IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 4, July 2026
Impact Factor: 8.2

Isolation, Characterisation, and Antifungal Activity of Zingiber Officinale Essential Oil

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Abstract: *The present study showed the capability of Zingiber oficinale (ginger) essential oil (EO) as an herbal fungicide and to help increase the shelf life of food commodities. Chemical characterisation of Ginger essential oil (ZEO) was performed by GC-MS, with citral (geranial) accounting for 11.45% as themajor compound.The minimum inhibitory concentration (MIC) of ZEO was 2.2 µl/ml. At this concentration, the synthesis of A. flavusmycelia was completely inhibited. The free radical scavenging activity of ZEO was good, and its IC50 value was 5.56µl/ml. In this study, ZEO gave good results in antifungal and antioxidant activity, which helps ZEO in future consideration as a good fungicide during post-harvest storage.*

Keywords: Ginger, Essential oil, Fungicide, Antioxidant.Phenolic content.

I. INTRODUCTION

Herbs and spices have played a significant role in human nutrition for centuries, primarily being utilised to improve the taste, appearance, and fragrance of various foods. Beyond their culinary contributions, these natural ingredients are also well recognised for their ability to extend the shelf life of food, fight harmful microorganisms, and offer a wide range of health benefits, making them one of the most ancient fields of human knowledge. The antimicrobial and antioxidant qualities of certain spices, herbs, and their active compounds have been scientifically studied and confirmed through experiments dating back to the late 1800s. Agricultural produce suffers significant damage from pests such as insects, fungi, rodents, and bacteria during storage. It is estimated that 30–35% of agricultural goods are lost between production and consumption (Anon, 2010). These massive losses could pose a grave threat in the coming years, given the rapidly increasing global population. Among the various spoilage agents, moulds are particularly concerning due to their widespread presence and their remarkable ability to contaminate food products at anystage, whether during production or storage. Their presence not only degrades the nutritional quality of food, making it unsuitable for human consumption, but also leads to the production of dangerous mycotoxins that pose serious health risks (Bluma and Etcheverry, 2008; Zabka et al., 2009).

Essential oils (EOs) derived from various traditionally used plant species have gained recognition as valuable components in the development of new plant-based preservatives, offering protection against the deterioration of food products caused by fungal growth, aflatoxin contamination, and the generation of free radicals (Kocić-Tanackov et al., 2012). One of the key advantages of plant-based formulations is their biodegradable nature, which makes them environmentally safe and sustainable. Among the wide variety of plant-derived products, EOs stand out as particularly favourable due to their volatile and short-lived characteristics, which also allow them to be effectively employed as fumigants under storage conditions (Prakash et al., 2012b).

Furthermore, the antifungal, antibacterial, antiviral, insect-repelling, antioxidant, and antiparasitic activities of various EOs have been extensively investigated by researchers worldwide (Dubey et al., 2010).

Indian ginger plant is an erect perennial, growing from 1–3 ft. in height. Mostly gingers in cultivation are sterile cultivars grown for the edible rhizomes and flowers are rarely seen. The rhizomes (spice of commerce) are aromatic, thick lobed,

DOI: 10.48175/IJARSCT-37974

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branched and scaly structures with a spicy lemon-like scent. It is well known that ginger rhizomes contain both aromatic and pungent components. The essential oil and oleoresins ex tracted from ginger rhizomes are very valuable products responsible for the characteristic ginger flavour and pungency. Both oil and oleoresins are used in many food items, soft drinks, beverages and many types of medicinal substances.

Ginger has been found to be abundantly rich in bioactive compounds, and numerous research studies have been conducted to investigate and explore the beneficial and therapeutic properties of ginger and its various extracts (Grzanna et al., 2005).

The essential oils derived from ginger have attracted considerable scientific interest owing to their high content of diverse functional compounds, predominantly terpenes, monoterpenes, and sesquiterpenes. It is these specific compounds that are largely responsible for conferring the oil withits notable biological activity and medicinal potential (Daferera et al., 2002).

