Bioactive compounds and pharmacological activities of *Zingiber cassumunar* from Eastern India

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Abstract

Zingiber cassumunar is a medicinal plant with valuable rhizome product used in pharmaceutical industry for wide beneficial health effects in curing various diseases. The current study aims to analyze compounds present in rhizome oil using Gas Chromatography-Time of Flight-Mass Spectrometry method. The plant rhizomes were collected for essential oil extraction using Clevenger apparatus by hydrodistillation method. From analysis, 99 constituents were found which showed major compounds as eucalyptol (10.61%), linalool (9.60%), l-Proline, n-propargyloxycarbonyl-, propargyl ester (8.58%), zerumbone (8.43%) and isoborneol (7.16%) respectively. The methanol, ethanol and aqueous plant extracts using Soxhlet apparatus of Zingiber cassumunar were assayed for antimicrobial and antioxidant content. Antimicrobial activities of plant extracts against Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis, Klebsiella pneumoniae and Candida albicans using disc diffusion method, revealed the highest zone of inhibition against Candida albicans in ethanol extract as 26 mm.

Antioxidant activity was most potent in ethanolic extract, showing better IC50 values than methanolic and aqueous extracts in both 2,2-diphenyl-1-picrylhydrazyl (81±0.97 µg/ml) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (69±0.25 µg/ml) assay. The rhizome extracts showed strong antimicrobial activity and rich antioxidants content. Hence, the present study revealed the presence of good quality oil and extracts that could be used to commercialize Zingiber cassumunar rhizome in the global market.

Keywords: *Zingiber cassumunar*, rhizome, gas chromatography-time of flight-mass spectrometry, antimicrobial, antioxidant.

Introduction

The herbaceous perennial plant *Zingiber cassumunar* Roxb. is a member of the Zingiberaceae family and is found across Southeast Asia[14], commonly known as plai in Thailand and bulei in China for use in treating both internal and external ailments[7]. The Zingiberaceae family offers a vital natural resource, offering a diverse range of bioactive substances and products with considerable use in food, spices, medical preparations, dyes, fragrances and aesthetic purposes. *Z. cassumunar* commonly known as "TekhaoYaikhu" in Manipur, India is a prominent species within this family, recognized for its diverse phytochemical and pharmacological properties. The perennial, tuberous root of the plant has jointed segments and long, white, fleshy fibers that resemble ginger but are considerably bigger. It is a bright yellow hue when fresh and it smells strongly of camphor and tastes warm, spicy and slightly bitter[18].

Traditionally, it has been employed to alleviate pain, sprains, as an antiseptic and to treat conditions like asthma, inflammation and rheumatism[7]. Terpenoids and terpenes, which are known to have analgesic and local anesthetic properties, make up the majority of the essential oil that is extracted from its rhizomes[5,11]. According to some studies, the rhizome extract contains various bioactive compounds such as curcuminoids, sesquiterpenoids, monoterpenoids, phenylbutenoids, benzaldehydes and quinones, contributing to its pharmacological potency having good antioxidant and antimicrobial propertiesagainst pathogenicorganisms[7,10,23]. Identifying the phytochemical makeup of *Z. cassumunar* rhizomes and assessing the antioxidant, antibacterial and antifungal qualities of rhizome extracts against a variety of pathogens are the goals of this study.

Material and Methods

Plant material collection: The plants were brought from Silviculture, Ghatikia, Bhubaneswar, Odisha and were grown in Centre for Biotechnology, Siksha 'O' Anusandhan University, Bhubaneswar, Odisha.

Rhizome oil and extract preparation: *Z. cassumunar* rhizomes were collected and rinsed for essential oil (EO) and extract preparation. Approximately 1000 grams of sliced rhizomes and 2000 ml distilled water are subjected to essential oil isolation using a Clevenger apparatus. For Soxhlet apparatus extraction. Rhizomes were dried and ground into powder at room temperature. It was then extracted using methanol, ethanol and aqueous as solvents. The extracts were first concentrated using a rotary evaporator after being run through Whatmann filter paper to get a semi solid mass. To guarantee repeatability, the extraction process was carried out in triplicate. For further examinations, the resultant residues and oils were kept at 4°C.

