Curcuma longa and Curcuma mangga leaves exhibit functional food property

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A B S T R A C T

Although leaves of *Curcuma mangga* and *Curcuma longa* are used in food preparations, the bioactive components in it are not known. In this study, antioxidant, antiinflammatory and anticancer activities of leave extracts and its isolates were investigated using established bioassay procedures in our laboratory. The leaf extracts of both plants gave similar bioassay and chromatographic profiles. The methanolic and water extracts of *C. mangga* (CMM and CMW) and *C. longa* (CLM and CLW), at 100 µg/mL, inhibited lipid peroxidation (LPO) by 78%, 63%, 81% and 43%, cyclooxygenase enzymes COX-1 by 55%, 33%, 43% and 24% and COX-2 by 65%, 55%, 77% and 69%, respectively. At same concentration, CMM, CMW, CLM and CLW showed growth inhibition of human tumour cell lines by 0–46%. Therefore, a bioassay-guided isolation of water and methanolic extracts of *C. longa* was carried out and afforded nine isolates. At 25 µg/mL, these compounds inhibited LPO by 11–87%, COX-1 and -2 enzymes by 0–35% and 0–82% and growth of human tumour cells by 0–36%, respectively.

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1. Introduction

Curcuma genus (zingiberaceae family) contains more than 80 species and among them *Curcuma mangga* and *Curcuma longa* are most used for the preparation of food, supplements and traditional medicine (Kurup, 1979). The rhizomes of these two plants have been investigated for antioxidant, antiinflammatory, insect antifeedant, antiviral, cytotoxic and trypanocidal activities and in the treatment of Alzheimer's disease, cancer, arthritis, and other clinical disorders. Curcuminoids, labdane, halimane and clerodane type diterpenoids are considered as the major biological constituents of *Curcuma* genus (Aggarwal, Kumar, & Bharti, 2003; Anand, Kunnumakkara, Newman, & Aggarwal, 2007; Hatcher, Planalp, Cho, Torti, & Torti, 2008; Kita, Imai, Sawada, & Seto, 2009; Roth, Chandra, & Nair, 1998; Silva, Gomes, & Rodilla, 2011). In south-east Asia, turmeric leaf is one of the ingredients added to various dishes for flavour, and believed to be beneficial for health. The aromatic leaves of *C. longa* and *C. mangga* are also used for flavouring steamed and baked fish. The turmeric leaves contained labda-8(17), 12-diene-15, 16 dial with antifungal and mosquitocidal activity (Roth et al., 1998) and several phenolic compounds. Ethanolic extract of *C. longa* leaves showed strong antioxidant activity and prevented accelerated oxidation of prepared food (Nor, Mohamed, Idris, & Ismail, 2009).

Although a limited number of publications report on leaf constituents of *C. longa*, studies on bioactive compounds in *C. mangga* leaves are not available. Also, the antioxidant, antiinflammatory and anticancer activities of leaf constituents of these plants in the context of functional food are not in the literature. In this study, the antioxidant activity was determined by MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] (Liu & Nair, 2010) and lipid peroxidation (LPO) inhibitory assays (Mulabagal, Lang, DeWitt, Dalavoy, & Nair, 2009). Similarly, the leaf extracts and its isolates were evaluated for antiinflammatory activity by cyclooxygenase enzymes (COX-1 and -2) inhibitory assays (Seeram, Cichewicz, Chandra, & Nair, 2003) and tumour cell proliferation inhibition by human breast, colon, gastric, lung, pancreas, prostate and central nervous system tumour cell lines (Jayaprakasam, Zhang, & Nair, 2004).

2. Materials and methods

2.1. General experimental procedures

All solvents used for isolation and purification were of ACS reagent grade (Sigma–Aldrich Chemical Company (St. Louis, MO, USA)). Preparative HPLC was performed on a recycling preparative HPLC (Japan Analytical Industry Co. model LC-20) with tandem C_{18} column (JAIGEL, 10 mm, 20 × 250 mm) at the flow rate of 4 ml/min. NMR spectra were recorded on a 500 (Varian Unity ± 500, ^{1}H NMR) and 125 (Varian Unity ± 500, ^{13}C NMR) MHz VRX instruments. MTT [3-(4,5-dimethylthiazole-2-yl)-2,

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5-diphenyltetrazolium bromide] for MTT antioxidant assay, tert-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) for lipid peroxidation assay, and positive controls aspirin, naproxen and ibuprofen used in COX inhibitory assay were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). COX-1 and -2 enzymes were prepared in our laboratory from ram seminal vesicles (Oxford Biomedical Research, Inc., Oxford, MI) and insect cells cloned with human PGHS-2 enzyme, respectively. Arachidonic acid was purchased from Oxford Biomedical Research, Inc. (Oxford Biomedical Research, Inc., Oxford, MI). Similarly, the nonsteroidal antiinflammatory drugs (NSAIDs) celeberex® and vioxx® (currently not sold as an NSAID) were physician's professional samples provided by Dr. Subhash Gupta, Sparrow Pain Center, Sparrow Hospital, Lansing, Michigan. All enzymes and reagents were stored in the Bioactive Natural Products and Phytoceuticals Laboratory at Michigan State University (East Lansing, MI).

