



Zederone from the rhizomes of *Zingiber zerumbet* and its anti-staphylococcal Activity

[Aislamiento de Zederona de los rizomas de *Zingiber zerumbet* y su actividad antiestafilocócica]

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Abstract

A sesquiterpene, zederone (1), was isolated from the crude ethanolic extract of the rhizomes of *Zingiber zerumbet* (L.) Smith. It is the first time report of isolation of this compound from the genus *Zingiber*. Its structure was established by a series of spectral data including high-field NMR (both 1D and 2D) and MS. The antibacterial activity of this compound was determined against a number of multi-drug resistant and methicillin-resistant *Staphylococcus aureus* strains (SA1199B, ATCC25923, XU212, RN4220 and EMRSA15) and minimum inhibitory concentration (MIC) values were found to be in the range of 64-128 µg/ml.

Keywords: *Zingiber zerumbet*; Zingiberaceae; Zederone; Antibacterial; *Staphylococcus aureus*

Resumen

Un sesquiterpeno, zederona (1), fue aislado del extracto crudo metanólico de los rizomas de *Zingiber zerumbet* (L.) Smith. Esta es la primera vez que se reporta este compuesto en el género *Zingiber*. Su estructura se estableció tras una serie de análisis espectrales incluyendo NMR de alto campo (1D y 2D) y espectrometría de masa. La actividad antibacteriana de este compuesto se determinó frente a varias cepas multi-fármaco resistentes y meticilina-resistentes *Staphylococcus aureus* (SA1199B, ATCC25923, XU212, RN4220 and EMRSA15) y las concentraciones inhibitorias mínimas se encontraron en el rango de 64-128 µg/ml.

Palabras Clave: *Zingiber zerumbet*; Zingiberaceae; Zederona; Actividad antibacteriana; *Staphylococcus aureus*.

Recibido | Received: December 01, 2009

Aceptado en Versión Corregida | Accepted in Corrected Version: December 15, 2009

Publicado en Línea | Published Online: December 17, 2009

Declaración de intereses | Declaration of interests: Authors have no competing interests.

Financiación | Funding: none declared

This article must be cited as: M. Golam Kader, M. Rowshanul Habib, Farjana Nikkon, Tanzima Yeasmin, Mohammad A. Rashid, M. Mukhlesur Rahman, Simon Gibbons. 2010. Zederone from the rhizomes of *Zingiber zerumbet* and its anti-staphylococcal Activity. Bol Latinoam Caribe Plant Med Aromat 9(1):63 – 68. {EPub December 17, 2009}.

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BLACPMA es una publicación de la [Cooperación Latinoamericana y Caribeña de Plantas Medicinales y Aromáticas](#)

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INTRODUCTION

Zingiber zerumbet (L.) Smith (Fam. Zingiberaceae) locally known as 'Bon Ada' is a vigorous ginger with leafy stems growing to about 1.2 m in height that is widely cultivated throughout the tropics including Southeast Asia, Korea, India and Bangladesh for its medicinal properties (Kritikar and Basu, 1984; Fansworth and Bunyapraphatsara, 1992; Saadiah and Halijah, 1995). The most common use of *Z. zerumbet* is as a shampoo and conditioner for the hair (Burkill, 1966; Petard, 1986). Its rhizomes are used in traditional medicine for the treatment of inflammation, swelling, loss of appetite, lumbago, diabetes, chest pain, rheumatic pains, bronchitis, dyspepsia and sore throat (Burkill, 1966; Kritikar and Basu, 1984; Farnsworth and Bunyapraphatsara, 1992). The juice of the boiled rhizomes has also been used in indigenous medicine for worm infestation in children (Petard, 1986). Previous phytochemical investigations on this plant have revealed the isolation of several sesquiterpenes, flavonoids and aromatic compounds (Matthes et al., 1980; Masuda et al., 1991; Dai et al., 1997; Jang et al., 2004; Jang et al., 2005). The volatile oil of the rhizomes has been shown to contain zerumbone, humulene, camphene α -caryophyllene and camphene (Hasnah, 1991; Srivastava et al., 2000; Bhuiyan et al., 2009). Zerumbone, a predominant sesquiterpene from this plant, has been studied intensively for its use as anti-inflammatory, and in chemoprevention and chemotherapy strategies (Dai et al., 1997; Kitayama et al., 2001; Murakami et al., 1999; Murakami et al., 2002; Tanaka et al., 2001). From the pharmacological point of view, *Z. zerumbet* has been reported to inhibit colon and lung carcinogenesis in mice (Kim et al., 2009) and CXCL12-induced invasion of breast and pancreatic tumor cells (Sung et al., 2008), suppresses phorbol ester-induced expression of multiple scavenger receptor genes in THP-1 human monocytic cells and inhibits Epstein-Barr Virus activation (Vimala et al., 1999; Murakami et al., 1999). As a part of our research focused on bioactive compounds from indigenous medicinal plants, we here report the isolation and identification of a compound (1) from the ethanolic extract of *Zingiber zerumbet* (L.) Smith and its ant-staphylococcal activity against a series of multi-drug resistant (MDR) and methicillin resistant *Staphylococcus aureus* strains: SA1199B, ATCC25923, XU212, RN4220 and EMRSA15.