GC x GC-TOF-MS analysis: The LECO Pegasus 4D GC×GC Time-of-Flight (TOF) mass spectrometry system

was used to analyze a 0.1 µL sample of rhizome oil. It required an Agilent 6890A gas chromatograph, an Agilent 7683B automatic liquid sampler, a KT-2007 cold jet modulator, a secondary oven and a TOF mass spectrometer that served as the detector. Researchers used a Restek Rtx-5MS (30 m × 0.25 mm I.D., 0.25 µm film thickness) as the primary column and a Restek Rxi-17 Sil MS (2 m × 0.25 mm I.D., 0.25 µm film thickness) as the secondary column. Helium was used as the carrier gas when the sample was delivered into the GC×GC inlet in split mode (1:700) at a flow rate of 1 mL/min.

With a detector voltage of 1620 V and operating in electron impact (EI) mode at 70 eV, the mass spectrometer scanned a mass-to-charge ratio range of 30–350 m/z. Both the ion source and the transfer line were kept at 250 °C in temperature. After starting at 50°C (held for one minute) and increasing at a rate of 5°C per minute to 230°C (kept for five minutes), the oven temperature program ramped up at a rate of 15°C per minute to 260°C (held for one minute). The temperature difference between the primary and secondary ovens was 10 °C. The modulation cycle was set at 5 seconds, comprising a 0.9-second hot pulse and a 1.6-second cold pulse. The TOF-MS system acquired data at a rate of 100 spectra per second. Compound identification was achieved through spectral matching with the NIST library using linear temperature-programmed retention indices (LTPRI). GC×GC peak area data was used to quantify the relative abundances of the chemicals.

Microbial sample collection and Minimum inhibitory concentration: The Microbial Type Culture Collection (MTCC), located in Chandigarh, provided the microbial cultures. The pure cultures were introduced onto plates of nutrient agar after the inoculum was produced aseptically and the plates were then incubated at 37 °C for 24 hours. Standardized method was used to evaluate the antibacterial activity of the plant-derived materials[2]. Minimum inhibitory concentration (MIC) was determined against bacterial strains like: *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 441), *Staphylococcus epidermidis* (MTCC 3086), *Klebsiella pneumoniae* (MTCC 4030) and one fungal strain, *Candida albicans* (MTCC 3017). Each well of 96-well microtiter plate received 100 µL of each of the test compounds' two-fold serial dilutions. The final test findings showed a range of 1000 µg/mL to 15.625 µg/mL. For seventy-two hours, the plates were incubated at 35 °C. Absorbance readings were measured using an ELISA plate reader. Statistical analyses were conducted using SPSS software[15].

Disc diffusion assay: 24-hour culture microbial colonies were suspended in 0.85% saline which was adjusted to meet the 0.5 McFarland threshold for turbidity. This produced suspensions containing approximately 1× 10^6 CFU/mL for bacteria and 1× 10^4CFU/mL for fungi. 0.1 mL aliquot of the microbial suspension was evenly spread onto Mueller-Hinton agar plates for bacterial strains. Potato dextrose agar plates were used for fungal strains. Test samples, prepared at concentrations corresponding to MIC results, were applied to the plates (100 µL per well). Solvents such as methanol, ethanol and water served as negative controls while norfloxacin (5 µg) was used as a positive control for clotrimazole (10 µg) for Candida species, cefoperazone–sulbactam (10 µg) according to EUCAST and CLSI criteria, both Gram-positive and Gram-negative microorganisms. The plates were incubated for a full day at 37 °C. The microbial growth's surrounding zones of inhibition were measured[22].