2.2. Extraction of C. longa and C. mangga leaves

C. longa (Accession No.: MSC 396404) and C. mangga (Accession No.: MSC 396405) plants were grown from rhizomes in 1-gallon pots containing sterile potting medium, fertilized every 2 weeks in the greenhouses of Bioactive Natural products and Phytoceuticals Laboratory at Michigan State University, East Lansing, MI. The plants were grown for about 10 months prior to the harvest of fresh leaves. The fresh leaves were then extracted immediately after harvesting.

Fresh leaves of C. longa (1 kg) were blended with water (2000 mL), and then transferred to a glass column (20 × 100 cm). The column was successively eluted with water (2000 mL × 3) and methanol (2000 mL × 3), respectively. The water extract was lyophilized to yield a powder (32.5 g). The methanolic extract was evaporated under vacuum to afford a greenish gum (8.5 g). Similarly, fresh C. mangga (500 g) was extracted to yield water extract (18.6 g) and methanolic extract (4 g). TLC profiles indicated C. longa and C. mangga extracts were identical and water extracts contained largely sugars with minor other compounds. These minor compounds were also found in the methanolic extracts. Hence, water and methanolic extracts were combined to avoid repeated isolation. An aliquot of the combined water and methanolic extracts (20 g) was successively stirred with hexane (200 mL × 3), chloroform (200 mL × 3), and methanol (200 mL × 3) to yield hexane-soluble (3.3 g), chloroform-soluble (2.3 g) and methanol-soluble (8.1 g) fractions and a residue (9.2 g), respectively. TLC profiles [CHCl₃/MeOH (4:1) and hexane–EtOAc (4:1)] showed that the chloroform soluble fraction contained primarily chlorophyll and hence was not investigated further.

2.3. Isolation of compounds in C. longa leaf extract

The extracts of C. mangga and C. longa leaves were analyzed by TLC under identical conditions [CHCl₃/MeOH (4:1) and hexane–EtOAc (4:1)] on the same plate and showed identical profile. Also, the extracts showed similar bioactivities in LPO and COX enzyme assays. Therefore, in our study, C. longa was selected for the isolation based on the availability of extract as well as the concentration of major constituents present in the extracts.

The hexane soluble fraction (3 g) was fractionated by using a silica gel column and eluted with hexane–acetone (15:1, v/v) to yield 25 fractions (15 mL/fraction). Pooling of these fractions, based on their TLC profiles, afforded fractions A: 200 mg; B: 1000 mg; C: 800 mg and D: 900 mg. Fraction B was purified to yield compound **1** (36 mg) by PTLC (Analtech, Inc., Newark, DE) (hexane–acetone, 15:1, v/v). Fraction C (500 mg) contained primarily chlorophyll by TLC and hence chromatographed on a Sephadex

LH-20 column. The column was eluted with methanol (500 mL) to yield chlorophyll-free fraction C (125 mg). It was further purified by PTLC (hexane–acetone, 10:1, v/v) to afford compounds **2** (R_f = 0.4; 42 mg) and **3** (R_f = 0.3; 21 mg). Fraction D, mainly chlorophyll, was not purified further.

The methanol-soluble fraction (2.5 g) was chromatographed on a Sephadex LH-20. The column was then eluted with methanol (1000 mL) to remove chlorophyll. The resulting chlorophyll-free fraction, eluted with methanol (100%, 1 g), was purified by preparative HPLC (Japan Analytical Industry Co. model LC-20; column: C18 (JAIGEL, 10 mm, 20 × 250 mm); solvent system: 30% methanol–70% water, v/v; flow rate: 4 mL/min; wavelength: 280 nm, 100 mg sample per injection) to yield compounds **4** (123 mg, R_t = 14.77 min), **5** (15 mg, R_t = 30.99 min), **6** (14 mg, R_t = 38.56 min), **7** (11 mg, R_t = 51.07 min), **8** (20 mg, R_t = 75.92 min) and **9** (10 mg, R_t = 10 8.51 min).

