

Chemical composition of *Alpinia zerumbet* (Pers.) essential oil and herbicidal activity on *Parthenium* and fungal spp

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ABSTRACT

Alpinia zerumbet (Pers.) shell ginger is a perennial plant valued for its essential oils (EO). We compared the chemical composition and biological activities of its rhizome and leaf essential oils extracted by hydro-distillation and analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The major constituents of rhizome oil were camphor (33.63 %), 1,8-cineole (13.91 %), β -linalool (9.95 %) and α -terpineol (5.75 %), whereas leaf oil contained 1,8-cineole (39.86 %), camphor (12.39 %), α -terpineol (9.67 %), β -linalool (6.42 %) and terpin-4-ol (3.81 %). Herbicidal activity was tested against *Parthenium hysterophorus* at concentrations of 50-200 μ L/mL. The rhizome oil inhibited seed germination by 93.33 % (IC₅₀ = 96.76 μ L/mL), root length by 97.77 % (IC₅₀ = 98.70 μ L/mL) and shoot length by 74.84 % (IC₅₀ = 110.43 μ L/mL) at 200 μ L/mL. The leaf oil showed stronger inhibition, with 91.66 % inhibition of seed germination (IC₅₀ = 88.09 μ L/mL), 98.14 % root length inhibition (IC₅₀ = 96.07 μ L/mL) and 90.00 % shoot length inhibition (IC₅₀ = 109.19 μ L/mL). Antifungal activity was assessed using the poisoned food technique against *Colletotrichum lindemuthianum* and *Curvularia lunata*. At 200 μ L/mL, the rhizome oil inhibited *C. lindemuthianum* and *C. lunata* growth by 91.48 % and 90.00 %, respectively, while leaf oil showed slightly higher inhibition of 92.22 % and 90.74 %, respectively. These findings highlight the potential of *A. zerumbet* essential oils as natural herbicides and antifungal agents, offering an eco-friendly alternative to synthetic chemicals.

Key words: Allelopathy, *Alpinia zerumbet*, chemical constituents, *Colletotrichum lindemuthianum*, *Curvularia lunata*, essential oil, fungicidal, GCMS, herbicidal, leaf oil, *Parthenium hysterophorus*, rhizome oil, shell ginger.

INTRODUCTION

Alpinia Zerumbet, (Pers.) B.L. Burt and R.M. Sm. (Zingiberaceae family) is a group of flowering plants with 1,300 species (23). *A. zerumbet*, commonly known as shell ginger or "getto" in Okinawa, Japan, is a perennial herbaceous plant, indigenous to East Asia and the Pacific Islands (25). It is found across tropical and subtropical regions and extensively cultivated for ornamental, culinary and medicinal purposes. In traditional medicine, its rhizomes, leaf and stems are used to treat various ailments due to their diverse pharmacological activities (2,8). Its leaves are used to make herbal tea and its rhizomes are used as spices. In Brazil, tea is made from its leaves, as it possess hypotensive, diuretic and anti-ulcerogenic properties (6,7,9). This species is rich in phytochemicals (phenolics, phenylpropanoids, flavonoids, pyrones, sterols, and terpenoids), contributing to its diverse medicinal properties (28,29). Recent studies have expanded the potential applications of *A. zerumbet*, particularly focusing on its essential oils as natural herbicidal agents. The chemical composition of *A. zerumbet* EOs varies depending on the plant part and geographic location. For example, essential oils extracted from the flowers of *A. zerumbet* collected in Martinique have significant insecticidal activity against *Aedes aegypti* mosquitoes, indicating their potential for broader pesticidal applications (27). Similarly, essential oils from other *Alpinia* species have shown notable biological activities (17,18). A

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comprehensive review highlighted that essential oils from various *Alpinia* species possess antimicrobial, antifungal and insecticidal properties, suggesting a rich potential for pest management (22,24,26). The herbicidal potential of *A. zerumbet* EOs can be attributed to their rich phytochemical composition, particularly the monoterpenes and sesquiterpenes (4). These compounds are phytotoxic, which suppresses the growth of weeds and other unwanted vegetation. Monoterpenes and sesquiterpenes interfere with key physiological processes in plants, such as seed germination and root elongation, leading to reduced growth and eventual plant death (27). The exploration of such natural herbicides is crucial for developing sustainable agricultural practices that minimize reliance on synthetic chemicals, which pose environmental and health risks.

The essential oils of *Alpinia zerumbet* have promising herbicidal properties, offering a natural alternative for weed management. Their potential for broad-spectrum activity against various weed species highlights their significance in sustainable agriculture. However, further research is warranted to fully elucidate the efficacy and mechanisms of action of *A. zerumbet* essential oils in different agricultural settings. Understanding the molecular basis of their herbicidal activity, optimizing their formulation and evaluating their environmental impact will be essential for their successful integration into modern agricultural systems.

MATERIALS AND METHODS

2.1. Plant material: The plant material for this study was collected in January 2024 from village Jhajra, Dehradun (altitude: 648 m, latitude 30.3461374° and longitude 77.918174°) Uttarakhand, India (Figure 1). The leaf and rhizome contains 1.2 and 0.6 % (v/w) oil.