MATERIALS AND METHODS

General experimental procedures

NMR spectra (both 1D and 2D) were acquired on a Bruker Avance 500 MHz NMR (500 MHz for ^1H and 125 MHz for ^{13}C) spectrometer using the residual solvent peak as internal standard. The IR spectra were recorded with a Perkin-Elmer Lambda spectrophotometer and the Mass spectra were recorded with HRTOF-MS in positive mode. The melting point were determined using a Digital Melting point Apparatus (model IA 8103, Electrothermal Engineering LTD, Southend-on-Sea, Essex, UK) and are uncorrected. All solvents used in this study were of analytical grade and purchased from BDH and Merck.

Plant materials

Fresh rhizomes of *Z. zerumbet* were collected from the hilly areas of Chittagong, Bangladesh in October 2007 and identified by a taxonomist, Dr. Mohammed Yusuf, BCSIR Laboratory, Chittagong, Bangladesh where a voucher specimen (No. 1061) of this collection has been maintained.

Extraction and isolation of compound (1)

The powdered plant material (800 g) of *Z. zerumbet* was extracted with ethanol (4 L) in an aspirator bottle for a week and then filtered and concentrated by using a rotary evaporator at 45°C under reduced pressure. The crude ethanol extract (2.0 g) was subjected to column chromatography over silica gel (Merck) eluting with petroleum ether and ethyl acetate of increasing polarity and finally with ethanol which yielded a total of 105 fractions. Based on TLC analysis, fractions 15-18 were combined together and then subjected to preparative TLC using the solvent system petroleum ether and ethyl acetate in a ratio of 20:1 to yield compound 1 (8.0 mg; white powder; R_f 0.45 in 5% EtOAc in petroleum ether). The structure of the compound (1) was confirmed by analysis of its IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and TOF- Mass spectral data at The School of Pharmacy, University of London, UK.

Compound 1: White powder; mp. 58-60°C; IR (KBr): 1662, 1521, 1533, 1558, 914, 861 cm^{-1} ; $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 5.49 (1H, dd, J = 12.0, 4.0 Hz, H-1), 2.25 (1H, m, H-2a), 2.53 (1H, m, H-2b), 1.29 (1H, m, H-3a), 2.31 (1H, dt, J = 13.0, 3.5 Hz, H-3b), 3.81 (1H, s, H-5), 3.69 (1H, d, J = 16.0 Hz, H-9a), 3.77 (1H, d, J = 16.0 Hz, H-9b), 7.10 (1H,

s, H-12), 1.61 (3H, s, H-13), 1.35 (3H, s, H-14), 2.12 (3H, s, H-15); ^{13}C -NMR (125 MHz, CDCl_3): 131.4 (C-1), 24.9 (C-2), 38.2 (C-3), 64.2 (C-4), 66.8 (C-5), 192.4 (C-6), 122.5 (C-7), 157.3 (C-8), 42.1 (C-9), 131.3 (C-10), 123.5 (C-11), 138.3 (C-12), 16.0 (C-13), 15.4 (C-14), 10.5 (C-15); HR-TOF-ESIMS $[\text{M}+\text{H}]^+$ m/z 247.0889.