2.3.1. Compound **1**

Colourless oil, ¹H NMR (500 MHz, CDCl₃): δ 7.20 (1H, s, H-12), 5.93 (1H, brs, H-5), 5.31 (1H, br d, J = 8.5 Hz, H-1), 3.82 (2H, brs, H-9), 2.11 (3H, brs, 14-Me), 2.12 (3H, s, 13-Me), and 1.42 (3H, s, 15-Me); ¹³C NMR (500 MHz, CDCl₃): δ 130.4 (C-1), 26.3 (C-2), 41.6 (C-3), 145.7 (C-4), 132.3 (C-5), 189.7 (C-6), 122.1 (C-7), 156.4 (C-8), 40.6 (C-9), 135.3 (C-10), 123.6 (C-11), 138.0 (C-12), 9.5 (C-13), 18.9 (C-14), and 15.7 (C-15). Therefore, the structure
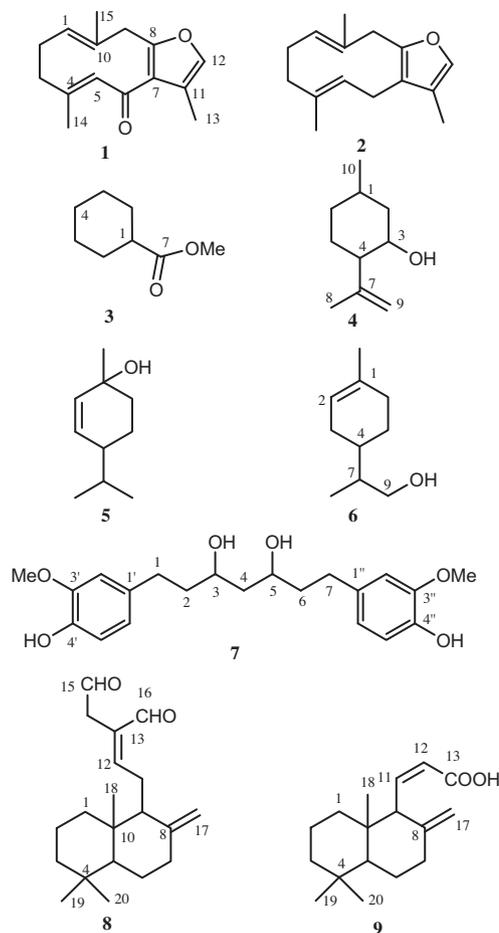

Fig. 1. Structures of 8,12-epoxygermacra-1(10), 4,7,11-tetraen-6-one (**1**), 8,12-epoxygermacra-1(10), 4,7,11-tetraene (**2**), cyclohexanecarboxylic acid methyl ester (**3**), isopulegol (**4**), 2-menthen-1-ol (**5**), menth-1-en-9-ol (**6**), octahydrocurcumin (**7**), labda-8(17)-12-diene-15, 16-dial (**8**), and coronadiene (**9**) isolated from C. longa and C. mangga leaves.

assigned to compound **1** was 8,12-epoxygermacra-1(10), 4,7,11-tetraen-6-one (Dekebo, Dagne, Hansen, Gautunb, & Aasen, 2000).

2.3.2. Compound **2**

Pale yellow oil, ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.39 (1H, s, H-12), 5.07 (1H, t, J = 6.0 Hz, H-1), 4.86 (1H, t, J = 7.5 Hz, H-5), 3.65 (1H, d, J = 16 Hz, H-9a), 3.58 (1H, 16 Hz, H-9b), 3.21 (2H, J = 6.5 Hz, H-6), 2.05 (3H, s, 13-Me), 1.73 (3H, s, 14-Me), and 1.34 (3H, s, 15-Me); ^{13}C NMR (500 MHz, CDCl$_3$): δ 129.0 (C-1), 24.4 (C-2), 26.8 (C-3), 128.8 (C-4), 127.6 (C-5), 39.5 (C-6), 118.9 (C-7), 149.7 (C-8), 40.9 (C-9), 134.3 (C-10), 121.9 (C-11), 136.0 (C-12), 8.9 (C-13), 16.5 (C-14), and 16.2 (C-15). From the spectral data, compound **2** was identified as 8,12-epoxygermacra-1(10), 4,7,11-tetraene (Baldovini, Tomi, & Casanova, 2001).

2.3.3. Compound **3**

Colourless oil, ^{1}H NMR (500 MHz, CDCl$_3$): δ 1.1–3.4 (10H, m, ring protons), and 3.6 (s, 3H, CH$_3$); 13C NMR (125 MHz, CDCl$_3$): δ 24.8 (C-3, 5), 25.71 (C-2, 6), 29.9 (C-4), 32.1 (C-1), 51.4 (OMe), and 174.2 (C-7). According to the spectral data, compound **3** was characterized as cyclohexanecarboxylic acid methyl ester (Kirk, Harman, & Blanksby, 2010).

2.3.4. Compound **4**

Colourless oil, ^{1}H NMR (500 MHz, MeOD): δ 4.64 (s, 1H, H-8a), 4.38 (s, 1H, H-8b), 1.78 (s, 3H, 9-Me), 0.98 (d, J = 6.5 Hz, 3H, 10-Me), and 0.88 (d, J = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, MeOD): δ 146.1 (C-7), 115.9 (C-8), 64.6 (C-3), 47.3 (C-4), 37.9 (C-2), 29.6 (C-6), 22.0 (C-1), 20.7 (C-10), 20.6 (C-5), and 15.6 (C-9). Therefore, compound **4** was characterized as isopulegol (Yadav, Vijaya Bhasker, & Srihari, 2010).

