Structure-function activity of dehydrozingerone and its derivatives as antioxidant and antimicrobial compounds

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Abstract Dehydrozingerone, structural half analogue of curcumin, is a phenolic compound isolated from ginger (*Zingiber officinale*) rhizomes. Dehydrozingerone and several of its derivatives such as glucopyranosides and its tetra acetate derivative and 4-*O*-acetyl and methyl derivatives of dehydrozingerone were synthesized in the present study. Dehydrozingerone, synthesised with improved yield was used for the synthesis of Dehydrozingerone 4-*O*- β -D-glucopyranoside (first time report) by modified Koenigs-Knorr-Zemplén method. Structures of all the compounds have been established using spectroscopic methods. These compounds were tested for radical scavenging activity by DPPH and FRAP method as well as for antibacterial and antifungal activities. The parent molecule exhibited better scavenging activity as compared to its derivatives indicating the significance of free phenolic hydroxyl group. Also, Dehydrozingerone and its derivatives exhibited antibacterial as well as antifungal activity due to the conjugation system present, which includes α,β -unsaturated carbonyl (C = O) group. This study gave an insight into structural requirements for dehydrozingerone activity.

Keywords Dehydrozingerone · Glucoside; NMR · Antioxidant · Antibacterial · Antifungal

Introduction

Plant isolates are used for food safety since antiquity. Concerns over the rise of antimicrobial resistance have sparked an interest in investigating the use of natural products to improve health and growth (Cowan 1999). Among natural food sources with antimicrobial activities, ginger rhizome (*Zingiber officinale* Roscoe; family Zingiberaceae) has been used widely grown as a spice for centuries. It has been used not only as food, but also as herbal medicine especially in East Asian countries. Moreover, it is certified as ‘generally recognized as safe’ (GRAS) in the Food and Drug Administration (FDA) of the United States. Dehydrozingerone (DZ), a phenolic compound, isolated from ginger rhizomes (De Bernardi et al. 1976; Motohashi et al. 1997) is the structural half analogue of curcumin (major compound from turmeric rhizomes). DZ have a similar structure as isoeugenol (acetyl in place of methyl group) and both are known to be prominent bioactive compounds (Rajkumar and Rao 1993). Recently, studies are focused on the structure-activity relationships (SARS) (Motohashi et al. 2000; Ohtsu et al. 2002). SARS of the dehydrozingerone and its derivatives [viz., Dehydrozingerone 4-*O*- β -D-Glucopyranoside (DZG) and its tetra acetate (DZGTA), 4-*O*-acetyl dehydrozingerone (ADZ), 4-*O* -methyl dehydrozingerone (MDZ)] are not reported so far.

The synthesis of glucosides of naturally occurring flavour compounds (viz., benzyl alcohol, ($\pm$)-menthol, (+)-borneol, thymol, carvacrol and eugenol) as well as bioactive compounds (viz., curcumin) has been reported

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(Gurudutt et al. 1996; Masteli et al. 2004; Parvathy and Srinivas 2008). In general, solubility of glycosides in aqueous media is more compare to parent compound, thereby rendering more activity. Synthesis of dehydrozingerone 4-*O*- β -D-glucopyranoside and its activity has not been reported to our knowledge. We found only a few literature reports on analogue syntheses and structure-activity relationships (SAR), in spite of its structural similarity with curcumin. Therefore, we report the syntheses of dehydrozingerone and its derivatives and their preservative potential with respect to antioxidative and antimicrobial properties to find application as food additives. Additionally, structure-function relationships are discussed.

Materials and methods

Apparatus and materials

All the solvents and reagents employed were of analytical reagent grade. All the chemicals and petriplates used for the microbial studies were procured from Hi Media Ltd., Mumbai, India. Melting points were determined by capillary method using melting point apparatus (Pathak electrical works, Mumbai, India) and are uncorrected. IR spectra were recorded using KBr pellets on a Nicolet 5700 FT-IR spectrometer (M/s Thermo electron scientific Instruments Corp., Madison, WI, USA). Ultraviolet spectra were measured in methanol and chloroform on a GBC Cintra 10, spectrophotometer (Victoria, Australia). Mass spectral analyses of the compounds were carried out using LC-MS/MS Waters Q-ToF Ultima (Waters Corporation, Manchester, UK) in the ES positive mode. Optical rotations were measured on a Perkin-Elmer 243 polarimeter containing sodium D-line 589 nm (M/s Bodensewerk, Perkin-Elmer & Co, Germany). Thin-layer chromatographic (TLC) analysis was performed on silica gel 60 F254 (Merck KgaA, 64271 Darmstadt, Germany) coated on aluminium sheet. Ultrasound device used for the reaction was Vibracell with a high intensity tapered probe of tip diameter –6.5 mm from Sonics and Materials Inc., Newtown, USA. For the reactions, the ultrasound reactor was set at 25% amplitude and pulse cycle of 25 s (on) and 5 s (off) with frequency of 40 KHz and an output power of 750 W. ^1H and ^{13}C NMR spectra were recorded on Bruker Avance spectrometer AV500 (Bruker, Germany) at 500 MHz and 125 MHz respectively using deuterated solvents, CDCl_3 (for DZ, ADZ, DZGTA), CD_3OD (for MDZ) and $\text{DMSO-}d_6$ for DZG). Coupling constants (J values) are given in Hz. Two-dimensional NMR experiments: The ^1H - ^1H -correlation spectroscopy (COSY) and ^1H - ^{13}C Heteronuclear Single Quantum Coherence (HSQC) experiments were carried out with an inverse probe.