Bacterial strains

The antibacterial assay was performed against a panel of multi-drug and methicillin-resistant strains of *Staphylococcus aureus*. *S. aureus* standard strain ATCC 25923 and tetracycline-resistant strain XU212 which possesses the TetK tetracycline efflux protein provided by Dr Edet Udo (Gibbons and Udo, 2000). Strain SA-1199B which overexpresses the *norA* gene encoding the NorA MDR efflux pump was provided by Professor Glenn Kaatz (Kaatz et al., 1993). Strain RN4220 which possesses the MsrA macrolide efflux protein was provided by Dr Jon Cove (Ross et al., 1989). EMRSA-15 (Richardson and Reith, 1993) was the generous gift of Dr Paul Stapleton.

Minimum inhibitory concentration (MIC) assay.

All five *S. aureus* strains were cultured on nutrient agar (Oxoid) and incubated for 24 h at 37°C prior to MIC determination. Norfloxacin was purchased from the Sigma Chemical Co. Mueller-Hinton broth (MHB; Oxoid) was adjusted to contain 20 and 10 mg/l of Ca^{2+} and Mg^{2+} , respectively. An inoculum density of 5×10^5 cfu of each of the test organisms was prepared in normal saline (9 g/l) by comparison with a 0.5 MacFarland standard. MHB (125 μl) was dispensed into 10 wells of a 96 well microtitre plate (Nunc, 0.3 ml volume per well). A stock solution of norfloxacin was prepared by dissolving the antibiotic in DMSO (Sigma) and dilution in MHB to give a final concentration of 0.625%. A DMSO control was included in all assays.

Compounds were serially diluted into each of the wells followed by the addition of the bacterial inoculum and the microtitre plate was incubated at 37°C for 18 h. The MIC recorded as the lowest concentration at which no growth was observed. This was facilitated by the addition of 20 μl of a 5 mg/ml methanolic solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma) to each of the wells and incubation for 20 minutes. A blue colouration indicated bacterial growth (Shiu and Gibbons, 2006).

RESULTS AND DISCUSSION

Identification of compound (1)

Compound (1) was isolated as an amorphous off-white powder from the crude ethanol extract of the rhizomes of *Zingiber zerumbet* (L.) Smith. The high-resolution TOFMS showed the pseudo molecular ion, $[\text{M}+\text{H}]^+$ at m/z 247.0889, corresponding to the molecular formula as $\text{C}_{15}\text{H}_{18}\text{O}_3$. The ^1H -NMR spectrum of compound 1 showed a downfield one proton singlet at δ 7.10, an olefinic proton signal at δ 5.49, another methine signal at δ 3.81, three methyl proton resonances at δ 1.35, 1.61, 2.12, and methylene proton resonances between δ 1.29-3.77 integrating for four protons. The ^{13}C -NMR spectrum displayed a total of 15 carbons while the DEPT-135 and HMQC experiments indicated that 9 out of 15 carbons had attached protons. Analysis of the ^{13}C and DEPT135 spectra allowed discernment of the carbon resonances into three methyls (δ_{C} 10.5, 15.4, 16.0), three methylenes (δ_{C} 24.9, 38.2 and 42.1), three methines (δ_{C} 66.8, 131.4, 138.3), and six quaternary carbons, including a carbonyl group (δ_{C} 192.4). The assignment of all carbons and protons and thereby the structure of the compound was resolved by 2D experiments, notably COSY, HMQC and HMBC experiments. In the COSY experiment, the olefinic proton at δ 5.49 (H-1; δ_{C} 131.4 from HMQC) showed strong interaction with H_2 -2 protons at δ 2.25 and 2.53 along with a weak connectivity with the methyl singlet at δ 1.61 (H_3 -15). The methylene protons (H_2 -2) showed coupling with H_2 -3 protons at δ 1.29 and δ 2.31 in the COSY experiment. The presence of a methine (66.8; δ_{H} 3.81 from HMQC) and a quaternary (64.2) in the ^{13}C experiment confirmed the presence of an epoxide in the molecule. The C-5 methine proton at δ 3.81 showed HMBC connectivities over two bonds (2J) to a quaternary carbon at δ 64.2 (C-4) and the carbonyl group at δ 192.4 (C-6). The methyl protons at 1.35 (δ_{C} 15.4 from HMQC) revealed 3J connectivities to δ 38.2 (C-3) and δ 66.8 (C-5) and thereby confirmed its linkage at C-4. The methylene protons at δ 3.70 and δ 3.77 (H_2 -9, δ_{C} 42.1 from HMQC) showed 2J correlations over two bonds to quaternary carbons at δ 131.3 (C-10) and δ 157.3 (C-8) and a 3J interactions to methyl (δ_{C} 16.0), olefinic (δ_{C} 131.4, C-1) and quaternary (δ_{C} 122.5, C-7) carbons. Furthermore, 3J connectivities from the methyl protons at δ 1.61 to δ 131.4 (C-1) and δ 42.1 (C-9) confirmed its placement

