Isolation and Derivatization of Bioactive Compounds from Zingiber zerumbet Essential Oil with Antifungal Activity Against Rice Pathogens

Navneet Kaur
Punjab Agricultural University
Ramandeep Kaur
ramansaini@pau.edu

Punjab Agricultural University
Urvashi Bhardwaj
Punjab Agricultural University
Parul Sharma
Oklahoma State University

Research Article

Keywords: Wild ginger, Zingiber zerumbet, Essential oil, Zerumbone, Antifungal potential

Posted Date: April 21st, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6116554/v1

Additional Declarations: No competing interests reported.

Abstract

This study examines the chemical composition and antifungal properties of essential oil extracted from the rhizomes of wild ginger (*Zingiber zerumbet* Smith) and evaluates its major constituents against three pathogenic fungi: *Bipolaris oryzae, Fusarium moniliforme*, and *Rhizoctonia solani*. The essential oil was obtained via hydrodistillation and analyzed using GC-MS to identify both major and minor components. Key constituents included endo-borneol (15.77%), limonene (11.95%), zerumbone (10.73%), and camphene (11.53%), along with minor compounds such as caryophyllene (1.80%), caryophyllene oxide (3.99%), 4-terpineol (2.01%), and borneol formate (2.14%). Bioactivity-guided isolation led to the identification of three prominent terpenoids: limonene, caryophyllene, and zerumbone. Among them, zerumbone exhibited the strongest antifungal activity and was further derivatized into its alcohol and epoxide forms to investigate structure-activity relationships. The alcohol derivative demonstrated enhanced antifungal efficacy against all tested fungi, comparable to a standard fungicide. These findings underscore the potential of wild ginger essential oil, particularly zerumbone, as a natural fungicide for managing fungal pathogens.

Introduction

Essential oils, also known as essences or volatile oils, are natural substances biosynthesized by living organisms. These oils can be extracted through various methods, including distillation, solvent extraction, or mechanical pressing, depending on the plant material and desired yield. Essential oils are valued for their aromatic and bioactive properties, making them indispensable in industries such as fragrance, food, cosmetics, and pharmaceuticals. The composition of these oils often varies based on factors like the region of cultivation and prevailing climate conditions, which can influence the presence and concentration of bioactive compounds.(1–3)

Zingiber zerumbet (L.) Smith, commonly known as wild ginger or shampoo ginger, is a perennial, aromatic herb belonging to the Zingiberaceae family. This plant is native to shaded areas and tropical regions in Southeast Asia, where it is widely cultivated for its medicinal properties.(1) The rhizome, the underground stem of the plant, is the most frequently used part for therapeutic purposes. Traditional uses of *Z. zerumbet* include treatments for fever, indigestion, sprains, constipation, toothaches, diarrhea, inflammation, and pain relief. It has also shown potential in addressing various types of cancer.(4–10)

Recent studies have highlighted the biological activities of *Z. zerumbet* essential oil and its constituents. These include antimicrobial, antioxidant, anticancer, and antidiabetic properties, which contribute to its growing popularity in natural medicine.(11) The plant's bioactive compounds span several classes, such as polyphenols, terpenes, and alkaloids, offering a wide range of therapeutic benefits. The chemical composition of *Z. zerumbet* essential oil varies depending on geographical factors. Zerumbone and α-caryophyllene are among the major constituents identified in the rhizome and leaf oils. Zerumbone, in particular, is a highly bioactive compound with notable potential for drug development due to its diverse biological properties.(12–15)

Fungal pathogens pose a significant threat to global agricultural production, contributing to substantial yield losses. Rice (*Oryza sativa* L.) is one of the world's most important food crops, serving as a staple for nearly half of the global population. Asia accounts for over 90% of rice cultivation and consumption, given that 55% of the world's population resides in this region.(16–20) Several fungal diseases impact rice crops, with *Bipolaris oryzae* (causing brown spot), *Rhizoctonia solani* (causing sheath blight), and *Fusarium moniliforme* (causing foot rot) being the most destructive. These diseases are prevalent across temperate, subtropical, and tropical regions, leading to significant yield losses. Various strategies have been employed to manage rice diseases, including cultural practices, biological control agents, chemical fungicides, and the development of resistant varieties. While chemical control remains effective, its continuous use raises concerns about environmental safety and the emergence of fungicide-resistant pathogens. These challenges have driven the search for natural, eco-friendly antifungal agents that can offer sustainable alternatives for disease management.(16, 20–22)

Essential oil from *Z. zerumbet* has demonstrated significant bioactive properties, including antifungal potential. Although its efficacy against several fungi has been documented. but its toxicity and effectiveness against rice pathogens such as *Bipolaris oryzae*, *Fusarium moniliforme*, and *Rhizoctonia solani* have not been thoroughly assessed.

This study focuses on the bioactivity-guided isolation and derivatization of compounds from *Z. zerumbet* essential oil and their antifungal activity against rice fungi. The findings underscore the importance of plant-derived compounds as sustainable solutions for managing fungal diseases in rice cultivation. The antifungal properties of *Z. zerumbet* essential oil, particularly its major component zerumbone, offer promising avenues for eco-friendly disease control. By reducing reliance on synthetic fungicides, these natural agents can mitigate environmental risks and address the growing challenge of fungicide resistance in agricultural practices.

Experimental

Plant materials:

Rhizomes of *Zingiber zerumbet* were collected in 2016 from the Punjab, India. The plant was formally identified and authenticated by the Principal Scientist and Head of the Department of Floriculture and Landscaping at PAU with voucher specimen number K0014928324. The precise location of the collection is at coordinates 30.900965°N latitude 75.857277°E longitude. The plant species was formally identified and authenticated by the Principal Scientist and Head of the Department of Floriculture and Landscaping at PAU.

Chemicals

Silica gel (60–120 mesh size, Qualigens Fine Chemicals, Mumbai) was utilized for column chromatography. Cerium chloride and sodium borohydride were procured from Loba Chemie Private

Limited, Mumbai. All solvents used were of analytical grade. FT-IR spectra were recorded using a Perkin Elmer RX-1 FT-IR spectrophotometer. NMR spectra, including both ^{1}H and ^{13}C, were obtained on a Bruker AC (400 MHz) spectrometer with CDCl3 as the solvent. Melting points were measured using a Buchi B-545 melting and boiling point apparatus with open capillaries and are reported as uncorrected. **Fungi Culture**

Pure cultures of wheat fungus (*Bipolaris oryzae*, *Fusarium moniliforme* and *Rhizoctonia solani*) were obtained from the Department of Plant Pathology, Punjab Agricultural University, Ludhiana. The pathogens were collected from diseased plants, maintained on potato dextrose agar (PDA), and incubated and stored at 25 ± 1°C in a BOD incubator.