2.3.5. Compound **5**

Colourless oil, ^{1}H NMR (500 MHz, MeOD): δ 0.83 (3H, d, J = 6.5 Hz, 8-Me), 0.94 (3H, d, J = 6.5 Hz, 9-Me), 1.27 (s, 3H, 10-Me), 5.68 (1H, d, J = 10 Hz, H-2), and 5.10 (1H, dd, J = 10, 15 Hz, H-1); ^{13}C NMR (125 MHz, MeOD): δ 18.4 (C-9), 18.5 (C-10), 22.7 (C-7), 28.2 (C-5), 31.2 (C-8), 37.3 (C-4), 40.6 (C-6), 72.1 (C-1), 130.6 (C-3), and 131.2 (C-2). Based on the spectral data, compound **5** was charecterized as 2-menthen-1-ol (Blair & Tuck, 2009).

2.3.6. Compound **6**

Colourless oil, ^{1}H NMR (400 MHz, MeOD): δ 5.39–5.35 (m, 1H, H-1), 3.05 (2H, d, H-8), 2.08–1.88 (m, 2H), 1.64 (s, 3H, 10-Me), and 0.91 (d, J = 6.5 Hz, 3H, 9-Me); ^{13}C NMR (125 MHz, MeOD): δ 138.6 (C-1), 127.4 (C-2), 62.2 (C-9), 43.0 (C-8), 35.5 (C-6), 35.2 (C-5), 30.0 (C-7), 28.9 (C-4), 26.7 (C-3), and 19.5 (C-10). Based on the spectral data, compound **6** was characterized as menth-1-en-9-ol (Serra, Fuganti, & Gatti, 2008).

2.3.7. Compound **7**

Colourless oil, ^{1}H NMR (500 MHz, CDCl$_3$): δ 1.61 (2H, m), 1.70 (4H, m), 2.52–2.66 (4H, m), 3.81 (6H, s, OCH$_3$ × 2), 3.71 (2H, brs), 6.59 (2H, d, J = 8 Hz), 6.63 (2H, bs), and 6.76 (2H, bd, J = 8 Hz); ^{13}C NMR (125 MHz, CDCl$_3$): δ 31.1 (C-1, 7), 39.8 (C-2, 6), 43.0 (C-4), 54.4 (OMe), 69.0 (C-3, 5), 111.2 (C-5′, 5″), 114.2 (C-2′, 2″), 119.9 (C-6′, 6″), 133.3 (C-1′, 1″), 143.6 (C-4′, 4″), and 146.9 (C-3′, 3″). From the spectral data, compound **7** was identified as octahydrocurcumin (Ohtsu et al., 2002).

2.3.8. Compound **8**

Wax-like solid, ^{1}H NMR (500 MHz, CDCl$_3$): δ 0.63, 0.78, 0.85 (3H, each s, H-18, 19 and 20). 4.36 (1H, d, J = 1.5 Hz, H-17a), 4.85 (1H, d, J = 1.5 Hz, H-17b), 6.65 (1H, d, J = 7 Hz, H-12), 9.53 (1H, s, H-16), and 9.65 (1H, t, J = 1.0 Hz, H-15); ^{13}C NMR (125 MHz, CDCl$_3$): δ 14.5 (C-20), 19.4 (C-2), 21.7 (C-19), 24.2 (C-6), 24.5 (C-11), 29.3 (C-14), 33.4 (C-18), 37.9 (C-7), 39.4 (C-1), 39.2 (C-4), 39.6 (C-10), 41.9 (C-3), 55.8 (C-5), 56.5 (C-9), 107.4 (C-17), 135.1 (C-13), 147.9 (C-8), 159.8 (C-12), 193.9 (C-16), and 197.5 (C-15). Based on the spectral data, compound **8** was identified as labda-8(17)-12-diene-15, 16-dial (Liu & Nair, 2011).

2.3.9. Compound **9**

Wax-like solid, ^{1}H NMR (500 MHz, CDCl$_3$): δ 6.67 (1H, dd, J = 3.5, 7 Hz, H-11), 5.44 (1H, dd, J = 3.5, 7 Hz, H-12), 4.80 (1H, brs, H-17a), 4.29 (1H, brs, H-17b), 0.80, 0.74, 0.64 (3H, each s, H-18, 19 and 20); ^{13}C NMR (125 MHz, CDCl$_3$): δ 171.2 (C-13), 148.3 (C-11), 144.3 (C-6), 124.7 (C-12), 107.7 (C-17), 56.4 (C-9), 55.6 (C-5), 42.2 (C-3), 39.7 (C-1), 39.5 (C-10), 38.0 (C-7), 34.5 (C-4), 33.8 (C-18), 25.7 (C-6), 24.3 (C-19), 21.9 (C-2), and 14.6 (C-20). Based on the spectral data, compound **9** was identified as coronadiene (Nakamura et al., 2008).
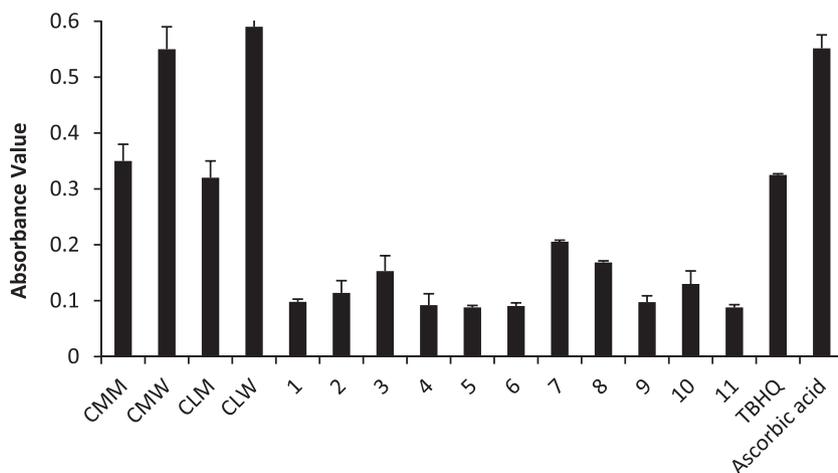