at C-10. The remaining methyl resonance at δ 2.12 (δ_C 10.5 from HMQC) showed connectivities to δ_C 122.5 (C-7) and a methine carbon at δ 138.3 (C-12) over three bonds. This allowed the placement of this methyl group at C-11. Accordingly, compound 1 was identified as zederone. Its NMR data were in agreement with those reported previously (Hikino et al., 1971). Although, it is a known natural product reported before from a number species of the genus *Curcuma* including *C. zedoaria* (Matthes et al., 1980), *C. aromatic* (Phan and Phan 2000), *C. comosa* (Qu et al., 2009), *C. kwangsiensis* (Zhu et al., 2009), *C. ochrorhiza*. (Sirat et al. 2009), *C. xanthorrhiza* (Sukari et al., 2008), this is the first isolation from the genus, *Zingiber*.

The compound was tested for the antibacterial activity against a panel of five strains of *Staphylococcus aureus*: SA1199B, ATCC25923, XU212, RN4220 and EMRSA15 and showed weak activity with minimum inhibitory concentration (MIC) values in the range of 64-128 μ g/ml (Table 2).

Figure 1. Structure of compound 1

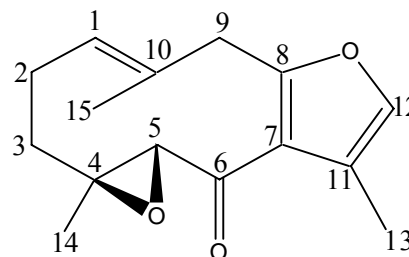


Table 1 ^1H NMR (500 MHz), ^{13}C NMR (125 MHz) and HMBC data of compound 1 in CDCl_3 .

Position	δ_{H}	δ_{C}	HMBC	
			2J	3J
1	5.49, dd, $J=12.0, 4.0$ Hz	131.4	C-2, C-10	C-9, C-15
2	2.25, br d; 2.53, m	24.9	C-3	-
3	1.29, m; 2.31, dt, $J=13.0, 3.5$ Hz	38.2	C-2, C-4	-
4	-	64.2	-	-
5	3.81, s	66.8	C-4, C-6	C-14
6	-	192.4	-	-
7	-	122.5	-	-
8	-	157.3	-	-
9	3.69, d, $J=16.0$ Hz; 3.77, d, $J=16.0$ Hz	42.1	C-8, C-10	C-7, C-15
10	-	131.3	-	-
11	-	123.5	-	-
12	7.10, s	138.3	C-11	C-7, C-8, C-13
13	1.61, s	16.0	-	C-7, C-12
14	1.35, s	15.4	C-4	C-3, C-5
15	2.12, s	10.5	-	C-1, C-9

