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Antioxidant, antimutagenic and antibacterial activities of curcumin-β-diglucoside

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ABSTRACT

Curcumin, the yellow colour constituent of turmeric, has several important biological activities but its use as a food colourant is restricted due to its insolubility in water. Curcumin-β-diglucoside (**III**), prepared by glycosylation of curcumin (**I**) at the phenolic hydroxyl group, was soluble in water at 10 mg/ml concentration. Studies of the radical-scavenging, as well as antioxidant properties, of **III** at different concentrations showed that these activities were higher than that of **I**. The mutagenicity studies showed that **I**, as well as **III**, afforded high protection against the mutagenicity of sodium azide to *Salmonella typhimurium* TA 1531 and TA 98. Also, **III** exhibited higher antibacterial properties against *Staphylococcus aureus* and *Escherichia coli* but showed lower activity against *Bacillus cereus* and *Yersinia enterocolitica* than did **I**. The results clearly demonstrate that conjugation of the phenolic hydroxyl group of curcumin to a sugar moiety rendered it water-soluble whilst retaining/enhancing its *in vitro* antioxidant, antimutagenic and antibacterial properties.

1. Introduction

Curcumin is a nutraceutical used worldwide for medicinal, as well as food, purposes. Curcumin exhibits potent antioxidant, antitumor and anticancer properties (Sharma, Gescher, & Steward, 2005). Antioxidant and wound-healing properties of curcumin, its anticancer and antiviral attributes, its effect on lymphocytes, platelet aggregation, detoxification mechanisms, cell cycle and apoptosis and its ameliorating role in diabetes and stress responses are well established (Joe, Vijaykumar, & Lokesh, 2004). It has attracted special attention due to its pharmacological activities, such as its potential as a neuroprotectant in neuro-degenerative diseases (Cole, Teter, & Frautschy, 2007). Studies have been carried out, extensively, on antioxidant and cleavage activities of curcumin and its derivatives on DNA (Ahsan, Parveen, Khan, & Hadi, 1999). Curcumin is shown to regulate the expression of inflammatory enzymes, cytokines, adhesion molecules and cell survival proteins. It down-regulates cyclin D1, cyclin E and MDM2 but up-regulates p21, p27 and p53. Cell culture and animal studies have shown that curcumin has potential as an antiproliferative and anti-angiogenic agent. Clinical trials have shown curcumin to be safe, even when consumed at a daily dose of 12 g for 3 months (Goel, Kunnumakkara, & Aggarwal, 2008). It suppresses cardiac hypertrophy through the disruption of p300 histone acetyltransferase-dependent (p300-HAT-dependent) transcriptional activation and affords protection against cardiac hypertrophy, inflammation, and fibrosis (Li et al., 2008).

With all these advantages, one of the drawbacks often associated with curcumin is its insolubility in water, which limits its usage in many food products, as well as in pharmaceuticals. Although curcumin is insoluble in water at acidic and neutral pH, it can be made soluble under alkaline conditions, but the colour of the chromophore changes to deep red and it also undergoes degradation. Higher plants accumulate a wide range of glycosides, as secondary metabolites and are capable of conjugating sugar residues to the targeted sites. The biological activities of glycosylated components have become more important because these are widely distributed in plants and they play a key role in the detoxification and stabilisation pathways. Glycosylation allows the conversion of water-insoluble compounds into corresponding water-soluble derivatives, which could probably improve their bioavailability and pharmacological properties. In fact, metabolism of curcumin involves its biotransformation into water-soluble glucuronides of reduced curcumin forms, namely tetrahydrocurcumin, hexahydro curcumin and octahydrocurcumin. The reduction of the conjugated double bonds in the enedione system lends stability to the molecule but renders it colourless (Pan, Huang, & Lin, 1999). Also, there is little information on the bioactivity of these metabolites.

