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Research Paper

Bioactive phenolics from mango leaves (*Mangifera indica* L.)

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ABSTRACT

Mango leaves (*Mangifera indica* L.) are a kind of crop waste and the present work is focusing on its bioactive constituents. Phytochemical study on the extract of mango leaves resulted in the isolation and identification of 5 benzophenones and 17 flavonoids. Among them, there were 4 new benzophenone glycosides, elucidated as 4′,6-dihydroxy-4-methoxybenzophenone-2-*O*-(2″),3-C-(1″)-1″-desoxy-β-fructopyranoside, 4,4′,6-trihydroxybenzophenone-2-*O*-(2″),3-C-(1″)-1″-desoxy-β-fructopyranoside, 4,4′,6-trihydroxybenzophenone-2-*O*-(2″),3-C-(1″)-1″-desoxy-β-fructofuranoside, and 2,4′,6-trihydroxy-4-methoxybenzophenone-3-C-(2-*O*-*p*-hydroxybenzoyl-*p*-hydroxybenzoyl)-α-D-galactoside, together with eighteen known compounds. The anti-inflammatory, antioxidant and α-glucosidase inhibitory activities of the isolated compounds were evaluated. The results displayed that some phenolics showed significant anti-inflammatory, antioxidant and antidiabetic activities. The study provided a phytochemical evidence for further development and utilization of mango leaves in health products.

1. Introduction

Mango (*Mangifera indica* L.) is one of the most economically important tropical plants (Barreto et al., 2008), which belongs to the family of Anacardiaceae. The reported studies found that the main active constituent of mango was mangiferin (Kulkarni and Rathod, 2014), which could be used to treat respiratory diseases and diabetes. With the continuous expansion and deepening of research, it has revealed a wide range of physiological and pharmacological activities of mango. Previous studies have shown that mango leaves is rich in phenolics, including phenolic acids (Ge et al., 2011), flavonoids (Berardini et al., 2005), and benzophenones (Berardini et al., 2004). The phenolics in mango have shown various bioactivities, such as antioxidant (Ribeiro et al., 2008; Chen et al., 2014), antidiabetic (Borbalán et al., 2003; Lin and Lee, 2014), antimicrobial, immunomodulatory, antipyretic, anti-inflammatory and analgesic activities (Lin and Lee, 2014; Xin et al., 2012).

Mango leaves are a kind of crop waste and rich in resources. In the continuing studies to find bioactive phytochemicals from high plants, detailed phytochemical studies designed to find anti-inflammatory (Feng et al., 2016), antioxidant (Xiang et al., 2016), anti-diabetes (Wang et al., 2016) constituents have been carried out. In this study, twenty-two phenolics, including four new, were isolated and identified from mango leaves. Some phenolics exhibited significant anti-inflammatory, antioxidant and antidiabetic activities. The current study suggested that mango leaves could be served as a good anti-metabolic disease for the development of healthy products.

2. Materials and methods

2.1. Apparatus and chemicals

All the 1D and 2D NMR were determined on a Bruker Avance III-400 (Bruker, Falanden, Switzerland). Positive-ion HR-ESI-TOF-MS were acquired on a Waters AQUITY UPLC/Q-TOF-MS (Milford, MA, USA). HPLC was performed on a C_{18} column (Cosmosil $5C_{18}$-MS-II, 10 ID × 250 mm, Nacalai Tesque, Kyoto, Japan). Silica gel for column chromatography (CC) was purchased from Anhui Liangchen Silicon Source Material Co., Ltd. (Lu'an, China). ODS (40–60 μm) for MPLC was product of Merck KGaA (Darmstadt, Germany). HPLC grade methanol was purchased from Oceanpak Alexative Chemical Co., Ltd. (Gothenburg, Sweden). All other analytical chemicals and reagents

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; DPPH, 2-diphenyl-1-picrylhydrazyl; FBS, fetal bovine serum; HMBC, ^{1}H-detected heteronuclear multiple-bond coherence spectroscopy; HPLC, high performance liquid chromatography; HR-ESI–MS, high-resolution electrospray mass spectrometry; HSQC, ^{1}H-detected heteronuclear single quantum coherence spectroscopy; IC_{50}, half of inhibition concentration; LPS, lipopolysaccharides; MPLC, middle pressure liquid chromatography; NMR, nuclear magnetic resonance spectroscopy; NO, nitric oxide; NOE, nuclear Overhauser effect; NOESY, nuclear overhauser enhancement spectroscopy; PDA, photodiode array detector; TFA, trifluoroacetate

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were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Plant material

The mango leaves were collected from the campus of Guangdong Pharmaceutical University in Guangzhou higher education mega center on May 2014, and identified to be *Mangifera indica* L. by Prof. X. J. He of Guangdong Pharmaceutical University. A voucher specimen (No. GDPU-NPR-201404) was deposited in the Lead Compounds Laboratory, School of Pharmacy, Guangdong Pharmaceutical University.