Table 2. MICs of 1 and standard antibiotic in $\mu\text{g/ml}$

	SA1199B	Xu212	ATCC 25943	RN 4220	EMRSA 15
Compound 1	128	64	128	64	128
Norfloxacin	16	4	0.5	0.5	0.5

REFERENCES

- Bhuiyan NI, Chowdhury JU, Begum J. 2009. Chemical investigation of the leaf and rhizome essential oils of *Zingiber zerumbet* (L.) Smith from Bangladesh. *Bangladesh J Pharmacol* 4: 9-12.
- Burkill HJ. 1966. Dictionary of the economic products of the Malay Peninsula. Ministry of Agric and Coop, Kuala Lumpur, 2345.
- Dai JR, Cardellina IJH, McMahon JB, Boyd MR. 1997. Zerumbone, an HIV-inhibitory and cytotoxic sesquiterpene of *Zingiber aromaticum* and *Z. zerumbet*. *Nat Prod Lett* 10:115-118.
- Fansworth NR, Bunyapraphatsara N. 1992. Thai Medicinal Plants. Prachachon, Bangkok, Thailand, 261-263.
- Gibbons S, Udo EE. 2000. The effect of reserpine, a modulator of multidrug efflux pumps, on the in vitro activity of tetracycline against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) possessing the tet(K) determinant. *Phytother. Research* 14: 139-140.
- Hasnah MS. 1991. Chemical constituents of some medicinal plants of zingiberaceae: Medicinal products from tropical rain forest. Proceedings of the Conference, Forest Research Institute Malaysia, Kuala Lumpur 2: 299-304.
- Hikino H, Tori K, Horibe I and Kuriyama K. 1971. Absolute configuration and conformation of zederone, a sesquiterpenoid of *Curcuma zedoaria*. *J Chem Soc* 37: 688-691.
- Jang DS, Han AR, Park G, Seo EK. 2004. Flavonoids and aromatic compounds from the rhizomes of *Zingiber zerumbet*. *Arch Pharm Res* 27: 386-389.
- Jang DS, Han AR, Park G, Seo EK. 2005. Potentially Bioactive Two New Natural Sesquiterpenoids from the Rhizomes of *Zingiber zerumbet*. *Arch Pharm Res* 28: 294-296.
- Kaatz GW, Seo SM, Ruble CA. 1993. Efflux-mediated fluoroquinolone resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 37: 1086-1094.
- Kim M, Miyamoto S, Yasui Y, Oyama T, Murakami A, Tanaka T. 2009. Zerumbone, a tropical ginger sesquiterpene, inhibits colon and lung carcinogenesis in mice. *Int J Cancer* 124:264-71.
- Kirtikar RK, Basu BD. 1984. Indian Medicinal Plant. Lalit Mohan Basu MB. Allahabad, India, vol. 4, pp 2415-2419.
- Kitayama T, Yamamoto K, Utsumi R, Takatani M, Hill RK, Kawai Y, Sawada S, Okamoto T. 2001. Chemistry of zerumbone. 2. Regulation of ring bond cleavage and unique antibacterial activities of zerumbone derivatives. *Biosci Biotechnol Biochem* 65:2193-2199.
- Masuda T, Jitoe A, Kato S, Nakatani N. 1991. Constituents of Zingiberaceae. Part 3. Acetylated flavonol glycosides from *Zingiber zerumbet*. *Phytochemistry* 30: 2391-2392.
- Matthes HWD, Luu B, Ourisson G. 1980. Chemistry and Biochemistry of Chinese drugs. Part VI. Cytotoxic components of *Zingiber zerumbet*, *Curcuma zedoaria* and *Curcuma domestica*. *Phytochemistry* 19: 2643-2650.