Fig. 2. Absorbance value of water and methanolic extracts (CLW: *C. longa* water extract; CLM: *C. longa* methanolic extract; CMW: *C. mangga* water extract; CMM: *C. mangga* methanolic extracts) and isolates **1–9** at 570 nm obtained after reaction with MTT at 37 °C at the concentration of 25 and 100 µg/mL, respectively. TBHQ and ascorbic acid were used as positive controls and tested at 25 µg/mL concentration. The standard error mean was represented for n = 4. (P < 0.05, t-test, paired, and two tailed).
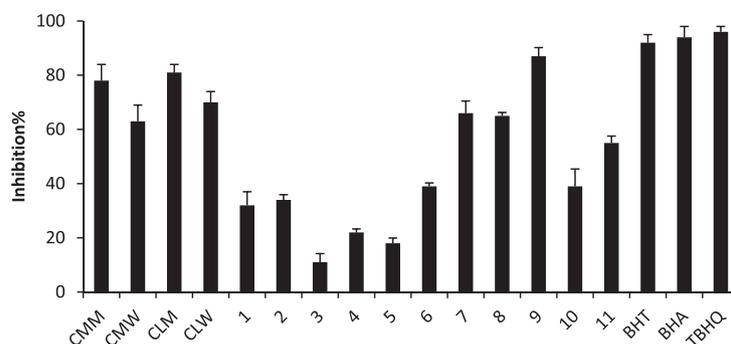
Fig. 3. Inhibition of LPO by extracts of *C. longa* and *C. mangga* leaves (CLW: *C. longa* water extract; CLM: *C. longa* methanolic extract; CMW: *C. mangga* water extract; CMM: *C. mangga* methanolic extracts) at 100 μg/mL, and by compounds **1**–**9** at 25 μg/mL. Commercial antioxidants BHA, BHT and TBHQ were tested at 1 μg/mL. The oxidation of lipid was initiated by the addition of Fe^{2+} ions and vertical bars represent the standard deviation of each data point ($n = 2$). The varying concentrations of positive controls used were to yield a comparable activity profiles between 0–100% by test extracts, compounds and positive controls alike. The standard error mean was represented for $n = 4$. ($P < 0.05$, *t*-test, paired, and two tailed).

2.4. MTT antioxidant assay

MTT assay was performed according to the reported procedure (Liu & Nair, 2010). Test compounds (**1**–**9**, Fig. 1) and extracts (CMM, CMW, CLM and CLW) were assayed at 25 and 100 μg/mL, respectively. The absorbance of reaction mixture was measured in a 96-well cell culture plate at 570 nm. The absorbance values represent the antioxidant potential of test sample or its ability to reduce an oxidizing agent (Liu & Nair, 2010). Each sample was assayed in duplicate. Ascorbic acid and TBHQ were used as positive controls.

2.5. Lipid peroxidation inhibitory assay

Large unilamellar vesicles (LUV) or liposome suspension was prepared and the test samples were assayed for their LPO inhibitory activities by using the fluorescence spectroscopy according to the reported procedure (Mulabagal et al., 2009). The decrease of relative fluorescence intensity of test solution over the time indicated the rate of peroxidation. The percentage of inhibition was calculated with respect to DMSO control. The extracts and compounds (**1**–**9**) were respectively tested at 100 and 25 mg/mL and the positive controls BHA, BHT and TBHQ were tested at 10 mM.