2.3. Extraction and isolation procedure

The air-dried leaves of mango (20 kg) were pulverized and then extracted three times with 70% ethanol-water (3 × 50 L) for 2 h at reflux. The concentrated solution (20 L) was successively extracted with cyclohexane, EtOAc, and *n*-BuOH to yield a cyclohexane-soluble fraction, an EtOAc-soluble fraction, and an *n*-BuOH-soluble fraction, respectively. The antioxidant, anti-inflammatory and α-glucosidase inhibitory activities were evaluated for each fraction. The results exhibited that the EtOAc and normal butanol fractions showed pronounced activities. The bioactive fractions were selected for further isolation and purification.

The EtOAc fraction (239 g) was subjected to a silica gel CC (200–300 mesh, 100 × 1200 mm) by using $CHCl_3$/MeOH (100:1 to 4:1, v/v) gradient elution to get 18 fractions. Fr. 7 (2.17 g) was applied to a silica gel CC (300–400 mesh, 25 × 370 mm) eluted with $CHCl_3$/acetone (30:1 to 3:1, v/v) to get four fractions. Fr. 7-1 (21.3 mg) was purified by a Sephadex LH-20 (15 × 460 mm) to afford compound **17** (3.3 mg, purity: 98.33%). Fr. 11 (18.94 g) was subjected to an ODS MPLC (20 × 250 mm) and eluted gradiently with MeOH/H_2O (10:90 to 100:0, v/v) to obtain seven fractions. Fr.11-3 (709.5 mg) was further separated with a Sephadex LH-20 (20 × 510 mm) by using MeOH/H_2O (90:10, v/v) to yield compound **16** (110.0 mg, purity: 99.54%). Fr.11-4 (1.09 g) was purified with a Sephadex LH-20 (20 × 510 mm) by using MeOH to afford compounds **20** (89.4 mg, purity: 98.83%) and **22** (150.1 mg, purity: 96.89%). Compound **21** (6.7 mg, purity: 97.57%) was obtained from Fr.11-6 (103.5 mg) through a Sephadex LH-20 column (15 × 460 mm) eluted with MeOH/H_2O (80:20, v/v). Fr.13 (13.91 g) was subjected to a silica gel CC (300–400 mesh, 40 × 420 mm) by using $CHCl_3$/MeOH (20:1 to 2:1, v/v) gradient elution to get eight fractions. Fr.13-2 (2.23 g) was isolated by an ODS MPLC (20 × 250 mm) eluted with MeOH/H_2O (30:70, v/v) and purified through the Rp-HPLC (Cosmosil $5C_{18}$-MS-II, 10 ID × 250 mm) eluted with MeOH/H_2O (40:60, v/v) to yield compound **4** (13.3 mg, t_R = 25.9 min, purity: 98.97%). Fr.13-5 (4.23 g) precipitated crystals to get Compound **6** (50.0 mg, purity: 97.35%), and the remaining fraction was further purified with Sephadex LH-20 eluted gradiently with MeOH/H_2O (60:40 to 100:0, v/v) and finally purified by the Rp-HPLC eluted with MeOH/H_2O (40:60, v/v) to get compounds **7** (68.0 mg, t_R = 32.9 min, purity: 99.48%), **8** (42.5 mg, t_R = 38.0 min, purity: 98.33%), and **9** (22.6 mg, t_R = 45.2 min, purity: 97.15%). Compound **10** (112.0 mg, purity: 98.72%) was crystalized from Fr.14 (18.82 g). Compound **18** (612.0 mg, purity: 97.88%) was purified by an ODS MPLC (20 × 250 mm) from Fr. 14 eluted gradient with MeOH/H_2O (10:90 to 100:0, v/v).

