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Chemical composition and biological activity of the essential oil of rhizome of *Zingiber zerumbet* (L.) Smith

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Abstract

The aim was designed to study the biological activity and chemical composition of essential oil of *Zingiber zerumbet* (L.) Smith. The essential oil extracted from the rhizome of the plant was analysed by gas chromatography-mass spectroscopy and its major components amounting to 98.4 % were found to be zerumbone (75.2%), α -caryophyllene (7.1%), camphene (5.1%), eucalyptol (2.4%), and camphor (3.0%). Antioxidant activity and total phenol content assay were studied using the DPPH and Folin-Ciocalteu colorimetric methods. Antimalarial, antileishmanial, antimicrobial assays were analysed for the essential oil and its major component, zerumbone. The essential oil and zerumbone exhibited antimalarial and antileishmanial activities, whereas only *Cryptococcus neoformans* showed antimicrobial activity of the essential oil.

Keywords: *Zingiber zerumbet*, Essential oil, Zerumbone, Antimalarial, Antileishmanial, Antimicrobial, Antioxidant

1. Introduction

Zingiber zerumbet (L.) Smith is a monocotyledonous perennial medicinal plant belonging to the Zingiberaceae family. It is known as shampoo ginger or pinecone ginger as its rhizomes produce foaming properties likes that of shampoo. It has many different local names depending on their area of vegetation and location. It grows in subtropical climates such as India, South-East Asian countries, South Pacific Islands and Okinawan Islands, and has been used for local folk medicine and gardening. It is called Shinkha in Manipur, North-East India. Traditionally, it is used for the treatment of stomach ache, toothache, fever, and indigestion [1]. It has been also used as a spice and for ulcerative colitis [2]. The essential oils of *Z. zerumbet* have been studied by different authors since 1944. The major component of the rhizome's oil varies from 12.7-73.1% [3, 4, 5, 6]. Zerumbone and α -caryophyllene has been reported as major constituents in rhizome and leaf oils [7]. Many authors have reported different bioactivities from the essential oils and extracts of *Z. zerumbet* such as anti-inflammatory [8, 9], antitumor [10, 2], free radical scavenging [11], colon and skin cancer [12], antialzheimer diseases and dementia activities [13, 14].

In the present work we aimed to evaluate the antileishmanial, antimalarial, antimicrobial and chemical composition of the essential oil. In this work we are reporting for the first time, the antileishmanial and antimalarial activities of essential oil from *Z. zerumbet* and the major compound zerumbone isolated from its rhizome essential oil.

2. Materials and Methods

2.1. Plant material

The plant materials were collected from Manipur, North-East India, 920 m from sea level, longitude 93°58' and latitude 24°44' in March, 2012. The plant was identified by the taxonomist of the institute and accession number as IBSD/Z-42-23.

2.2. Solvents and chemicals

Gallic acid, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ciprofloxacin, amphotericin-B chloroquine, pentamidine were obtained from Sigma Aldrich. Analytical grade solvents were used during the experiments obtained from the Merck chemicals, Mumbai, India and Sigma-Aldrich.

2.3. Extraction of essential oil

Fresh rhizomes were collected and washed thoroughly with tap water. These were cut into 5-6 mm slices and put into the Clevenger type oil extractor. The oil was dried over anhydrous sodium sulphate and stored at 4 ± 2 °C.

2.4. Gas chromatography-mass spectrometry (GC-MS)

The oil was analysed by GC-MS on a Varian CP-3800 GC coupled to a Varian Saturn 2000 MS/MS. The GC was equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm, with film thickness of 0.25 μ m) operated using the following conditions: injector temperature, 240 °C, column temperature, 60-240 °C at 3 °C per minute, then held at 240 °C for 5 min; carrier gas, He; injection volume, 1 μ l (splitless). The MS mass ranged from 40 to 650 m/z , filament delay of 3 min, target TIC of 20,000, a prescan ionization time of 100 μ sec, an ion trap temperature of 150 °C, manifold temperature of 60 °C, and a transfer line temperature of 170 °C.