Figure 1. *Alpinia Zerumbet* Wild cultivated plant (A) leaf and (B) rhizomes

2.2. Oil extraction: The essential oils from the fresh rhizome (0.9 kg rhizome) and leaves (1.2 kg) of *Alpinia Zerumbet* were extracted for 4-5 h using the hydro distillation method by the Clevenger-type apparatus (10). The obtained essential oils were dried over anhydrous sodium sulphate before filtered and stored in dark glass vials at 4 °C for further use.

2.3. GC-MS analysis: The phytochemical composition of essential oils was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) with a PerkinElmer GCMS-SQ8 system, which featured a PE5 column (30.0 m × 250 μm i.d., 0.25 μm film thickness). A 1 μL sample of the oil was injected into the instrument, with the injector temperature set at 280 °C. Helium was used as the carrier gas, flowing at a rate of 1 mL/min with a split ratio of 50:1. The temperature programme for the GC oven began at 50 °C for 3 min, then increased to 200 °C at a rate of 3 °C/min. It then ramped up to 250 °C at 6 °C/min, where it was held for 2 min, followed by an additional hold for 11 minutes. To identify the compounds in the essential oils, their mass fragmentation patterns and relative retention index (RI) values were compared against entries in the NIST (version 2.1) and WILEY (7th edition) mass spectral libraries. Additionally, the data were corroborated with known fragmentation patterns from the literature (1). Experimental retention indices were determined by injecting a series of n-alkanes ranging from C7 to C20. The compounds were quantified from the peak areas, with each compounds abundance expressed as a percentage of the total peak area.

HERBICIDAL ACTIVITIES

Allelopathic activity: The allelopathic herbicidal activity of essential oil extracted from *Alpinia zerumbet* was assessed against the seed germination and seedlings growth of *Parthenium hysterophorus* L. seeds as per Sahu and Devkota (21). Essential oil solutions were prepared in an aqueous solution containing 1 % Tween-20 at varying concentrations (50-200 μL/mL) to evaluate their effects on seed germination. Prior to the experiment, the *P. hysterophorus* L. seeds were surface sterilized for 15 min in 5 % NaOCl solution. For the assay, 7-sterilized *Parthenium* seeds were placed in each petri plate lined with filter paper to maintain the optimal moisture level for germination. Each plate was then treated with 4 mL of respective essential oil concentration and incubated at 25±1 °C for 24 h to allow for seed germination. The experiment was concluded when all seeds in control had germinated. The effectiveness of the essential oil allelopathic activity was determined by comparing it to distilled water (Negative control) and paraquat herbicide (positive control). The following formulas were used for the calculations.

Inhibition of seed germination	Inhibition of shoot length	Inhibition of root length
Inhibition (%) = $100 \times (1 - Gt/Gc)$	Inhibition (%) = $100 \times (1 - Ct/Cc)$	Inhibition (%) = $100 \times (1 - Rt/Rc)$
Where, Gt: no. of seeds germinated in treatment Gc: no. of seeds germinated in control	Where, Ct: Shoot length in treatment Cc: Shoot length in control	Where, Rt: Root length in treatment Rc: Root length in control

2.4.2 Antifungal activity: To assess the antifungal properties of the *Alpinia zerumbet* essential oils, two plant pathogenic fungi, *Curvularia lunata* and *Colletotrichum lindemuthianum*, were cultured in the Plant Pathology Laboratory in our University. The evaluation followed the poisoned food technique developed by Grover and Moore (15). In this process, fungal colonies were aseptically transferred onto petri dishes containing Potato

Dextrose Agar (PDA) medium to revive and culture the phytopathogenic fungi. The Petri dishes were incubated at 26 ± 2 °C for one week. Cultures of the test 7-days old fungi, were then used to prepare assay discs. To determine the antifungal efficacy of the essential oils, various concentrations (50, 100, 150, and 200 $\mu\text{L}/\text{mL}$) were aseptically added to the prepared PDA plates. A control plate, without any essential oils, was prepared under the same conditions to serve as a baseline for comparison. The fungal growth on the control plate was allowed to extend to the edge of the dish. The antifungal activity of the essential oils was evaluated by measuring the clear zones of inhibition around the fungal colonies on the Petri plates. These inhibition zones indicated areas where the growth of the fungal mycelium was suppressed by the essential oils. The percentage of radial growth inhibition for each fungal strain was calculated relative to the control plate. As a benchmark, the standard fungicide carbendazim was used and the inhibition (%) was determined by McKinney's method (19).

$$\text{Inhibition (\%)} = 100 \times (X - X/Y)$$

Where, X : Growth in control, Y : Growth in treatment.