Synthesis of dehydrozingerone

Vanillin (4-Hydroxy-3-methoxybenzaldehyde, 16 mM) was dissolved in acetone (800 mM; 50 ml) and stirred on a magnetic stirrer; aqueous potassium hydroxide (10% w/v; 70 ml) was added drop wise and stirring continued overnight at room temperature. The crude mixture was acidified with hydrochloric acid (10%), yellow powder precipitated out, which was filtered through whatmann No.1 filter paper placed in a buchner funnel under vacuum. It was crystallized from aqueous hot ethanol, filtered and washed with water and dried to get shiny light yellow crystals of dehydrozingerone (DZ) [4-(4-hydroxy-3-methoxyphenyl) but-3-en-2-one]: Yellow crystalline solid, mp 129–131°C (128–129°C reported Agarwal et al. 2001; MS (ESI) m/z 193.08 $[\text{M}+\text{H}]^+$; HRMS found, 192.21 (calculated for $\text{C}_{11}\text{H}_{12}\text{O}_3$: 192.08); Anal. ($\text{C}_{11}\text{H}_{12}\text{O}_3$) C, 68.74; H, 6.29; O, 24.97; UV λ_{max} (MeOH) nm: 272; IR (KBr) ν_{max} / cm^{-1} : 3463 ($-\text{OH}$), 1679 (α,β -unsaturated C = O), 1580, 1519 ($-\text{Ar}$).

Synthesis of dehydrozingerone 4-*O*- β -D-glucopyranoside (DZG)

By bromination of per acetylated sugar with hydrobromic acid in glacial acetic acid, 2, 3, 4, 6-tetra-*O*-acetyl- α -D-glucopyranosyl bromide (ABG) was obtained (Conchie and Levvy 1963). Dehydrozingerone (0.48 g; 2.5 mM in chloroform) was added to aqueous solution of potassium hydroxide (0.2 g; 3.6 mM) followed by the addition of a solution of 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl bromide (1.02 g, 2.4 mM) in chloroform (20 ml). To the above reaction mixture, an aqueous solution of benzyltributylammonium chloride (0.4 g, 1.25 mM) was added and the mixture was sonicated until the completion of the reaction (5 h), which was indicated by the phase separation followed by TLC analysis. The organic layer was washed with water, dried over anhydrous sodium sulphate and distilled under reduced pressure in rotavapour. Crystals were separated by addition of hexane followed by methanol to the residue. The crystals were filtered, washed with methanol and dried in desiccator. DZGTA [4-*O*- β -D-glucopyranosyl dehydrozingerone tetra acetate]: white crystals, mp 170–173°C; MS (ESI) m/z 523.18 $[\text{M}+\text{H}]^+$; HRMS found, 522.50 (calculated for $\text{C}_{25}\text{H}_{30}\text{O}_{12}$, 522.17); Anal. ($\text{C}_{25}\text{H}_{30}\text{O}_{12}$) C, 57.47; H, 5.79; O, 36.75. UV λ_{max} (MeOH) nm: 297; IR (cm^{-1}): 1670 (α,β -unsaturated C = O), 1580, 1513 ($-\text{Ar}$).

Dehydrozingerone glucoside tetra acetate (DZGTA, 0.26 g; 0.5 mM) was dissolved in dry methanol (10 ml) and added with stirring to a solution of sodium hydroxide in methanol (0.4 g in 5 ml) placed in an ice water bath. The deacetylation reaction was monitored on TLC. At the end of the reaction, freshly regenerated Dowex (IR-120) H^+

resin was added to the reaction mixture. The resin was filtered and the solvent was distilled under reduced pressure to afford pure 4-*O*- β -D-glucopyranosyl dehydrozingerone (DZG). Brownish yellow solid, $[\alpha]_D^{25} -54.5^\circ$ (1:1 MeOH: H₂O, c:1.6); mp 160–162°C; MS (ESI) *m/z* 377.46 [M+Na]⁺; HRMS found, 354.35. (Calculated for C₁₇H₂₂O₈, 354.13); Anal. (C₁₇H₂₂O₈) C, 57.62; H, 6.26; O, 36.12. UV $\lambda_{\max}$ (MeOH) nm: 328; IR (cm⁻¹): 1644 (α,β -unsaturated C = O), 1580, 1514 (–Ar).

Acetylation

Dehydrozingerone (100 mg; 0.5 mM) was dissolved in pyridine (0.25 ml, 4.75 mM) and then acetic anhydride (0.5 ml; 5 mM) was added and kept aside for 24 h. The reaction mixture was poured into ice water, extracted with chloroform, washed with dilute hydrochloric acid and water successively to remove excess pyridine and hydrochloric acid respectively. The chloroform extract was dried over anhydrous sodium sulphate, concentrated and was crystallized from methanol to give colourless needles. ADZ [2-methoxy-4-(3-oxobut-1-enyl) phenyl acetate]: White crystalline solid, mp 112–115°C; MS (ESI) *m/z* 235.09 [M+H]⁺; HRMS found, 234.25 (calculated for C₁₃H₁₄O₄, 234.09); Anal. (C₁₃H₁₄O₄) C, 66.66; H, 6.02; O, 27.32. UV $\lambda_{\max}$ (CHCl₃) nm: 287; IR (cm⁻¹): 1664 (α,β -unsaturated C = O), 1580, 1508 (–Ar).