2.5. Identification of components

The constituents of the oil were identified using retention times, Kovats indices and mass spectra. Confirmed integrated peaks were then used for the percentage of each chemical constituents present in the essential oil. Kovats indices were calculated using the equation:

$KI(x) = 100 \left[\frac{\log RT(x) - \log P_z}{\log RT(P_{z+1}) - \log RT(P_z)} \right]$
Where: $RT(P_z) \leq RT(x) \leq RT(P_{z+1})$, and P_4, \dots, P_{25} are n paraffins.

2.6. Determination of total phenolic content

The total phenolic content of the essential oil was determined by the Folin-Ciocalteu colorimetric method [15]. An aliquot (0.125 ml) of a diluted acetone sample was added to 0.5 ml of deionized water and 0.125 ml of the Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before adding 1.25 ml of 7% sodium carbonate solution. The solution was adjusted with deionized water to a final volume of 3 ml and mixed thoroughly. After incubation for 90 min at 23 °C, the absorbance versus prepared blank was read at 725 nm with a UV-Visible spectrophotometer (Multiskan spectrum, Thermo Scientific). Total phenolic content was expressed as milligrams of gallic acid equivalents per 100 gram of essential oil through the calibration curve with gallic acid.

2.7. Antioxidant activity assay

The DPPH free radical scavenging was assessed of the essential oil [16]. 0.1 mM DPPH radical solution in ethanol was prepared. After 30 min, the absorbance was measured at 517 nm on UV-Visible spectrometer (Multiskan spectrum, Thermo Scientific). Decreasing the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. The radical scavenging activity, expressed as percentage of inhibition was calculated using the equation:

$$\text{Inhibition Concentration \%} = \left[\frac{(A-B)}{A} \right] \times 100$$

Where A is the absorbance of control (DPPH solution without the sample), B is the absorbance of DPPH solution in the presence of the sample.

IC₅₀ value is the concentration of the sample required to

scavenge the 50% DPPH free radical.

2.8 Biological activity

2.8.1. Antimicrobial assay

The oil and the isolated compound, zerumbone were tested for antibacterial activity against *Staphylococcus aureus* ATCC 29213, methicillin resistant *S. aureus* ATCC 33591(MRS), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC27853, *Mycobacterium intracellulare* ATCC 23068 and antifungal activity against *Candida albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113, *Aspergillus fumigatus* ATCC 204305. Ciprofloxacin and Amphotericin-B were used as positive control for bacteria and fungi respectively [17].

2.8.2. Antimalarial activity

In vitro antimalarial activity was determined against chloroquine sensitive (D6, Sierra Leone) and resistant (W2, Indo China) strains of *P. falciparum* by measuring plasmodial LDH activity. Chloroquine was used as positive control [18].

2.8.3. Antileishmanial activity

The antileishmanial activity was tested against *Leishmania donovani* promastigotes; pentamidine and Amphotericin-B were used as positive controls [19].

2.8.4 Statistical analysis

All IC₅₀ values were calculated using the XL Fit curve fitting software.

3. Results and Discussion

3.1. Extraction yield and GC-MS analyses

The yield of essential oil was 0.12%. GC-MS analyses of the essential oil led to the identification of ten major compounds accounting for the 98.4% of the oil (Table 1). Zerumbone (75.2%), α -caryophyllene (7.1%), camphene (5.1%), eucalyptol (2.4%), and camphor (3.0%) were the major components of the oil. Zerumbone was isolated in pure form and its structure was confirmed by ¹H-NMR, ¹³C-NMR, DEPT, HR-ESIMS and comparison with literature data.

3.2. Antioxidant activity and total phenolic content

The DPPH free radical scavenging activity together with Folin-Ciocalteu colorimetric assay was performed on the essential oil [15]. The results were given in table 2. The antioxidant activity in the DPPH radical scavenging test is due to the hydrogen donating ability of the test material. The capability of substances to donate hydrogen to convert DPPH into the non-radical form of DPPH can be followed spectrophotometrically. The oil exhibited DPPH scavenging activity (IC₅₀ 1.60 μ g/ml) which is comparable with the standard ascorbic acid (IC₅₀ 1.59 μ g/ml). The high scavenging activity was attributed to the high content of zerumbone (IC₅₀ 2.55 μ g/ml). Total phenolic content of the oil was 5.05 mg/100 g and it doesn't play much role in the high scavenging activity of the oils [16].

