Chemical profile, antiproliferative and antioxidant activities of rhizome oil of *Zingiber anamalayanum* from Western Ghats in India

Mohamed Salim, T.K. Ahmedul Kabeer, S. Ajikumaran Nair, Mathew Dan, M. Sabu and Sabulal Baby

ABSTRACT
Volatile oil from fresh rhizomes of *Zingiber anamalayanum* was isolated by hydrodistillation and characterised by GC–FID and GC–MS. Twenty-one out of 24 constituents comprising 99.47% of the oil were identified. Major components in *Z. anamalayanum* rhizome oil were δ-2-carene (52.83%), camphene (9.83%), endo-fenchol (9.42%), iso-dihydrocarveol (6.44%) and cis-p-mentha-2,8-dien-1-ol (5.19%). Monoterpene hydrocarbons in the rhizome oil were 65.81%, followed by oxygenated monoterpenes (23.78%) and sesquiterpene hydrocarbons (9.87%). Physical parameters of rhizome oil were $n_{20}D$ 1.4031, $[\alpha]_D^{20} = -16.097^\circ$ (c = 1, CHCl3) and $d_{20} 0.9202$. *Z. anamalayanum* rhizome oil showed significant anti-Dalton's Lymphoma Ascitic activity.

1. Introduction
Genus *Zingiber* Boehm. of Zingiberaceae family is one important group of rhizomatous herbs constituting spices, herbs and ornamentals. The term 'Zingiber' is derived from the Sanskrit term 'Shringavera' meaning 'horn-shaped', referring to the protrusions on their rhizomes. The genus comprises over 90 species distributed in tropical and subtropical regions in Southeast Asia.
Asia (Sabu 2006; Ali et al. 2008). *Zingiber officinale* is the commercial ginger species best known for its ethnomedical, pharmacological activities and spice value (Ravindran & Babu 2005; Sabu 2006; Ali et al. 2008; Singh et al. 2008; Sasidharan et al. 2012; Marrelli et al. 2015). Zingiberene and ar-curcumene are the major volatile constituents in *Z. officinale* rhizomes (Afzal et al. 2001). *Z. officinale* rhizome oil is used in beverage and fragrance industries (Sakamura et al. 1986). *Zingiber zerumbet* rhizomes are used as flavourings in cooking and are traditionally used as a decoction for relieving stomach ache, as an anaesthetic for tooth ache and for the treatment of swellings, strains, bruises and cuts (Duñg et al. 1993; Lechat-Vahirua et al. 1993). Zerumbone is the major constituent in the rhizome oil of *Z. zerumbet* (Sabulal et al. 2009). It is a sesquiterpene with potential anticancer, anti-inflammatory, anti-HIV and other biological activities (Sabulal et al. 2009). We recently reported chemical profiles of the rhizome volatile oils of two endemic ginger species, *Zingiber nimmonii* and *Zingiber neesanum*, from south India (Sabulal et al. 2006; Sabulal et al. 2007). β-Caryophyllene and α-humulene were the major constituents in *Z. nimmonii* rhizome oil and phenylbutanoids were the major components in *Z. neesanum* rhizome oil (Sabulal et al. 2006; Sabulal et al. 2007). Major constituents in *Zingiber montanum* (*Zingiber cassumunar*) rhizome oil were terpinen-4-ol and the phenylbutanoid, (E)-1-(3,4-dimethoxyphenyl)buta-1,3-diene (Bordoloi et al. 1999; Sabulal et al. 2007; Bua-in & Paisooksantivatana 2009; Sabulal et al. 2009).

*Zingiber anamalayanum* Sujanapal & Sasidh. is a recently discovered species from the southern Western Ghats in India. It is a sturdy herb of 75–170 cm tall with a basal cylindrical spike, sparsely distributed in high-altitude grasslands and edges of woodlands (Sujanapal & Sasidharan 2010). *Z. anamalayanum* is a perennial, rhizomatous herb and its rhizomes are thick, fleshy, yellowish and aromatic with many roots. Here, we report (i) the chemical composition of essential oil from fresh rhizomes of *Z. anamalayanum* and (ii) its antioxidant and antiproliferative activities.

### 2. Results and discussion

Hydrodistillation of fresh rhizomes (464 g) of *Z. anamalayanum* afforded 1.4 mL (0.3%, v/w, fr. wt.) colourless, pleasant smelling oil. Refractive index ($n_20^D$), specific rotation ([α]_D^20) and specific gravity ($d_20$) of rhizome oil were 1.4031, −16.097° (c = 1, CHCl_3) and 0.9202, respectively. GC–MS of *Z. anamalayanum* rhizome oil showed 24 components of which 21 (99.47%) were identified (Table 1). Monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpene hydrocarbons in rhizome oil were 65.81, 23.78 and 9.87%, respectively. δ-2-Carene (52.83%), camphene (9.83%), endo-fenchol (9.42%), iso-dihydrocarveol (6.44%), cis-p-metha-2,8-dien-1-ol (5.19%), trans-daumca-(11), 7-diene (5.18%) and α-ylangene (4.27%) were the major constituents in *Z. anamalayanum* rhizome oil (Table 1). *Z. anamalayanum* has a unique volatile chemical profile compared to other previously investigated *Zingiber* species such as *Z. officinale*, *Z. zerumbet*, *Z. nimmonii*, *Z. neesanum*, *Z. cassumunar* and *Z. montanum* (Sakamura et al. 1986; Duñg et al. 1993; Lechat-Vahirua et al. 1993; Bordoloi et al. 1999; Afzal et al. 2001; Ravindran & Babu 2005; Sabulal et al. 2006; Sabulal et al. 2007; Bua-in & Paisooksantivatana 2009; Sabulal et al. 2009).