Isolation of Essential Oil

The rhizomes (500 g) were thoroughly cleaned by washing under running water and rinsing twice with sterilized water to remove soil and debris. They were then crushed and soaked overnight in 2.5 L of water within a 5-liter round-bottom flask. Essential oil extraction was performed using steam distillation with a Clevenger apparatus for 4 hours. This procedure was repeated five times to obtain a sufficient quantity of essential oil for further analysis, including the study of bioactive compounds and antifungal properties. The oily layer, along with water, was collected and extracted with diethyl ether. The organic phase was dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure to obtain the crude essential oil. The oil was stored in dark-colored vials at 4°C until it was ready for analysis. (20)

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Essential Oil

The chemical composition of essential oil extracted from the rhizomes of *Zingiber zerumbet* was analyzed using a Shimadzu QP2010 Plus gas chromatography-mass spectrometry (GC-MS) system. A single injection was performed with the split valve closed for the first minute to optimize sample introduction. The injector temperature was set at 280°C, and helium was used as the carrier gas at a constant pressure of 69 kPa. Separation of components was achieved using an Rtx-5 MS capillary column (30 m length, 20 mm internal diameter, 0.25 µm film thickness). The column temperature program began at 50°C (held for 2 minutes), then increased at 3°C/min to 180°C, followed by a rise to 280°C at 10°C/min. Mass spectrometric detection was conducted under electron ionization conditions at 70 eV, with the interface temperature also set at 280°C. The scan range spanned from 40 to 600 amu, enabling a comprehensive analysis of the oil's constituents. Retention indices were calculated for the detected peaks using a series of n-alkanes (C9-C33) under identical conditions. These indices were cross-referenced with reference values obtained from the ADAM RI system and databases such as NIST08, WILEY8, and specialized fragrance and flavor libraries. Additionally, the retention indices were validated against the NIST Chemistry WebBook. This rigorous analytical procedure ensured accurate

identification of the oil's components, forming a critical basis for evaluating its antifungal properties. (20)

Isolation of pure compounds

A sample of *Zingiber zerumbet* essential oil (10 g) was dissolved in a minimal amount of hexane and adsorbed onto silica gel to form a free-flowing powder. This prepared material was loaded into a pre-packed silica gel column (60–120 mesh size, activated by heating at 110°C for 1 hour) containing a slurry of silica gel in hexane. The column was eluted using hexane and dichloromethane as solvents, and fractions were collected for further analysis.(23–30)

Limonene (1). Limonene (**1**, 1.5g), Colourless liquid, b.p.175 ºC, was isolated using pure petroleum ether as eluting solvent. IR spectrum (Nujol, v_{max} cm^{-1}): 3082, 2919, 1643, 1437 and 1376. ^{1}HNMR signals ($CDCl_3$, 400MHz, δ, ppm, J/Hz**)**: 1.65 (3H, s, C_{10}), 1.73 (3H, s, C_9), 4.7 (2H, d, *J* = 0.92 Hz, C_8) and 5.39–5.40 (1H, m, C_2). ^{13}CNMR signals ($CDCl_3$, 100MHz, δ, ppm): 133.76 (C_1), 120.65 (C_2), 30.81 (C_3), 41.09 (C_4), 27.92 (C_5), 30.6 (C_6), 150.28 (C_7), 108.36 (C_8), 20.82 (C_9) and 23.47 (C_{10}). **(See SI for the NMR)**

Caryophyllene (2). Caryophyllene (**2**, 0.2 g), colourless oily liquid, b.p. 130 ºC, was also isolated from the petroleum ether fraction after complete removal of limonene. IR spectrum (Nujol, v_{max} cm^{-1}): 3068.1, 2926.7, 2858.1, 1631.3, 1449.5 and 885.9. ^{1}HNMR signals ($CDCl_3$, 400MHz, δ, ppm, J/Hz**)**: 5.32 (1H, t, C_{11}), 4.85 (1H, d, C_{15}), 4.93 (1H, d, C_{15}), 1.65 (3H, s, C_{12}), 1.05 (3H, s, C_{13}), 1.04 (3H, s, C_{14}), 1.76 (2H, t, C_9). ^{13}CNMR signals ($CDCl_3$, 100MHz, δ, ppm): 154.50 (C_{15}), 135.26 (C_1), 124.41 (C_{11}), 111.73 (C_8), 53.62 (C_4), 48.5 (C_5), 40.41 (C_6), 40.02 (C_2), 34.84 (C_9), 30.09 (C_5), 22.72 (C_{13}). **(See SI for the NMR)**

Zerumbone (3). Zerumbone (3, 1.2g), yellow colored needle crystals, m.p. 65ºC was isolated using pure dichloromethane as eluting solvent. IR spectrum (KBr, v_{max} cm^{-1}): 2958.8, 2923.9, 2852.8, 1735.6 and 1658.6. ^{1}HNMR signals ($CDCl_3$, 400MHz, δ, ppm, J/Hz): 1.09 (3H, s, C_{15}), 1.23 (3H, s, C_{14}), 1.56 (3H, s, C_{13}), 1.82 (3H, s, C_{12}), 5.91 (1H, d, *J* = 16.2 Hz, C_{11}), 5.87 (1H, d, *J* = 16.2 Hz, C_{10}), 1.90 (2H, m, C_8), 5.29 (1H, m, C_7), 2.59 (2H, m, C_5), 2.28 (2H, m, C_4), 6.02 (1H, m, C_3). ^{13}CNMR signals ($CDCl_3$, 100MHz, δ, ppm): 11.8 (C_{12}), 15.2 (C_{13}), 24.2 (C_{15}), 24.4 (C_{14}), 29.4 (C_4), 37.8 (C_9), 39.4 (C_5), 42.3 (C_8), 124.9 (C_7), 127.1 (C_{11}), 136.3 (C_6), 137.9 (C_2), 148.9 (C_3), 160.8 (C_{10}), 204.4 (C_1). **(See SI for the NMR)**

Derivatization of Zerumbone

Zerumbol (4). In a conical flask, cerium chloride (25 mg) was added to zerumbone (1.0 g) dissolved in 3 mL of methanol. The mixture was stirred for 15 minutes, after which sodium borohydride (1 g) was gradually added in small portions over 5 minutes with continuous stirring. (23) Upon completion of the reaction, the mixture was poured into water, and the product was extracted using dichloromethane. The solvent was then evaporated under reduced pressure, yielding pure white crystals of zerumbol. (4). Yield

80%, mp 77.5–78 ºC. IR spectrum (KBr, v_{max} cm^{-1}): 1664, 2929, 3340.7. ^{1}HNMR signals (**(**$CDCl_3$, 400MHz, δ, ppm, J/Hz): 1.06 (3H, s, C_{14}), 1.08 (3H, s, C_{15}), 1.43 (3H, s, C_{13}), 1.66 (3H, s, C_{12}), 4.63 (1H, d, C_{10}), 4.80 (1H, m, C_7) and 5.52–5.85 (1H, m, C_3). ^{13}CNMR signals ($CDCl_3$, 100MHz, δ, ppm):12.68 (C_2), 15.06 (C_6), 22.93 (C_4), 24.17 (C_9), 29.46 (C_9), 37.15 (C_9), 39.15 (C_5), 41.93 (C_8), 78.66 (C_1), 124.70 (C_3), 124.89 (C_7), 131.41 (C_{11}), 132.99 (C_6), 139.29 (C_{10}), 141.92 (C_2). **(See SI for the NMR)**

Epoxy zerumbone (5). Zerumbone (1.0 g) dissolved in chloroform (20 ml) was treated with perbenzoic acid as per the method used by Urvashi *et al* (2018).(43) Upon Vacuum evaporation of solvent, white colored crystals epoxy zerumbone were obtained (5). Yield 80%, m.p. 76–78 ºC. IR spectrum (KBr, v_{max} cm^{-1}): 2958.9, 2925.5, 2869.4, 1734.0, 1649.0, 1458.8, 1116. ^{1}HNMR signals **(**$CDCl_3$, 400MHz, δ, ppm, J/Hz): 1.18 (3H, s, C_{15}), 1.25 (3H, s, C_{14}), 1.32 (3H, s, C_{13}), 1.85 (3H, s, C_{12}), 1.95 (2H, m, C_8), 2.17 (2H, m, C_4), 2.63 (2H, m, C_5), 2.75 (1H, d, C_7), 6.03 (1H, m, C_{10}), 6.112 (1H, m, C_{11}), 6.11 (1H, m, C_{13}). ^{13}CNMR signals ($CDCl_3$, 100MHz, δ, ppm): 12.09 (C_{12}), 15.62 (C_{13}), 24.68 (C_{14}), 24.01 (C_{15}), 29.27 (C_4), 35.97 (C_9), 37.39 (C_5), 42.65 (C_8), 61.39 (C_7), 62.82 (C_6), 128.28 (C_{11}), 139.45 (C_2), 147.71 (C_3), 159.44 (C_{10}), 202.91 (C_1). **(See SI for the NMR)**

