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Synthesis and quorum sensing inhibitory activity of key phenolic compounds of ginger and their derivatives

N. Vijendra Kumar, Pushpa S. Murthy, J.R. Manjunatha, B.K. Bettadaiah *

Plantation Products, Spices and Flavour Technology Department, CSIR-Central Food Technological Research Institute, Mysore 570 020, India

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ABSTRACT

Phenolic components of ginger (*Zingiber officinale* Roscoe) *viz*. [6]-gingerol, [6]-shogaol and zingerone exhibited quorum sensing inhibitory activity (QSI) against *Chromobacterium violaceum* and *Pseudomonas aeruginosa*. The inhibitory activity of all the compounds was studied by zone inhibition, pyocyanin, and violacein assay. All the compounds displayed good inhibition at 500 ppm. [6]-Azashogaol, a new derivative of [6]-shogaol has been synthesized by Beckmann rearrangement of its oxime in the presence of $ZnCl_2$. The structure elucidation of this new derivative was carried out by 1D (^{1}H NMR and ^{13}C NMR) and 2D-NMR (COSY, HSQC and NOESY) spectral studies. This compound showed good QSI activity against *P. aeruginosa*. An isoxazoline derivative of [6]-gingerol was prepared and it exhibited good QSI activity. Present study illustrated that, the phenolic compounds of ginger and their derivatives form a class of compounds with promising QSI activity.

1. Introduction

The ginger rhizome (*Zingiber officinale* Roscoe), belonging to *Zingiberaceae* family, is consumed throughout the world as a spice. It is a common ingredient in foods & beverages and is valued for its pungency. It is used in food preparations both in fresh and dry forms (Zachariah, 2008, chap. 5). [6]-Gingerol is the active pungent principle of fresh ginger whereas [6]-shogaol, the dehydrated form of [6]-gingerol, is the pungent principle of dry ginger. Ginger is also valued as a medicinal herb for its bio-active attributes in the treatment of ailments like catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation, and diabetes (Rahath & Rao, 2012; Tapsell et al., 2006). The health benefits of ginger are mainly due to the presence of key phenolic compounds such as [6], [8], [10]-gingerols, [6], [8], [10]-shogaols, zingerone, paradols etc. (Ali, Bluden, Tanira, & Nemmar, 2008). [6]-Azagingerol, a derivative of [6]-gingerol, is protective against development of metabolic syndrome in mice fed a high fat diet (Okamoto et al., 2011).

The phenomenon of quorum sensing or cell-to-cell communication relies on the principle that when a single bacterium releases autoinducers (AI's) into the environment, their concentration is too low to be detected. However, when sufficient bacteria are present, AI concentrations reach a threshold level that allows the bacteria to sense a critical cell mass and to activate target genes (Vasil, 2003). In Gram negative bacteria AI's are *N*-acyl homoserine lactones (AHL's), which interact with cellular receptors and triggers the expression of genes including virulence, bio-luminescence, bio-film formation, mobility and swarming, a process called quorum sensing (Manefield et al., 2002). Quorum sensing is employed by a diverse group of bacteria including those commonly associated with food. It results in pathogenesis, cellular dissemination or dispersal, DNA transfer, metabolism and microbial biofilm development (Thiba, Wai-Fong, & Kok-Gan, 2012). Thus, food spoilage and biofilm formation by food-related bacteria is significant problem in food industry (Houdt & Michiels, 2010; Jamuna & Ravishankar, 2011). The molecules, natural and synthetic, capable of quenching QS have been an intense focus recently in combating bacterial pathogenesis (Kalia, 2013).

We investigated the QSI activity of phenolic components of ginger and their derivatives as QSI is an important in the area of food processing environment. Long alkyl chain containing structural motifs resembling *N*-acyl-homoserine lactone are known to inhibit the LasR-dependent gene expression, which is important in quorum sensing activity, and molecules having such long chains are believed to be scaffolds for identification of new quorum sensing modulators. One such compound containing amide spacer between a phenyl ring and an alkyl chain having 12-carbons binds to LasR with IC_{50} at 10 μmols (Müh et al., 2006). QS inhibitory activity of ginger was identified for ginger conserve but, no active

* Corresponding author. Address: Department of Plantation Products, Spices and Flavour Technology, Central Food Technological Research Institute, Cheluvamba Mansion, Mysore 570 020, India. Tel.: +91 821 2512352; fax: +91 821 2517233.
E-mail address: bettadaiah@cftri.res.in (B.K. Bettadaiah).

