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Zingiber zerumbet (L.) Roscoe ex Sm. from northern India: Potential source of zerumbone rich essential oil for antiproliferative and antibacterial applications

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ABSTRACT

Zingiber zerumbet (L.) Roscoe ex Sm. (Zingiberaceae), commonly known as bitter ginger, has long been used in traditional herbal medicine for the treatments of inflammations, rheumatism, sprains, colic pain, diarrhea, tonsillitis and various other ailments. The aim of the present study was to assess the chemical composition of the rhizome essential oil of *Zingiber zerumbet* grown in the foothills of northern India using GC-FID, GC–MS, IR and NMR, and to evaluate the antibacterial and antiproliferative potential of the rhizome essential oil and its major constituent. Altogether, thirty-four constituents were identified representing 98.0% of the essential oil composition. Zerumbone (72.86%), α-humulene (7.09%), camphene (5.04%), humulene oxide I (2.45%), humulene oxide II (1.8%), and camphor (1.41%) were the major constituents. The potential of the rhizome essential oil of *Z. zerumbet* and zerumbone was tested against nine pathogenic bacterial strains. The results showed that both essential oil and zerumbone, possessed significant antagonist activity against *Staphylococcus aureus*-96 (MIC: 52.0–166.6 μg/mL), *Streptococcus mutans* (MIC: 62.5–208.0 μg/mL), and *Escherichia coli* (MIC: 104.1–208.0 μg/mL). Zerumbone was found more active compared to the essential oil. Moreover, the antiproliferative potential of *Z. zerumbet* oil and zerumbone was evaluated against various human cancer and normal cell lines (A549, MDAMB-231, A431, K562, WRL-68, COLO-205, HaCaT, and HEK-293). Results showed that, both essential oil and zerumbone possessed antiproliferative activity against tested cell lines, where zerumbone was more competent then essential oil.

1. Introduction

Plants synthesise and preserve a variety of secondary metabolites useful for human being for diverse applications. Among them, essential oils and aroma constituents extracted from aromatic plants, represents a major component of various industrial products such as food and beverages, perfume, fragrance, nutraceutical and pharmaceuticals (Hadian et al., 2014; Padalia, 2012). Zingiberaceae, the largest monocotyledonous family in India, includes various rhizomatous plants of economic importance characterised by the presence of essential oils and oleoresins of export value. It included 52 genera and 1400 species distributed in Indo-Malaysian region of Asia. Among them, 22 genera and 178 species were reported in north eastern and peninsular region of India (Jen and Ved, 1995). Several members of this family were used as spices, perfumes, medicines, flavouring agents, as well as the source of certain dyes and other economic uses (Burkill, 1966; Dai et al., 2013). *Zingiber zerumbet* (L.) Roscoe ex. Sm. considered as one of the important member of this family. It is the native of Southeast Asia, but has been now widely cultivated in tropical and subtropical regions around the world. It is mainly distributed in India, Bangladesh, Malaysia, Nepal and Sri Lanka. Traditionally this plant is known as 'Asian ginger' or 'bitter ginger' that grows naturally in damp, shaded parts of the lowland or hill slopes (Baby et al., 2009; Madegowda et al., 2016). It is also known as 'Pinecone ginger' and 'Shampoo ginger' because of the foaming properties of its pine like inflorescence (Tushar et al., 2010; Yob et al., 2011). *Z. zerumbet* is widely used in foods, beverages and for ornamental purposes (Koga et al., 2016; Singh et al., 2014). All plant parts of *Z. zerumbet* are utilised in traditional medicines for treatments