Methylation

Dehydrozingerone (500 mg; 2.6 mM) in 50 ml of acetone was placed in a round bottom flask with condenser and anhydrous potassium carbonate (500 mg; 3.6 mM) was added followed by methyl iodide (2 ml; 32 mM). The mixture was refluxed on a water bath maintained at 50°C for about 5 h and after the completion of the reaction, filtered under vacuum to remove potassium carbonate and the filtrate was concentrated using rotavapour to obtain a semisolid mass. The crystals were separated by addition of chloroform. MDZ [4-(3,4-dimethoxyphenyl) but-3-en-2-one]: Pale yellow solid, mp 80–82°C; MS (ESI) *m/z* 207.1 [M+H]⁺; HRMS found, 206.24 (Calculated for C₁₂H₁₄O₃, 206.09); Anal. (C₁₂H₁₄O₃) C, 69.88; H, 6.84; O, 23.27. UV $\lambda_{\max}$ (MeOH) nm: 272; IR (cm⁻¹): 1680 (α,β -unsaturated C = O), 1596 (–Ar).

Radical scavenging activity (DPPH model system)

The radical scavenging activity of the compounds was evaluated as per the method of Blois (1958). In brief, different concentrations (each 500 μ l) of test compounds (0.1–50 mM) and trolox (10–200 μ M) were prepared in different test tubes respectively. Five hundred microlitre of

methanolic solution of DPPH (0.2 mM) was added to these tubes. Then, these mixtures were vortexed vigorously and allowed to stand in the dark for 20 min. Finally, the absorbance was measured by using a spectrophotometer at 517 nm. The control was prepared as above without any test compounds and methanol was used for the baseline correction. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

Radical scavenging activity(%)

$$= (\text{Abs}_{\text{control}} - \text{Abs}_{\text{Sample}} / \text{Abs}_{\text{control}}) \times 100.$$

The sample concentration providing 50% of radical scavenging activity (IC₅₀) was obtained through interpolation of linear regression analysis. The lower IC₅₀ indicates higher radical scavenging activity and vice versa.

Reducing ability (FRAP assay)

The determination of the total antioxidant activity (FRAP assay) of the synthesized compounds was evaluated by modified method of Benzie and Strain (1996). The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-S-triazine) solution in 40 mM HCl, and 20 mM FeCl₃·6H₂O solution. The fresh working solution was prepared by mixing acetate buffer (25 ml), TPTZ (2.5 ml), and FeCl₃·6H₂O (2.5 ml). The temperature of the solution was raised to 37°C before use. All the five compounds with varying concentrations were allowed to react with 1,500 μ l of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 50 and 1,000 μ M FeSO₄. Results are expressed in μ M Fe (II)/g dry mass and compared with that of trolox.

Measurement of reactivity of compounds with the folin-ciocalteu reagent

A slightly modified version of the method of Singleton et al. (1999) was used. Synthesized compounds (50 mg) were dissolved in ethanol and the following dilutions were made 1:10, 1:20, 1:30, 1:50, and 1:100. Three replicates for each of the dilutions were prepared. To each tube, 1.58 ml of water, 0.1 ml of F-C reagent, and 20 μ l of the proper dilution of test compound were added. The contents were stirred and allowed to stand 5 min. Aqueous sodium carbonate solution (20%; 0.3 ml) was added, stirred and incubated at 45°C for 30 min in a dry bath. Absorbance of solutions was read at 765 nm. Graphs of concentration versus absorbance were plotted. The activities of the compounds are expressed in terms of gallic acid equivalents

(GAE). GAE defined as the slope of the test compound curve divided by the slope of the gallic acid standard curve.

Antibacterial activity

The antibacterial assay of the synthesized compounds was tested using well diffusion method against *Bacillus subtilis*, *Listeria monocytogenes* Scott-A, *E.coli* MTCC 118, and *Salmonella typhi* (Perez et al. 1990). One hundred microliters of culture broth (18–24 h) was spread on nutrient agar plate by spread plate method. Wells were bored (8 mm diameter) in the agar plates and filled with different concentration of compounds and were incubated at 37°C for 24 h. At the end of the incubation period, the susceptibility of the test organisms was determined by measuring the radius of the zone of inhibition around the well. Zone of inhibition was taken from average of triplicate experiments. Standard Erythromycin (15 µg/disc) and Ciprofloxacin (5 µg/disc) were used as positive control for comparison of the antibacterial activity.

Antifungal activity

The antifungal activities of the compounds were also studied by agar diffusion assay according to Singh et al. (2008). The antifungal activities of the synthesized compounds were studied against various fungi *Fusarium* sp Gt-1019, *Aspergillus flavus*, *Aspergillus oryzae*, *A.ochraceus*, *A. niger* and *Penicillium* sp. Fluconazole (25 µg/disc) was used as a positive control for antifungal activity. Each test was carried out in triplicates and fungal toxicity was measured in terms of mycelial inhibition zones measured in mm.

Statistical analysis

All experiments were performed in triplicates and the results were expressed as mean $\pm$ standard deviation. Analyses of variance were performed by ANOVA test and significant differences between the means were determined by Duncan's multiple range tests. P values <0.05 were regarded as significant.