DPPH Assay: With a few minor adjustments, the radical scavenging test was performed using the Jena et al[8] technique. The combination was let to stand at room temperature for half an hour. Then one milliliter of the extract at a different concentration was combined with one milliliter of a 0.1 mM methanolic DPPH solution. The absorbance of the solution was recorded at 517 nm. Using the following formula, the proportion of DPPH radical inhibition was determined:

$$Inhibition\% = 100\ x \frac{\text{Absorbance of control (Ac)} - \text{Absorbance of test sample(As)}}{\text{Absorbance of control (Ac)}}$$

The conc. of extract required to attain 50% inhibition of DPPH radicals, or the IC50 value, was calculated[8].

ABTS Assay: With minor adjustments, Re et al[17] procedure was used to evaluate the rhizome extracts' ABTS radical scavenging activity. A stock solution of ABTS was made by combining 2.45 mM ammonium persulfate with 7 mM ABTS, then letting it sit at room temperature for sixteen hours to produce ABTS radicals. To get an absorbance of 734 nm, this solution was then diluted using methanol. 1 mL of the extract at different conc. was combined with 1 mL of the ABTS solution to test the scavenging activity and the absorbance at 734 nm was recorded. The same methodology used for the DPPH test was used to determine the percentage of ABTS radical inhibition. The extract concentration needed to attain 50% inhibition of ABTS radicals was evaluated as the IC_{50} value[17].

Results and Discussion

The EO from *Z. cassumunar* rhizomes exhibited a whitish color with a camphor-like aroma, yielding 0.7 mL per 100 g of fresh weight. A comprehensive analysis of its volatile composition using GCxGC-TOF-MS identified total 99 compounds among which the major constituents are identified as eucalyptol (10.61%), linalool (9.60%), l-proline, n-propargyloxycarbonyl-, propargyl ester (8.58%), zerumbone (8.43%) and isoborneol (7.16%) (Figures 1, 2 and table 1). These results are in contrast with earlier research that found distinct key components. For instance, Bhuiyan et al[1] discovered that the primary components of leaf oil were caryophyllene (9.47%), caryophyllene oxide (13.85%), β-pinene (14.32%) and sabinene (14.99%).

Rhizome oil included terpinen-4-ol (18.45%), (Z)-ocimene (21.97%) and triquinacene 1,4-bis (methoxy) (26.47%). The primary constituents were sabinene, terpinen-4-ol and (E)-1-(3,4-dimethoxyphenyl) butadiene, according to Yingngam and Brantner[24]. Tyas et al[21] found the following compounds as significant: benzoene, 4-(1Z)-1,3-butadien-1-yl-1,2-dimethoxy and (E)-4-(3,4-Dimethoxyphenyl)-but-3-en-1-ol. According to Kamazeri et al[9], *Z. cassumunar* oil has large concentrations of α-caryophyllene (23.92%) and 2,6,9,9-tetramethyl-2,6,10-cycloundecatrien-1-one (60.77%).

According to Brophy and Zwaving[4], trans-1-(3,4-dimethoxyphenyl)-but-1-ene, trans-1-(3,4-dimethoxy phenyl)-butadiene, sabinene, terpinen-4-ol and trans-4-(3,4-dimethoxyphenyl)but-3-ene-1-yl acetate comprised of about 46% of the EO of *Z. cassumunar* from Indonesia that was extracted using light petroleum. 50.5% terpinen-4-ol, (E)-l-(3,4-dimethoxyphenyl). However, *Z. cassumunar* from northeast India contained buta-1,3-diene (19.1%), (E)-1-(3,4-dimethoxyphenyl)but-1-ene (6.0%) and β-sesquiphell andrene (5.9%)[3]. However, as seen in table 1, our investigation discovered the same compounds at varying amounts.