2.5. Statistical Analysis

Each experiment was conducted in triplicate, and the results were presented as the mean \pm standard deviation (SD). Statistical analysis was performed using two-way or three-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) to evaluate the differences between treatment means. These analyses were carried out using RStudio (version 2021.09.2). A p-value of less than 0.05 was considered to be statistically significant, suggesting that the observed differences between treatments were unlikely due to chance. For a more detailed examination of the data, SRPLOT (<http://www.bioinformatics.com.cn/en?keywords=heatmap>) was utilized for principal component analysis (PCA) and circular heat map clustering. These advanced techniques were applied to analyze the chemical composition and biological activity data of the essential oils. PCA helped identify the main components that contributed to the variation within the dataset, while the circular heat map clustering provided a visual summary, highlighting the most significant features and their interrelationships within the data.

RESULTS AND DISCUSSION

Chemical composition of essential oils from *Alpinia zerumbet*, the phytoconstituents in the rhizome and leaf oil were identified using GC-MS on a PE-5 column (Table 1).

(i). Rhizome oil: In rhizome essential oil, 16 compounds were identified, accounting for 74.34 % of total oil. The dominant component in the rhizome was camphor. Components contributing > 1.00 % of the oil were classified as major, while those contributing < 1.00 % were considered minor constituents. The rhizome essential oil was predominantly composed of oxygenated monoterpenes (71.47 %), followed by oxygenated sesquiterpenes (0.96 %), monoterpene hydrocarbons (0.6 %), and other compounds (1.31 %). Several key constituents were identified in the rhizome oil, including oxime-methoxy-phenyl, 1,8-cineole, β -linalool, camphor, terpin-4-ol, α -terpineol and 2 α -hydroxy-1,8-cineole. Additionally, some compounds were unique to the rhizome oil, such as borneol alcohol, trans-sabinol, cis-piperitone epoxide, o-cymen-

5-ol, p-menth-6-en-2-one, (E)-ethyl cinnamate, p-meth-2-en-ol, globulol and α -epi-7-epi-5-eudesmol.

(ii). Leaf oil: In the leaf essential oil, 13 compounds were identified, making up 79.45 % of total oil. The dominant component in the leaf was 1,8-cineole. Similar to the rhizome oil, compounds contributing > 1.00 % were classified as major, while those < 1.00 % were considered minor. The leaf essential oil was rich in oxygenated monoterpenes (74.48 %), followed by sesquiterpene hydrocarbons (1.22 %), monoterpene hydrocarbons (0.74 %), oxygenated sesquiterpenes (0.45 %) and various other compounds (2.56 %). The leaf oil shared several key constituents with the rhizome oil, including oxime-methoxy-phenyl, 1,8-cineole, β -linalool, camphor, terpin-4-ol, α -terpineol and 2 α -hydroxy-1,8-cineole. However, some compounds were unique to the leaf oil, such as p-mentha-1,5,8-triene, cis-verbenol, humulene, γ -muurolene, α -elemol and β -eudesmol.

Table 1. Chemical composition (%) of essential oils from leaf & rhizome part of *Alpinia Zerumbet* L.

S.No	Compound	RI ^c Value	RI ^L Value	Leaf	Rhizomes
1.	β -Linalool (OM)	1092	1096	6.42	9.95
2.	1,8 Cineole (OM)	1032	1031	39.86	13.91
3.	p-Meth-2-en-ol (OM)	1116	1118	-	0.51
4.	p-Mentha-1,5,8-triene (MH)	1123	1128	0.74	-
5.	<i>trans</i> -Sabinol (OM)	1140	1142	-	0.76
6.	Camphor (OM)	1149	1146	12.39	33.63
7.	Cis-verbenol (OM)	1143	1144	1.23	-
8.	Borneol alcohol (OM)	1150	1148	-	0.74
9.	Terpin-4-ol (OM)	1177	1178	3.81	2.59
10.	α -Terpineol (OM)	1183	1188	9.67	5.75
11.	p-Menth-6-en-2-one (MH)	1236	1236	-	0.6
12.	Cis-piperitone epoxide (OM)	1253	1253	-	0.64
13.	<i>o</i> -Cymen-5-ol (OM)	1334	1340	-	1.63
14.	(E)-Ethyl cinnamate (O)	1460	1462	-	0.41
15.	Humulene (SH)	1461	1458	0.73	-
16.	γ -Muurolene (SH)	1477	1478	0.49	-
17.	α -Elemol (OM)	1551	1549	0.59	-
18.	Globulol (OS)	1589	1590	-	0.52
19.	α -Epi-7-epi-5-eudesmol (OS)	1616	1617	-	0.44
20.	Oxime-, methoxy-phenyl (O)	1625	1630	2.56	0.9
21.	β -Eudesmol (OS)	1652	1650	0.45	-
22.	2 α -Hydroxy-1,8-cineole (OM)	1836	1838	0.51	1.36
Chemical classes				Leaf	Rhizome
Monoterpene Hydrocarbons (MH)				0.74	0.6
Oxygenated Monoterpene (OM)				74.48	71.47
Sesquiterpene Hydrocarbons (SH)				1.22	0.00
Oxygenated Sesquiterpene (OS)				0.45	0.96
Others (O)				2.56	1.31
Total				79.45	74.34

RI^c Value: Literature Retention Indices value on PE5 column; RI^L Value Literature Retention Indices value in reference (1).