- Murakami A, Takahashi D, Kinoshita T, Koshimizu K, Kim HW, Yoshihiro A, Nakamura Y, Jiwajinda S, Terao J, Ohigashi H. 2002. Zerumbone, a Southeast Asian ginger sesquiterpene, markedly suppresses free radical generation, proinflammatory protein production, and cancer cell proliferation accompanied by apoptosis: The α,β -unsaturated carbonyl group is a prerequisite. *Carcinogen* 23: 795-802.
- Murakami A, Takahashi M, Jiwajinda S, Koshimizu K, Ohigashi H. 1999. Identification of zerumbone in *Zingiber zerumbet* Smith as a potent inhibitor of 12-O-tetradecanoylphorbol-13-acetate-induced Epstein-Barr virus activation. *Biosci Biotechnol Biochem* 63: 1811-2.
- Phan MG, Phan TS. 2000. Isolation of Sesquiterpenoids from the Rhizomes of Vietnamese *Curcuma aromatica* Salisb. *Tap Chi Hoa Hoc* 38: 96-99.
- Petard P. 1986. Quelques plantes utiles de la Polynesie et Ra'au Tahiti. Papeete, Haere Po no Tahiti, 1876.
- Qu Y, Xu F, Nakamura S, Matsuda H, Pongpiriyadacha Y, Wu L, Yoshikawa M. 2009. Sesquiterpenes from *Curcuma comosa*. *J Nat Med* 63: 102-104.
- Richardson JF, Reith S. 1993. Characterization of a strain of methicillin-resistant *Staphylococcus aureus* (EMRSA-15) by conventional and molecular methods. *J. Hosp. Infect.* 25: 45-52.
- Ross JI, Farrell AM, Eady EA, Cove JH, Cunliffe WJ. 1989. Characterisation and molecular cloning of the novel macrolide streptogramin B resistance determinant from *Staphylococcus epidermidis*. *J. Antimicrob. Chemother.* 24: 851-862.
- Saadiah MS, Halijah I. 1995. Proceedings of the National Convention on Herbal medicine. 21, Kuala Lumpur, Forest Research Institute Malaysia, 205-07.
- Shiu WKP, Gibbons S. 2006. Anti-staphylococcal acylphloroglucinols from *Hypericum beani*. *Phytochemistry*. 67: 2568-2572.
- Sirat, HM, Jamil, S, Rahman AA. 2009. Sesquiterpenes from the rhizomes of *Curcuma ochrorhiza*. *Nat Prod Comm* 4: 1171.
- Srivastava AK, Srivastava SK, Shah NC. 2000. Essential Oil Composition of *Zingiber zerumbet* (L.) Sm. from India. *J Essent Oil Res* 12: 595-97.
- Sukari M A, Rashid NY, Tang SW, Rahmani M, Lajis NH, Khalid K, Yusuf UK. 2008. Chemical constituents and bioactivity of *Curcuma xanthorrhiza* roxb. *J Ultra Sci Phy Sci* 20: 605-610.
- Sung B, Jhurani S, Ahn K. S, Mastuo Y, Yi T, Guha S, Liu M, Aggarwal BB. 2008. Zerumbone down-regulates chemokine receptor CXCR4 expression leading to

- inhibition of CXCL12-induced invasion of breast and pancreatic tumor cells. *Cancer Res* 68: 8938-44.
- Tanaka T, Shimizu M, Kohno H, Yoshitani SI, Tsukio Y, Murakami A, Safitri R, Takahashi D, Yamamoto K, Koshimizu K, Ohigashi H, Mori H. 2001. Chemoprevention of azoxymethane-induced rat aberrant crypt foci by dietary zerumbone isolated from *Zingiber zerumbet*. *Life Sci* 69:1935-1945.
- Vimala S, Norhanom AW, Yadav M. 1999. Anti-tumour promoter activity in Malaysian ginger rhizobia used in traditional medicine. *Br J Cancer* 80: 110-6.
- Zhu K, Li J, Luo H, Li J, Qiu F. 2009. Chemical constituents from the rhizome of *Curcuma kwangsiensis*. *Shenyang Yaoke Daxue Xuebao* 26: 27-29.