Additionally, extracts of *Z. cassumunar* have demonstrated antimicrobial properties. In our study, the aqueous extract demonstrated weak antimicrobial effects against the majority of the strains tested. At doses ranging from 62.5 to 250 µg/mL, the ethanolic extract had wider antibacterial efficacy against bacteria and fungi, whereas the methanol and ethanol extracts showed sensitivity against *K. pneumoniae* and *C. albicans*. In contrast, methanol extract exhibited weaker antimicrobial effects [Table 2]. Throughout the study, co-trimoxazole (23.75/1.25) mcg (Himedia SD010-50DS) and Fluconazole (25 mcg) (Himedia SD232-1Vl) were used as positive control for bacteria and fungus respectively.

Despite the essential oil's antibacterial efficacy against *S. aureus* and *E. coli*, other studies, including that of Kamazeri et al[9] reported minimal antimicrobial activity for *Z. cassumunar* EO. It has been also reported that the methanolic extract of the plant rhizome is inactive against *E.coli* and *C.albicans*[6]. Both Gram-positive and Gram-negative bacteria and fungi are susceptible to the antibacterial activity of *Z. cassumunar*[16]. Nonetheless, we are studying *S. aureus, B. subtilis, K. pneumoniae, S. epidermis and C. albicans* separately.