Therefore, the primary aim of this study was to examine the chemical composition of ginger essential oil and to evaluate its antifungal potential against fungal strains responsible for causing storage rot. Furthermore, the study sought to investigate the effect of the Minimum Inhibitory Concentration (MIC) of Zingiber officinale essential oil on the mycelial growth of Aspergillus flavus.

II. MATERIALS AND METHODS

2.1 Materials

Hydrodistillation unit, UV spectrophotometer, Centrifuge, laminar unit, Incubator.

2.2 Collection and Extraction of Ginger

Ginger was bought from local market and identified by the herbarium and pesticide lab, Department of Botany, B.H.U Varanasi. Then after that the cleaned ginger roots cut in small pieces and put into a Clevenger unit, which round shaped with narrow neck flask and filled with distilled water for extraction of oil. The mixture was subjected to hydrodistillation for 4-5 hours during which volatile constituents were vaporised, condensed, and collected as a biphasic distillate. After that oil layer was separated and stored in the oil collection vial at 4◦c.

2.3 Chemical characterisation of oil

The chemical composition of ginger essential oil was determined using Gas Chromatography-Mass Spectrometry (GC-MS) analysis, which is widely regarded as the most reliable technique for identifying volatile compounds in essential oils (Adams, 2007). The compounds were identified by matching their retention indices and mass spectral data with standard reference libraries (McLafferty and Turecek, 1993). GC-MS analysis revealed that ginger essential oil is predominantly composed of terpenes, monoterpenes and sesquiterpenes, which are responsible for its characteristic biological activities (Daferera et al., 2002).

2.4. The total phenolic content of the oil

The total phenolic content (TPC) of the essential oils was determined spectrophotometrically using the Folin-Ciocalteu method as described by Gholivand et al. (2010). A measured quantity of essential oil was dissolved in DMSO, diluted with distilled water, and treated with Folin-Ciocalteu reagent followed by sodium carbonate solution. After incubation at room temperature for 4 hours, absorbance was measured at 760 nm using a UV-visible spectrophotometer. The TPC was calculated by comparing the absorbance values against a gallic acid standard curve and expressed as µg gallic acid equivalent per mg of oil.

Absorbance = 0.0012 x gallic acid (µg) + 0.024

2.5 Antioxidant activity of essential oil

The DPPH free radical scavenging activity of the essential oils of C. longa, Z. officinale and their combination was evaluated spectrophotometrically according to the method of Prakash et al. (2010) with slight modifications. Various concentrations (1–600 µL/mL) of the test samples were added to 0.004% methanolic DPPH solution and incubated for 30 minutes at room temperature (25 ± 2°C). BHA and BHT were used as positive controls while methanolic DPPH solution served as blank. After incubation, absorbance was measured at 517 nm and the antioxidant activity was expressed

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as IC_{50} value, representing the concentration required to neutralise 50% of DPPH radicals, calculated from the percentage inhibition versus concentration graph using the formula

% Inhibition = (A blank − A sample / A blank) × 100.

2.6 Antifungal activity for determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of cumin essential oil (CUEO) against *Aspergillus flavus* was determined by the poisoned food technique following the methods described by Singh et al. (2008) and Kumar et al. (2016) with slight modifications.(SMKY) medium was prepared and sterilized at 121°C for 15 min. Diffenent concentration of ZEO were prepared with 5% tween 20 and then pour diffentent concentration of essential oil put in the different conical flasks. 25 mL of the amended medium was poured into sterile conical flask and mix with oil. Conical flasks containing ZEO but lacking essential oil served as the control. And after that 25 µl spore suspension pour into the conical flask. The inoculated conical were incubated at 27 ± 2°C for 7 days in a Biological Oxygen Demand (BOD) incubator.The MIC was defined as the lowest concentration of ZEO that completely inhibited visible fungal growth during the incubation period. Each treatment was performed in triplicate, and the experiment was repeated independently to ensure reproducibility.