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Sugar derivatives of curcumin have been shown to increase its water solubility, as well as to reduce toxicity of xenobiotic phenols (Kaminaga, Sahin, & Mizukami, 2004). Curcumin glucoside is prepared by Koenigs–Knorr synthesis of glycosides under classical conditions, and this involves formation of glycosyl halides, followed by the glycosyl transfer to curcumin in the presence of heavy metal salts. Known methods of preparation of curcumin sugar derivatives employ the reaction of α-D-tetraacetohaloglucose with curcumin under biphasic conditions in the presence of a phase transfer catalyst at higher temperatures but give very low yields (Hergenhahn, Bertram, Wiessler, & Sorg, 2003; Mishra, Narain, Mishra, & Misra, 2005). A condensation reaction of arylaldehyde with acetyl acetone–B_2O_3 complex also gives a curcumin glycoside boron complex (Mohri et al., 2003). Attempts have also been made to synthesise curcumin glucoside by enzymatic means using amyloglucosidase (Vijay Kumar & Divakar, 2005) and *Catharanthus roseus* cell cultures by supplying curcumin exogenously (Kaminaga et al., 2003). Recent reports are available on synthesis of oligosaccharide conjugates of curcumin (Shimoda, Hara, Hamada, & Hamada, 2007) and synthesis of PEG curcumin (Safavy et al., 2007). Curcumin glycoside, taken for the present work, was prepared by reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with the potassium salt of curcumin under biphasic conditions, using a phase transfer catalyst under the influence of ultrasound (Parvathy & Srinivas, 2008).

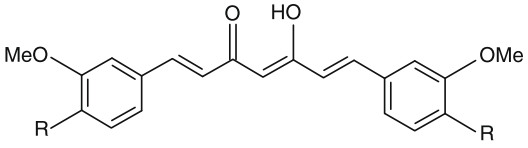
In the present study, we evaluate the biological properties associated with curcumin glucoside with a view to extending the use of curcumin in water-based food and pharmacological formulations. Accordingly, the curcumin-β-diglucoside synthesised was subjected to *in vitro* evaluation of its antioxidant, antimutagenic and antibacterial properties.

2. Materials and methods

2.1. Apparatus and materials

All the solvents and reagents used for the synthesis were of analytical grade. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and β-carotene were procured from Sigma Chemical Co. (St. Louis, MO, USA). Spectrophotometric studies were done on a UV–Visible spectrophotometer (GBC Cintra 10, Australia). ^{1}H NMR spectra for the compounds were recorded on a Bruker Avance 500 MHz spectrometer, using $CDCl_3$ and DMSO-d_6 solvents. Coupling constants (*J* values) are given in Hz. Mass spectral analyses of the synthetic compounds were carried out using MS (Waters Q-Tof Ultima) in the ES positive mode. The ultrasound device used for the reaction was a Vibracell-750 with a tapered probe of tip diameter 6.5 mm from Sonics and Materials Inc., Newtown, USA. It was employed at 25% amplitude with ultrasound frequency of 40 kHz and power of 750 W. Thin-layer chromatographic (TLC) analysis was performed on silica gel 60 F_{254} (Merck, Germany) coated on an alumina sheet and 3% methanol in chloroform was used as the developing solvent. Isolation of the products was carried out by column chromatography on silica gel (100–200 mesh) with chloroform as the eluent. High performance liquid chromatography of samples was carried out on a reverse phase C-18 column with methanol: water (70:30) containing trifluoroacetic acid (0.1%) as the mobile phase at a flow rate of 1 ml/min and monochromatic detection at 423 nm. All the chemicals and Petri plates used for microbial studies were procured from Hi Media Ltd., Mumbai, India.

Table 1
Curcumin and its glucoside derivatives



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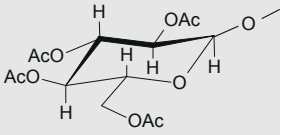
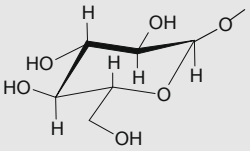
Compound	Structure number	R
Curcumin	**I**	OH
Curcumin di-β-glucoside tetraacetate	**II**	
Curcumin di-β-glucoside	**III**	