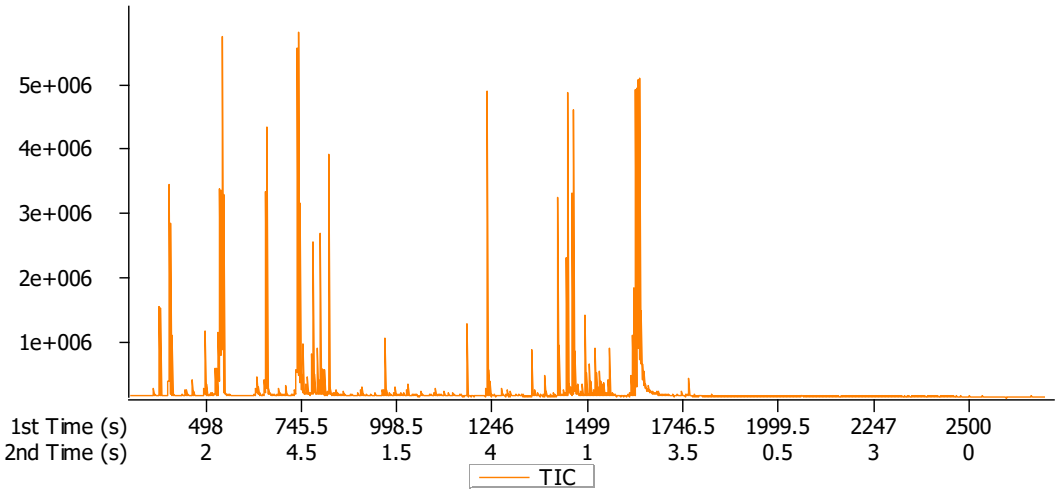

Figure 1: GCxGC-TOF-MSanalysis of *Zingiber cassumunar* rhizome oil

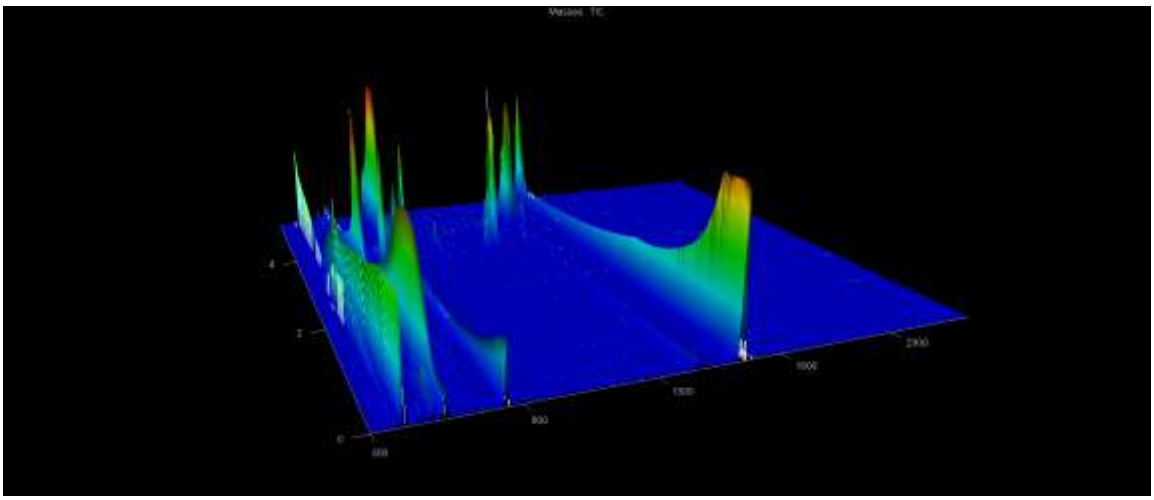

Figure 2: GCxGC-TOF-MS3D analysis of *Zingiber cassumunar* rhizome oil

Table 1
Chemical composition of *Zingiber cassumunar*rhizome essential oil

S.N.	Compound Name	RT (min, sec)	RI EXP	Molecular Formula	Area (%)
1.	(-)-α-Pinene	3.77 , 5.36	914	$C_{10}H_{16}$	0.59
2.	Camphene	4.10 , 0.04	947	$C_{10}H_{16}$	4.39
3.	α-Sabinene	4.37 , 2.89	752	$C_{10}H_{16}6$	0.02
4.	á-Myrcene	4.65 , 0.12	913	$C_{10}H_{16}$	0.27
5.	Paraethylphenol	4.92 , 4.27	783	$C_8H_{10}O$	0.03
6.	trans-á-Ocimene	5.03 , 0.27	868	$C_{10}H_{16}$	0.3
7.	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	5.14 , 0.94	709	$C_{10}H_{18}O_2$	0.05
8.	β-Cymene	5.25 , 3.40	953	$C_{10}H_{14}$	1.56
9.	Limonene	5.31 , 0.43	922	$C_{10}H_{16}$	0.92
10.	**Eucalyptol**	5.36 , 4.29	533	$C_{10}H_{18}O$	10.61
11.	Linalool oxide B	6.02 , 0.83	800	$C_{10}H_{18}O_2$	0.02
12.	ç-Terpinene	6.24 , 3.40	800	$C_{10}H_{16}$	0.04
13.	Camphenilone	6.24 , 4.70	807	$C_9H_{14}O$	0.04
14.	Fenchone	6.30 , 1.96	896	$C_{10}H_{16}O$	1.23
15.	Perillen	6.46 , 3.51	767	$C_{10}H_{14}O$	0.02
16.	**Linalool**	6.52 , 3.40	867	$C_{10}H_{18}O$	9.60
17.	1,5-Decadiyne	6.52 , 3.75	751	$C_{10}H_{14}$	0.02
18.	α-Ocimene	6.57 , 1.19	794	$C_{10}H_{16}$	0.06
19.	Muramic acid	6.57 , 3.14	733	$C_9H_{17}NO_7$	0.99
20.	2-Nonanol	6.63 , 3.06	866	$C_9H_{20}O$	0.06
21.	Fenchol, exo-	6.85 , 3.75	781	$C_{10}H_{18}O$	0.25
22.	α-Thujene	6.96 , 1.34	800	$C_{10}H_{16}$	0.04
