In vitro Anticancer and Antioxidant potential of Essential oil of *Zingiber zerumbet* (L.) Simth grown in Western Ghats region-South India

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Abstract

Anticancer and antioxidant potentials of essential oil of Zingiber zerumbet (L.) Simth grown in Western Ghats region-South India were examined. In vitro antioxidant potential was evaluated by DPPH and superoxide radical scavenging methods. Moreover, in vitro anticancer activity against MOLT-3 (T-cell acute lymphoblastic leukemia cancer cell line) using MTT assay was carried out. A total of 20 compounds were present in the essential oil obtained from hydro distillation of Z. zerumbet leaves. The major constituents were Zerumbone (37.44%), Camphor (9.93%), 1, 8 Cineole (9.55%), iso borneal (5.36%) and α-pinene (5.07%).

The in vitro antioxidant results demonstrated that the essential oil of Z.zerumbet showed a concentration dependent antiradical activity by inhibiting DPPH radical with IC_{50} value of 19.53 µg/ml and showed significant SO inhibiting activity with IC_{50} being 28.56 µg/ml. Ascorbic acid was used as the standard with IC_{50} values of 5.45 µg/ml and 12.42 µg/ml for both assays respectively. The essential oil exhibited significant anticancer activity against MOLT-3 cancer cell line with IC_{50} of 62.5 µg/ml.

Keywords: Anticancer, Antioxidant, *Z. zerumbet*, MOLT-3, DPPH and SOs activity.

Introduction

The research of volatile oil bearing plants in the westernghat region, which are made available, is continuosly investigated. One of them is *Zingiber zerumbet* (L.) which belongs to Zingiberaceae family, it is known as shampoo ginger or bitter ginger as its rhizomes are very useful part in the plant. It is widely distributed in India, South-East Asian countries and Reunion Island. It is used for the treatment of stomachache, toothache, fever and indigestion[1]. It has been also used as a spice and for ulcerative colitis[2]. It contained essential oils which are used as medicinal drugs, spices and raw materials for industry[3]. The plants are also used for ornamental purposes[4]. They have a wide variety of biological uses like anti-inflammatory, antimicrobial, *in vitro* cytotoxic and antinoceptive[1,5-8]. There are many reports available for the essential oil of *Z. Zerumbet* rhizomes and contain major compounds[9-11]. Zerumbone and α-caryophyllene have been reported as major constituents in rhizome and leaf oils[12]. Our present investigation aimed to evaluate the essential oil of *Z. zerumbet* leaves in the Westernghats region and its *in vitro* antioxidant activity using DPPH and SOs assay and also tested its anticancer potential.

Material and Methods

Plant materials: Fresh leaves of *Z. zerumbet* were collected from home garden in Pollachi between the periods of June-July 2016. The plant material was identified and authenticated by Department of Botany, NGM College, Pollachi, Coimbatore, Tamil Nadu. The voucher specimen (16CHE004) was preserved in the Chemistry department.

Isolation of essential oil: About 500g of fresh leaves were subjected to hydro distillation using Clevenger type apparatus for 4h. The oil obtained was dried over anhydrous sodium sulphate and stored in a container and kept in freezer until GC-MS analysis.

GC-MS analysis: GC–MS analysis of the phytoconstituents of *Z. zerumbet* was carried out using thermo GC - trace ultra-version: 5.0 coupled with thermo MS DSQ II instrument. Compounds were separated on DB-45, MS capillary standard non - polar column (40m x 0.25 mm), film thickness 0.25 µm. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 70˚C and held for 2 minutes and the temperature of the oven was raised to 260°C for 10min and raised 6°C per minute and final temperature was 400°C for 10min. The sample of 100mL was dissolved in 1mL of acetone and injected with splitless mode. Mass spectra were recorded over 50–500amu range with electron impact ionization energy 70eV while injector and MS transfer line temperature were set at 240°C and 280°C respectively.

DPPH radical scavenging assay: The free radical scavenging capacity of the essential oil of *Z. zerumbet* was determined using the DPPH method described by Brand-Williams with small modifications. DPPH (200 µm) solution was prepared in 95% methanol. Both extracts (1.0 mg/ml) were diluted to final concentrations of 20, 40, 60, 80 and 100 µg/ml in ethanol taken in five test tubes and one ml of freshly prepared 0.3 mm DPPH solution was added and incubated with both extracts and standard ascorbic acid was used as reference. After 30 minutes, decrease in absorbance was taken at 518 nm using spectrophotometer and percentage of DPPH activity was expressed by the following formula:

AA % = 100 − {[(ABS SAMPLE - ABS BLANK) × 100]/ABS CONTROL}

Superoxide scavenging activity: Superoxide scavenging was determined by the nitroblue tetrazolium reduction method[13]. The assay was based on the capacity of the essential oil to inhibit formazone formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. The reaction mixture contains ethylene diamine tetra acetic acid (EDTA, 6 µM), sodium cyanide (3µg), riboflavin (2 µM), nitroblue tetrazolium (50 µM), various concentrations of *Z. zerumbet* essential oil (10, 20, 40, 60, 80 and 100 µg/ml) and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 ml. The tubes were uniformly illuminated with an incandescent visible light for 15 minutes and the optical density was measured at 530 nm before and after the illumination. Ascorbic acid was used as standard[14].