2.2. Preparation of curcumin glucoside tetraacetate (CGTA)

Curcumin (**I**, 1 g, 2.7 mmol) was taken in chloroform (40 ml) and, to this, a solution of KOH (0.6 g, 10.8 mmol) in water (20 ml) was added, followed by the addition of a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (2.2 g, 5.35 mmol) in chloroform (40 ml). To the reaction mixture, an aqueous solution of benzyltributylammonium chloride (0.5 g, 1.6 mmol) in water (20 ml) was added. Reaction was subjected to sonication and was monitored by TLC. The product was isolated by normal work-up and the compound purified by chromatographic separation on silica gel (100–200 mesh) with $CHCl_3$ as the eluant. The solvent was evaporated to obtain curcumin di-β-glucoside tetraacetate in 68% yield (**II**, Table 1). ^{1}H NMR ($CDCl_3$), 2.09 (s, 6H, $COCH_3$), 2.11 (s, 6H, $COCH_3$), 2.12 (s, 6H, $COCH_3$), 2.13 (s, 6H, $COCH_3$), 3.97 (s, 6H, OCH_3), 4.25 (dd, 2H, *J* = 3 Hz and 7 Hz), 4.40 (dd, 2H, *J* = 3 Hz and 6 Hz), 4.45 (dd, 2H, *J* = 7 Hz and 12 Hz), 5.25 (dd, 2H, *J* = 4.5 Hz and 5 Hz), 5.58 (d, 2H, *J* = 4 Hz), 5.82 (s, 1H), 5.92 (br, s, 2H), 6.66 (s, 2H), 6.95 (d, 2H, *J* = 8 Hz), 7. 07 (d, 2H, *J* = 2 Hz), 7.14 (dd, 2H, *J* = 2 and 8 Hz), 6.50 (d, 2H, *J* = 16 Hz), 7.61 (d, 2H, *J* = 16 Hz); ESI-MS, *m*/*z* 1051.48 $[M+Na]^+$.

2.3. Preparation of curcumin glucoside (CG)

Curcumin di-β-glucoside tetraacetate (**II**, 1 g) was dissolved in dry methanol (10 ml). To this, sodium methoxide [sodium (60 mg) in dry methanol (8 ml)], was added and the mixture was stirred. The deacetylation reaction was monitored by TLC. At the end of the reaction (30 min), the solution was neutralised by the addition of freshly regenerated Dowex (IR-120) H^+ resin. The resin was filtered and the solvent distilled under reduced pressure to afford pure curcumin di-β-glucoside in 95% yield (**III**). ^{1}H NMR (DMSO-d_6), 3.1–3.4 (m, 8H), 3.6 (m, 3H), 3.77 (s, 6H, OCH_3), 4.31 (br, s, 1H), 4.5 (t, 2H, *J* = 5.5Hz), 4.93 (d, 2H, *J* = 7 Hz), 4.98 (d, 2H, *J* = 5 Hz), 5.06 (br, s, 2H), 5.25 (d, 2H, *J* = 4 Hz), 6.04 (s, 1H), 6.81(d, 2H, *J* = 16 Hz), 7.05 (d, 2H, *J* = 8.5 Hz), 7.18 (d, 2H, *J* = 8 Hz), 7.32 (s, 2H), 7.52 (d, 2H, *J* = 16 Hz); ESI-MS, *m*/*z* 715 $[M+Na]^+$.

2.4. Radical-scavenging activity by DPPH· method

Radical-scavenging activity of curcumin glucoside was analysed by the DPPH**.** method (Moon & Terao, 1998). Individual samples

were prepared by dissolving each compound in DMSO. The samples were assayed at different concentrations, making them up to 1 ml with 100 mM Tris–HCl buffer (pH 7.4), followed by addition of 4 ml of 2,2-diphenyl-1-picrylhydrazyl (0.1 mM solution in methanol) and mixing of the contents by vigorous shaking. A control in these experiments was prepared by same protocol except that the compound was not included. The tubes were incubated in the dark at room temperature for 20 min. The absorbance was then recorded at 517 nm using DMSO for baseline correction. The% radical-scavenging activity was calculated using the following formula:

$$\text{Radical-scavenging activity}(\%) = [(\text{Control OD} - \text{Sample OD})/\text{Control OD}] \times 100 \quad (1)$$

The experiments were carried out in triplicates.

2.5. Antioxidant activity by β-carotene bleaching method

In this assay, antioxidant activity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Adegako et al., 1998). A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.2 mg of β-carotene was dissolved in 0. 2 ml of chloroform (HPLC grade), to which 20 mg linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 40 ml of distilled water saturated with oxygen (30 min, 100 ml/min) were added with vigorous shaking; 4 ml of this stock were dispensed into test tubes and 0.2 ml portions of the samples were added and the emulsion system was incubated for 2 h at 50 °C temperature. The same procedure was repeated for curcumin as positive control and a blank. After this incubation period, absorbance of the mixtures was measured at 470 nm at zero time ($t = 0$) and continued, at intervals of 15 min, until the colour of β-carotene in the control tubes disappeared (180 min) and the antioxidant activity was determined with the formula:

$$\text{Antioxidant property} = 100[1 - (A_0 - A_t)/(A_0^o - A_t^o)] \quad (2)$$

where A_0 and A_0^o are the absorbances measured at zero time for the incubation of the test sample and control, respectively, and A_t and A_t^o are the absorbances measured in the test sample and control after incubation for 180 min.