23.	Dehydrosabinene	7.01 , 0.96	777	$C_{10}H_{14}$	0.57
24.	Cosmene	7.23 , 1.60	717	$C_{10}H_{14}$	0.09
25.	Camphore	7.29 , 4.69	898	$C_{10}H_{16}O$	0.27
26.	(+)-2-Bornanone	7.34 , 4.71	932	$C_{10}H_{16}O$	0.17
27.	**l-Proline, n-propargyloxycarbonyl-, propargyl ester**	7.40 , 4.72	745	$C_{12}H_{13}NO_4$	8.58
28.	α-Thujol	7.78 , 1.85	818	$C_{10}H_{18}O$	0.12
29.	**Isoborneol**	7.78 , 4.08	882	$C_{10}H_{18}O$	7.16
30.	Cyclofenchene	7.78 , 4.14	860	$C_{10}H_{16}$	3.91
31.	Terpinen-4-ol	7.95 , 3.90	861	$C_{10}H_{18}O$	2.62
32.	α-Terpinolene	7.95 , 3.93	881	$C_{10}H_{16}$	0.87
33.	Cymenene	8.00 , 4.76	828	$C_{10}H_{12}$	2.41
34.	Crypton	8.00 , 5.19	886	$C_9H_{14}O$	0.12
35.	Terpineol	8.17 , 4.08	908	$C_{10}H_{18}O$	3.70
36.	β-Terpinyl acetate	8.17 , 4.11	892	$C_{12}H_{20}O_2$	3.17
37.	Carveol	8.28 , 4.26	787	$C_{10}H_{16}O$	0.09
38.	Isobutylbenzene	8.33 , 4.30	757	$C_{10}H_{14}$	0.03
39.	trans-Pipertiol	8.39 , 4.01	797	$C_{10}H_{18}O$	0.04
40.	Levoverbenone	8.39 , 5.17	858	$C_{10}H_{14}O$	0.09
41.	3-Carene	8.55 , 4.00	769	$C_{10}H_{16}$	0.02
42.	β-Citral	8.88 , 4.33	750	$C_{10}H_{16}O$	0.02
43.	10-Undecyn-1-ol	8.94 , 4.35	712	$C_{11}H_{20}O$	1.03
44.	(-)-Carvone	8.99 , 4.96	919	$C_{10}H_{14}O$	0.29
45.	2-Nonenenitrile	9.05 , 5.32	702	$C_9H_{15}N$	0.02
46.	Car-3-en-5-one	9.05 , 5.38	836	$C_{10}H_{14}O$	0.1248
47	Piperitone	9.16 , 4.92	855	$C_{10}H_{16}O$	0.05
48.	3-Decyn-2-ol	9.27 , 3.77	720	$C_{10}H_{18}O$	0.03
49.	3-Isopropyl-4-methyl-1-pentyn-3-ol	9.32 , 4.96	731	$C_9H_{16}O$	0.05

