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Curcuma longa and *Curcuma mangga* leaves exhibit functional food property

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ABSTRACT

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.

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 [$CHCl_3$/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 [$CHCl_3$/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, ^{1}H NMR (500 MHz, $CDCl_3$): 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); ^{13}C NMR (500 MHz, $CDCl_3$): 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

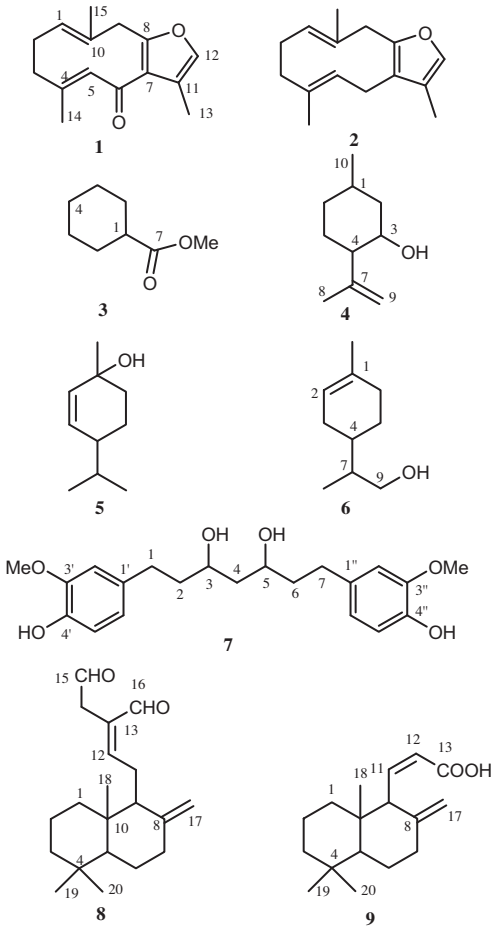

Fig. 1. Structures of 8,12-epoxygermacra-1(10), 4,7,11-tetraen-6-one **(1),** 8,12-epoxygermacra-1(10), 4,7,11-tetraene (**2**), cyclohexanecarboxylic acid methyl ester (**3**), isopulegol (**4**), 2-menthen-1-ol (**5**), menth-1-en-9-ol (**6**), octahydrocurcumin (**7**), labda-8(17)-12-diene-15, 16-dial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