2.6. Antimutagenicity by Ames test

The standard plate incorporation test was carried out according to Maron and Ames (1983). In the antimutagenicity test, the inhibition of mutagenic activity of the sodium azide by the test samples was determined. Two millilitres of top agar (0.6% agar and 0.25 ml of 0.5 mM histidine–biotin mixture) were dispersed to a 13 × 100 mm capped culture tube held at 45 °C in a water bath and, to it, different concentrations (625, 1250, 2500 and 5000 μg) of the test sample made in DMSO and 0.1 ml of 10-h old culture of either *Salmonella typhimurium* TA 1531 (MTCC 1254, Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India) or *S. typhimurium* TA 98 (MTCC 1251, Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India) were added. It was mixed by vortexing at low speed and then poured onto the minimal glucose agar (1.5% agar with 40% glucose and VB salt) plate to form a uniform thin layer. Positive and negative controls were included in each assay. Sodium azide was used as a diagnostic mutagen (1.5 μg/plate) in positive control plates. Negative control plates were prepared with equivalent amounts of DMSO without sodium azide and the test sample, and used as a check for the number of colonies that arise spontaneously. Only the sample without mutagen was also taken as control. The number of histidine$^+$ (His^+) revertant colonies was counted after incubation of the plates at 37 °C for 48 h. Each sample was assayed using duplicate plates and the data presented as means ± SD of three independent assays. The mutagenicity of sodium azide in the absence of test samples was defined as 100% or 0% inhibition. The calculation of per cent inhibition was done according to the formula given below (Ong, Wang, Stewart, & Brockman, 1986):

$$\%\ \text{Inhibition} = [1 - T/M] \times 100 \quad (3)$$

where T is number of revertants per plate in the presence of mutagen (sodium azide) and test samples and M is number of revertants in the positive control (sodium azide). The number of spontaneous revertants was subtracted from the numerator and denominator. The antimutagenic effect was considered moderate when the inhibitory effect was 25–40% and strong where, it was more than 40%. Inhibitory effects of less than 25% were considered as weak and were not recognised as a positive result (Ilken et al., 1999).

2.7. Antibacterial activity

The antibacterial assay of curcumin and curcumin glucoside was tested by the pour plate method against *Bacillus cereus* (F 4810, Public Health Laboratory, London, UK), *Staphylococcus aureus* (FRI 722, Public Health Laboratory, The Netherlands), *Escherichia coli* (MTCC 108, Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India) and *Yersinia enterocolitica* (MTCC 859, Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India) by the method of Negi, Jayaprakasha, Rao, and Sakariah (1999). Samples were prepared by dissolving the compound in acetone. To flasks containing 20 ml of melted agar, different concentrations of test material in acetone were added. Equivalent amounts of acetone were used as controls. One hundred μl (about 10^3 cfu/ml) of culture were inoculated into the flasks under aseptic conditions. The media were then poured into sterilised Petri plates and incubated at 37 °C for 24 h for growth. The inhibitory effect was calculated using the following formula:

$$\%\text{Inhibition} = (1 - T/C) \times 100 \quad (4)$$

where T is cfu/ml of test sample and C is cfu/ml of control.

Each experiment was done in duplicate and repeated three times. The minimum inhibitory concentration (MIC) was reported as the lowest concentration of the compound capable of inhibiting the complete growth of the bacterium being tested.

2.8. Statistical analysis

All the experiments were repeated three times and the data were calculated as means ± SD. The data of all the assays were analysed by one-way ANOVA. Duncan's multiple range test (DMRT) was used to make the comparisons (Gomez & Gomez, 1984).

