potent antioxidant, broad antimicrobial, anti-inflammatory, insecticidal, anti-aflatoxigenic, gut-protective, possible neuroprotective (Sharma *et al.,* 2016) [29]	Strong inhibition of *A. flavus*, *A. niger*, *A. fumigatus* growth and aflatoxin B_1 production; effective in vapor and contact assays (Kim *et al.*, 2018) [14]
		Eugenol	15.956	7.74	Phenylpropanoid phenolic ether	Strong antioxidant, broad-spectrum antimicrobial, analgesic, anti-inflammatory, antitumor; food-preservative potential (Ulanowska and Olas, 2021) [34]	Active against *Aspergillus* spp., *Candida* and dermatophytes; inhibits cell envelope and virulence factors (Didehdar *et al.,* 2022) [9]
		Cinnamyl acetate	17.110	3.97	Phenylpropanoid ester	Fragrance component; contributes to antioxidant and antimicrobial effects of cinnamon phenylpropanoids (Shinde *et al.,* 2023) [30]	Limited direct data; activity usually attributed to co-occurring cinnamaldehyde/eugenol; may support overall antifungal effect (Sharma *et al.*, 2016) [29]
		Eugenyl acetate	17.862	0.52	Phenylpropanoid ester	Biotransformation product of eugenol; shares aromatic backbone, contributes to aroma and mild antimicrobial/antioxidant effects (Shinde *et al.,* 2023) [30]	Specific anti-*Aspergillus* data scarce; usually discussed with eugenol-rich EOs (Pandey *et al.,* 2017) [22]
		Benzyl benzoate	21.181	10.24	Aromatic ester	Acaricidal and insecticidal; used clinically for scabies/lice; repellent activity; some antimicrobial contribution in EOs (Saltan *et al.*, 2024) [27]	Primarily insecticidal; EOs rich in benzyl benzoate show activity vs yeasts and *Saccharomyces*; direct anti-*Aspergillus* data limited (Farjam, 2012) [11]
		Benzaldehyde	8.916	0.15	Aromatic aldehyde	Flavoring and preservative; antimicrobial, antioxidant, anti-inflammatory, sometimes enhances	Used as antibacterial /anti-fungal preservative; component of antimicrobial EOs, but usually as

						antibiotic activity (Aljaafari *et al.*, 2022) [4]	minor contributor (Xiao *et al.*, 2024) [36]
		Benzyl alcohol	10.474	0.23	Aromatic alcohol	Widely used bacteriostatic/antifungal preservative in pharmaceuticals and cosmetics; local anesthetic properties (European Medicines Agency, 2017) [10]	Provides preservative-level antimicrobial activity; usually supportive rather than primary antifungal agent in EOs (European Medicines Agency, 2017) [10]
		Others	–	1.63	Mixed minor constituents	Mixture of low-abundance terpenes/phenolics that may synergize with major compounds in antimicrobial and antioxidant activity (Pandey *et al.*, 2017) [22]	Often contribute to synergy but individual roles not defined
2	*L. nobilis*	β-Pinene	6.300	3.17	Monoterpene hydrocarbon	Antimicrobial, antioxidant, anti-inflammatory; contributes to respiratory and bronchodilatory effects of coniferous EOs (Bhavaniramya *et al.,* 2019) [7]	EOs rich in α/β-pinene show antifungal activity vs plant pathogens and *Aspergillus* spp. *(Kim et al.*, 2018) [14]
		1,8-Cineole	7.158	2.63	Monoterpene oxide (cyclic ether)	Mucolytic, bronchodilatory, anti-inflammatory, analgesic, antioxidant and antimicrobial; key respiratory EO component (Caputo *et al.*, 2017) [8]	Inhibits growth and aflatoxin production of *A. flavus* and *A. parasiticus*; active vs *A. niger*and other storage fungi (Kim *et al.,* 2018) [14]
		Limonene	7.402	3.71	Monoterpene hydrocarbon	Antioxidant, antimicrobial, anti-inflammatory; gastroprotective and potential anxiolytic; widely used as flavor/fragrance (Bhavaniramya *et al.*, 2019) [7]	Contributes to antifungal activity of citrus and laurel EOs; inhibits storage molds at higher concentrations (Bhavaniramya *et al.,* 2019) [7]
		β-Linalool	7.925	9.90	Monoterpene alcohol	As above for linalool: antimicrobial, antioxidant, anti-inflammatory, CNS-active (sedative, anxiolytic, antidepressant) (Kamatou and Viljoen, 2008) [13]	Inhibits *A. flavus* and other fungi; effective as biofumigant; antifungal vs *Candida* spp. (Li *et al.*, 2022) [17]
		1-Terpinene (terpinene-type monoterpene)	9.275	2.10	Monoterpene hydrocarbon/terpenoid	Part of terpinene/terpineol family; antioxidant, antimicrobial, anti-inflammatory; contributes to respiratory and skin-care effects (Bhavaniramya *et al.*, 2019) [7]	Terpinen-rich EOs show fungicidal activity vs *Aspergillus* and dermatophytes, usually synergistic with other monoterpenes (Pandey *et al.,* 2017) [22]
		Benzaldehyde	9.467	10.96	Aromatic aldehyde	See row above: antimicrobial, antioxidant, food preservative, sometimes enhances antibiotic effects (Aljaafari *et al.*, 2022) [4]	Antibacterial and antifungal preservative; part of EO antimicrobial spectrum, but rarely dominant antifungal driver (Xiao *et al.,* 2024) [36]
		Eugenol	11.525	50.40	Phenylpropanoid phenolic ether	As above: strong antioxidant, antimicrobial, analgesic, anti-inflammatory, potential neuroprotective; major contributor to clove/bay activity (Ulanowska and Olas, 2021) [34]	Significant antifungal action against *Aspergillus* spp. and other pathogens; damages cell envelope and inhibits virulence factors (Didehdar *et al.,* 2022) [9]
		Caryophyllene (β-caryophyllene)	12.358	10.23	Sesquiterpene hydrocarbon	Analgesic, antioxidant, antimicrobial, potent anti-inflammatory and immunomodulatory via CB2 agonism; neuroprotective and cardioprotective potential (Gushiken *et al.*, 2022) [12]	Contributes to antifungal and antibacterial effects of spice EOs; often synergistic with phenylpropanoids(Gushiken *et al.,* 2022) [12]
		Others	–	7.99	Mixed minor constituents	Monoterpenes, sesquiterpenes and phenylpropanoids that modulate aroma, volatility and contribute to antioxidant/antimicrobial synergy (Caputo *et al.*, 2017) [8]	Likely to enhance or modulate antifungal action in combination with major components

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