The observed differences in the chemical profiles between rhizome and leaf essential oils can be attributed to their distinct exposure to sunlight and their differing physiological roles within the plant. Previous studies suggest that factors like light exposure, seasonal changes, rainfall, dry conditions and circadian rhythms can significantly influence the chemical composition of essential oils (11).

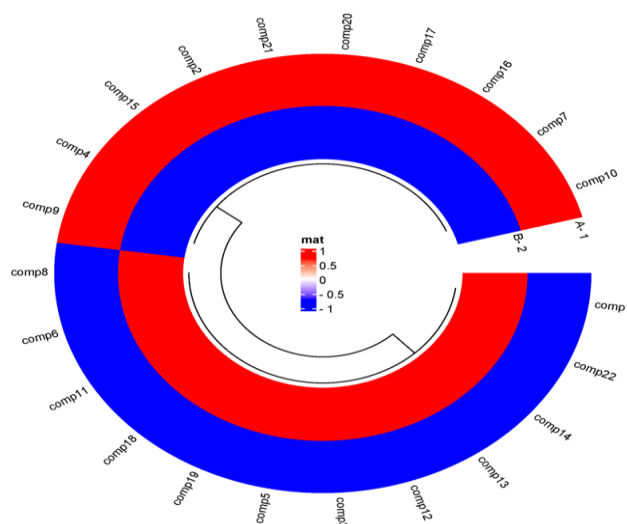


Figure 2. Circular Clustered heat map of the chemical composition of essential oil from different parts of *Alpinia Zerumbet* L.; A-1= *Alpinia Zerumbet* leaf essential oil; B-2= *Alpinia Zerumbet* rhizome essential oil.

The chemical composition of essential oils from *Alpinia zerumbet* rhizome and leaf was visualized using a circular heat map (Figure 2). This map highlights the volatile components of the essential oils, with colour gradients representing the abundance of each metabolite. In the circular clustered heat map, red indicates high levels of metabolites, blue shows the lowest levels and varying shades of light red reflect intermediate values, with the intensity corresponding to metabolite abundance. The heat map clearly distinguishes between the rhizome and leaf essential oils. Notably, the rhizome essential oil is rich in camphor (compound 6), whereas the leaf essential oil has a higher concentration of 1,8-cineole (compound 2). Several compounds were common to both essential oils (compounds 1, 2, 6, 9, 10, 20, 22), while distinct differences were evident in other compounds (compounds 3,4, 5,7, 8, 9, 11-19, 21). The rhizome oil shows higher levels of camphor, 1,8-cineole, β -linalool, α -terpineol and terpin-4-ol. Similarly, the leaf oil is rich in 1,8-cineole, camphor, α -terpineol, β -linalool, terpin-4-ol and oxime-methoxy-phenyl. This analysis highlights both the similarities and the differences in the overall profiles of rhizome and leaf essential oils. Despite some specific variations in compound concentrations, the general profiles of the two essential oils showed significant similarity.

HERBICIDAL ACTIVITIES

RHIZOME OIL HERBICIDAL ACTIVITY

We investigated the allelopathic effects of essential oil derived from the rhizome part of *Alpinia zerumbet* on seed germination, root length, and shoot length inhibition of *Parthenium* seeds at various concentrations (50-200 $\mu\text{L}/\text{mL}$) (Insert Table 2 and Figures 3-6). The results showed that rhizome essential oil exhibited moderate to strong allelopathic herbicidal activity in a dose-dependent manner.

(a) Seed Germination: The percent inhibition of *Parthenium* seed germination by rhizome essential oil was 91.66 % at the highest concentration tested, with an IC_{50} value of $96.76 \pm 1.78 \mu\text{L}/\text{mL}$.

(b) Root Length: Rhizome oil inhibited root length by 97.77 %. The IC_{50} value for root length inhibition was $98.70 \pm 1.31 \mu\text{L}/\text{mL}$.

(c) Shoot Length: At the highest concentration of rhizome oil (200 $\mu\text{L}/\text{mL}$), shoot length inhibition was 74.84 %. The IC_{50} value for shoot length inhibition was $110.43 \pm 2.47 \mu\text{L}/\text{mL}$.

LEAF OIL HERBICIDAL ACTIVITY

We also investigated the allelopathic effects of essential oil derived from the leaf part of *Alpinia zerumbet* on seed germination, root length, and shoot length inhibition of *Parthenium* seeds at various concentrations (50–200 $\mu\text{L}/\text{mL}$). The results showed that leaf essential oil exhibited stronger allelopathic activity than rhizome oil in a dose-dependent manner.

(a) Seed Germination: The percent inhibition of *Parthenium* seed germination by leaf essential oil was **93.33 %** at the highest concentration tested, with an IC_{50} value of $88.09 \pm 3.42 \mu\text{L}/\text{mL}$.

(b) Root Length: Leaf oil inhibited root length by 98.14%. The IC_{50} value for root length inhibition was $96.07 \pm 1.10 \mu\text{L}/\text{mL}$.

(c) Shoot Length: At the highest concentration of leaf oil (200 $\mu\text{L}/\text{mL}$), shoot length inhibition was 90.00 %. The IC_{50} value for shoot length inhibition was $109.19 \pm 1.90 \mu\text{L}/\text{mL}$.