Scavenging effect % = $[(A_0-A_1)/A_0]$ x 100

where A_0 was the absorbance of the control and A_1 was the absorbance of the extract.

***In vitro* anticancer screening:** An *in vitro* cytotoxicity test was performed for the given test sample as per standard procedure using MTT assay[15]. The culture medium from the MOLT-3 monolayer was replaced with fresh medium. Test sample in duplicate was added to the cells. After incubation at 37±1°C for 18 hrs, MTT was added in all the wells and incubated for 4 hrs. After incubation, DMSO was added in the wells and read at 570 NM using a microplate reader. Cytotoxicity was calculated by using the formula:

Cytotoxicity = [(Control — Treated)/ Control] * 100

Result and Discussion

Chemical composition of essential oil of *Z. zerumbet*

GC-MS analysis: A total of 20 components were identified from the leaves of the essential oil of *Z. zerumbet* representing 90.38% of the oil content. The results are given in table 1 and GC-MS profile of essential oil is given in fig. 1. Zerumbone (37.44%), Camphor (9.93%), 1, 8 Cineole (9.55%), iso borneal (5.36%), α-pinene (5.07%) were major compounds and other minor components were Terpineol (4.28%), Trans-a-Bergamole (2.60%), α-humulene (2.7%), Farnesene (1.74%), 6(1-hydroxy ethyl)-7-Methoxy-2,2-Dimethyl-2H-Cromene (1.73%), Calarene (1.62%), phytol (1.37%), Camphene (1.14%).

Very minor components were 1-(3,5-Dimethoxyphenyl)pent-1-ene (1.08%), Trimethylcholesta-8,14,Dien-3yl-acetate (0.98%), Bornyl acetate (0.91%), a-Sesquiphellandrene (0.51%), α-Guaiene (0.35%), 2-hexyl-1-decen-3-yne (0.34%),6-methyl-5methylidene-anti-3-nortricyclanol (0.30%), 4,5-Methanchrysene (0.24%), Ethyl 3,4-diphenyl-6, 7, 8, 9- tetrahydropyridazino [4, 3: 4, 5] thieno [3, 2- b] [1,6naphthyridine-8-carboxylate (0.13%), Methyl ester of 1-(Dimethylamino)-1,2-dihydro-3(methoxycarbonyl)-5-[E-2-methoxycarbonyl)ethynyl]6-methyl-2-pyrdineacetc acid (0.12%), Stigmast-s-en-3-ol (0.11%), Heptacosane (0.10%). In Asian countries, the Zerumbone (15%-75%) was the major components.

The investigation from Asia, USA and Nigeria revealed that the essential oils of *Z. zerumbet* rhizomes and leaf oil were different in the concentration of zerumbone. Z-citral (26.1%) camphene (16.3%), α-pinene (3.3%), camphene (16.3%) as the major composition in Nigerian essential oil which is similar to the results of Vietnam oil. (Z) - citral (30.1%), camphene (9.7%), *β*-phellandrene (7.5%) and 1, 8-cineole (7.0%). Zingiberene (5.3%)[16]. Except these two countries, there were no reports about Z-citrol and sabanine. Moreover, in Malaysia, α-humulene (5.9%), Camphene (2.8%) and Caryophyllene (2.7%), were the major components[17]. But Zerumbone (75.2%), α-caryophyllene (7.1%), camphene (5.1%), Eucalyptol (2.4%) and camphor (3.0%) were the major compounds in the USA[18].

Similar results were shown in Bangladesh with zerumbet as major compound (46.83%), caryophyllene oxide (3.70%), 4-terpineol (0.23%)[19]. The essential oil from leaves in the Western Ghats region, India has similar chemotype. Trans caryophyllene (19.62%), β-elemene (12.34%), 1, 5, cyclodecadiene (7.74%), zerumbone (11.4%), 1,4 terpineol (1.41%), (-)- caryophyllene (6.93%) constituted the leaf oil from Indonesia[20]. Curzerenone (14.4%), zerumbone (12.6%), camphor (12.8%), isoborneol (8.9%) and 1, 8-cineole (7.1%), were found as major compounds in north India[21]. But zerumbone (74.82%), humulene (6.02%) and β-copaen-4α-ol (4.32%) were identified as major compounds. E-Bisabol-11-ol (4.32%), E-bisabol-11-ol (0.92%), β-sinensal (0.57%), and α-himachalene oxide (0.19) also were reported in rhizome essential oil[22]. Zerumbone was low content compared to our results. 1,8-cineole was another noted compound in the leaf oil obtained from India.