3. Results and discussion

3.1. Synthesis of curcumin glucoside (CG)

CG was prepared in high yields under ultrasonic conditions by a Koenigs–Knorr-type reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-1-bromoglucose with curcumin, which is facilitated by the combined effect of ultrasound and phase transfer catalyst that promotes proper mixing, along with better mass transfer of reactants between the two phases. MS analysis of the product [ESI-MS] showed a molecular mass ion at m/z 1051 $[M+Na]^+$ corresponding to the

diglucoside tetracetate of curcumin. Further, ^{1}H NMR confirmed it as curcumin-β-diglucoside. The nucleophilic substitution takes place by inversion of the configuration at the anomeric position of the sugar moiety, resulting in the formation of β-glucoside. Further, the diglucoside tetracetate of curcumin was subjected to deacetylation and the product formed had a molecular ion peak at 715 $[M+Na]^+$ corresponding to curcumin-β-diglucoside.

3.2. Solubility of curcumin glucoside

The solubility of curcumin in water was always a matter of concern because of its insolubility at neutral and acidic pH. The modification of curcumin by introducing a sugar moiety to its phenolic hydroxyl group enhanced its water solubility. CG showed a solubility of 10 mg/ml. The antioxidant, antimutagenic and antibacterial activities of the synthesised CG was evaluated by *in vitro* methods.

3.3. Radical-scavenging and antioxidant activities of curcumin glucoside

Radical-scavenging assay was done by taking curcumin as a reference for comparison with the water-soluble CG. Curcumin was dissolved in DMSO at 10–50 ppm levels, which corresponded to 0.027–0.135 μM concentrations; similarly CG was dissolved in DMSO at 10–50 ppm levels, which corresponded to 0.015–0.072 μM concentrations. The radical-scavenging activity (RSA) by the DPPH**.** method showed 28% and 44% activity at 0.027 μM curcumin and 0.029 μM of CG, respectively (Fig. 1). At 0.05 μM, RSA values of curcumin and CG were 41% and 50%, respectively. However, RSA values of curcumin and CG at both of these concentrations were not significantly different from each other ($p < 0.05$).

The antioxidant activity by the β-carotene bleaching method (Fig. 2) was carried out. Curcumin showed 27% activity at 0.027 μM and CG had 46% activity at 0.029 μM, which were statistically ($p < 0.05$) different. At 0.058 μM, CG showed significantly ($p < 0.05$) higher activity (56%) than did curcumin at 0.054 μM (41%); similarly, at 0.07 μM concentration, CG showed much higher antioxidant activity (75%) than did curcumin at 0.08 μM (42%). These results clearly showed that CG had higher antioxidant activity than had curcumin at all concentrations.

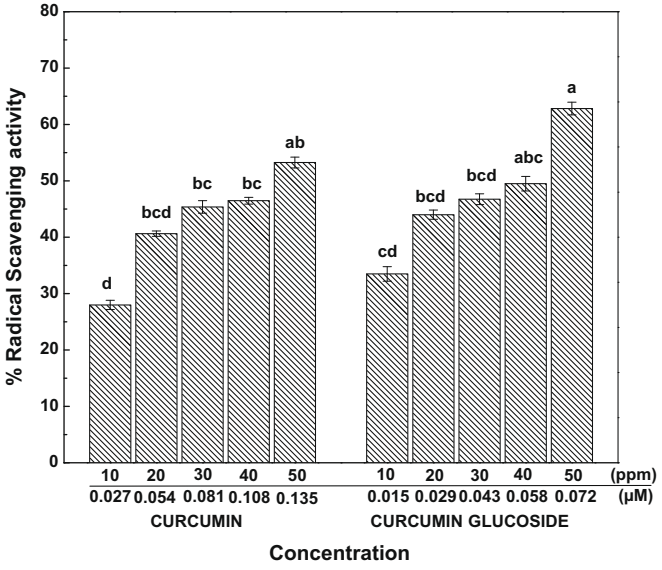


Fig. 1. Radical-scavenging activity of curcumin and curcumin glucoside by DPPH**.** method.