Our research highlighted the strong allelopathic properties of *Alpinia zerumbet* essential oils, which significantly inhibited the *Parthenium* seed germination and the growth of both roots and shoots. At high concentrations, these oils effectively disrupt the early developmental stages of plants, aligning with previous findings on the herbicidal effects of similar compounds. For instance, 1,8-cineole, a major component in eucalyptus oils, possess herbicidal activity against species like annual ryegrass (*Lolium rigidum*) and radish (*Raphanus sativus*) in pre-emergence bioassays (3). Similarly, camphor, found in *Satureja cuneifolia* essential oil, significantly inhibits the growth of weeds *Amaranthus hybridus* and

Conyza canadensis (13). Linalool, present in Bergamot oil, was inhibitory against *Amaranthus retroflexus* L., *Convolvulus arvensis* L. and *Rumex crispus* L. (5).

Our results also align with studies on *Hedychium spicatum* essential oil, where compounds like camphor, 1,8-cineole, isoborneol and linalool were effective against radish seeds (*Raphanus raphanistrum*). These bioactive compounds interfere with seed germination and growth by disrupting membrane integrity, enzyme activities and hormonal balances, leading to inhibition or plant death. The complex chemical composition of *Alpinia zerumbet* essential oils, including phytotoxic compounds like 1,8-cineole, camphor and linalool showed their allelopathic effects. These compounds disrupt cellular processes and metabolic pathways in target plants, affecting their ability to germinate and grow. Future research should focus on isolating and identifying the specific components responsible for these allelopathic effects in *Alpinia zerumbet* essential oils. The significant allelopathic activity, making them promising candidates for developing natural herbicides. Their ability to inhibit seed germination and growth of roots and shoots presents new opportunities for sustainable weed management practices.

Table 2. Inhibitory effects of *Alpinia zerumbet* oils doses on of seed germination, root and shoot length of *Parthenium* over control

Seed germination inhibition (%)				
Samples	50 µL/mL	100 µL/mL	150 µL/mL	200 µL/mL
Rhizome	33.33±1.52 ^a	50.33±0.57 ^b	65.33±0.57 ^c	93.33±1.15 ^d
Leaf	31.66±1.52 ^a	50±1.73 ^b	72.33±1.52 ^c	91.66±0.57 ^d
* Paraquat herbicide	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00

Shoot length inhibition (%)				
Sample	50 µL/mL	100 µL/mL	150 µL/mL	200 µL/mL
Rhizome	28.88±1.92 ^a	51.11± 2.22 ^b	62.59±1.69 ^c	74.84±1.69 ^d
Leaf	27.40±0.64 ^a	40.37±1.69 ^b	70.74±1.69 ^c	90.00±1.11 ^d
* Paraquat herbicide	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00

Root length inhibition (%)				
Sample	50 µL/mL	100 µL/mL	150 µL/mL	200 µL/mL
Rhizome	32.22±1.11 ^a	45.55±1.11 ^b	71.85±1.28 ^c	97.77±1.11 ^d
Leaf	34.81±0.64 ^a	45.55±1.11 ^b	70.37±1.28 ^c	98.14±0.64 ^d
* Paraquat herbicide	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00

According to DMRT (Duncan multiple range test) test ($p < 0.05$), mean values in a column that are followed by same letter do not substantially differ from each other.

$$\text{Inhibition (\%)} = 100 \times (1 - Gt/Gc)$$

Where, Gt: no. of seeds germinated in treatment, Gc: no. of seeds germinated in control.

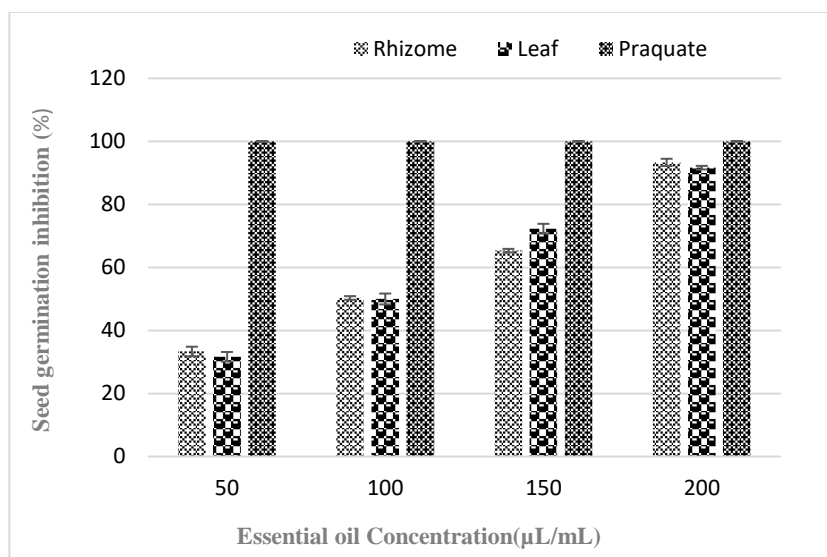


Figure 3. Inhibitory effects of *Alpinia Zerumbet* oils doses on *Parthenium* seed germination