Antifungal assay

The antifungal activity of all components was assessed against *Bipolaris oryzae*, *Fusarium moniliforme*, and *Rhizoctonia solani* using the poisoned food technique at various concentrations: 100, 250, 500, and 1000 µg/mL. (16, 28, 29) The Department of Plant Pathology, PAU, Ludhiana provided pure cultures of the rice fungi. The fungal strains were sub-cultured on Potato Dextrose Agar (PDA) slants and stored at 4°C for maintenance during the experiments. The test mixture (PDA + antifungal material) was poured into sterilized 90 mm Petri dishes and allowed to solidify. Each treatment was performed in triplicate. A 5 mm mycelial disk, cut from a seven-day-old fungal culture, was placed at the center of each PDA plate. The plates were incubated in the dark at 25 ± 1°C for seven days. Colony growth diameter was recorded once the control treatments exhibited full growth across the Petri dishes. Carbendazim and propiconazole were used as reference standards. Mycelial growth (in cm) was measured along three diametric directions for both treated (T) and control (C) groups.(20, 30–35) The percentage inhibition of growth (PI%) was calculated using the following formula:

$$\text{Percent mycelial growth inhibition} = \frac{\text{Average increase in mycelial growth in control} - \text{Average increase in mycelial growth in treatment}}{\text{Average increase in mycelial growth in control}} \times 100$$

The graphs were plotted for different compounds at different concentrations. Effective dose/concentration (ED_{50}) where 50% inhibition takes place was obtained from graph.

Result and Discussion

Isolation and GC-MS analysis

The essential oil of *Zingiber zerumbet* rhizomes was extracted using the hydro-distillation method with a Clevenger apparatus, yielding 0.4% (w/v) oil. This yield aligns with previous reports.(35–40) The essential oil appeared as a pale yellow, viscous liquid with a strong characteristic odor. Its measured properties included a pH of 5.5, a refractive index of 1.50, and a specific gravity of 0.887. The oil was insoluble in water, sparingly soluble in non-polar solvents such as hexane and benzene, and completely soluble in polar solvents like acetone, methanol, dichloromethane, and ethanol. GC-MS analysis identified 30 components, representing 99.93% of the total oil (Table 1). Sesquiterpenes dominated the composition, with 60–70% as oxygenated sesquiterpenes, 15–20% as sesquiterpene hydrocarbons, and only trace amounts of fatty acids. The major compounds identified included endo-borneol (15.77%), limonene (11.95%), zerumbone (10.73%), and camphene (11.53%). Minor constituents included caryophyllene (1.80%), caryophyllene oxide (3.99%), 4-terpineol (2.01%), and borneol formate (2.14%).

Table 1
GC-MS analysis of *Z. zerumbet* essential oil

Sr. No.	Compounds	Retention Time (min)	Area (%)	Chemical Formula
1.	Nonane	4.269	0.95	C_9H_{20}
2.	Tricyclene	4.778	1.67	$C_{10}H_{16}$
3.	1-Decene,2,4-dimethyl	4.993	0.55	$C_{12}H_{24}$
4.	α-Pinene	4.992	2.83	$C_{10}H_{16}$
5.	Camphene	5.363	11.53	$C_{10}H_{16}$
6.	Mesitylene	5.744	0.61	C_9H_{12}
7.	Benzene,1,2,3-trimethyl	6.325	0.67	C_9H_{12}
8.	Decane	6.422	2.47	$C_{10}H_{22}$
9.	Decane,4-methyl	6.965	1.07	$C_{11}H_{24}$
10.	β-Cymene	7.085	1.05	$C_{10}H_{14}$
11.	Limonene	7.225	11.95	$C_{10}H_{16}$
12.	Camphenilone	8.697	1.04	$C_{10}H_{16}O$
13.	Undecane	9.082	4.32	$C_{11}H_{24}$
14.	Camphor	10.476	1.41	$C_{10}H_{16}O$
15.	Endo-borneol	11.264	15.77	$C_{10}H_{18}O$
16.	4-Terpineol	11.440	2.01	$C_{10}H_{18}O$
17.	Cuminyl acetate	11.635	0.80	$C_{12}H_{16}O_2$
18.	3-Cyclohexene-1-methanol,α,α,4-trimethyl	11.872	2.97	$C_{10}H_{18}O$
19.	Dodecane	11.934	2.87	$C_{12}H_{26}$
20.	Undecane,2,5-dimethyl	12.287	0.62	$C_{13}H_{28}$

Chemical constituents are categorize as follows: Hydrocarbons: Alkanes (Saturated Hydrocarbons): Nonane (4.269); Decane (6.422); Decane, 4-methyl (6.965); Undecane (9.082); Dodecane (11.934); Tetradecane (14.782); Pentadecane (20.163) **Alkenes (Unsaturated Hydrocarbons)**: 1-Decene, 2,4-dimethyl (4.993) **Cyclic Hydrocarbons**: Tricyclene (4.778); α-Pinene (4.992); Camphene (5.363) **Aromatic Hydrocarbons**: Mesitylene (5.744); Benzene, 1,2,3-trimethyl (6.325); β-Cymene (7.085); Benzene, 1,2,4-trimethyl-5-(1-propenyl) (21.397) **Alcohols**: Endo-borneol (11.264); 4-Terpineol (11.440); 3-Cyclohexene-1-methanol, α,α,4-trimethyl (11.872) **Ketones**: Camphor (10.476); Camphenilone (8.697); Zerumbone (25.914) **Esters**: Cuminyl acetate (11.635); Borneolformate (12.797); Trans-bornylacetate (14.363) **Chlorinated Hydrocarbons**: Octadecane-1-chloro (17.538) **Terpenoids**: Limonene (7.225); Caryophyllene oxide (22.340); α-Caryophyllene (28.353)

Sr. No.	Compounds	Retention Time (min)	Area (%)	Chemical Formula
21.	Borneolformate	12.797	2.14	$C_{11}H_{18}O_2$
22.	Nonane,3-methyl	13.970	0.55	$C_{10}H_{22}$
23.	Trans-bornylacetate	14.363	0.92	$C_{12}H_{20}O_2$
24.	Tetradecane	14.782	2.041	$C_{14}H_{30}$
25.	Octadecane-1-chloro	17.538	2.47	$C_{18}H_{37}Cl$
26.	Pentadecane	20.163	0.58	$C_{15}H_{32}$
27.	Benzene,1,2,4-trimethyl-5-(1-propenyl)	21.397	7.61	$C_{12}H_{16}$
28.	Caryophyllene oxide	22.340	3.99	$C_{15}H_{24}O$
29.	Zerumbone	25.914	10.73	$C_{15}H_{22}O$
30.	α-caryophyllene	28.353	1.80	$C_{15}H_{24}$