Results and discussion

Synthesis of dehydrozingerone and its glucoside along with related derivatives

Vanillin undergoes a facile crossed-aldol reaction with acetone to give DZ [4-(4'-hydroxy-3'-methoxyphenyl)-3-buten-2-one], which can be hydrogenated to zingerone [4-

(4'-hydroxy-3'-methoxyphenyl)-2-butanone], a substance identified as one of the major flavour compounds of ginger (Nomura 1917). The earlier reported method required 48 h to complete the reaction (Denniff et al. 1981), which was modified to obtain the product within 24 h by changing the alkali from sodium hydroxide to potassium hydroxide. This change improved the yield of the product also from 83% to 92%. Although both are strong bases, the reactivity of potassium ions being different from sodium ions. 4-*O*-Acetyl dehydrozingerone (ADZ; yield 96%) was prepared by treating the DZ with acetic anhydride in presence of pyridine. Methyl ether of dehydrozingerone (MDZ) was prepared by treating DZ with methyl iodide and potassium carbonate in a yield of 62%.

Synthesis of dehydrozingerone 4-*O*- β -D-glucopyranoside tetra acetate (DZGTA) was reported first time (Fig. 1a). Synthesis of DZGTA (Yield 32%) involved the reaction of DZ (organic phase) in potassium hydroxide (aqueous phase) with α -2, 3, 4, 6-tetra-*O*-acetyl-1-bromoglucose using a phase transfer catalyst (viz., benzyltributylammonium chloride) in presence of ultrasound, an modified Koenigs-Knorr procedure (Parvathy and Srinivas 2008). 2, 3, 4, 6-tetra-*O*-acetyl- α -D-glucosyl chloride or bromide has been an important and versatile synthetic intermediates used as electrophiles in glycosidic bond formation in carbohydrate chemistry. The glucosylation reaction was a nucleophilic substitution at saturated carbon where the substrate was α -acetobromoglucose, and alcohols, phenols or phenolates are the nucleophiles. It has mainly bimolecular S_N2 nucleophilic substitution with total inversion of the product configuration. The phase transfer catalyst (PTC) causes homogenization of the two phases, ultrasound has known to effect cavitation and facilitate the mixing of two phases, thereby, providing high interfacial area with the formation of fine emulsion and enhancing interfacial contact between the reactants (Sivakumar et al. 2001). Koenigs-Knorr type reaction of 2, 3, 4, 6-tetra-*O*-acetyl- α -D-1-bromoglucose with dehydrozingerone has been facilitated by combined effect of ultrasound and phase transfer catalyst (PTC), which promote proper mixing along with better mass transfer of reactants between the two phases. Completion of the reaction was indicated by separation of the layers and the discoloration of the aqueous phase and confirmed by analysis of the product by TLC. The product was isolated from the reaction mixture by separation of the organic phase, on treatment with water, drying followed by distillation to obtain crude product. The oily product was dissolved in a small volume of warm ethanol and crystallized to give DZGTA. The obtained tetra acetate was deacetylated to dehydrozingerone 4-*O*- β -D-glucopyranoside (DZG; Yield 84%) using the standard procedure of Zemplén (Parvathy and Srinivas 2008).

Fig. 1 (a). Scheme for the synthesis of Dehydrozingerone 4-*O*- β -D-glucopyranoside. (b). Structure of Dehydrozingerone and its derivatives

Characterisation of the compounds

All the compounds were purified prior to analysis and purity evaluated by chromatographic methods. Structural elucidation (Fig. 1b) of these compounds was carried out by spectral techniques (viz., UV, IR, ^1H - and ^{13}C -NMR and mass).

IR spectrum of DZ showed absorption bands for hydroxyl group ($3,463\text{ cm}^{-1}$), α,β -unsaturated carbonyl

($1,679\text{ cm}^{-1}$) and an aromatic ring ($1,580, 1,519\text{ cm}^{-1}$). The structure was further indicated by ES-MS, which showed a base peak at m/z 193 corresponding to the protonated molecular ion $[\text{M}+\text{H}^+]$ and the other characteristic peaks at m/z 215 and 175 corresponding to $[\text{M}+\text{Na}^+]$ and $[\text{M}+\text{H}^+-\text{H}_2\text{O}]$ ion respectively. The ^1H NMR spectrum (Table 1) of DZ showed ABX pattern for the presence of three aromatic protons at $\delta(\text{ppm})$ 7.10 (dd, $J=1.5, 8.0\text{ Hz}$, 1H), 6.94(d,