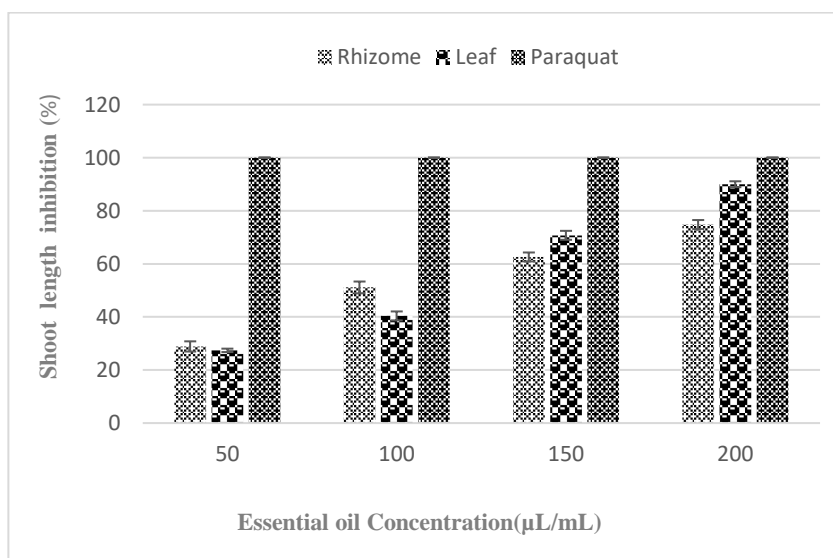


Figure 4. Inhibitory effects of *Alpinia Zerumbet* oils doses on shoot length of *Parthenium*

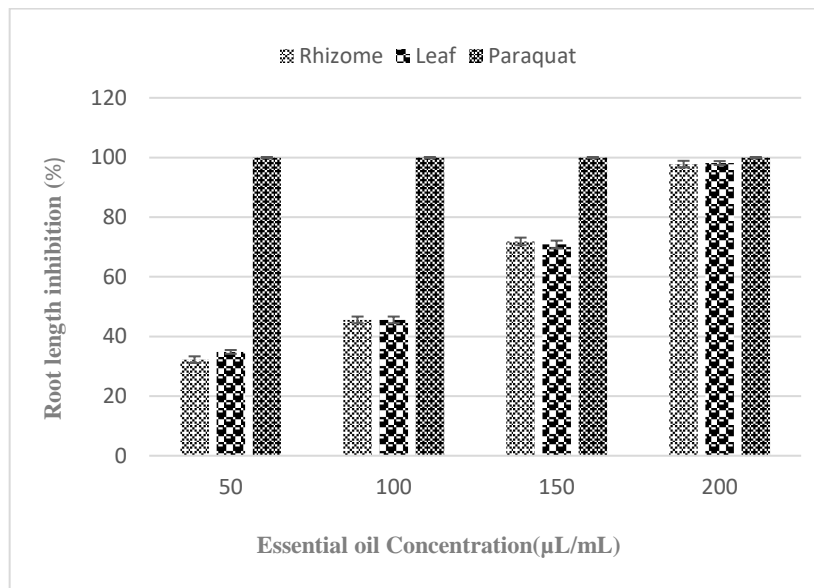


Figure 5. Inhibitory effects of *Alpinia Zerumbet* oils doses on root length of *Parthenium*

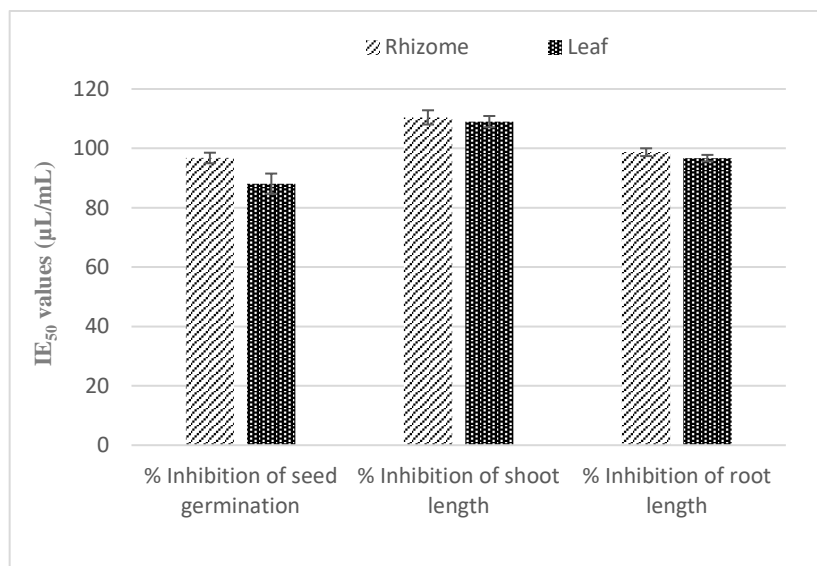


Figure 6. IC_{50} value of *Alpinia Zerumbet* essential oil for allelopathy activity