Chemical constituents are categorize as follows: Hydrocarbons: Alkanes (Saturated Hydrocarbons): Nonane (4.269); Decane (6.422); Decane, 4-methyl (6.965); Undecane (9.082); Dodecane (11.934); Tetradecane (14.782); Pentadecane (20.163) **Alkenes (Unsaturated Hydrocarbons)**: 1-Decene, 2,4-dimethyl (4.993) **Cyclic Hydrocarbons**: Tricyclene (4.778); α-Pinene (4.992); Camphene (5.363) **Aromatic Hydrocarbons**: Mesitylene (5.744); Benzene, 1,2,3-trimethyl (6.325); β-Cymene (7.085); Benzene, 1,2,4-trimethyl-5-(1-propenyl) (21.397) **Alcohols**: Endo-borneol (11.264); 4-Terpineol (11.440); 3-Cyclohexene-1-methanol, α,α,4-trimethyl (11.872) **Ketones**: Camphor (10.476); Camphenilone (8.697); Zerumbone (25.914) **Esters**: Cuminyl acetate (11.635); Borneolformate (12.797); Trans-bornylacetate (14.363) **Chlorinated Hydrocarbons**: Octadecane-1-chloro (17.538) **Terpenoids**: Limonene (7.225); Caryophyllene oxide (22.340); α-Caryophyllene (28.353)

Isolation of pure compounds:

The essential oil of *Zingiber zerumbet* was chromatographed on silica gel, isolating three significant compounds: limonene (1), caryophyllene (2), and zerumbone (3). These compounds were characterized using IR, 1H NMR, and 13C NMR spectroscopy, revealing detailed structural information. Additionally, two derivatives, zerumbol (4) and zerumbone epoxide (5), were synthesized from zerumbone and analyzed for structural confirmation.(34, 36, 39)

Limonene (1) was identified as a monoterpene. Its IR spectrum displayed characteristic bands at 3082 cm^{-1} for = C–H stretching, 2919 cm^{-1} for C–H stretching of methylene, 1437 cm^{-1} for C–H bending, and 1643 cm^{-1} for C = C stretching. The 1H NMR spectrum showed two singlets at δ 1.65 ppm (3H, C10) and δ 1.73 ppm (3H, C9), a doublet at δ 4.7 ppm (2H, J = 0.92 Hz, C8), and a multiplet at δ 5.39–5.40 ppm (1H, C2). The 13C NMR spectrum exhibited ten signals corresponding to the carbons of limonene, with notable peaks at δ 150.28 and 108.36 ppm indicating an exocyclic double bond, and δ 133.76 and

120.65 ppm confirming an endocyclic double bond. These spectral features confirmed the structure of limonene as a cyclic monoterpene with both exocyclic and endocyclic double bonds.

Caryophyllene (2), a sesquiterpene, showed characteristic IR bands at 3068.1 cm^{-1}, 2926.7 cm^{-1}, and 2858.1 cm^{-1} due to -CH_2 and -CH_3 stretching, as well as a distinct band at 1631.3 cm^{-1} corresponding to C = C stretching. The absence of aromatic Csp^2-H stretching confirmed the non-aromatic nature of the double bonds. The 13C NMR spectrum revealed 15 signals, consistent with a sesquiterpene structure, with δ 53.62 ppm (cyclobutane carbon) and δ 48.5 ppm (cyclononene carbon) standing out. Terminal olefinic carbons were identified at δ 111.73 ppm (C8) and δ 154.5 ppm (C15). The 1H NMR spectrum further supported the structure with a multiplet at δ 5.28–5.30 ppm (C11 alkene proton) and signals at δ 4.93 ppm and δ 4.80 ppm for terminal ethylene protons. The data confirmed the bicyclic nature of caryophyllene.

Zerumbone (3) was characterized as a sesquiterpene with an α,β-unsaturated ketone group. Its IR spectrum showed significant bands at 2958.8 cm^{-1} and 2923.9 cm^{-1} for sp^3 C–H stretching, 1658.6 cm^{-1} for the conjugated C = O group, and 1735.6 cm^{-1} for a C = C bond. The 1H NMR spectrum indicated germinal dimethyl groups at δ 1.23 ppm and δ 1.09 ppm (C14, C15), a methyl group on an isolated double bond at δ 1.56 ppm (C13), and additional methyl groups at δ 1.82 ppm (C12). Multiplet signals at δ 1.90 ppm (C8) and δ 2.28–2.59 ppm (C4, C5) were also observed. Olefinic protons appeared as doublets at δ 5.29 ppm (C7), δ 5.87 ppm (C10), and δ 5.91 ppm (C11), with a multiplet at δ 6.02 ppm (C3). The 13C NMR spectrum identified olefinic carbons (δ 148.9, 124.9, 160.8, and 127.1 ppm) and a carbonyl carbon (δ 204.4 ppm), confirming the presence of the conjugated ketone group.**(See SI for the NMR).**

Zerumbol (4), a reduced derivative of zerumbone, was characterized by the disappearance of the carbonyl signal in the IR spectrum and the appearance of a broad hydroxyl band at 3340.7 cm^{-1}. The 1H NMR spectrum showed a doublet at δ 4.63 ppm for the hydroxyl proton, confirming the reduction of the carbonyl group while the double bonds remained unaffected. Zerumbone epoxide (5) was identified by a new IR band at 1116 cm^{-1} for C–O stretching, indicating epoxidation. The 1H NMR spectrum displayed shifts, with the olefinic proton at C7 replaced by a doublet at δ 2.75 ppm (J = 15.6 Hz), confirming epoxide formation. The 13C NMR spectrum further supported the structure, with signals at δ 62.8 ppm and δ 61.4 ppm corresponding to epoxidized carbons (C6 and C7). These detailed characterizations not only confirm the structures of the isolated compounds and their derivatives but also highlight their potential reactivity and functional group diversity, essential for further chemical and biological investigations. **(See SI for the NMR)**

Antifungal effects

The antifungal activity of the essential oil and its isolated components from *Zingiber zerumbet* rhizomes was evaluated against three phytopathogenic fungi: *Bipolaris oryzae*, *Fusarium moniliforme*, and *Rhizoctonia solani*. The essential oil exhibited significant inhibitory effects, with ED50 values of 1.23,

1.39, and 1.59 mg/mL, respectively, for the three fungi.(41–46) This activity can be attributed to its complex chemical composition, including both major and minor terpenoid constituents, which are known to disrupt fungal cell walls and interfere with enzymatic processes essential for fungal growth and morphogenesis. Previous studies suggest that terpenoids reduce mitochondrial activity, leading to increased reactive oxygen species (ROS) and disrupted ATP generation, which ultimately affect fungal viability.(6, 13, 15)

Among the isolated compounds, zerumbone (3) demonstrated the highest antifungal activity, with ED50 values of 0.77, 0.92, and 1.07 mg/mL against *B. oryzae*, *F. moniliforme*, and *R. solani*, respectively. The superior activity of zerumbone may be attributed to its α,β-unsaturated carbonyl group, which enhances its reactivity with fungal cell components. The literature supports this finding, highlighting zerumbone as a highly bioactive sesquiterpene with potential applications in managing fungal diseases in plants. For example, when used as a seed treatment, zerumbone has been reported to control up to 85.7% of damping-off disease in *Phaseolus aureus* caused by *R. solani*. **(**Table 2**)**

Caryophyllene (2) showed moderate antifungal activity with ED50 values of 1.43, 1.61, and 1.92 mg/mL against the three fungi, respectively. While not as potent as zerumbone, caryophyllene's sesquiterpene nature contributes to its antifungal properties, as documented in earlier studies. However, limonene (1), a monoterpene, was the least effective, with ED50 values of 1.99, 2.35, and 2.64 mg/mL, despite showing moderate activity against *B. oryzae*. The reduced effectiveness of limonene may be due to its simpler molecular structure and lower chemical reactivity compared to sesquiterpenes like caryophyllene.