2.6. COX-1 and -2 enzymes inhibitory assay

The inhibitory effects of test samples on COX-1 and -2 were measured by monitoring the initial rate of O_2 uptake using an oxygen electrode (Instech laboratories, Plymouth Meeting, PA) attached to a biological oxygen monitor (Yellow Spring Instrument, Inc., Yellow Spring, OH) at 37 °C as per published procedure (Liu & Nair, 2011; Seeram et al., 2003). The extracts and compounds (**1**–**9**) were tested at 100 and 25 μg/mL concentrations, respectively. The positive controls, commercial NSAIDs aspirin, celebrex, vioxx, naproxen, and ibuprofen were tested at 108, 1, 1, 15 and 12 μg/mL or 60, 26, 32, 11 and 10 μM, respectively. The potency of enzyme inhibition varied with each non-steroidal anti-inflammatory agent (NSAIDs). Therefore, a varying concentration of the positive controls was necessary to optimize their COX enzyme inhibitory activity or not to exceed 100%.

2.7. Tumour cell proliferation assay

The extracts (CMM, CMW, CLM and CLW) and compounds **1**–**9** were assayed for tumour cell growth inhibition as per published method (Jayaprakasam et al., 2004). MCF-7 (breast), SF-268 (CNS), NCI-H460 (lung), HCT-116 (colon), AGS (gastric), MI aPaCa-2 and BxPc-3 (pancreatic), and LNCaP and DU-145 (prostate) human
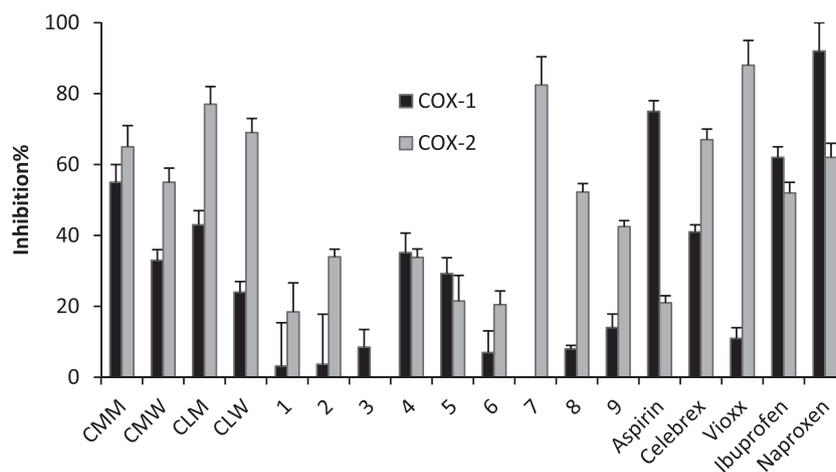

Fig. 4. COX-1 and COX-2 enzyme inhibitory activities of extracts of *C. longa* and *C. mangga* leaves (CLW: *C. longa* water extract; CLM: *C. longa* methanolic extract; CMW: *C. mangga* water extract; CMM: *C. mangga* methanolic extracts) at 100 μg/mL, compounds **1**–**9** at 25 μg/mL and commercial NSAIDs aspirin, celebrex®, vioxx®, naproxen and ibuprofen used as positive control at 108, 1, 1, 12, and 15 μg/mL, respectively. Vertical bars represent the standard deviation of each data point ($n = 2$). The varying concentrations of positive controls used were to yield a comparable activity profiles between 0–100% by test extracts, compounds and positive controls alike. The standard error mean was represented for $n = 4$. ($P < 0.05$, *t*-test, paired, and two tailed).

tumour cells were cultured in RPMI-1640 medium containing pen-icillin–streptomycin and 10% foetal bovine serum (FBS). Aliquots of 100 μL of test compounds were added to each well containing the test tumour cell line (100 μL). After incubation for 48 h, an aliquot (25 μL) of MTT solution was added and the plates incubated for 3 h at 37 °C. The medium was removed from each well and cells treated with DMSO (200 μL). The plates were then shaken and optical density was measured using a microplate reader at 570 nm. Adriamycin was used as positive control in this assay. The samples were assayed in duplicate.