3.1.2. Principal component analysis (PCA)

After identifying the components and biological effects of each oil, the variability in their allelopathic activity against *Parthenium* seeds was assessed (Figure 7). Principal component analysis (PCA) was used to examine differences in allelopathic activity between two essential oils: *Alpinia zerumbet* rhizome essential oil and *Alpinia zerumbet* leaf essential oil. The study focussed on three parameters: (i). Percentage inhibition of seed germination, (ii). Root length inhibition and (iii). Shoot length inhibition, based on the plant part from which the essential oils were extracted. The cumulative contribution of variance from the first two principal components (PC1 and PC2) in the PCA was 100 %, explaining the entire range of differences in allelopathic activity. PC1 explained 98.3 % of the total variance and was positively associated with shoot inhibition. PC2 contributed 1.3 % to the variance and showed positive correlations with seed germination inhibition and root inhibition. This analysis effectively characterized the distinct allelopathic activities of rhizome and leaf essential oils from *Alpinia zerumbet*.

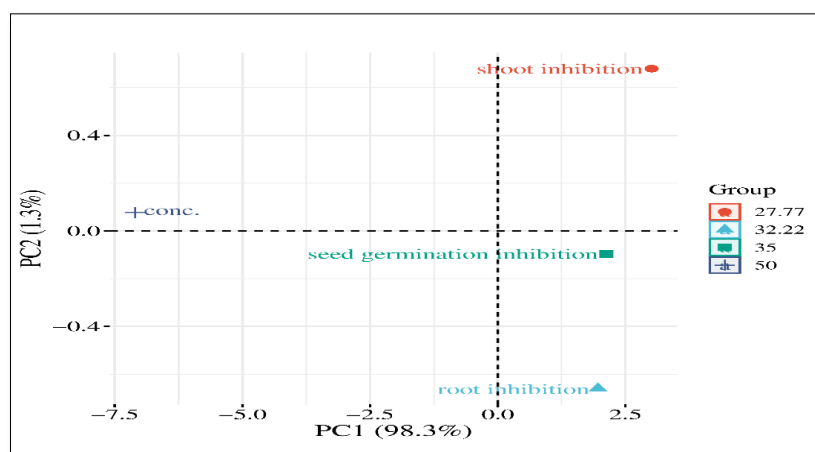


Figure 7. PCA of *Alpinia zerumbet* rhizome and leaf essential oil

3.1.3. Antifungal activity

The antifungal activity of essential oils from *Alpinia zerumbet* rhizomes and leaf was investigated against the plant pathogenic fungi *Colletotrichum lindemuthianum* and *Curvularia lunata*. These oils possess moderate inhibitory effects on mycelial growth of 50 to 200 $\mu\text{L}/\text{mL}$ concentrations (Figure 8 and 9). At the highest concentration tested (200 $\mu\text{L}/\text{mL}$), leaf sample exhibited the most substantial antifungal activity against *Colletotrichum lindemuthianum* (92.22 %), followed by rhizome sample (91.48 %). Similarly, leaf exhibited the most significant antifungal activity against *C. lunata* (90.74 %), followed by rhizome sample (90.00 %). Despite this significant inhibition, it is crucial to note that the effectiveness of these essential oils, even at their highest concentrations, was lower than standard fungicide carbendazim, which achieved 100 % inhibition under similar conditions. This observations suggested that while *Alpinia zerumbet* essential oils are potent,

they may not fully replace conventional fungicides but could serve as valuable components in integrated pest management strategies. The antifungal properties of rhizome and leaf essential oil can be attributed to their complex chemical compositions, rich in bioactive compounds (α -terpineol, trans-verbenol, borneol alcohol, cyclotetrasiloxane, trans-sabinol, and 2,4-dimethyl-1,3-cyclopentanedione). These compounds inhibit the fungal growth. For instance, α -terpineol and 1,8-cineole prominent in *Alpinia zerumbet* oils have antifungal efficacy across various studies (12,26).

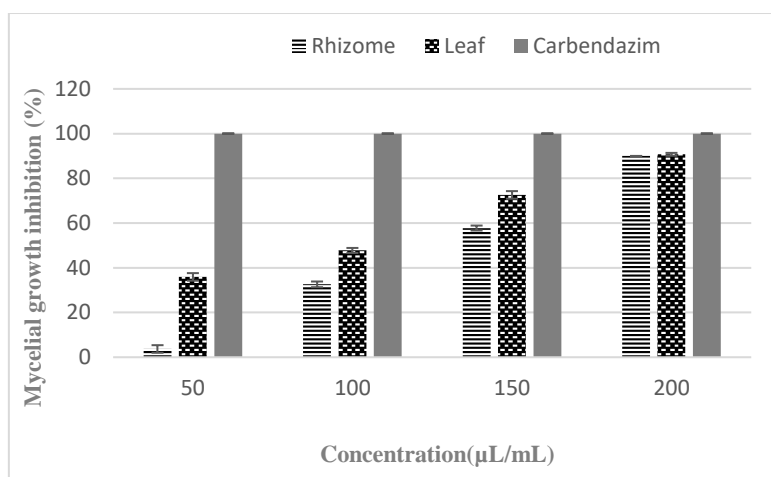


Figure 8. Mycelial growth Inhibitory effects (%) of *Alpinia zerumbet* oils doses on *Colletotrichum lindimuthianum*.