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constituent was tested (Vattem, Mihalik, Crixell, & McLean, 2007). Since phenolic constituents of ginger posses the structural requirement of long alkyl side chain for QS inhibitory activity, we have selected [6]-gingerol and [6]-shogaol for screening studies. Zingerone, one of the most important bioactive components of ginger has also been selected for its QS inhibitory activity. Also, in this study two compounds *viz*., [6]-azashogaol and isoxazoline derivative of [6]-gingerol were synthesized and their QSI activity tested. The structure of compounds used in the present study is provided in Fig. 1.

2. Materials and methods

2.1. Materials and equipment

All the solvents and reagents used for the synthesis were of analytical grade. Zinc chloride, DIMCARB, and hydroxylamine hydrochloride were procured from Sigma Chemical Co. (St. Louis, MO, USA). NMR spectra (^{1}H and ^{13}C) for the compounds and intermediates were recorded on a 500 MHz NMR spectrometer (Bruker Avance, Reinstetten, Germany) using $CDCl_3$ as solvent. The chemical shift values (ppm) and coupling constants (J) are given in δ and Hz respectively. Mass spectral analyses were carried out in the ESI positive mode using HRMS mass spectrometer (Waters Q-Tof Ultima, Manchester, UK). OD of samples was measured using UV/Vis spectrophotometer, UV-1800 Shimadzu, Tokyo, Japan. Thin-layer chromatographic (TLC) analyses were performed on silica gel 60 F_{254} (Merck, Germany) coated on alumina sheet by eluting with 2–10% ethyl acetate in n-hexane. The crude products were purified by column chromatography on silica gel (200–400 mesh) with a mixture of ethyl acetate and petroleum ether (60–80 °C) as eluting medium. All the chemicals and petri-plates used for QS inhibitory studies were procured from Hi Media Ltd., Mumbai, India.

2.2. Synthesis of [6]-shogaol (**4**)

To a magnetically stirred solution of zingerone (0.5 g, 2.57 mmol) and dimethylammonium dimethyl carbamate (DIMCARB, 0.52 g, 3.86 mmol), hexanal (0.51 g, 5.15 mmol) was added drop wise at 52 °C over a period of 1 h. The progress of the reaction was monitored by TLC using 20% ethyl acetate in hexane. After completion of reaction (5 h), it was acidified with 10% aqueous HCl and extracted with CH_2Cl_2 (10 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product thus obtained was purified by column chromatography over silica gel (200–400 mesh). Pure [6]-shogaol was obtained in 70% yield (0.46 g).

^{1}H-NMR (500 MHz, $CDCl_3$); δ = 0.91 (t, 3 H, J = 6.9 Hz, H-1), 1.29–1.35 (m, 4 H, H-2 & H-3), 1.46 (p, 2H, J = 7.35 Hz, H-4), 2.21 (q, 2H, J = 7.16 Hz, H-5), 2.90–2.84 (m, 4H, H-9 & H-10), 3.89 (s, 3 H, H-17), 6.10 (dt, 1 H, J = 15.81, 1.41 Hz, H-6), 6.69 (dd, 1 H , J = 8.04, 1.75 Hz, H-16), 6.72 (d, 1 H, J = 1.77 Hz, H-12), 6.86–6.80 (m, 2 H, H-15 & H-6). ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 199.54 (C-8), 147.57 (C-6) , 146.13 (C-13) , 143.61 (C-14), 132.90 (C-11) , 130.00 (C-7), 120.48 (C-16), 114.04 (C-15), 110.85 (C-12), 55.55 (C-17), 41.65 (C-9), 32.12 (C-5), 31.01 (C-3), 29.57 (C-10), 27.45 (C-4), 22.09 (C-2), 13.61 (C-1).