*Z. anamalayanum* rhizome oil showed very low (% inhibition) activity in superoxide radical scavenging and DPPH assays, whereas in hydroxyl radical scavenging assay it showed comparable activity to quercetin (Table S1). Percentage cell viability of DLA cells was tested
by trypan blue exclusion method. *Z. anamalayanum* rhizome oil showed 89 and 100% cell deaths at 50 and 100 μg/mL, respectively (Table S2). High contents of monoterpenes, their oxygenated derivatives (89.59%, δ-2-carene 52.83%) and possible synergism between these constituents justify the cytotoxic and antioxidant activities of *Z. anamalayanum* rhizome oil (Bayala, Bassole, Gnoula et al. 2014; Bayala, Bassole, Scifo et al. 2014).

### 3. Conclusions

This is the first report of the chemical composition of the rhizome volatile oil of the rare ginger, *Z. anamalayanum*. On in vitro cytotoxicity assay, rhizome oil showed significant anti-DLA activity.

### Supplementary material

Experimental details relating to this article are available online, alongside Tables S1 and S2.

### Acknowledgement

AK is thankful to Department of Science and Technology, Government of India for INSPIRE fellowship (No. IF 130643).
Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
This work was supported by the Department of Biotechnology, Government of India [grant number BT/PR15278/NDB/52/195/2011 dated 28-02-2012].

References
Adams RP. 2007. Identification of essential oil components by gas chromatography/ quadrupole mass spectroscopy. Carol Stream (IL): Allured Publication Corporation.
Afzal M, Al-Hadidi D, Menon M, Pesek J, Dhami MSI. 2001. Ginger: an ethnomedical, chemical and pharmacological review. Drug Metabol Drug Interact. 18:159–190.
Ali BH, Blunden G, Tanira MO, Nemmar A. 2008. Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): a review of recent research. Food Chem Toxicol. 46:409–420.
Bayala B, Bassole IHN, Gnoula C, Nebie R, Yonli A, Morel L, Figueredo G, Nikiema J-B, Lobaccaro J-MA, Simpore J. 2014. Chemical composition, antioxidant, anti-inflammatory and anti-proliferative activities of essential oils of plants from Burkina Faso. PLoS ONE. 9:e92122.
Bayala B, Bassole IHN, Scifo R, Gnoula C, Morel L, Lobaccaro JM, Simpore J. 2014. Anticancer activity of essential oils and their chemical components – a review. Am J Cancer Res. 4:591–607.
Bordoloi AK, Sperkova J, Leclercq PA. 1999. Essential oils of Zingiber cassumunar Roxb. from Northeast India. J Essent Oil Res. 11:441–445.
Bua-in S, Paisooksantivatana Y. 2009. Essential oil and antioxidant activity of cassumunar ginger (Zingiberaceae: Zingiber montanum (Koenig) Link ex Dietr.) collected from various parts of Thailand. Kasetsart J Nat Sci. 43:467–475.
Duñg NX, Chińh TD, Rañg DD, Leclercq PA. 1993. The constituents of the rhizome oil of Zingiber zerumbet (L.) Sm. from Vietnam. J Essent Oil Res. 5:553–555.
Lechat-Vahirua IV, François P, Menut C, Lamaty G, Bessiere JM. 1993. Aromatic plants of French Polynesia. I. Constituents of the essential oils of rhizomes of Three Zingiberaceae: Zingiber zerumbet Smith, Hedychium coronarium Koenig and Etlingera cevuga Smith. J Essent Oil Res. 5:55–59.
Marrelli M, Menichini F, Conforti, F. 2015. A comparative study of Zingiber officinale Roscoe pulp and peel: phytochemical composition and evaluation of antitumour activity. Nat Prod Res. 29:2045–2049.
Ravindran PN, Babu KN. 2005. Ginger: the genus Zingiber. Boca Raton (FL): CRC Press.
Sabu M. 2006. Zingiberaceae and Costaceae of South India. Calicut: Indian Association for Angiosperm Taxonomy.
Sabulal B, Dan M, Anil JJ, Kurup R, Chandrika SP, George V. 2007. Phenybutanoid-rich rhizome oil of Zingiber nesanan from Western Ghats, southern India. Flavour Fragr J. 22:521–524.
Sabulal B, Dan M, Anil JJ, Kurup R, Pradeep NS, Valsamma RK, George V. 2006. Caryophyllene-rich rhizome oil of Zingiber nimmonii from South India: chemical characterization and antimicrobial activity. Phytochemistry. 67:2469–2473.
Sabulal B, Dan M, Thaha ARM, Anil JJ, Kurup R, Balakrishnapillai P, Lim CK. 2009. High content of zerumbone in volatile oils of Zingiber zerumbet from southern India and Malaysia. Flavour Fragr J. 24:301–308.
Sakamura F, Ogiharat K, Suga T, Taniguchi K, Tanaka R. 1986. Volatile constituents of Zingiber officinale rhizomes produced by in vitro shoot tip culture. Phytochemistry. 25:1333–1335.
Sasidharan I, Venugopal VV, Menon AN. 2012. Essential oil composition of two unique ginger (Zingiber officinale Roscoe) cultivars from Sikkim. Nat Prod Res. 26:1759–1764.
Singh G, Kapoor IP, Singh P, de Heluani CS, de Lampasona MP, Catalan CA. 2008. Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of Zingiber officinale. Food Chem Toxicol. 46:3295–3302.
Sujanapal P, Sasidharan N. 2010. Zingiber anamalayanum sp. nov. (Zingiberaceae) from India. Nord J Bot. 28:288–293.