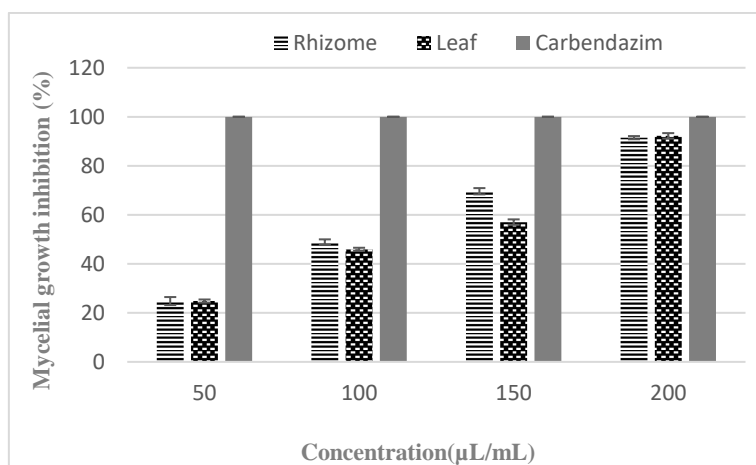


Figure 9. Mycelial growth Inhibitory effects (%) of *Alpinia zerumbet* oils doses on *Curvularia lunata*

Hmiri *et al.* (14) reported that 1,8-cineole from *Myrtus communis* and *Rosmarinus officinalis* completely inhibited the growth of *Botrytis cinerea*, *Penicillium expansum* and *Alternaria alternata* at varying concentrations. Similarly, Ghasemi *et al.* (14) demonstrated the antifungal activity of camphor and 1,8-cineole derived from *Artemisia sieberi* against *Botrytis cinerea*. Sethi *et al.* (22) highlighted the antifungal activity of camphor from *Alpinia malaccensis* leaves against pathogens (*Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*) at various concentrations. These findings align with the current study's results, where Camphor significantly contributed to the antifungal potential of rhizome. Terpinen-4-ol, obtained from the steam distillation of *Alpinia zerumbet* var. *exelsa* leaves, demonstrated antifungal activity against *Aspergillus brasiliensis*, with a minimum inhibitory concentration of 0.4 % (20). This supported the idea presence of such compounds in *Alpinia zerumbet* oils enhances their antifungal effectiveness.

These findings underscore the potential of rhizome and leaf essential oil as natural antifungal agents. While they may not completely replace synthetic fungicides, their use could reduce the reliance on chemical treatments, promoting a more sustainable approach to manage fungal diseases in agriculture. The moderate efficacy observed against *Colletotrichum lindemuthianum* and *Curvularia lunata* suggested that these oils could be integrated into pest management strategies, possibly in combination with other treatments to achieve synergistic effects. The variations in antifungal activity between rhizome and leaf essential oil can be linked to their distinct chemical compositions. Rhizome, with higher camphor content and leaf, rich in 1,8-cineole, exhibit different levels of efficacy, highlighting the importance of understanding the specific roles of these compounds in antifungal mechanisms. Future research could focus on isolating and testing these individual compounds to determine their precise contributions to the overall antifungal activity.

CONCLUSIONS

The essential oils from the rhizomes and leaves of *Alpinia zerumbet*, were rich in oxygenated monoterpenes like camphor and 1,8-cineole. These compounds, known for their diverse biological activities, were comprehensively evaluated. Rhizome oil predominantly contained camphor (33.63 %), 1,8-cineole (13.91 %), β -linalool (9.95 %) and α -terpineol (5.75 %), whereas, leaf oil was mainly composed of 1,8-cineole (39.86 %), camphor (12.39 %), α -terpineol (9.67 %), β -linalool (6.42 %), benzylacetone (4.86 %) and terpin-4-ol (3.81 %). These findings indicated significant variability and a rich diversity in bioactive compounds between the two oils. The heat map analysis highlighted the higher concentrations of camphor in rhizome and 1,8-cineole in leaf, likely influenced by sunlight and physiological differences. This is the first detailed study of chemical analysis of *Alpinia zerumbet* essential oils from the Indian Garhwal region, India. The unique chemical compositions and biological activities of these oils make them a promising natural resource for development of eco-friendly pesticides for sustainable alternatives to synthetic chemicals.

ACKNOWLEDGEMENTS

The authors acknowledge the Dev Bhoomi Uttarakhand University (Dehradun), Uttarakhand, India, for providing academic support and Sophisticated Industrial Materials Analytic Labs Pvt. Ltd. in Haridwar, for providing facility for GC-MS analysis.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration with all authors. All authors finally approved and drafted the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest. All authors agree to publish it.

DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

ETHICAL STATEMENT

This is to inform you that in this study, we have not been involved in any animal and human studies.

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