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of a variety of ailments. The leaves of *Z. zerumbet* are used in the treatments of joint pain and skin diseases, whereas the young shoots and inflorescence are used as condiments (Devi et al., 2014; Murakami et al., 2002). The cone-shaped flowers were employed in craft arrangements for ornamental purposes, while the viscous juice present in the mature inflorescence, rich in surfactants, used as a natural shampoo (Devi et al., 2014; Murakami et al., 2002; Yob et al., 2011). However, the rhizomes of the plant are the most economic part used as food flavouring and appetizer in Malay and Indian cuisines, and as drug in Indian, Asian, Chinese, and Arabic folk medicine (Norulaini et al., 2009; Sreevani et al., 2013; Yob et al., 2011). Rhizomes are used in folk medicine for the treatment of various ailments, such as cough, cold, swelling, stomachache, ear inflammation, colic pain, diarrhea, tonsillitis, sore throat, worm infestations, and skin diseases as well as antispasmodic, antirheumatic, antiflatulent and diuretic agents (Koga et al., 2016; Rana et al., 2016; Tushar et al., 2010; Yob et al., 2011). Phytochemical studies on different plant parts of *Z. zerumbet* from different origins reported the presence of diverse secondary metabolites, such as polyphenols, alkaloids and terpenoids (Koga et al., 2016). Most of the earlier studies focused on rhizome essential oil showed that the essential oil was a complex mixture of terpenoids, with a predominance of sesquiterpenoids, mainly zerumbone, humulene, humulene oxides, β-caryophyllene, α-caryophyllene as major constituents; followed by varying proportions of monoterpenoids viz. camphene, sabinene, myrcene, etc. (Baby et al., 2009; Batubara et al., 2013; Bhuiyan et al., 2009; Dai et al., 2013; Koga et al., 2016; Madegowda et al., 2016; Rana et al., 2008, 2016). Contrarily to rhizome oil, the essential oil extracted from the aerial parts (leaf, stem and inflorescence) of *Z. zerumbet* was dominated by nerolidol, *trans*-phytol, β-caryophyllene, linalool, pinenes, with lesser content of zerumbone (Bhuiyan et al., 2009; Chane-Ming et al., 2003; Dung et al., 1995; Rana et al., 2016). However, the quantitative composition of the essential oils (content of the reported respective constituents) of different plant parts of *Z. zerumbet* was found to be highly variable according to origin of plant. The rhizome essential oil and its main bioactive constituent (zerumbone) have been shown to possess significant anti-tumor, anti-inflammatory, anti-oxidant, anti-diabetic, antimalarial, antisecretory, anti-microbial, anti-proliferative, antiviral, anti-allergic, anti-pyretic, analgesic and cyclooxygenase-2 suppressant properties (Abdul et al., 2008; Joseph et al., 2015; Murakami et al., 2002; Sakinah et al., 2007; Somchit et al., 2012; Sulaiman et al., 2010). Thus, with the background of antimicrobial and anticancerous potential of the essential oil of *Z. zerumbet* and no previous report about chemical composition of *Z. zerumbet* grown in foothill agroclimatic conditions in northern India, the present study was planned to explore the composition and biological activity of the rhizome essential oil of Z. *zerumbet* from northern India. The present study was designed to evaluate the following objectives: (i) to explore the rhizome essential oil composition of *Z. zerumbet* grown in the foothills of northern India; (ii) to isolate and characterise the major constituent by chromatographic and spectrometric analysis; (iii) to evaluate the antiproliferative activity against various organ specific cell lines; (iv) and to evaluate the antimicrobial activity against nine pathogenic bacterial strains to have an inclusive view to use the rhizome oil of *Z. zerumbet*, as alternatives, in microbial and cancer control therapy in humans.