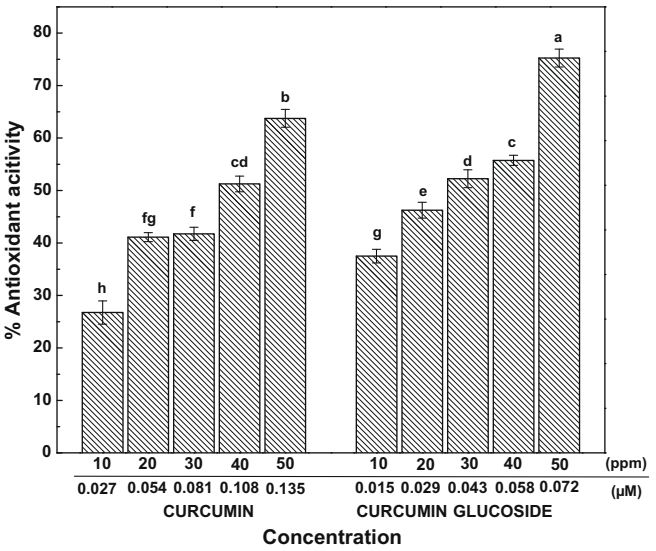


Fig. 2. Antioxidant activity of curcumin and curcumin glucoside by β-carotene bleaching method.

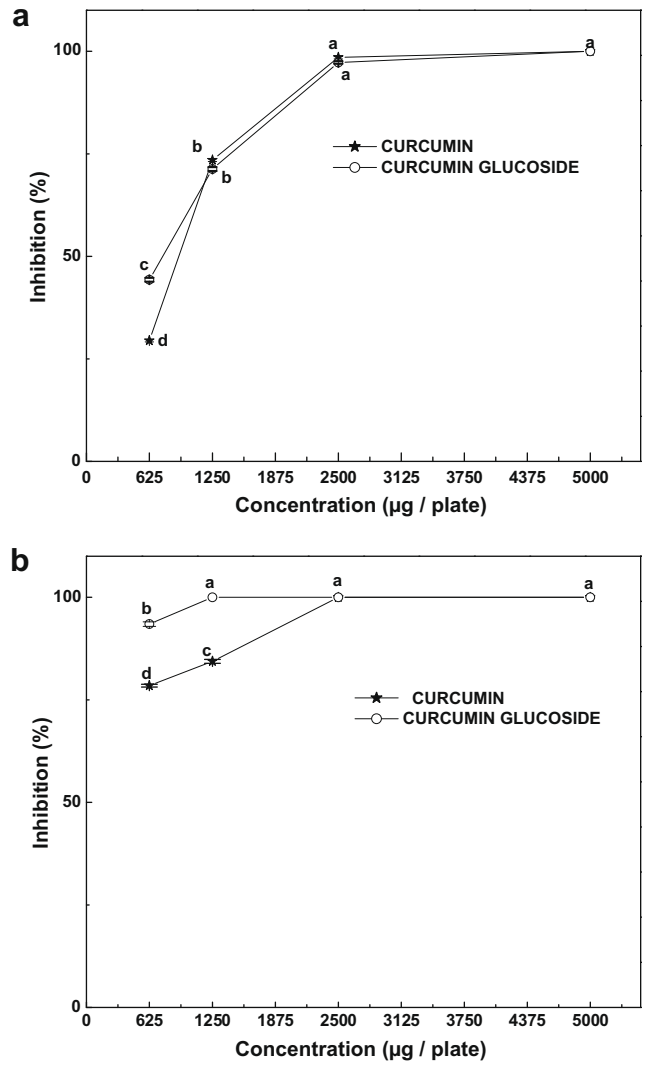


Fig. 3. Inhibitory effect of curcumin and curcumin glucoside against the mutagenicity of sodium azide to (a) *S. typhimurium* TA 1531 and (b) *S. typhimurium* TA 98.

Table 2
Minimum inhibitory concentration (μM)* of curcumin and curcumin glucoside.

Bacteria	MIC of Curcumin (μM)	MIC of Curcumin glucoside (μM)
Bacillus cereus	0.135	0.181
Staphylococcus aureus	0.081	0.051
Escherichia coli	0.611	0.469
Yersinia enterocolitica	0.679	0.867

* The results of four experiments performed in duplicates.