Table 1 NMR spectral data of DZ, ADZ and MDZ

C/H no.	SEFT	DZ	ADZ		MDZ					
			HMBC ^b		HMBC ^b					
			HSQC ^a	HSQC ^a	HSQC ^a	HSQC ^a				
		¹³ C (δ)	¹ H (δ)	¹³ C (δ)	¹ H (δ)	¹³ C (δ)	¹ H (δ)			
1	↓	143.4	7.46 (d, 16.0 Hz)	2', 6', 1', 2, 3	142.3	7.48 (d, 16.0 Hz)	1', 2', 6', 2, 3	146.0	7.57 (d, 16.0 Hz)	2', 6', 2, 3
2	↓	124.7	6.60 (d, 16.0 Hz)	1', 3, 4	127.0	6.68 (d, 16.0 Hz)	1', 3	125.7	6.66 (d, 16.0 Hz)	1', 3, 4, 1
3	↑	198.1	—	—	197.8	—	—	201.5	—	—
4	↓	27.0	2.38(s)	2, 3	27.2	2.40 (s)	2, 3	27.3	2.34 (s)	1, 2, 3
1'	↑	126.6	—	—	133.0	—	—	128.9	—	—
2'	↓	109.1	7.06 (d, 1.5 Hz)	6', 1	111.0	7.15 (d, 2.0 Hz)	1, 3',4', 6'	111.9	7.20 (d, 1.5 Hz)	1', 3', 6', 1
3'	↑	148.0	—	—	151.2	—	—	153.1	—	—
4'	↑	146.6	—	—	141.4	—	—	150.8	—	—
5'	↓	114.5	6.94 (d, 8.0 Hz)	6'	121.2	7.08 (d, 8.0 Hz)	1', 4', 3'	112.8	6.96 (d, 8.0 Hz)	1', 3', 4', 6'
6'	↓	123.2	7.10 (dd, 1.5, 8.0 Hz)	1, 2'	123.0	7.16 (dd, 2.0, 8.0 Hz)	1, 4'	124.5	7.15 (dd, 1.5, 8.0 Hz)	2'
-OCH ₃	↓	55.7	3.93 (s)	3'	55.4	3.89 (s)	3'	56.6	3.852 (s)	4'
-OCOCH ₃	↑	—	—	—	168.4	—	—	56.5	3.854 (s)	3'
-OCOCH ₃	↓	—	—	—	20.3	2.35(s)	OCOCH ₃	—	—	—

^a HSQC Connectivities^b Key HMBC Connectivities

8.0 Hz, 1H) and 7.06 (d, 1.5 Hz, 1H). Also showed signals for olefinic protons [δ 6.60 (d, $J=16.0$ Hz, 1H) and 7.46 (d, $J=16.0$ Hz, 1H) ppm] characteristic of α,β -unsaturated ketonic system, and acetyl methyl protons (δ 2.38 ppm). The singlet at δ (ppm) 3.93 (3H) showed the presence of aromatic methoxyl group. ^{13}C NMR spectrum (Table 1) showed the presence of eleven peaks, indicating an eleven-carbon skeleton of the molecule. The signals at δ (ppm) 198.1, 143.4 and 124.7 confirmed the presence of α,β -unsaturated carbonyl system. The chemical shifts at δ (ppm) 148.0, 146.6, 126.6, 123.2, 114.5 and 109.1 were assigned for C-3', 4', 1', 6', 5' and 2' of aromatic ring. Methoxyl carbon and acetyl methyl carbon appeared at δ 55.7 and 27.0 ppm respectively. SEFT experiment confirmed assignment of these signals. HSQC spectrum showed the correlations between Carbons and respective protons. Strong correlation was found between H-1 (δ 7.46) and H-2 (δ 6.60) signals in COSY experiment confirming the α,β -unsaturated protons. The C-H long-range correlations between H1-C1', H2'-C1 and H6'-C1 in HMBC spectrum, confirmed the connectivity between C1 of the aliphatic chain to C1' of the aromatic ring.

IR spectrum of ADZ showed additional absorption band for acetyl carbonyl at $\nu_{\text{max}}^{\text{KBr}}$ 1,757 cm^{-1} . ^1H NMR spectrum (Table 1) of ADZ showed signals at δ (ppm) 2.35 (s, 3H) and 2.40 (s, 3H) indicated the presence of two acetyl methyl groups. The signal at δ (ppm) 2.35 (s, 3H) was assigned for the acetoxyl methyl group in the aromatic ring, while the other signal at δ (ppm) 2.40 (s, 3H) assigned for acetyl methyl in the aliphatic chain as indicated from HMBC correlations. Further, the ^{13}C NMR signals at δ (ppm) 197.8 and 168.4 corresponds to two carbonyl carbons i.e., one for the α,β -unsaturated carbonyl carbon and the other related to the acetate carbonyl carbon respectively. The remaining parts of the spectra were similar to that of DZ.

The proton NMR of MDZ showed two singlet signals at δ 3.852 (3H) and 3.854 (3H) and indicated the presence of two aromatic methoxyl groups, which in turn supported by two carbon signals at δ 56.5 and 56.6 ppm appearing in the ^{13}C NMR spectrum. The remaining part of the spectra was similar to that of DZ.

The ES-MS of DZGTA exhibiting a pseudo molecular ion peak $[\text{M}+\text{Na}]^+$ at m/z 545 revealed the molecular formula of $\text{C}_{25}\text{H}_{30}\text{O}_{12}$. The ^1H NMR spectrum of DZGTA displayed signals at: δ 7.12 ppm (d, $J=8.0$ Hz, H-5'), δ 7.10 ppm (d, $J=2.0$ Hz, H-2') and δ 7.10 ppm (dd, $J=2.0, 8.0$ Hz, H-6') showing the presence of 1, 3, 4-trisubstituted benzene ring. A singlet at 3.87 ppm (s, 3H) indicates that one of the substituents was a methoxy group. The signal at δ 5.04 ppm (d, $J=7.5$ Hz, H-1'') assigned to a β -coupled anomeric proton was in agreement with the presence of a β -D-glucopyranoside moiety. Signals corresponding to the olefinic protons appear as doublets at δ (ppm) 7.46 (d, H-1) and