50.	α-Citral	9.38 , 4.41	746	$C_{10}H_{16}O$	0.02
51.	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethenyl)-, (S)-	9.43 , 0.10	812	$C_{10}H_{14}O$	0.16
52.	Acetic acid, bornyl ester	9.65 , 3.96	858	$C_{12}H_{20}O_2$	0.87
53.	t-Carenol	9.71 , 4.42	541	$C_{10}H_{16}O$	0.14
54.	2-Decanone	9.76 , 3.52	866	$C_{10}H_{20}O$	0.04
55.	Bicyclo[4.2.0]oct-1-ene, 7-endo-ethenyl-	9.82 , 4.06	720	$C_{10}H_{14}$	0.02
56.	2-Dodecanol	9.93 , 3.20	890	$C_{12}H_{26}O$	0.30
57.	2-Isopropylidene-3-methylhexa-3,5-dienal	9.93 , 4.66	704	$C^{10}H_{14}O$	0.03
58.	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1à,3à,5à)]-	10.09 ,4.45	782	$C_{10}H_{16}O$	0.33
59.	Bicyclo[2.2.1]heptane-2-carboxylic acid, 3,3-dimethyl-	10.15 ,4.41	826	$C_{10}H_{16}O_2$	0.05
60.	Cephreine	10.64 ,3.57	863	$C_{12}H_{22}O_2$	0.02
61.	cis-p-Mentha-2,8-dien-1-ol	11.41 ,5.36	733	$C_{10}H_{16}O$	0.05
62.	Hexadecane	11.47 ,2.50	904	$C_{16}H_{34}$	0.05
63.	1,3-Dioxolane, 2-(3-bromo-5,5,5-trichloro-2,2-dimethylpentyl)-	11.47 ,4.81	713	$C_{10}H_{16}BrCl_3O_2$	0.05
64.	Caryophyllene	11.80 ,3.62	909	$C_{15}H_{24}$	0.55
65.	Octan-2-one, 3,6-dimethyl-	11.91 ,5.21	700	$C_{10}H_{20}O$	0.02
66.	Carvone hydrate	11.96 ,0.74	882	$C_{10}H_{16}O_2$	0.03
67.	(Z)-α-Farnesene	11.96 ,3.29	896	$C_{15}H_{24}$	0.02
68.	1-Ethenyl-3-(1-hexenyl)-4-trimethylsilylcyclopentane	12.18 ,4.91	731	$C_{16}H_{30}Si$	0.06
69.	cis-á-Farnesene	12.24 ,3.30	829	$C_{15}H_{24}$	0.05
70.	Humulene	12.29 ,3.77	907	$C_{15}H_{24}$	0.68
71.	Myrcenol	12.68 ,0.26	546	$C_{10}H_{18}O$	0.04
72.	Patchulane	12.68 ,4.32	761	$C_{15}H_{26}$	0.12
73.	Epoxy-à-terpenyl acetate	12.84 ,5.04	712	$C_{12}H_{20}O_3$	0.02
74.	(1S,2S,4S)-Trihydroxy-p-menthane	12.95 ,0.26	774	$C_{10}H_{20}O_3$	0.02
75.	Germacrene D	12.95 ,3.77	816	$C_{15}H_{24}$	0.03
76.	δ-Cadinene	13.23 ,3.87	859	$C_{15}H_{24}$	0.05
77.	Calamenene	13.28 ,4.26	797	$C_{15}H_{22}$	0.01
78.	Bicyclo[6.1.0]nonane, 9-(1-methylethylidene)-	13.50 ,4.89	801	$C_{12}H_{20}$	0.73
79.	Caryophyllene oxide	13.72 ,4.41	769	$C_{15}H_{24}O$	5.44
80.	Bicyclo[4.1.0]heptane,-3-cyclopropyl,-7-hydroxymethyl, trans	13.78 ,4.62	733	$C_{11}H_{18}O$	0.02
81.	Nerolidol	13.83 ,3.68	884	$C_{15}H_{26}O$	0.26
82.	(3R,4aS,8aS)-8a-Methyl-5-methylene-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene	14.44 ,4.75	824	$C_{15}H_{22}$	1.14
83.	Humulene epoxide 2	14.60 ,4.71	920	$C_{15}H_{24}O$	6.89
84.	D-Alanine, N-(4-butylbenzoyl)-, isohexyl ester	14.88 ,4.50	744	$C_{20}H_{31}NO_3$	0.04
85.	Ledene oxide-(II)	14.88 ,4.69	798	$C_{15}H_{24}O$	1.02
86.	3-Bromo-7-methyl-1-adamantanecarboxylic acid	14.93 ,4.81	758	$C_{12}H_{17}BrO_2$	0.07
87.	Cyclobutene, 4,4-dimethyl-1-(2,7-octadienyl)-	14.99 ,5.15	815	$C_{14}H_{22}$	0.51
88.	Panaxydol	14.99 ,5.22	584	$C_{17}H_{24}O_2$	0.07
89.	Germacrene D	15.04 ,4.44	813	$C_{15}H_{24}$	0.05
90	Preg-4-en-3-one, 17à-hydroxy-17á-cyano-	15.04 ,4.76	777	$C_{20}H_{27}NO_2$	0.09
91.	α-Limonene diepoxide	15.21 ,0.35	660	$C10H_{16}O_2$	0.25
92.	Doconexent	15.21 ,4.65	605	$C_{22}H_{32}O_2$	0.05
93.	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	15.26 ,5.03	767	$C_{13}H_{20}O_2$	0.47
94.	trans-Arbusculone	15.98 ,5.32	673	$C_9H_{14}O_2$	0.07
95.	**Zerumbone**	16.25 ,0.22	866	$C_{15}H_{22}O$	8.43
96.	2-Amino-4-cyanomethyl-6-piperidino-1,3,5-triazine	16.36 ,0.12	593	$C_{10}H_{14}N_6$	1.97
97.	Myrtenol	16.86 ,0.06	597	$C_{10}H_{16}O$	0.03
98.	cis-Z-à-Bisabolene epoxide	17.57 ,5.17	707	$C_{15}H_{24}O$	0.41
99.	3-Methyl-2-cyclopentenone	17.79 ,0.54	769	C_6H_8O	0.06