HRMS: Mass (ESI): [M$^+$+Na] for $C_{17}H_{25}O_3Na$, Calculated: 299.1622; Found: 299.1682.

2.3. Synthesis of [6]-shogaol oxime (**5**)

To a solution of [6]-shogaol (0.5 g, 1.71 mmol) in methanol (3 mL), hydroxylamine hydrochloride (0.24 g, 3.43 mmol) was added at room temperature. The mixture was magnetically stirred, and progress of the reaction was monitored by TLC using 20% ethyl acetate in hexane. After completion of reaction (12 h), the mixture was filtered and the residue was washed with methanol. The filtrate was concentrated under reduced pressure to afford a syrupy mass and it was dissolved in dichloromethane (10 mL). The organic layer was washed with water and dried over anhydrous Na_2SO_4. The resultant clear solution was concentrated to afford material (0.5 g, 95%).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 6.86 (d, 1H, J = 8.47 Hz, H-15), 6.75–6.78 (m, 2H, H-12 & 16), 6.10–5.99 (m, 2H, H-6 & H-7), 3.90 (s, 3H, H17), 2.79 (s, 4H, H-9 & H-10), 2.16 (q, 2H, J = 6.8 Hz, H-5), 1.45–1.38 (m, 2H, H-4), 1.35–1.28 (m, 4H, H-2 & H-3), 0.91 (t, 3H, J = 7.05, H-1). ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 159.15 (C-8), 146.10 (C-13), 143.66 (C-14), 133.29 (C-11), 136.39 (C-6), 125.90 (C-7), 114.05 (C-15), 110.85 (C-12), 55.66(C-17), 32.56 (C-5), 31.86 (C-9), 31.04 (C-3), 28.33 (C-4), 26.36 (C-10), 22.16 (C-2), 13.68 (C-1).

HRMS: Mass (ESI): [M$^+$+1] for $C_{17}H_{25}NO_3$, Calculated: 292.1834; Found: 292.2115.

2.4. Synthesis of [6]-azashogaol (**6**)

[6]-shogaol oxime (0.2 g, 0.68 mmol) was dissolved in dry acetonitrile (5 ml). To this solution was added anhydrous $ZnCl_2$ (0.05 g, 0.34 mmol). The reaction mixture was stirred at room temperature under an inert atmosphere. The progress of the reaction was monitored by TLC using ethyl acetate in hexane (20:80). After completion of reaction (24 h), solvent was evaporated, and the residue was taken in dichloromethane (20 mL). The organic extract was washed with water (20 ml × 3) followed by brine (5 mL) and then dried over anhydrous sodium sulphate. The crude product obtained was purified by column chromatography over silica gel

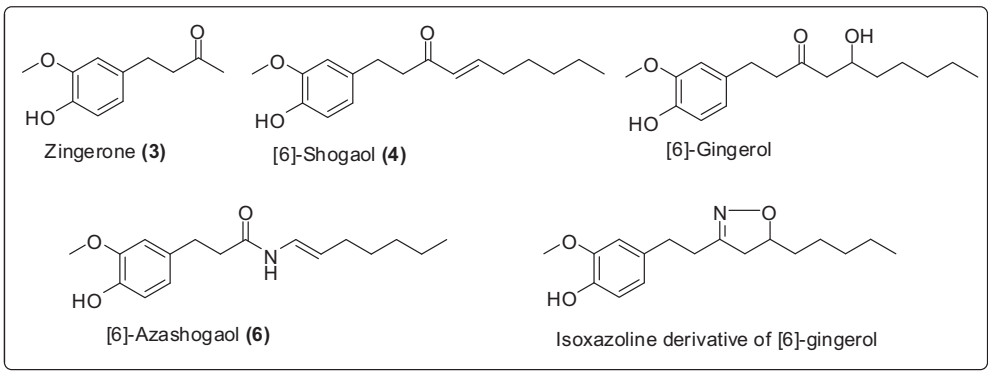

Fig. 1. Structure of phenolic components of ginger and their derivatives.