6.64 (d, H-2) with J value of 16.0 Hz, in addition to the acetoxyl signal observed at δ 2.39 ppm, indicated the presence of a $\text{CH}=\text{CH}-\text{CO}-\text{CH}_3$ moiety. In addition four acetyl signals appeared at δ (ppm) 2.05, 2.06, 2.09, 2.10 (each 3H and singlets). This hypothesis was confirmed by the ^1H - ^1H -correlation spectroscopy (COSY) spectrum revealing the connectivity of H-1 to H-2. The location of the methoxy group at C-3' was proved by the long range coupling between H_{OMe} (δ 3.87 ppm) and C-3' (δ 150.6 ppm) deduced from the HMBC spectrum. The analysis of COSY data sets showing correlations $\text{H}_{1''}-\text{H}_{2''}$; $\text{H}_{2''}-\text{H}_{3''}$; $\text{H}_{4''}-\text{H}_{5''}$; $\text{H}_{5''}-\text{H}_{6''\text{a}}$; $\text{H}_{5''}-\text{H}_{6''\text{b}}$ and $\text{H}_{6''\text{a}}-\text{H}_{6''\text{b}}$ as well as the observation on the HMQC spectrum of only one anomeric carbon C-1'' (δ 99.9 ppm) made it possible to establish the skeleton of the monoglucopyranoside. The linkage of the monosaccharide moiety at C-4' of the aglycone was deduced from the HMBC spectrum showing the characteristic correlation between the anomeric proton H-1'' (δ 5.04 ppm) and carbon C-4' (δ 147.7 ppm). Comparison of these spectral data to 4-*O*- β -D-glucopyranosyl zingerone tetra acetate (Hammami et al. 2006) confirmed the structure.

The ^1H NMR spectrum of DZG indicated deacetylation by the absence the four singlets in the range of δ (ppm) 2.05–2.10 corresponding to the acetyl groups seen in the spectrum, for the corresponding tetra acetate (Table 2). ES-MS analysis of dehydrozingerone 4-*O*- β -D-glucopyranoside exhibited molecular mass ion at 377 $[\text{M}+\text{Na}]^+$. The β -pyranoside structure of the prepared glucoside was confirmed by one- and two-dimensional homo and heteronuclear ^1H and ^{13}C NMR (Table 2) measurements in $\text{DMSO}-d_6$. This was determined on the basis of specific carbon and proton chemical shifts, H–H spin-spin couplings. The assignment was substantiated by connectivities in homo- (COSY) and heteronuclear (HSQC, HMBC) correlation spectra as well. The number of signals and their integrals showed the existence of only one anomeric proton (4.99, d, $J=7.0$ Hz) indicated the attachment of the anomeric carbon to the aromatic ring. The signals at 3.81 (s), 7.35 (bd, 2.0 Hz), 7.11 (d, 8.5 Hz) and 7.22 (dd, 2.0; 8.5 Hz) correspond to the methoxyl and aromatic protons of the DZ moiety, while signals corresponding to the olefinic protons appear as doublets at 6.76 and 7.56 with J value of 16.5 Hz. The remaining glucose protons showed signals in the range of δ (ppm) 3.17 to 3.65, which was similar to reported for zingerone glucoside (Kim et al. 2005). ^{13}C NMR spectral analyses further substantiated for aglycone unit similar to DZ. In addition, it showed the presence of signals for one sugar moiety (viz., glucose).

In-vitro antioxidant activity studies

In this study, free radical scavenging activities (RSA) of compounds were determined at different concentrations

Table 2 NMR spectral data of DZGTA and DZG

C/ H no.	DZGTA			DZG		
	HSQC ^a		HMBC ^b	HSQC ^a		HMBC ^b
	¹³ C (δ)	¹ H (δ)		¹³ C (δ)	¹ H (δ)	
1	142.4	7.46 (d, 16.0 Hz)	2', 6', 1', 2, 3	143.5	7.56 (d, 16.5 Hz)	2',6',3
2	126.3	6.64 (d, 16.0 Hz)	1', 3, 4	125.8	6.76 (d, 16.5 Hz)	1'
3	197.7	—	—	198.1	—	—
4	27.1	2.39 (s)	2, 3	27.4	2.31 (s)	3
1'	130.7	—	—	128.4	—	—
2'	111.3	7.10 (d, 2.0 Hz)	1, 3', 4', 6'	111.6	7.35 (bd, 1.5 Hz)	1',3'
3'	150.6	—	—	149.3	—	—
4'	147.7	—	—	148.8	—	—
5'	119.3	7.12 (d, 8.0 Hz)	1', 3', 4'	115.2	7.11 (d, 8.5 Hz)	1',4'
6'	121.7	7.10 (dd, 2.0 & 8.0 Hz)	1, 4'	122.7	7.22 (dd, 2.0 & 8.5 Hz)	1,4'
OMe	55.8	3.87(s)	3'	55.9	3.81(s)	3'
1''	99.9	5.04(d, 7.5 Hz)	5'', 4'	99.9	4.99 (d, 7.0 Hz)	4'
2''	72.2	3.82 (m)	—	73.3	3.28 (m)	1'',3'',4''
3''	71.9	5.29–5.33 (m)	1'', 2'', 5'', -OCOCH ₃	77.2	3.31(m)	2'',4''
4''	70.9	5.29–5.33 (m)	2'', 3'', 5'', -OCOCH ₃	69.8	3.17(m)	3'',5'',6''
5''	68.1	5.18 (m)	3'',4''	77.0	3.31(m)	4'',6''
6''	61.6	4.28–4.31; 4.18–4.21	5''	60.8	3.65(m)	—
[–OCOCH ₃] X4	168.9, 169.0, 169.9, 170.1	—	—	—	—	—
[–OCOCH ₃] X4	20.2, 20.3	2.05, 2.06, 2.09, 2.10	–OCOCH ₃	—	—	—