Table 2
Minimum Inhibitory Concentration (MIC) and Zone of Inhibition (ZOI) of *Zingiber cassumunar* rhizome extracts

Test Extracts	Test Organisms				
	MIC (µg/ml)				
	S. aureus	***B. subtilis***	***S. epidermis***	***K.pneumonae***	***C. albicans***
Methanol extract	>500	>500	>500	>125	>500
Ethanol extract	>250	>125	>125	>62.5	>62.5
Aqueous extract	-	-	-	-	-
	ZOI (in mm)				
Methanol extract	12	10	12	20	14
Ethanol extract	23	23	24	20	26
Aqueous extract	-	-	-	-	-

Table 3
Antioxidant Activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Assay in *Zingiber cassumunar* rhizome extracts

Sample/Control	DPPH IC_{50} (µg/ml)	ABTS IC50 (µg/ml)
Methanol extract	121±0.42	102±0.35
Ethanol extract	81±0.97	69±0.25
Aqueous extract	149±0.16	131±0.78
Ascorbic acid	34±0.12	28±0.92

In terms of biological activity, *Z. cassumunar* is recognized for its significant antioxidant properties. Studies have demonstrated the antioxidant effects of its rhizome extract, particularly in scavenging DPPH and ABTS radicals. The IC50 value for DPPH was 149 ± 0.16 µg/ml, 121 ± 0.42 µg/ml, 81 ± 0.97 µg/ml of aqueous, methanolic and ethanolic extracts respectively. In a similar vein, the ABTS assay's IC_{50} value was determined to be 131 ± 0.78 µg/ml for aqueous, 102 ± 0.35 µg/ml for methanolic and 69 ± 0.25 µg/ml for ethanolic extracts. The study found that the ethanolic extract outperformed the aqueous and methanolic extracts in terms of IC_{50} activity (Table 3). Studies done by Sukati et al[20] reported the IC_{50} value to be 73.11±3.80%[20]. With an IC_{50} of 22.96 ± 0.87 µg/mL, *Z. cassumunar* extracts showed high DPPH radical scavenging activity[12]. *Z. cassumunar* showed significant DPPH scavenging activity in spite of variable water deprivation[13].

Conclusion

This study underscores the antimicrobial and antioxidant properties present in *Z. cassumunar* rhizome and its major constituent was eucalyptol and linalool. Eucalyptol commonly found in mouthwashes and cough suppressants, is known for its ability to control mucus hypersecretion and to manage asthma. Linalool, on the other hand, has anti-inflammatory and anti-cancer effects. Despite these findings, there is limited research on identifying phytochemicals present in essential oil of *Z. cassumunar* from Eastern India, especially through advanced techniques like GCxGC-TOF-MS.

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