The obtained normal butanol fraction (412 g) was subjected to a HP-20 resin column (60 × 1100 mm) eluted with MeOH/H_2O (0:100 to 100:0, v/v). The 40% MeOH elution (122.4 g) was subjected to a silica gel CC (200–300 mesh, 100 × 950 mm) by using $CHCl_3$/MeOH (30:1 to 2:1, v/v) gradient elution to get twelve fractions. Fr.8 (6.34 g) was separated with a Sephadex LH-20 column (30 × 550 mm) eluted gradiently with MeOH/H_2O (50:50 to 100:0, v/v) to get six fractions. Fr.8-1 was further purified with Rp-HPLC eluted with MeOH/H_2O (20:80, v/v) to get compounds **1** (108.0 mg, t_R = 22.0 min, purity: 98.89%) and **5** (12.1 mg, t_R = 31.5 min, purity: 98.56%). Compound **3** (15.5 mg, purity: 98.52%) was obtained from Fr.8-3 by a Sephadex LH-20 (20 × 510 mm) eluted with MeOH/H_2O (80:20, v/v). Compound **2** (7.5 mg, purity: 99.63%) was get from Fr.8-4 by Rp-HPLC eluted with MeOH/H_2O (30:70, v/v). Fr.8-5 was recrystallized with methanol to obtain compound **12** (29.5 mg, purity: 97.49%). Fr.9 (6.45 g) was separated on Sephadex LH-20 column (30 × 550 mm) eluted with MeOH/H_2O (60:40, v/v) to afford three fractions. Compound **13** (44.0 mg, purity: 98.71%) was obtained by recrystallized with methanol from Fr.9-3. Compound **11** (37.6 mg, t_R = 26.5 min, purity: 98.62%) was finally purified by Rp-HPLC eluted with MeOH/H_2O (25:75, v/v). Fr.10 (1.61 g) was recrystallized with methanol to afford compound **14** (28.0 mg, purity: 99.41%). Fr.11 (4.77 g) was separated by Sephadex LH-20 column (30 × 550 mm) eluted gradiently with MeOH/H_2O (55:45 to 100:0, v/v), and further purified by Rp-HPLC eluted with MeOH/H_2O (35:65, v/v) to obtain compounds **15** (17.6 mg, t_R = 46.3 min, purity: 98.81%) and **19** (42.1 mg, t_R = 32.1 min, purity: 98.78%).

2.4. Qualitative and quantitative analysis of the phenolics in mango leaves by HPLC-PDA

In this study, the HPLC chromatograms of 70% ethanol extract from the leaves of mango were recorded at 254 nm. Under the optimized chromatographic conditions, the extract of mango leaves had a good separation by performing on an Amethyst C_{18} column (4.6 × 250 mm, 5 μm, Sepax Technologies Inc., Newark, NJ, USA) using the Waters HPLC system at temperature of 40 °C and a flow rate of 1.0 mL/min. The mobile phase was consisted of solvent A (methanol) and solvent B (methanol: water: TFA = 5:95:0.1). The linear gradient elution (0 − 55) was performed as below: 0–13 min, 5–10% A; 14–23 min, 20–30% A; 24–33 min, 30% A; 34–45 min, 50–90% A and keep 90% A for 10 min.

2.5. Inhibitory activity on LPS-induced NO production

Macrophage RAW 264.7 cells were cultured in DMEM including 10% FBS, 100 μg/mL streptomycin and 100 U/mL penicillin with a humid atmosphere of 5% CO_2 at 37 °C. The NO produced in the culture medium was evaluated by measuring the NO_2^- level in the Griess reaction (Pan et al., 2016).

2.6. DPPH radical scavenging activity

The DPPH radical scavenging activity assay was measured by using the modified protocol in our lab (Zeng et al., 2012). All compounds were dissolved in methanol at the concentrations ranged from 5–300 μM and measured in triplicate. Ascorbic acid and gallic acid were used as the positive controls. The results were represented as the concentration of the tests required to scavenge 50% of the DPPH radical.

2.7. α-Glucosidase inhibitory activity

The α-glucosidase inhibitory assay was measured on a 96-well plate by spectrophotometry using the reported method (Wang et al., 2013). All compounds were dissolved in 50% methanol-water at the concentrations ranged from 50 to 1000 μM and measured in triplicate. Acarbose were dissolved in water and used as positive control. Results were represented as the concentration of the test samples that inhibited 50% of the enzymatic activity.

2.8. Statistical analysis

All experimental data were represented as mean ± SD. Statistical values were assessed by using the SPSS software with a one-way ANOVA, $p < 0.05$ expected to indicate statistical significance.