III. RESULT AND DISCUSSION

3.1Chemical characterisation and phenolic content of ZEO

The essential oil was extracted from of Zinger. The sample Zinger was identified by the herbarium of the lab of herbal pesticides Dept. of Botany, Banaras Hindu University, Varanansi, India. The chemical characterisation of ZEO by GCMS revealed 46components, accounting for 84.16% of the oil (table No.1, Figure No. 1). Citral (Geranial) (11.45%), Neral (cis-Citral / Citral b) (8.91%), Eucalyptol (1,8-Cineole) (8.29%), o-Cymene (o-Cymol) (8.03%), Camphene (5.56%) and benzene/cymene derivative (5.33%) was major components of the ZEO.

The phenolic content in the ZEO were 395.83µg/mg gallic acid equivalent, respectively.

Figure No. 1. GC-MS analysis of ZEO

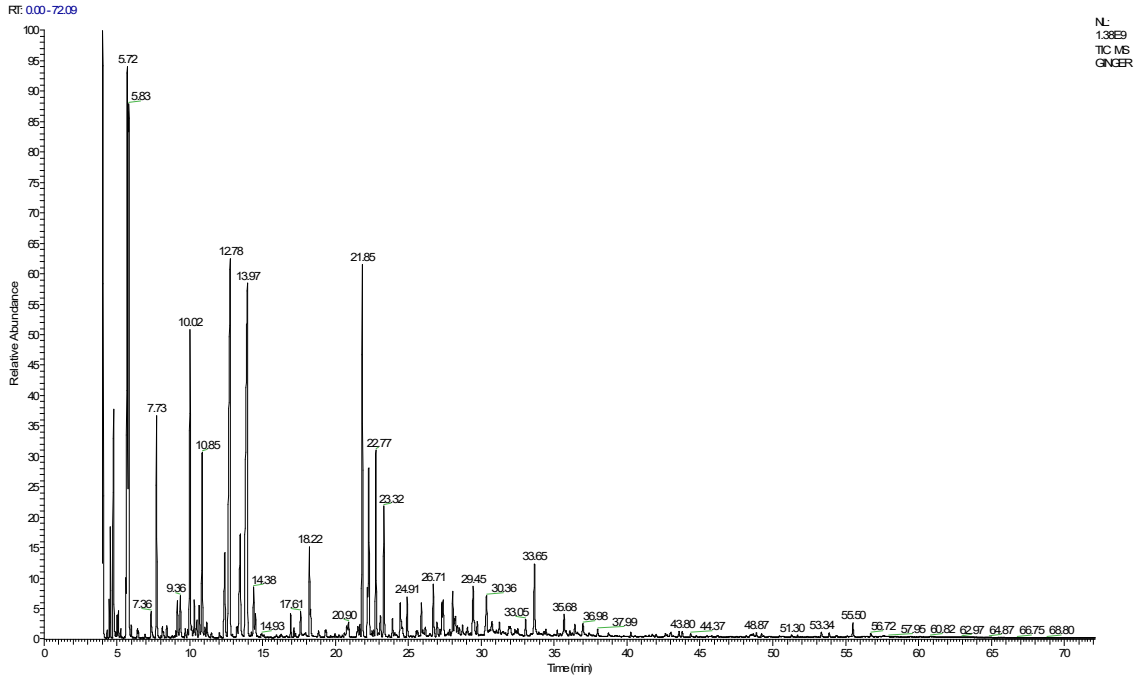

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Table No. 1 Chemical composition of ZEO