Although not much data on antioxidant activity of *Z. zerumbet* is available, solvent extracts (ethanol, methanol and isopropanol) of *Z. zerumbet* spent have shown fair DPPH scavenging activity [20] which is similar with what we are reporting. However, other authors reported low DPPH

scavenging activity which may be due to difference in extraction methods, cultivars, maturity and other environmental factors [21].

Table 1: Chemical composition of *Zingiber zerumbet* essential oil

Compounds	Retention time	Kovat Index	% Components
Cyclone	6.050	927.04	0.11
Alpha pinene	6.338	938.31	0.59
Camphene	6.744	953.35	5.1
Eucalyptol	9.250	1037.09	2.4
Camphor	13.506	1150.84	3.0
Humulene	26.455	1458.66	1.2
Caryophyllene Oxide	31.514	1582.35	1.91
2,6,6-trimethylundecan-1,3-dien-9-yn-5-one	32.122	1595.85	1.96
α -caryophyllene	32.526	1606.61	7.1
Zerumbone	37.209	1740.71	75.2

Table 2: Antioxidant and Total phenol content assay of *Zingiber zerumbet* essential oil and zerumbone

Samples	IC ₅₀ (μ g/ml)	TPC (mg/100 g)
Rhizome oil	1.60	5.05
Ascorbic acid	1.59	ND
Zerumbone isolated	2.55	ND

ND-not determined

3.3. Antimicrobial activity

Antimicrobial study was done for both the isolated compounds and the essential oil against five fungi and five bacteria. The oil was found to have good activity against *Cryptococcus neoformans* ATCC 90113 with IC₅₀ value of 8 μ g/ml. However, no antimicrobial activity was shown against zerumbone (Table 3) [17].

The essential oil of *Z. zerumbet* exhibited a negative antifungal effect using broth microdilution and disc gel diffusion methods [22]. The antifungal activity was assessed against five dermatophytes (*Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum canis*, *M. nanum* and *Epidermophyton floccosum*), three filamentous fungi (*Aspergillus niger*, *A. fumigatus* and *Mucor* spp.) and five strains of yeast (*Saccharomyces cerevisiae*, *C. neoformans*, *Candida albicans*, *C. tropicalis* and *Torulopsis glabrata*). This report is similar with what we are reporting, however we found the essential oil *Z. zerumbet* with IC₅₀ value of 8 μ g/ml against *C. neoformans*.

3.4. Antimalarial activity

Antimalarial activity was studied both for the oil and the compound zerumbone against chloroquine sensitive *Plasmodium falciparum* (D6, Sierra Leone) and resistant (W2, Indo China). The oil showed moderate antimalarial activity (IC₅₀ 17.5 μ g/ml and 20.0 μ g/ml against *P. falciparum* D6 and *P. falciparum* W2). Zerumbone showed good activity with IC₅₀ values of 3.9 μ g/ml and 4.4 μ g/ml against *P. falciparum* D6 and *P. falciparum* W2 (Table 3) [18].

3.5. Antileishmanial activity

Antileishmanial evaluation was done on the oil and the compound zerumbone against *Leishmania donovani*. The oil showed moderate activity with IC₅₀ and IC₉₀ values of 4.6 μ g/ml and 18.0 μ g/ml. Zerumbone showed good activity with IC₅₀ and IC₉₀ <1.6 μ g/ml and 4.7 μ g/ml [19].

Table 3: Biological activity of essential oil of *Zingiber zerumbet* and zerumbone

Biological activity	Test parasite	Rhizome essential oil (μ g/ml)	Zerumbone (μ g/ml)
Antileishmanial activity	<i>L. donovani</i> IC ₅₀	4.62	<1.6
	IC ₉₀	18.00	4.69
Antimalarial activity	<i>P. falciparum</i> D6 IC ₅₀	17.47	3.94
	W2 IC ₅₀	20.03	4.44
Antimicrobial	<i>C. neoformans</i>	8.00	No activity

4. Conclusion

In conclusion, the biological activity of the essential oil of *Z. zerumbet* is attributed to zerumbone. To the best of our knowledge, this is the first study of antimalarial and antileishmanial properties of zerumbone and its rhizome essential oil. A further continuation of this research work for

its antimalarial and antileishmanial properties is on its way which may lead to further information about the properties of zerumbone.

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