***In vitro* antioxidant activity:** DPPH is relatively stable and hence it is less reactive free radical, so it can be reduced primarily by more reactive reducing components such as phenolic substance, which has been widely accepted as a tool for estimating free radicals scavenging activities of antioxidants. SOs is known to be a very harmful species to cellular compounds as a precursor of more reactive species[23-26].

The present study examines the antioxidant activity of essential oil of *Z. zerumbet* by DPPH and SOs methods and showed a concentration dependent anti radical activity by inhibiting DPPH radical with IC_{50} values of 19.53 µg/ml (Acorbic acid used as the control with IC_{50} values of 5.45 µg/ml). DPPH is a purple coloured free radical which on reaction with the plant extracts changes to the yellow colored stable compound and the extent of the reaction depending on the hydrogen releasing capacity of the antioxidant.

The essential oil of *Z. zerumbet* exhibited a significant scavenging activity with an IC_{50} value of 28.56 µg/ml and Ascorbic acid used as standard with IC_{50} values of12.42 µg/ml. The essential oil of *Z. zerumbet* showed dose dependant activity. The results revealed that the essential oil of *Z. zerumbet* has the ability to stop the chain reactions of free radicals by forming the stable compounds given in the figure 2 and 3.

***In vitro* anticancer activity:** The essential oil was evaluated for its anticancer activity against MOLT-3 cancer cell line by MTT assay, it was found that the anticancer activity was a dose dependent pattern and 50 percent cancer cells were killed in the concentration of 62.5 µg/ml. Few reports are available for the anticancer activity of the various extracts of *Z. zerumbet* plant, the extracts showed significant anticancer activity. According to Norfazlina et al,[17] the hexane extract has potent cytotoxicity against HL60 and inhibited 50% at 63.75 µg/ml. The zerumbone was isolated and analyzed for its anticancer potential using HeLa cell line by MTT assay and IC_{50} value was determined to be 11.3 µg/ml[27].

The various extracts of *Z. zerumbet* were tested with MCF-7 cancer cell line and all the tested extract exhibited significant antiproliferative activity[28]. Recent studies have shown a significant protective activity of zerumbone in colon cancer[29], pancreas cancer[30], liver cancer[31] and leukemia[32]. But there is no report available for anticancer activity of essential oil of *Z. zerumbet* in Western Ghats region.

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

S.N.	Name of the compound	R.T	(%) of compounds
1	α-pinene	3.57	5.07
2	Camphene	3.91	1.14
3	Camphor	4.38	9.93
4	1,8-cineole	5.41	9.55
5	Terpineol	8.03	4.28
6	Bornyl acetate	9.49	0.91
7	Calarene	10.21	1.62
8	α-Humulene	12.27	2.79
9	Farnesene	12.86	1.74
10	α-Sesquiphellandrene	13.53	0.51
11	Zerumbone	14.61	37.44
12	α-Patchoulene	15.09	0.17
13	α-Guaiene	16.83	0.35
14	caryophyllene oxide	17.41	0.43
15	1-(3,5-Dimethoxyphenyl)pent-1-ene	17.91	1.08
16	Trans-a-Berfamotol	18.42	2.60
17	2-hexyl-1-decen-3-yne	20.51	0.34
18	6-(1-hydroxyethyl)-7-Methoxy-2,2-Dimethyl-2H-	21.70	1.73
19	Phytol	25.18	1.37
20	4,5-Methanchrysene	26.57	0.24

Table 2
***In vitro* anticancer activity of *Z.zerumbet* essential oil**

S.N.	Concentration (µg/ml)	% of cytotoxicity
1	5	27
2	25	33
3	50	42
4	75	68
5	100	81

Table 3
IC_{50} value of *Invitro* anticancer activity of *Z.zerumbet* essential oil