S.N.	Compound name	RT	AREA%
1	Camphene	4.02	5.56
2	Sabinene	4.48	0.26
3	Alpha-Pinene	4.78	3.16
4	Alpha-Thujene	5.11	0.22
5	o-Cymene (o-Cymol)	5.72	8.03
6	Eucalyptol (1,8-Cineole)	5.83	8.29
7	Likely Beta-Phellandrene — unconfirmed	7.36	0.41
	Linalool	7.87	2.56
8	Pinocarveol (trans-Pinocarveol)	8.43	0.19
9	Camphor	9.15	0.44
10	Citronellal	9.36	0.58
11	Borneol	10.02	4.25
12	Terpinen-4-ol	10.31	0.42
13	Citronellal (related dienal isomer)	10.49	0.24
14	Cryptone (p-Menth-1-en-3-one)	10.65	0.43
15	Alpha-Terpineol	10.85	2.49
16	Unconfirmed — likely a terpene epoxide aldehyde	12.4	1.52
17	Neral (cis-Citral / Citral b)	12.78	8.91
18	Geraniol	13.47	2.21
19	Citral (Geranial)	13.97	11.45
20	Methyl Nonyl Ketone	14.51	0.29
21	Citronellyl Acetate	16.92	0.29
22	Alpha-Copaene	17.61	0.32
23	Geranyl Acetate	18.22	1.1
24	benzene/cymene derivative	21.85	5.33
25	Alpha-Selinene / Beta-Eudesmene type	22.28	2.79
26	Alpha-Bisabolene	22.77	2.47
27	Globulol / Spathulenol type alcohol	23.09	0.36
28	terpene-derived cyclohexene	23.32	1.72
29	Eudesmol-type sesquiterpene alcohol	23.91	0.26
30	Perillyl Alcohol type	24.44	0.49
31	Nerolidol	24.91	0.56
32	Sesquisabinene Hydrate	25.89	0.44
33	Alpha-Bisabolol type	26.71	0.74
34	Eudesmol (Beta- or Gamma-Eudesmol)	26.95	0.28

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35	Aromadendrene Oxide	27.3	0.42
36	Alpha-Bisabolol (isomer)	27.39	0.56
37	sesquiterpene naphthalenol derivative	28.05	0.56
38	Alpha-Bisabolol (duplicate of #37)	29.45	1.02
39	Viridiflorol / Cedrol type	29.73	0.18
40	Thunbergol	30.36	0.75
41	Farnesal	31.24	0.18
42	Bisabolene Oxide	31.91	0.17
43	Spirovetivane-type ketone (Acorone-related)	33.05	0.25
44	Diepicedrene Oxide	33.65	1.1
	TOTAL		**84.16**

Antioxidant activity of test ZEO

The radical scavenging activity of ZEO was measured by a commonly used method viz. DPPH. ZEO showed strong free radical scavenging activity with IC50 value5.56µL/ml against DPPH. Figure No. 2 and table no.2. The highest percentage of free radical scavenging activity was shown at 16.67 µl/ml.