^a HSQC Connectivities^b Key HMBC Connectivities

(0.1–50 millimolar) using DPPH (1, 1'- diphenyl- 2- picryl-hydrazyl) a stable free radical (Fig. 2) and compared with Trolox (standard). The model of scavenging the stable DPPH radical has been widely used for relatively rapid evaluation of antioxidant activities compared to other methods. The reduction capability of the DPPH radical was determined by its absorbance decrease at 517 nm, as induced by natural antioxidants. IC₅₀ value of DZ was found to be 0.3 mM and comparable to Trolox (0.26 mM), whereas the IC₅₀ value of ADZ, MDZ, DZGTA and DZG were found to be 40, 20, 10 and 7.5 mM respectively. Antioxidant activity assays of derivatives with diverse substituent inferred that the presence of hydroxyl substituents on the phenyl nucleus enhanced activity, whereas substitution by groups like methoxyl and acetoxyl groups decreased antioxidant activity remarkably. Dehydrozingerone, which contains an extended conjugated system, was active. This observation was in agreement with the structural requirement for activity on flavonoids (Pietta 2000); the 3'-O-methoxy- 4'-hydroxy substitution on the aromatic ring was also demonstrated to exhibit radical scavenger activity. Dehydrozingerone showed mild inhibi-

tion of lipid per oxidation by acting as free radical scavenger (Rajkumar and Rao 1993). The antioxidant activity has been attributed to various mechanisms, among which are the prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the prevention of continued hydrogen abstraction, the reductive capacity and radical scavenging. DZ was found to be a free radical scavenger (total reactive antioxidant potential TRAP=229 μM) while zingerone, a hydrogenated product of DZ showed no activity which confirms the activity due to an extended ketone conjugation in DZ (Desmarchelier et al. 2005). While, the hydroxyl radical scavenging activity of DZ (ES₁₅ value=48.36 mM) was reported to be lower than curcumin (25.01 mM) (Nugroho et al. 2006). The antioxidant activity of DZ may be attributed to the presence of -OH and C = O group, which are in conjugation, as reported in structurally similar types of compounds (Nikolaos et al. 2003).

The antioxidant potentials of DZ and its derivative were estimated from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II). The reducing ability of the DZ was 179.7±5.78 μM of Fe (II)/g while that of the

Fig. 2 Radical scavenging activity of the derivatives of DZ. Scavenging capacities of the derivatives were measured at different concentrations (2.5, 5, 10 and 20 mM) and the results are expressed as percentage inhibition of the DPPH radicals. Results are average of three measurements $\pm$ standard deviation; values with different superscripts were significantly different ($p < 0.05$)

derivatives was drastically decreased in the order MDZ ($2.14 \pm 0.47 \mu\text{M}$) > DZG ($0.91 \pm 0.04 \mu\text{M}$) > DZGTA ($0.25 \pm 0.06 \mu\text{M}$) > ADZ ($0.07 \pm 0.01 \mu\text{M}$) respectively. The ferric reducing/antioxidant power (FRAP assay) has been widely used in the evaluation of the antioxidant component in dietary polyphenols (Luximon-Ramma et al. 2005). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Recently, Tatsuzaki and co-workers (2006) reported the synthesis of compounds related to isoeugenol, 2-hydroxychalcone and dehydrozingerone, and evaluated in vitro against human tumour cell replication.

Also, our study found DZ (0.701 molar GAE), BHA (0.438 molar GAE) and trolox (0.427 molar GAE) showed reactivity toward the Folin-Ciocalteu (F-C) reagent. No reactivity was observed for the compounds (viz., ADZ, MDZ, DZGTA and DZG). This study confirmed that the non-phenolic compounds did not show reactivity toward the F-C reagent (Everette et al. 2010).

Antibacterial and antifungal activity of the compounds

Generally, phenolic compounds target the cytoplasmic membrane and partition into the lipid bilayer due to their hydrophobic nature. Further the interactions between both lipids and membrane embedded proteins along with the phenolic compound results in destabilization of the membrane and causes loss of integrity of the cell (Sikkema et al. 1995). DZ and its derivatives were evaluated for their antibacterial activity against food-borne pathogenic bacteria. Significant diversity in the antibacterial activity of the synthesized compounds was observed amongst the gram positive and gram-negative organisms. Erythromycin (15 μg /disc) was found to produce maximum (26 ± 1.0 mm) and minimum (15 ± 2.0 mm) zones of inhibition against *Listeria monocytogenes* and *Bacillus subtilis*, respectively, whereas Ciprofloxacin (5 μg /disc) produced maximum (25 ± 2.0 mm) and minimum (13 ± 2.2 mm) inhibition zones against *Escherichia coli* and *Salmonella typhi* respectively. Graphical

representations of antibacterial activity as zone of inhibition (mm) on y-axis versus the different test compounds at different concentration (2–20 micromoles) on x-axis was plotted (Fig. 3). All the compounds showed strong to moderate inhibition against the tested organism at different concentrations. In case of *Bacillus subtilis*, DZGTA showed highest activity, while in case of *Salmonella typhi* MDZ showed highest inhibition. In case of *Listeria monocytogenes* and *Escherichia coli*, both DZ and MDZ showed marginally higher inhibition, when compared to other derivatives. DZ and its derivatives exhibited antibacterial activity due to the conjugation system existed in the compounds, which includes α,β -unsaturated carbonyl ($\text{C}=\text{O}$) group. The inhibitory activity of DZ appears to be similar to vanillin, which detrimentally affects the integrity of the cytoplasmic membrane with the loss of ion gradients, pH homeostasis and inhibition of respiratory activity (Fitzgerald et al. 2004).