S.N.	Name	IC_{50}	cell line
1	*Z. zerumbet* essential oil	62.5µg/ml	MOLT-3

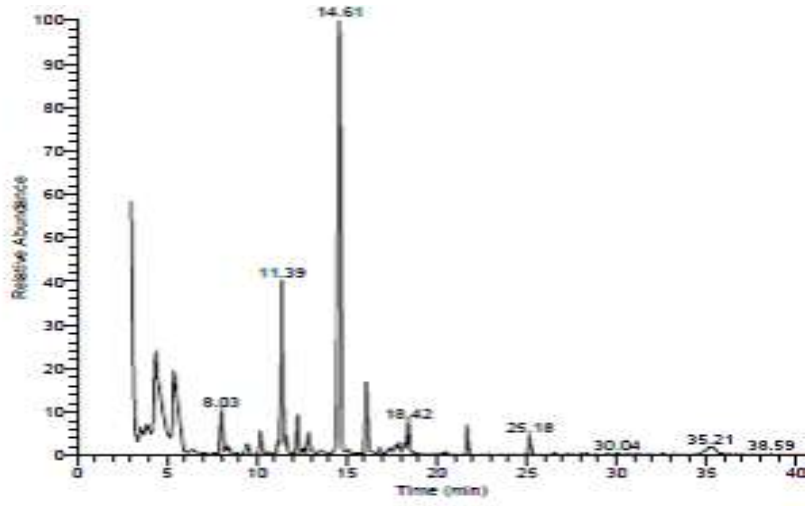

Figure 1: GC-MS chromatogram of *Z .zerumbet* essential oil.

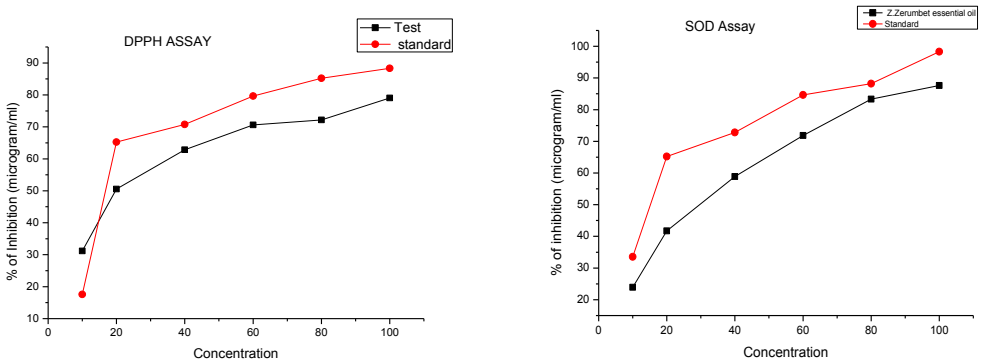

Figure 2 and 3: DPPH and SOs assay of *Z. zerumet* essential oil

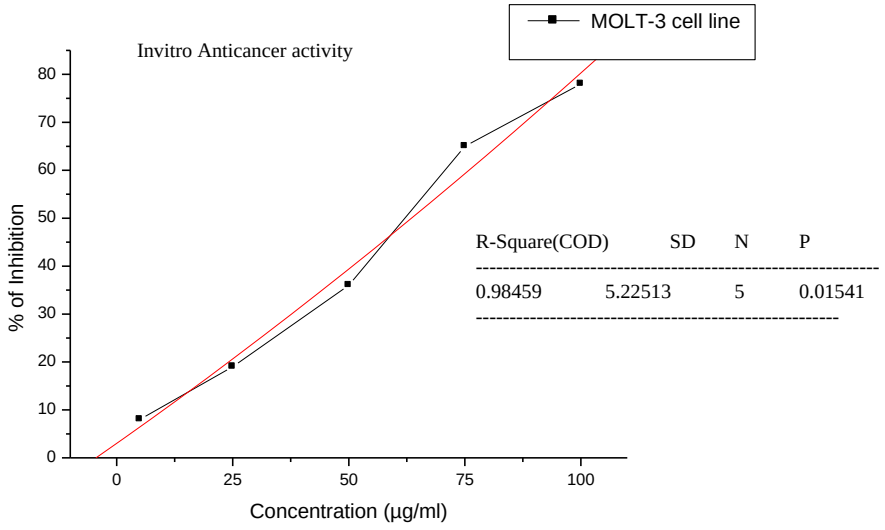

Figure 4: *In vitro* anticancer activity of *Z.zerumbet* essential oil

Conclusion

In vitro anticancer and antioxidant capability of the essential oil of *Z. zerumbet* grown in the Western Ghat region was analyzed. The GC-MS analysis of essential oil of the leaves showed 20 compounds. Zerumbone, Camphor, 1, 8 Cineole, Iso-borneal and α-Pinene are the major components of *Z. zerumbet* leaf oil. There are several differences in the composition of essential oil from the Western Ghats region compared to the other parts of the world, it may be due to climatic and geographical variations.

In vitro antioxidant activity was tested by DPPH and SO scavenging methods. All the two tested methods have significant antioxidant activity with IC_{50} values of DPPH 19.50µg/ml and SOs 28.56 µg/ml respectively. The essential oil exhibited significant anticancer activity against MOLT-3 cancer cell line with IC_{50} of 62.5µg/ml. The present study demonstrated the efficiency of essential of *Z. zerumbet* leaves as an antioxidant and anticancer agents. The essential oil may be used as alternate for commercial drugs in cancer prevention and oxidative stress.

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