2. Materials and methods

2.1. Plant material and extraction of essential oil

The fresh rhizomes of *Z. zerumbet* were collected from the crop raised at the experimental field of CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Pantnagar (Udham Singh Nagar) Uttarakhand. The experimental site is located between coordinates 29°N, 79.38°E at 243 m above mean sea level at foothills of northern India. The maximum temperature ranges between 35 and 45 °C, and minimum between 2 and 5 °C. The soil of the experimental site was sandy-loam in texture, with neutral pH. Voucher specimen and herbarium record of the plant have been maintained CSIR-CIMAP, Research Centre, Pantnagar. Fresh rhizomes were taken for the study after digging the crop in spring season (March 2016). The rhizomes were crushed and hydrodistilled in a Clevenger type apparatus for 4 h, in triplicate, for extraction of essential oil. The essential oil was measured directly in the extraction burette and content (%) was determined as volume (mL) of essential oil per 100 g of fresh biomass. The oil was dehydrated over anhydrous Na_2SO_4 and kept in a cool and dark place prior to analysis.

2.2. Analysis and characterization of essential oil constituents

The chemical composition of essential oil was analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC–MS) techniques. GC analysis was done on a DB-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) fixed inside the oven of NUCON Gas Chromatograph (model 5765). The column oven temperature was programmed from 60 to 230 °C, at the rate of 3 °C min^{-1}, using Hydrogen as carrier gas at constant flow rate of 1.0 mL min^{-1}. The injection volume was 0.02 μL neat (syringe: Hamilton 0.5 μL capacity, Alltech USA) in split mode (1:40). The injector and detector (FID) temperatures were maintained at 220 °C and 230 °C, respectively. GC–MS analysis was carried on Perkin-Elmer Turbomass Mass Spectrometer (Shelton, USA) fitted with Equity-5 fused silica capillary column (60 m × 0.32 mm; 0.25 μm film thickness; Supelco Bellefonte, PA, USA). The column temperature was programmed 70 °C, with initial hold time of 2 min, to 250 °C at 3 °C min^{-1} with final hold time of 3 min, using helium as carrier gas at a flow rate of 1.0 mL min^{-1}. The injector, ion source and transfer line temperatures were maintained at 250 °C. The injection volume was 0.04 μL neat with split ratio 1:30. Mass analysis was carried out in electron ionization (EI) mode at 70 eV with the mass scan range of 40–400 amu. The identification of the individual compounds was carried out using retention index (RI) determined using a homologous series of *n*-alkanes (C_7–C_{30}, Supelco Bellefonte, PA, USA) and by comparing mass spectra with those of authentic sample as well as mass spectra library search in NIST and WILEY Mass Spectral Library, and by comparing with the mass spectral literature data (Adams, 2007).

2.3. Isolation and characterisation of zerumbone

The major constituent of the essential oil was isolated by column chromatography and crystallisation process. The essential oil (2 mL) was dissolved in hexane (5 mL), adsorbed onto silica gel (100–200 mesh, 20 g), and dried at room temperature for 1 h. The adsorbed material was then column chromatographed in glass column packed with silica gel (40 g, 230–400 mesh, Merck) by using hexane followed by ethyl acetate/hexane mixture as eluting agent. Elution of the column with 5% ethyl acetate/hexane mixture (ratio 1:19) yielded 1.0 g of pure white compound, which was purified by slow crystallisation in petroleum ether at low temperature. The purity of the isolated compound was checked using thin layer chromatography (TLC) and gas chromatography (GC) (Purity > 98.0%). The compound was characterised as zerumbone by IR, MS, ^{1}H NMR and ^{13}C NMR spectral data. Mass spectrum was recorded in Perkin-Elmer Turbomass Mass Spectrometer (Shelton, USA), while NMR spectra of the isolated compound was recorded on a 500 MHz NMR spectrometer (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR, Bruker, Germany) using $CDCl_3$ as dissolving agent and tetramethylsilane (TMS) as an internal standard. IR spectrum was recorded on Perkin-Elmer Spectrum BX FT-IR spectrophotometer. A thin film of the solution made by dissolving 2 mg of isolated compound in carbon tetra-chloride (CCl_4), was developed between KBr plate and the spectrum was taken at room temperature in the range between 4000 cm^{-1} to 650 cm^{-1}. Spectral data of zerumbone is