The remarkable antifungal potential of zerumbone prompted the synthesis of two derivatives: zerumbol (alcohol derivative) and zerumbone epoxide. Both derivatives exhibited improved antifungal activity compared to the parent compound. Zerumbol showed the highest activity, with ED50 values of 0.47, 0.57, and 0.38 mg/mL, surpassing even zerumbone epoxide, which had ED50 values of 0.63, 0.77, and 0.91 mg/mL against the respective fungi. The enhanced activity of zerumbol may be attributed to its hydroxyl group, which can disrupt fungal RNA and modify membrane permeability, leading to cell death. Similar findings have been reported for other alcohol derivatives, such as carotol and daucol, which exhibit strong antifungal activity against various fungal pathogens. **(**Figs. 2 and 3**) (See SI for the images)**

Despite the promising activity of the natural compounds and their derivatives, their antifungal potential was lower than that of commercially available fungicides, such as carbendazim and propiconazole, which demonstrated much lower ED50 values. These results suggest that while natural compounds like zerumbone and its derivatives have significant potential as eco-friendly alternatives for managing fungal pathogens, further optimization and formulation are needed to match the efficacy of synthetic fungicides.

In conclusion, the study highlights the potential of *Z. zerumbet* essential oil and its components as effective antifungal agents. The findings underscore the importance of chemical derivatization in

enhancing the bioactivity of natural compounds, paving the way for the development of sustainable fungicidal agents for agricultural applications.

Table 2
Fungicidal activity of Z. zerumbet components against three phytopathogenic plant fungi

Components	Fungal Strains					
	B. oryzae		*F. moniliforme*		*R. solani*	
	ED_{90}	ED_{50}	ED_{90}	ED_{50}	ED_{90}	ED_{50}
Essential oil	4.28	1.23	5.03	1.39	5.43	1.59
Limonene	7.36	1.99	8.59	2.35	9.69	2.64
Caryophyllene	5.28	1.43	5.75	1.61	5.99	1.92
Zerumbone	3.76	0.77	4.20	0.92	4.21	1.07
Zerumbol	3.31	0.47	3.53	0.55	3.83	0.38
Zerumbone epoxide	3.57	0.63	3.89	0.77	4.24	0.91
Carbendazim	-	-	0.038	0.012	0.16	0.022
Propiconazole	0.42	0.04	0.021	0.006	0.08	0.018

Structure-activity relationship

The structure-activity relationship (SAR) of the antifungal compounds derived from *Zingiber zerumbet* was examined by evaluating the antifungal potential of the isolated and derivatized compounds. Limonene, a monoterpene, contains one exocyclic and one endocyclic double bond, both of which are important for its antifungal activity. However, limonene exhibited relatively modest antifungal activity compared to other compounds. This can be attributed to its simpler molecular structure, as it lacks functional groups such as a carbonyl or hydroxyl group that could enhance its reactivity with fungal cells. The presence of these unsaturated bonds is necessary for activity but not sufficient alone for optimal inhibition. (10, 18, 34)

Caryophyllene, a sesquiterpene and structural isomer of zerumbone, showed marginal antifungal activity. While it did not possess the key α,β-unsaturated carbonyl moiety found in zerumbone, its antifungal activity was greater than limonene's. The higher activity of caryophyllene can be attributed to its larger molecular structure, which includes more carbon chains. These chains likely contribute to its ability to interact with fungal cell membranes or interfere with fungal metabolism. Nevertheless, caryophyllene's lack of a functional group like a carbonyl group, which plays a crucial role in the activity of zerumbone, limited its overall effectiveness.

Zerumbone itself demonstrated the highest antifungal potential among the compounds tested. The presence of an unsaturated α,β-carbonyl group in zerumbone is likely responsible for its potent antifungal activity. This structure, characterized by a conjugated double bond system and an isolated double bond, enhances the compound's reactivity, making it more effective at disrupting fungal cell membranes and interfering with enzymatic processes crucial to fungal growth.(11, 22)

In an effort to explore the impact of structural modifications on zerumbone's antifungal potential, the compound was derivatized into two forms: zerumbol and zerumbone epoxide. The transformation of zerumbone into zerumbol involved replacing the α,β-carbonyl group with a hydroxyl group while maintaining the double bonds in the structure. The alcohol derivative, zerumbol, exhibited even greater antifungal activity than the parent compound, suggesting that the hydroxyl group enhances the compound's ability to interact with fungal cell components. The presence of the alcohol group likely improves the compound's ability to interfere with fungal metabolism, inhibiting growth more effectively.

Epoxidation of zerumbone, which involved attaching an epoxide group to the isolated double bond without affecting the other two double bonds, also increased its antifungal activity compared to zerumbone. The resulting epoxide derivative retained the unsaturated α,β-carbonyl group and gained the additional reactivity of the epoxide moiety. This modification further enhanced the compound's ability to disrupt fungal cell structures and metabolic pathways, as epoxide groups are known to be highly reactive and capable of forming covalent bonds with biological molecules, including proteins and nucleic acids.

Overall, the structure-activity relationship indicates that the presence of functional groups such as the α,β-carbonyl group, hydroxyl group, and epoxide moiety significantly contribute to the antifungal activity of these compounds. Among all the derivatives tested, zerumbol, which contains the hydroxyl group, exhibited the most potent antifungal activity. This suggests that the introduction of a hydroxyl group enhances the compound's ability to interfere with fungal metabolism, making it the most effective antifungal agent in this study. These findings highlight the importance of chemical derivatization in improving the bioactivity of natural compounds and underscore the potential of zerumbol as a candidate for the development of novel antifungal agents.

Conclusion

In conclusion, the essential oil and its isolated components from *Zingiber zerumbet* demonstrated significant antifungal activity against the three phytopathogenic fungi, *Bipolaris oryzae*, *Fusarium moniliforme*, and *Rhizoctonia solani*. Among the tested compounds, zerumbone exhibited the highest antifungal potential, which was further enhanced through chemical derivatization to form its alcohol (zerumbol) and epoxide derivatives. The structure-activity relationship revealed that the presence of key functional groups, such as the α,β-unsaturated carbonyl group, hydroxyl group, and epoxide moiety, played a critical role in the antifungal efficacy of these compounds. Notably, zerumbol, the alcohol derivative, demonstrated the most potent antifungal activity, suggesting that the hydroxyl group significantly enhances the compound's ability to interfere with fungal metabolism. While the natural

compounds showed promising antifungal properties, their activity was still lower than that of commercially available fungicides, indicating room for further optimization. These findings highlight the potential of *Zingiber zerumbet* derivatives as valuable candidates for the development of natural antifungal agents, offering an alternative to synthetic fungicides for sustainable agricultural practices. Further studies are needed to explore the detailed mechanisms of action and to assess the environmental and economic feasibility of these compounds for use in crop protection.

Declarations

Acknowledgments

The authors are thankful to the Department of Floriculture and Landscaping, Punjab Agricultural University (Ludhiana), for the identification and authentication of the plant specimen and the Department of Plant Pathology for the rice fungi culture.

Ethics Declaration

Funding : There are no funders to report for this submission

Availability of data and materials

We have carried out the research work and assure you that it can be provided whenever required.

Author's Contribution

Navneet Kaur: Searched and worked on the project, extracted the data, wrote the original draft, given final approval of the version to be published.

Ramandeep Kaur: Conceptualization, supervision, drafting and revising the manuscript, and giving the final approval of the version to be published.

Urvashi Bhardwaj: Reviewed and final approval of the version to be published.

Parul Sharma: drafting and revising the manuscript and giving the final approval of the version to be published.

All authors have read and approved the final version of the manuscript submitted for publication and are responsible for the final content.

Data availability: The manuscript contains all the data, and more information will be provided upon request.

Declarations Ethics: Rhizomes of *Zingiber zerumbet* were collected in 2024 from the Ludhiana, Punjab, India. The plant was formally identified and authenticated by the Principal Scientist and Head of the

Department of Floriculture and Landscaping at PAU. Therefore, no license or authorization is needed to gather plant material for this research work.