3. Results and discussion

Initially, the extracts (CMM, CMW, CLM and CLW) of *C. mangga* and *C. longa* leaf were analyzed by TLC under identical conditions on the same plate. The TLC profiles showed that both leaf extracts contained identical compounds with slight variation in their concentrations. Therefore, in our study, *C. longa* was selected for the isolation based on the availability of extract as well as the concentration of major constituents present in the extracts. The mass balance showed that the extracts contained about 40% of chlorophyll. Hence, removal of chlorophyll was critical prior to isolation and purification of compounds. After trying different chromatographic techniques, the chlorophyll was successfully removed by a Sephadex LH-20 column with methanol as the eluant. Further purification of chlorophyll-free *C. longa* leaf extract by chromatographic techniques such as MPLC, PTLC, CombiFlash column chromatography and preparative HPLC afforded compounds **1–9** (Fig. 1). The structures of these compounds were assigned as 8,12-epoxyger-macra-1(10), 4,7,11-tetraen-6-one **(1)**, 8,12-epoxygermacra-1(10),
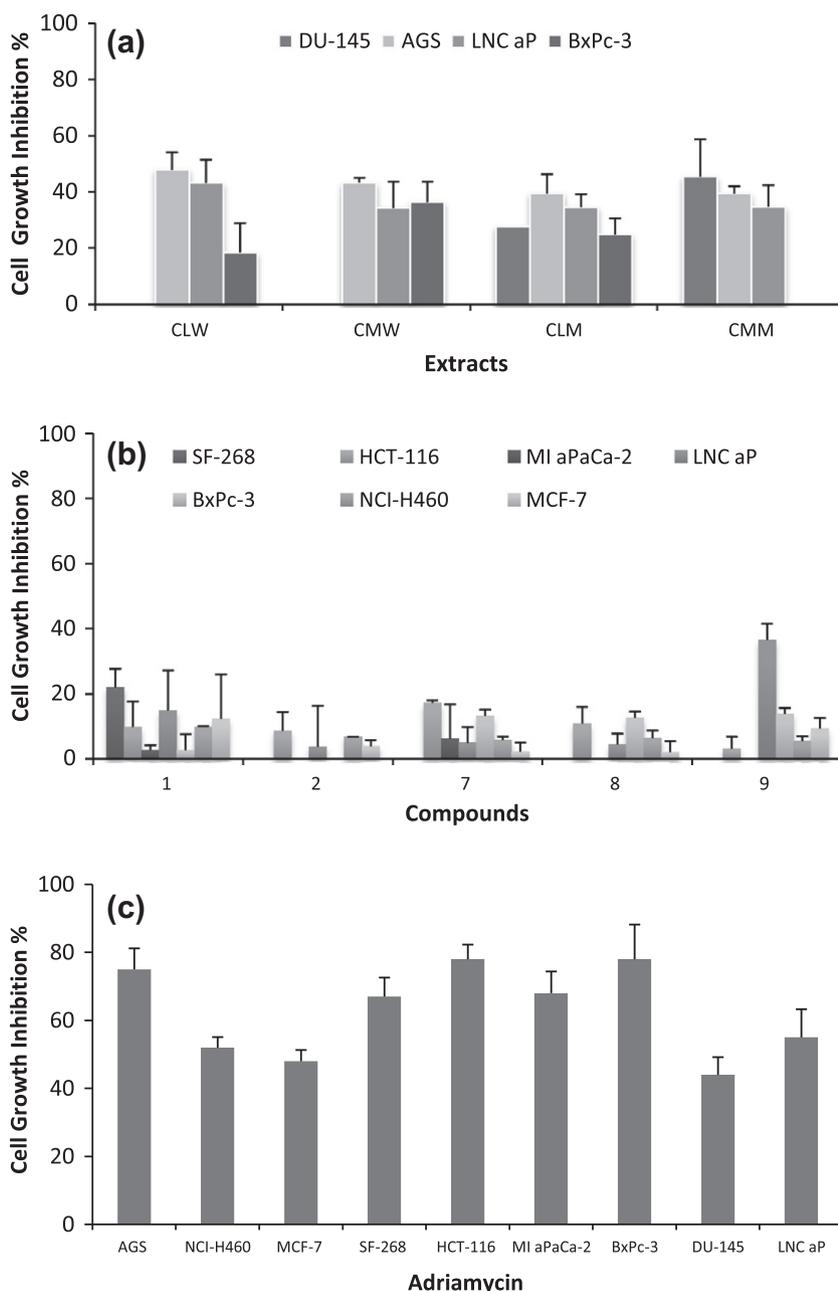

Fig. 5. Tumour cell (MCF-7, SF-268, NCI-H460, HCT-116, AGS, MI aPaCa-2, BxPc-3, LNC aP and DU-145) proliferation inhibitory activities of extracts of *C. longa* and *C. mangga* leaves (CLW: *C. longa* water extract; CMW: *C. mangga* water extract; CLM: *C. longa* methanolic extract; CMM: *C. mangga* methanolic extracts) at 100 μg/mL; compounds **1, 2** and **7–9** at 25 μg/mL, respectively. Compounds **3–5** were inactive in this assay. Adriamycin (1.6 μg/mL) was used as positive control. Vertical bars represent the standard deviation of each data point (*n* = 2). The standard error mean was represented for (*n* = 4). (*P* < 0.01, *t*-test, paired, and two tailed).

4,7,11-tetraene (**2**), cyclohexanecarboxylic acid methyl ester (**3**), isopulegol (**4**), 2-menthen-1-ol (**5**), menth-1-en-9-ol (**6**), octahydrocurcumin (**7**), labda-8(17)-12-diene-15, 16-dial (**8**), and coronadiene (**9**) by comparison of their NMR spectral data with published reports.