3.4. Antimutagenicity of curcumin glucoside by Ames test

Antimutagenicity studies of curcumin and CG were carried out with *S. typhimurium* TA 1531, as well as TA 98, by Ames test against sodium azide as a mutagen. Curcumin is known to be a good antimutagen (Goud, Polasa, & Krishnaswamy, 1993). The results indicated that CG possesses as strong an antimutagenic property as does curcumin (Fig. 3). Both curcumin and CG showed strong antimutagenic activities with TA 98 against sodium azide at all the concentrations tested. In the case of TA 1531 (Fig. 3a), curcumin had moderate activity at 625 μg/plate, whereas CG showed strong and significantly higher ($p < 0.05$) antimutagenic activity at a similar concentration. Curcumin had strong antimutagenic activities at higher concentrations, which were statistically similar to those of CG. In the case of TA 98 (Fig. 3b), CG had significantly higher ($p < 0.05$) antimutagenic activity at 625 and 1250 μg/plate concentrations than had curcumin, but, at higher concentrations, they both showed similar activities. Our results indicated higher antimutagenic potential of CG than of curcumin. Free radicals are implicated in many physiological disorders, such as inflammation, ageing and carcinogenicity (Namiki, 1990), but oxygen-scavenging can reduce these effects (Surh, 1999). Hochstein and Atallah (1988) suggested that compounds having antioxidant potential could also inhibit mutagenicity and cancer. Our results support these observations, as reflected by the antioxidant and antimutagenic activities of curcumin and CG.

The antioxidant activity and antimutagenicity studies showed that CG was as potent as was curcumin. In the former, the phenolic hydroxyl of curcumin was substituted with glucose, blocking the route of the HAT (hydrogen atom transfer) mechanism of