Consent for publication: All authors have seen the latest version of the manuscript and agree to its publication.

Competing interests: The authors declare no competing interests.

Funding: The work received no external funding**.**

Clinical Trial: Not applicable

Consent to Participate declaration: Not applicable

References

1. R.O. Prakash, R.K. Kumar, A. Rabinarayan, and M.S. Kumar, Pharmacognostical and phytochemical studies of *Zingiber zerumbet* (L.) Sm. rhizome, *Int. J. Res. Ayurveda Pharm.*, **2**, 698–703 (2011).
2. M.N.I. Bhuiyan, J.U. Chowdhury, and J. Begum, Chemical investigation of the leaf and rhizome essential oils of *Zingiber zerumbet* Smith from Bangladesh, *Bangladesh J. Pharmacol.*, **4**, 9–12 (2009).
3. E.S. Carmo, E. de Oliveira Lima, and E.L. de Souza, The potential of *Origanum vulgare* L. (Lamiaceae) essential oil in inhibiting the growth of some food-related *Aspergillus* species, *Braz. J. Microbiol.*, **39**, 362–367 (2008).
4. M.J. Chane, R. Vera, and J.C. Chalchat, Chemical composition of the essential oil from rhizomes, leaves, and flowers of *Zingiber zerumbet* Smith from Reunion Island, *J. Essent. Oil Res.*, **15**, 202–205 (2003).
5. N.A.N. Norulaini, O. Anuar, A.K.M. Omar, A.F.M. Alkarkhi, W.B. Setiano, M.O. Fatehah, F. Sahena, and I.S.M. Zaidul, Optimization of SC–CO2 extraction of zerumbone from *Zingiber zerumbet* (L.) Smith, *Food Chem.*, **114**, 702–705 (2009).
6. H.T. Chang, Y.H. Cheng, C.L. Wu, S.T. Chang, T.T. Chang, and Y.C. Su, Antifungal activity of essential oil and its constituents from *Calocedrus macrolepis* var. *formosana* Florin leaf against plant pathogenic fungi, *Bioresour. Technol.*, **99**, 66–70 (2008).
7. S.Y. Chung, D.S. Jang, A. Han, J.O. Jang, Y. Kwon, E. Seo, and H.J. Lee, Modulation of P-glycoprotein-mediated resistance by kaempferol derivatives isolated from *Zingiber zerumbet*, *Phytother. Res.*, **21**, 565–569 (2007).
8. S. Combrinck, T. Regnier, and G. Kamatou, *In vitro* activity of eighteen essential oils and some major components against common postharvest fungal pathogens of fruit, *Ind. Crops Prod.*, **33**, 344–349 (2011).
9. N. Dikbas, R. Kotan, F. Dadasoglu, and F. Sahin, Control of *Aspergillus flavus* with essential oil and methanol extract of *Satureja hortensis*, *Int. J. Food Microbiol.*, **124**, 179–182 (2008).

10. A.M. Elgorban, A.H. Bahkali, M.A. El-Metwally, M. Elsheshtawi, and M.A. Abdelwahab, *In vitro* antifungal activity of some plant essential oils, *Int. J. Pharmacol.*, **11**, 56–61 (2015).
11. T. Kitayama, T. Masuda, Y. Kawai, R.K. Hill, M. Takatani, S. Sawada, and T. Okamoto, The chemistry of zerumbone. Part 3: Stereospecific creation of five stereogenic centers by double Sharpless oxidation, *Tetrahedron: Asymmetry*, **12**, 2805–2810 (2001).
12. A.Y. Koga, F.L. Beltrame, and A.V. Pereira, Several aspects of *Zingiber zerumbet*: A review, *Braz. J. Pharmacogn.*, **26**, 385–391 (2016).
13. W.T. Liu and C.L. Chu, Thymol and acetic acid vapors reduce postharvest brown rot of apricots and plums, *Hort. Sci.*, **37**, 151–156 (2002).
14. B.H. Madegowda, P. Rameshwaran, N.P. Nagaraju, and P.S. Murthy, *In vitro* mycological activity of essential oil from *Zingiber zerumbet* rhizomes, *J. Essent. Oil Res.* (2015). DOI: 10.1080/10412905.2015.107927.
15. M. Moghaddam and L. Mehdizadeh, Essential oil and antifungal therapy, *Recent Trends in Antifungal Agents and Antifungal Therapy*. Springer, DOI: 10.1007/978-81-322-2782-3_2 (2016).
16. Y.L. Nene and B.W. Thapliyal, *Fungicides in Plant Disease Control*, Oxford & IBH Publisher House, New Delhi (1979).
17. E. Pinto, M.J. Gonçalves, C. Cavaleiro, and L. Salgueiro, Antifungal activity of *Thapsia villosa* essential oil against *Candida*, *Cryptococcus*, *Malassezia*, *Aspergillus*, and dermatophyte species, *Molecules*, **22**, 1595 (2017).
18. P. Sharma, N. Singla, R. Kaur, and U. Bhardwaj, A review on phytochemical constituents and pharmacological properties of *Catharanthus roseus* (L.) G. Don, *J. Med. Plants Stud.*, **12**(3), 131–156 (2024). DOI: 10.22271/plants.2024.v12.i3b.1675.
19. P. Sharma and R. Kaur, Comprehensive analysis and therapeutic potential of ginger essential oil, *J. Pharmacogn. Phytochem.*, **13**(3), 420–425 (2024). DOI: 10.22271/phyto.2024.v13.i3e.14984.
20. P. Sharma, R. Kaur, U. Bhardwaj, and J. Kaur, Chemical composition and antifungal potential of *Vinca rosea* leaf essential oil and extracts from Northern India, *Cogent Food Agric.*, **10**(1), DOI: 10.1080/23311932.2024.2382317 (2024).
21. M.N. Gallucci, M.E. Carezzano, M. Oliva, M.S. Demo, R.P. Pizzolitto, M.P. Zunino, J.A. Zygadlo, and J.S. Dambolena, *In vitro* activity of natural phenolic compounds against fluconazole-resistant *Candida* species: A quantitative structure-activity relationship analysis, *J. Appl. Microbiol.*, **116**, 795–804 (2014).
22. E. Haque, S. Irfan, M. Kamil, S. Sheikh, A. Hasan, A. Ahmad, V. Lakshmi, A. Nazir, and S.S. Mir, Microbiology, **85**, 436 (2016). https://doi.org/10.1134/S0026261716040093.
23. R.A. Holley and D. Patel, Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials, *J. Food Microbiol.*, **22**, 273–292 (2005).
24. G. Kader, F. Nikkon, M.A. Rashid, and T. Yeasmin, Antimicrobial activities of the rhizome extract of *Zingiber zerumbet* Linn., *Asian Pac. J. Trop. Biomed.*, **1**, 409–412 (2011).