Figure No. 2. Percent radical scavenging activity of test ZEO through DPPH assay

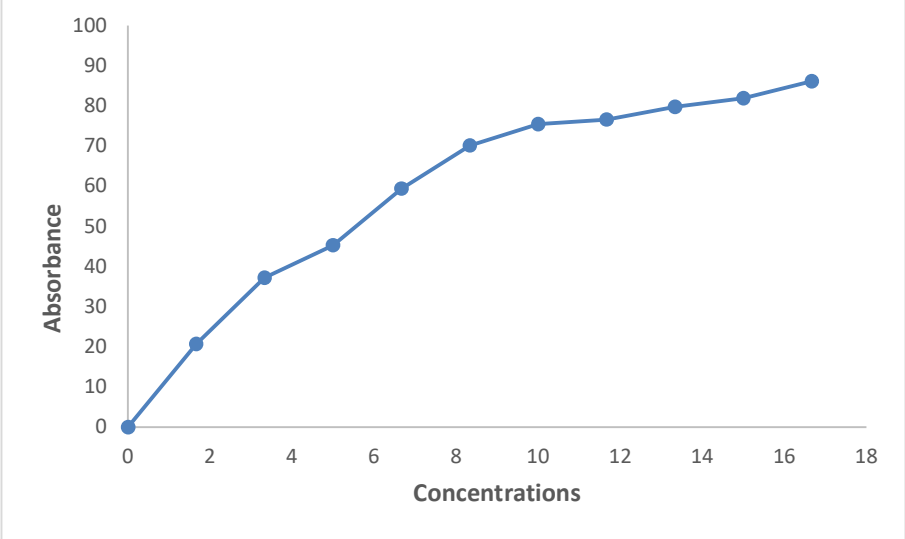

Table No.2. % radical scavenging activity of test ZEO through DPPH assay

S.N.	Concentration (µl/ml)	% Radical scavenging activity
1.	0	0 ± 0
2.	1.66	20.681 ± 7.45
3.	3.33	37.187 ± 10.35
4.	5	45.257 ± 10.05
5.	6.67	59.374 ± 6.75

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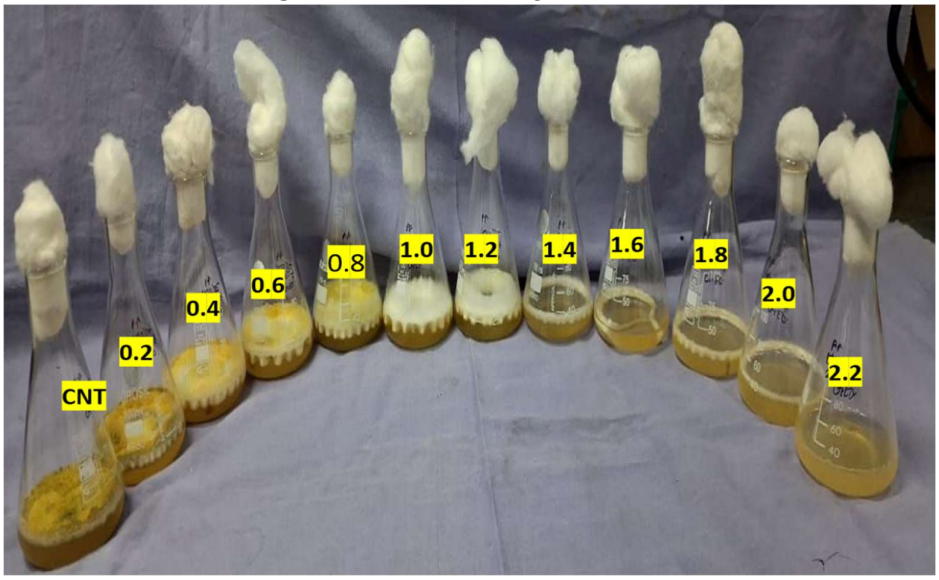
6.	8.33	70.075 ± 6.61
7.	10	75.407 ± 4.89
8.	11.67	76.566 ± 4.36
9.	13.33	79.681 ± 4.24
10.	15	81.875 ± 3.51
11.	16.67	86.099 ± 1.84

MIC, Ergosterol Inhibition and AFB_1 Inhibition of CUEO

MIC of CUEO against *A. flavus* was found at 2.2 µl/ml. This concentration resulted in complete inhibition of mycelial growth (Figure no. 2). The ergosterol inhibition % is also 2.2 µl/ml**Figure No.3.** Effect of different concentrations of test EOs on the growth of *A. flavus.*

Figure no.3 MIC of ZEO against A.flavus



IV. CONCLUSION

In the present study,*A.flavus* was chosen because of its strong capacity to contaminate various food commodities, which is caused by its secretion of hydrolytic enzymes (DeSouza, Lima, Freire, & De Sousa, 2005). Antifungal activity of Ginger oil was tested.The results of this study recommended ZEO as an antifungal essential oil.The ZEO showed good antifungal activity against *A.flavus*. The results of the present study showed that the oil of ginger is a free radical scavenger showing antioxidant activity. The characterisation and phenolic content of ZEOwere also calculated. All studies of ZEO that have been performed, including antifungal activity, antioxidant activity and characterisation of oil, indicate this essential oil has the possibility to be used as plant-based fungicides.

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