The inhibitory action of natural products from spices on fungi generally involves cytoplasmic membrane rupture, granulation within cytoplasm, and inactivation and/or inhibition of intercellular and extracellular enzymes. These biological events might occur separately or concomitantly culminating with mycelium germination (Cowan 1999). Fluconazole (25 μg /disc) was used as a positive control for antifungal activity and found to produce maximum (30 ± 0.5 mm) and minimum (10 ± 1.0 mm) zones of inhibition against *Fusarium sp.* and *A. ochraceus* respectively. DZ and its derivatives were tested for anti fungal activity against *Aspergillus niger*, *A. ochraceus*, *A. oryzae*, *A. flavus*, *Penicillium sp.* and *Fusarium sp.* All the compounds developed inhibition zones and the zone diameter was in the range of 15.0–31.5 mm (Table 3). DZG and DZGTA showed no inhibition zones against *Aspergillus niger* and *A. flavus*. DZ exhibited highest inhibition zones with *A. niger*, *A. ochraceus*, *A. flavus*, and *Penicillium sp.* However, excellent inhibition observed against *Penicillium sp.* and *A. niger*. DZG showed excellent inhibition against *A. oryzae*, while ADZ exhibited highest inhibition against *Fusarium sp.* However, the diameter of the inhibition zones varies

Fig. 3 Antibacterial activity of DZ and its derivatives against (a) *Bacillus subtilis* (b) *Listeria monocytogenes* Scott-A (c) *E.coli* MTCC 118 and (d) *Salmonella typhi* by agar well diffusion assay. Standard Erythromycin (15 μg/disc) and Ciprofloxacin (5 μg/disc) were used as

positive control. Values for the Zone of growth inhibition are presented as mean ± standard deviation exhibited by different bacteria against various concentrations (2, 5, 10 and 20 μM). Values with different superscripts were significantly different ($p < 0.05$)

with the organism and the compound structure. The antifungal activity of the DZ and its derivatives suggests that the carbonyl moiety plays a key role in the antifungal activity. The presence of the polarized carbon-oxygen double bond of aldehyde/keto groups, which probably accounts for their activity forming covalent bonds with DNA and proteins and interfere with their metabolism (Feron et al. 1991). Our results are similar to the studies reported on vanillin (Fitzgerald et al. 2005). DZ have also shown significant antifungal activity (EC_{50} 86.49 mg litre⁻¹) against *Rhizoctonia solani* (Agarwal et al. 2001).

Conclusions

In conclusion, Dehydrozingerone 4-*O*-β-D-glucopyranoside has been synthesized for the first time via a simple synthetic route (two steps) from DZ, which was prepared expeditiously with improved yield from commercially available vanillin. DZ showed highest radical scavenging activity (IC_{50} =0.3 mM) similar to that of Trolox. Among the other compounds prepared, DZGTA showed highest antibacterial activity against *Bacillus subtilis*, while DZG showed excellent antifungal activity against *A. oryzae*.

Table 3 Antifungal activity of DZ and its derivatives

Fungi	Zone of Inhibition (mm)*				
	DZ	DZGTA	DZG	ADZ	MDZ
<i>A. niger</i>	31.0±1.0 ^d	NI ⁺	NI ⁺	21.0±1.0 ^b	19.0±1.0 ^b
<i>A. flavus</i>	20.5±2.5 ^a	NI ⁺	NI ⁺	15.0±1.0 ^a	16.0±2.0 ^a
<i>A. oryzae</i>	25.0±5.0 ^b	16.5±1.5 ^a	30.0±0.2 ^b	25.0±0.4 ^c	24.5±0.5 ^c
<i>A. ochraceus</i>	28.5±1.5 ^c	25.0±4.0 ^c	16.5±3.5 ^a	27.5±2.5 ^d	25.0±0.4 ^c
<i>Penicillium sp.</i>	31.5±3.5 ^d	21.0±1.0 ^b	17.5±0.5 ^a	30.5±0.5 ^e	26.5±0.5 ^c
<i>Fusarium sp.</i>	18.5±3.5 ^a	19.5±0.5 ^a	19.0±1.0 ^a	24.0±1.0 ^c	21.0±1.0 ^b

⁺ NI no inhibition; Positive control: Fluconazole (25 μg/disc)

* Values for zone of growth inhibition (1 mg level) are presented as mean ± standard deviation. Means of the same column followed by different letters are significantly different ($p < 0.05$)

Conjugation system existed in these compounds, which includes α,β -unsaturated carbonyl ($C=O$) group might be responsible for these activities. Overall, the results of in vitro biological assays demonstrated that DZ to be an effective antioxidant, antibacterial and antifungal hence finds application as food additives, while other compounds possess good antibacterial and antifungal activities. These results warrant further research into the role of these compounds as antimicrobial agents in food systems as well as study to find respective binding sites and mode of action. These may be useful as lead products for quantitative structure-activity relationships studies.

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