25. D. Kalemba and A. Kunicka, Antibacterial and antifungal properties of essential oils, *Curr. Med. Chem.*, **10**, 813–829 (2003).
26. D. Kataria, K.K. Chahal, A. Kumar, and R. Singh, Antifungal activity and molecular docking studies of sesquiterpenoids from *Daucus carota*, *Pest. Res. J.*, **29**, 188–195 (2018).
27. S.A. Khayyat and M.Y. Sameeh, Bioactive epoxides and hydroperoxides derived from naturally monoterpene geranyl acetate, *Saudi Pharm. J.*, **26**, 14–19 (2018).
28. N. Kishore and R.S. Dwivedi, Zerumbone: A potential fungitoxic agent isolated from *Zingiber cassumunar* Roxb., *Mycopathologia*, **120**, 155–159 (1992).
29. T. Kitayama, R. Nagao, T. Masuda, R.K. Hill, M. Morita, M. Takatani, S. Sawada, and T. Okamoto, The chemistry of zerumbone IV: Asymmetric synthesis of zerumbol, *J. Mol. Catal. B: Enzymatic*, **17**, 375–379 (2002).
30. V.S. Rana, V. Ahluwalia, N.A. Shakil, and L. Prasad, Essential oil composition, antifungal, and seedling growth inhibitory effects of zerumbone from *Zingiber zerumbet* Smith, *J. Essent. Oil Res.*, **29**, 320–329 (2016).
31. A. Rancic, M. Sokovic, G.L. Van, and J. Vukojevic, Antimicrobial activity of limonene, *Agris*, **23**, 83–88 (2003).
32. C. Romagnoli, R. Bruni, E. Andreotti, M.K. Rai, C.B. Vicentini, and D. Mares, Chemical characterization and antifungal activity of essential oil of capitula from wild Indian (*Tagetes patula* L.), *Protoplasma*, **225**, 57–65 (2005).
33. B. Sabulal, M. Dan, A.R.M. Thaha, A.J. Johnson, R. Kurup, P. Balakrishnapillai, and C.K. Lim, High content of zerumbone in volatile oils of *Zingiber zerumbet* from Southern India and Malaysia, *Flavour Fragr. J.*, **24**, 301–308 (2009).
34. S. Savary, P.S. Teng, L. Willocquet, and F.W. Nutter, Quantification and modeling of crop losses: A review of purposes, *Annu. Rev. Phytopathol.*, **44**, 89–112 (2006).
35. S. Savary, L. Willocquet, F.A. Elazegui, N.P. Castilla, and P.S. Teng, Rice pest constraints in tropical Asia: Quantification of yield losses due to rice pests in a range of production situations, *Plant Dis.*, **84**, 357–369 (2000).
36. Y.M. Shabana, G.M. Abdel-Fattah, A.E. Ismail, and Y.M. Rashad, Control of brown spot pathogen of rice (*Bipolaris oryzae*) using some phenolic antioxidants, *Braz. J. Microbiol.*, **39**, 438–444 (2008).
37. N. Sharma and A. Tripathi, Effects of *Citrus sinensis* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem, *Microbiol. Res.*, **163**, 337–344 (2008).
38. H.M. Sidahmed, N.M. Hashim, M.A. Abdulla, H.M. Ali, S. Mohan, S.I. Abdelwahab, M.M. Taha, L.M. Fai, and J. Vadivelu, Antisecretory, gastroprotective, antioxidant, and anti-*Helicobacter pylori* activity of zerumbone from *Zingiber zerumbet* (L.) Smith, *PLoS One*, **10**, e0121060 (2015).
39. C.B. Singh, K. Nongalleima, S.B. Singh, N. Swapana, and C.D. Singh, *Zingiber zerumbet* Smith - An important medicinal plant of *Zingiberaceae* family, *NeBIO*, **2**, 9–13 (2012).

40. A.K. Srivastava, S.K. Srivastava, and N.C. Shah, Essential oil composition of *Zingiber zerumbet* (L.) Sm. from India, *J. Essent. Oil Res.*, **12**, 595–597 (2000).
41. A.C. Tavares, M.J. Gonçalves, M.T. Cruz, C. Cavaleiro, M.C. Lopes, J. Canhoto, and L.R. Salgueiro, Essential oils from *Distichoselinum tenuifolium*: Chemical composition, cytotoxicity, antifungal and anti-inflammatory properties, *J. Ethnopharmacol.*, **130**, 593–598 (2010).
42. P. Thobunluepop, C. Jatisatienr, E. Pawelzik, and S. Vearasilp, *In vitro* screening of the antifungal activity of plant extracts as fungicides against rice seed-borne fungi, *Acta Hort.*, **837** (2009).
43. Urvashi, Isolation and characterization of terpenoids from vetiver oil and their evaluation as pesticides, Ph.D. dissertation, PAU, Ludhiana, India (2016).
44. N.J. Yob, S.M. Jofrry, M.M.R. Affandi, L.K. Teh, M.Z. Salleh, and Z.A. Zakaria, *Zingiber zerumbet* (L.) Smith: A review of its ethnomedicinal, chemical and pharmacological uses, *Evid. Based Complement. Alternat. Med.*, **2011**, 543216 (2011). DOI: 10.1155/2011/543216.
45. A. Zheng, R. Lin, D. Zhang, P. Qin, L. Xu, P. Ai, L. Ding, Y. Wang, Y. Chen, Y. Liu, Z. Sun, H. Feng, X. Liang, R. Fu, C. Tang, Q. Li, J. Zhang, Z. Xie, Q. Deng, S. Li, S. Wang, J. Zhu, L. Wang, H. Liu, and P. Li, The evolution and pathogenic mechanisms of the rice sheath blight pathogen, *Nat. Prod. Commun.*, **4**, Article number: 1424 (2013).
46. N. Kaur, K.K. Chahal, R. Singh, A. Kumar, and U. Bhardwaj, Antioxidant activity of *Anethum graveolens* L. essential oil constituents and their chemical analogues, *J. Food Biochem.*, DOI: 10.1111/jfbc.12782 (2019).

Schemes

Schemes 1 and 2 are available in the Supplementary Files section.