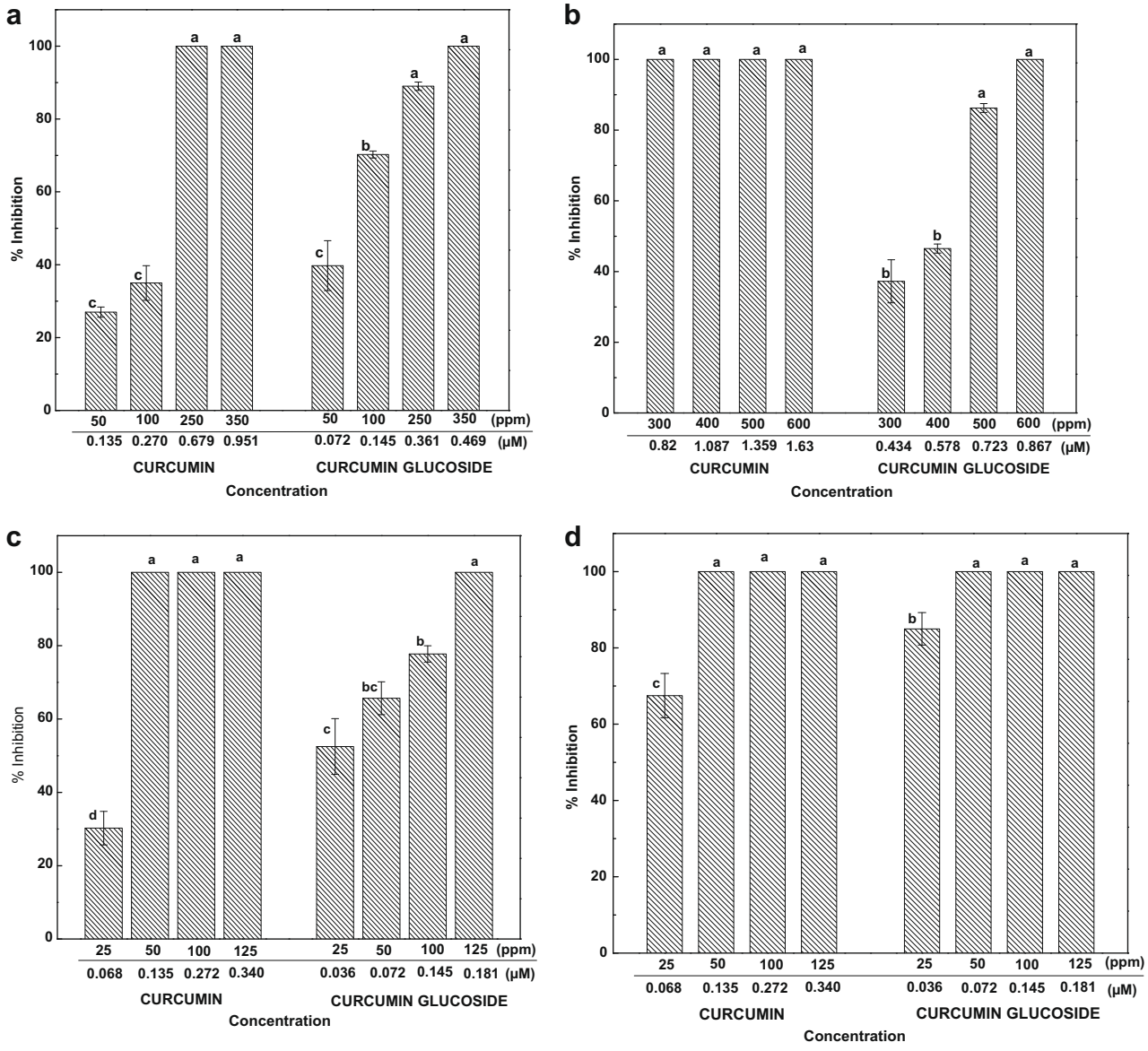


Fig. 4. Anti-bacterial activities of curcumin and curcumin glucoside against (a) *Escherichia coli*, (b) *Yersinia enterocolitica*, (c) *Bacillus cereus* and (d) *Staphylococcus aureus*.

antioxidant activity. CG was found to have greater antioxidant and antimutagenic properties after its modifications at the phenolic hydroxylic positions. Here, the mechanism involved may be sequential proton loss electron transfer (SPLET), which is supposed to be faster than other mechanisms, which means that the keto–enol moiety of curcumin has a more easily dissociable proton, the enolic proton being more acidic than the other two hydroxyls of the phenol rings in curcumin (Litwinienko & Ingold, 2004). This hypothesis of sequential proton loss electron transfer (SPLET) in curcumin was also supported by some theoretical calculations, e.g. density functional theory (DFT) and time-dependent density functional theory (TD-DFT) (Shen & Ji, 2007).

3.5. Antibacterial activity of curcumin glucoside

Further, curcumin and CG were tested for their antimicrobial potential against two Gram-positive (*B. cereus* and *S. aureus*) and two Gram-negative bacteria (*E. coli* and *Y. enterocolitica*). MIC of the compounds is presented in Table 2. Fig. 4a–d shows the inhibition of bacteria at different concentrations of curcumin and CG. Curcumin and its glucoside showed 27% and 70% inhibition in the case of *E. coli* at 0.135 μM and 0.145 μM concentrations, respectively, which were statistically different ($p < 0.05$). CG showed 100% inhibition at 0.469 μM but, for curcumin, 100% inhibition was obtained only at 0.611 μM. In the case of *Y. enterocolitica*, 0.679 μM curcumin showed 100% inhibition whereas a similar inhibition with CG was observed at 0.867 μM.

The growth inhibition by these compounds against *B. cereus* showed that curcumin inhibited 30% of growth, whereas CG showed 66% inhibition at 0.07 μM, which were significantly different ($p < 0.05$). At 0.135 μM, curcumin showed 100% inhibition whilst CG showed only 77% inhibition at 0.14 μM, which was statistically lower ($p < 0.05$) than the inhibition shown by curcumin at slightly lower concentrations. In the case of *S. aureus*, curcumin showed 67% inhibition at 0.07 μM, whilst CG showed complete inhibition at 0.051 μM.

Certain curcumin bio-conjugates containing esters and peptides showed enhancement in antifungal and antibacterial activities, which was attributed to better cellular uptake, increased cellular concentration and better receptor binding (Kapoor, Narain, & Misra, 2007), as observed in higher antimicrobial activity of CG against *S. aureus* and *E. coli*. However, an enhancement of bacterial growth in the case of *B. cereus* and *Y. enterocolitica* was observed when the growth medium was supplemented with glucose (Rhee, Diaz Ricci, Bode, & Schugerl, 1994). The high MIC value found for *B. cereus* and *Y. enterocolitica* may be due to the fact that the bacterial enzyme system can cleave the glycosidic linkage and the bacteria can then utilise the released glucose.

In conclusion, the results of these *in vitro* biological assays demonstrate that CG has antioxidant, antimutagenic and antibacterial properties similar to or better than those of curcumin. The molecule may be a good candidate for use in water-based food formulations and pharmacological applications. The results show that the modification carried out with curcumin at its phenolic hydroxyl positions renders the curcumin molecule water-soluble but does not affect its antioxidant, antimutagenic or antibacterial properties.

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