The isolates from extracts were assayed along with the respective extracts to determine their antioxidant and antiinflammatory activities at 25 and 100 μg/mL concentrations, respectively. The MTT assay detects compounds that are capable of reducing an oxidizing agent (Liu & Nair, 2010). At 100 μg/mL concentrations, the extracts (CMM, CMW, CLM, CLW) gave absorbance values of 0.35, 0.55, 0.32 and 0.58, respectively. At 25 μg/mL, compounds **1–9** gave absorbance values of 0.09, 0.11, 0.15, 0.09, 0.09, 0.09, 0.21, 0.17, 0.10, 0.13, and 0.09 at 570 nm in the MTT assay, respectively (Fig. 2). Although the extracts showed good activity in this assay when compared to Vitamin C, the pure isolates showed weak activity. This could be explained as synergism or additive effect of compounds present in the extract. The LPO assay detects compounds that are potential free radical scavengers (Liu & Nair, 2011). The methanolic and water extracts of *C. mangga* and *C. longa* (CMM, CMW, CLM, and CLW) at 100 μg/mL showed LPO inhibition by 78%, 63%, 81% and 43%, respectively. Similarly, compounds **1–9** showed inhibition against LPO enzyme by 11%, 22%, 18%, 39%, 66%, 65%, 87%, 39% and 55%, at 25 μg/mL concentrations, respectively (Fig. 3). Compounds **7–9** showed strong LPO inhibition and accounted for the activity exhibited by the extracts.

The antiinflammatory activity of extracts and isolates of *Curcuma* spp. studied was carried out by measuring the inhibition of COX-1 and -2 enzymes. This assay determines the ability of COX enzymes to covert arachidonic acid to prostaglandins. Prostaglandins initialize the inflammatory process in the body (Liu & Nair, 2011). The extracts CMM, CMW, CLM, and CLW inhibited COX-1 enzyme by 55%, 33%, 43% and 24%, and COX-2 by 65%, 55%, 77% and 69%, respectively, at 100 μg/mL. Similarly, compounds **1–9** inhibited COX-1 enzyme by 3%, 4%, 7%, 35%, 29%, 7%, 0%, 8% and 14%, and COX-2 by 18%, 34%, 0%, 34%, 22%, 20%, 82%, 52% and 42%, respectively, at 25 μg/mL (Fig. 4). Although the extracts in general showed inhibition against COX-1 and COX-2 enzymes, compounds **1**, **2**, **7**, **8** and **9** inhibited COX-2 enzyme similar to non-steroidal antiinflammatory drugs (NSAIDs) used in the assay as positive controls (Fig. 4). Compound **7**, octahydrocurcumin, one of the curcuminoids present in the rhizomes of *C. longa*, showed the strongest COX-2 enzyme inhibition with little or no activity against COX-1 enzyme. This is of particular importance since selective COX-2 enzyme inhibitors are often preferred to treat inflammatory pain in many patients.

The tumour cell proliferation inhibitory activity of Curcuma leaf extracts and compounds were determined by using MCF-7 (breast), SF-268 (CNS), NCI-H460 (lung), HCT-116 (colon), AGS (gastric), MIAPaCa-2 and BxPc-3 (Pancreatic), and LNCaP and DU-145 (Prostate) human tumour cell lines (Fig. 5). At 100 μg/mL concentration, water extracts of *C. mangga* and *C. longa* showed inhibition against DU-145 by 28% and 46%, respectively, while their methanolic extracts showed little or no inhibition against. Water and methanolic extracts of *C. longa* and *C. mangga* (WCL, WCM, MCL, and MCM) inhibited the growth of prostate tumour cell line LNCaP by 43%, 34%, 34% and 34%, respectively. Similarly, water and methanolic extracts of both plants revealed growth inhibition against pancreatic tumour cell line BxPc-3 by 18% and 36%, respectively. All isolates, except for compound **9**, showed weak inhibition against the human tumour cell lines tested. Compound **9** inhibited the proliferation of prostate cell line LNCaP by 36% at 25 μg/mL. Overall, all isolates showed weak inhibition against the tumour cell lines tested (Fig. 5).

Previous reports indicated that *C. longa* and *C. mangga* rhizomes contained compounds belonging to polyphenols, curcuminoids and labdanes. The leaf extracts of the *Curcuma* spp. grown in the greenhouse gave identical chemical and activity profiles. However, compound **7**, the curcuminoid, was a minor constituent in *C. mangga* leaf when compared to the *C. longa* leaf. The higher COX-2 enzyme inhibition demonstrated by *C. longa* leaf extract when compared to the *C. mangga* leaf extract can therefore be attributed to the higher concentration of compound **7**, octahydrocurcumin, in *C. longa*.

4. Conclusion

The edible leaves of *C. longa* and *C. mangga* showed similar biological activity and chemical constituent profiles. The leaves of *C. longa* afforded a combined 364 mg of compounds **1–9** and 1.8 g of chlorophyll per 100 g of fresh leaves under present extraction method. Bioassay-guided isolation afforded primarily bioactive terpenoidal compounds. Leaves of both species contained very little curcuminoids, the primary bioactive compounds present in their rhizomes. This is the first report of the isolation of compounds **1–7** and **9** in *C. mangga* and *C. longa* leaves and their potential health benefits. Although the in vitro bioassay results support the anecdotal biological activity of *C. longa* and *C. mangga* leaves, further research on the leaves is needed to provide more information on its daily consumption as a functional food.

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