Figures

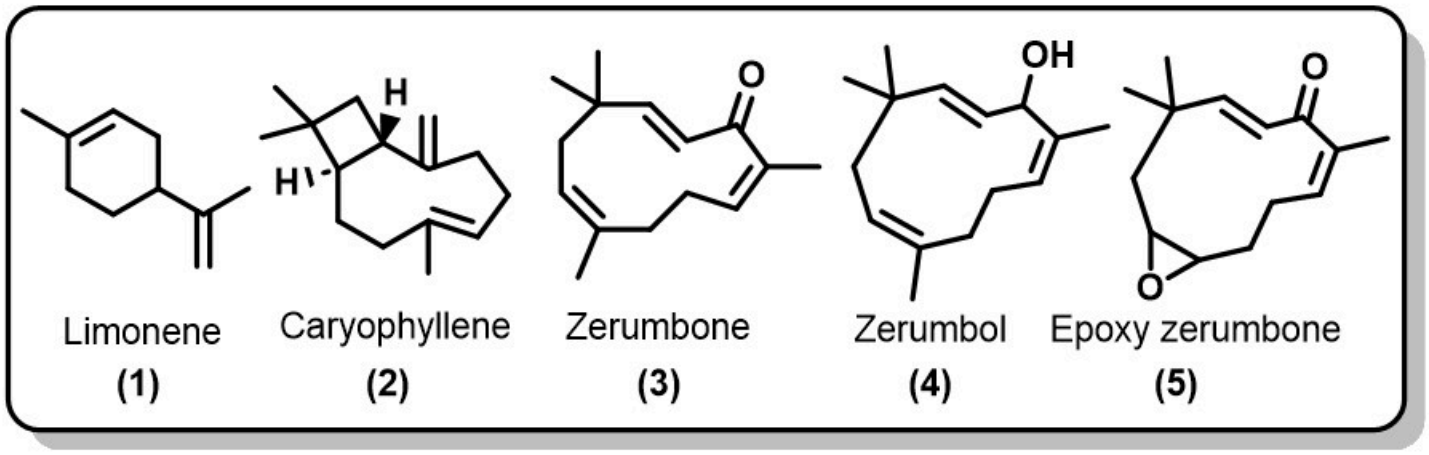

Figure 1

Compounds isolated from the essential oil from *Zingiber zerumbet*

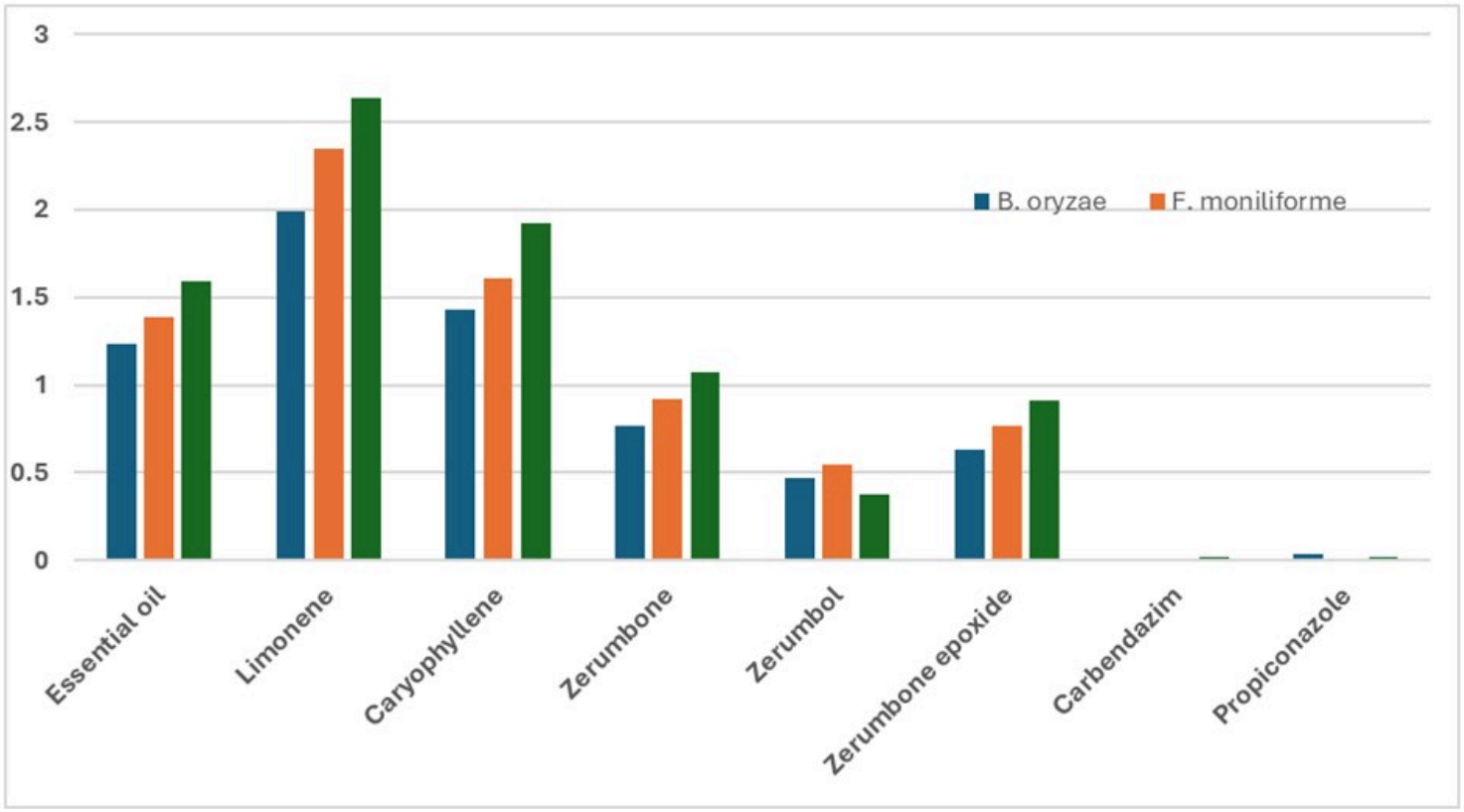

Figure 2

ED_{50} value Antifungal activity of essential oil and its isolated compound

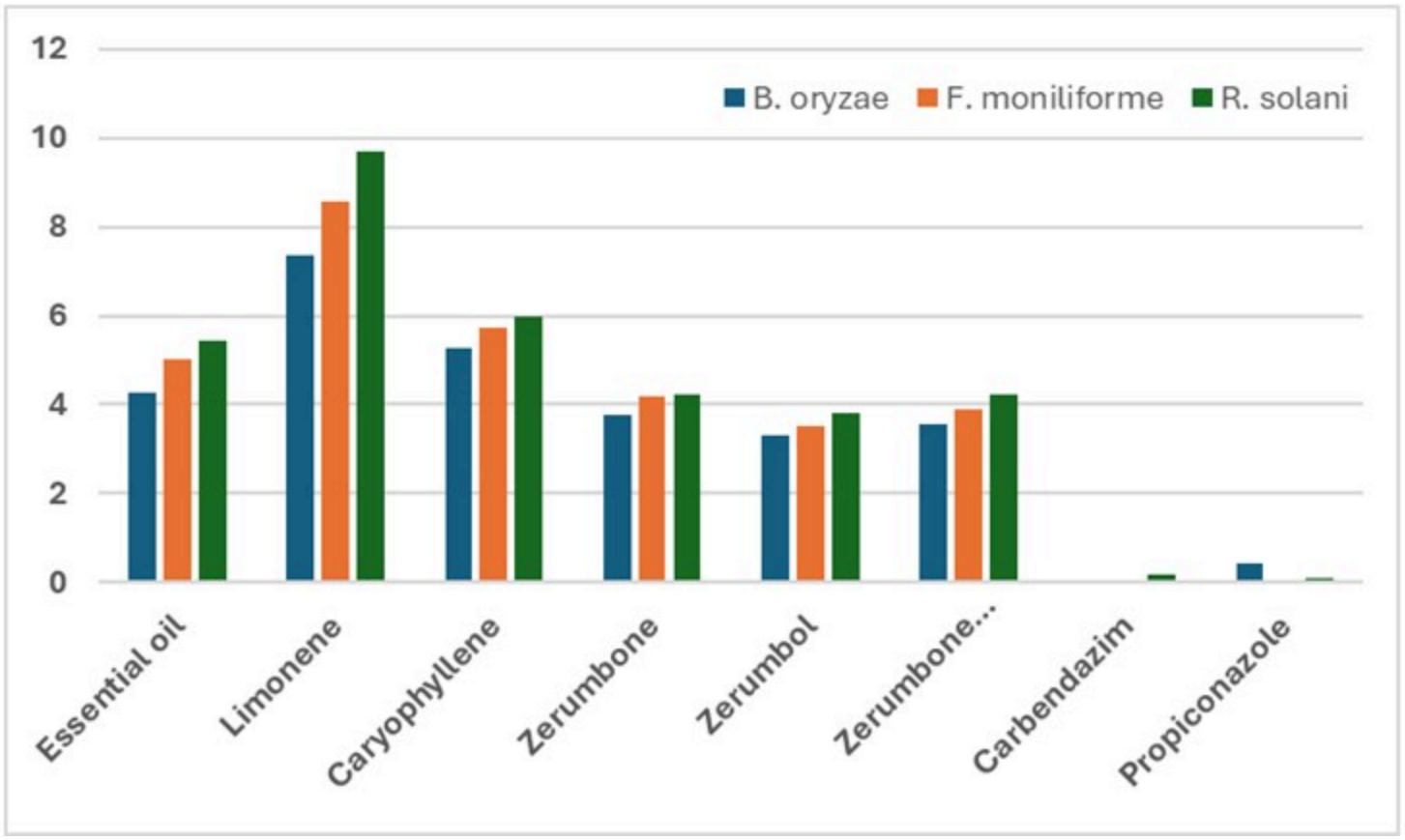

Figure 3

ED_{90} value Antifungal activity of essential oil and its isolated compound

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Abstract.jpg
- SupportingInformation.docx
- scheme1.jpg
- scheme2.jpg