Food Chemistry 156 (2014) 408–415

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Evaluation of the antioxidant and anti-osteoporosis activities of chemical constituents of the fruits of *Prunus mume*

Xi-Tao Yan [a], Sang-Hyun Lee [b], Wei Li [a], Ya-Nan Sun [a], Seo-Young Yang [a], Hae-Dong Jang [b,*], Young-Ho Kim [a,*]

[a] College of Pharmacy, Chungnam National University, Daejeon 305-764, Republic of Korea
[b] Department of Food and Nutrition, Hannam University, Daejeon 305-811, Republic of Korea

ARTICLE INFO

Article history:
Received 19 October 2013
Received in revised form 20 January 2014
Accepted 23 January 2014
Available online 6 February 2014

Keywords:
Prunus mume
Rosaceae
Acylated sucrose
Antioxidant activity
Anti-osteoporosis activity

ABSTRACT

The present study investigated the antioxidant and anti-osteoporosis activities of phytochemicals in the fruits of *Prunus mume*. From the methanol extract, three new acylated sucroses, mumeoses P–R (**1**–**3**), were isolated together with 20 known compounds (**4**–**23**). Compounds **1**–**3** showed potent peroxyl radical-scavenging activities and **12**–**19** showed both potent peroxyl radical-scavenging and reducing activities. The anti-osteoporosis activity was evaluated using murine pre-osteoblastic MC3T3-E1 cells and pre-osteoclastic RAW 264.7 cells. Compounds **2** and **3** (*cis*–*trans* isomers), **5**, **7**, **8**, and **10** significantly stimulated the differentiation of pre-osteoblastic MC3T3-E1 cells to increase collagen synthesis or mineralization functions of osteoblasts, while compounds **5**, **6**, **9**, **10**, **12**, **14**–**16**, **18**, **20**, and **22** significantly suppressed tartrate-resistant acid phosphatase activity in receptor activator of nuclear factor-κB ligand-induced osteoclastic RAW 264.7 cells. These results indicated that the fruits of *P. mume* are an excellent source of antioxidant and anti-osteoporosis phytochemicals.

1. Introduction

The fruit of *Prunus mume*, commonly known as Maesil in Korea, is a common commercial product and a valuable source of food and medicinal material in Eastern Asian countries due to its flavour and aroma, wide availability, and its nutritional and therapeutic benefits. *P. mume* Sieb. et Zucc. belongs to the Rosaceae family and is one of the most popular fruit trees in Korea, China, and Japan. Its fruit has been used as a food garnish (pickled plum), sauce, juice, and liquor in these countries. In addition, processed immature fruits of *P. mume* (Chinese name "Wu Mei") are used in traditional Chinese medicines for antitussive, expectoration, antiemetic, antidiarrheal, anthelmintic, and antipyretic treatments (Xiao, Li, & Yang, 2002).

Oxidative stress is caused by an imbalance between the generation of reactive oxygen species (ROS) and the activity of antioxidant defence. Severe oxidative stress has been implicated in many chronic and degenerative diseases, including osteoporosis, cancer, ageing, and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (Barnham, Masters, & Bush, 2004; Benz & Yau, 2008; Rao & Rao, 2013). Much research is focused on the use of natural phytochemicals, such as those found in vegetables and fruits, for their antioxidant and functional properties (Kaur & Kapoor, 2001). The natural antioxidants in foods have also attracted interest because of their safety and potential nutritional and therapeutic effects. There are several methods that have been developed to estimate the antioxidant capacity of phytochemicals in foods. The most popular and most well characterised is the peroxyl radical scavenging assay (Huang, Ou, & Prior, 2005; Zulueta, Esteve, & Frigola, 2009). In addition, reducing capacity is also a popular assay, frequently used for measuring the electron-donating capacity of food extracts or antioxidants (Apak, Guclu, Ozyurek, Bektasoglu, & Bener, 2008; Huang et al., 2005).

Recently, the possibility that naturally occurring phytochemicals from edible materials, especially fruits and vegetables, may reduce the risk of age-related degenerative diseases has gained considerable interest. Osteoporosis, one of the most frequently encountered degenerative diseases in ageing communities, is characterised by a decrease in bone mass and density, causing bones to become fragile and more prone to fracture. Bone development in vertebrate animals is maintained by the coordinated actions of osteoblasts (bone formation) and osteoclasts (bone resorption). Inadequate formation of new bone or excessive bone resorption

* Corresponding authors. Tel.: +82 42 629 8796; fax: +82 42 629 8805 (H.-D. Jang). Tel.: +82 42 821 5933; fax: +82 42 823 6566 (Y.-H. Kim).
E-mail addresses: haedong@hnu.kr (H.-D. Jang), yhk@cnu.ac.kr (Y.-H. Kim).

http://dx.doi.org/10.1016/j.foodchem.2014.01.078
0308-8146/

is often association with osteoporosis disease. A recent report showed that an extract of *P. mume* fruit affects the proliferation and differentiation of pre-osteoblastic MC3T3-E1 cells. The water-soluble fraction of *P. mume* fruits stimulated the proliferation and osteoblast differentiation of cells and may have potential to treat osteoporosis (Kono et al., 2011). During our search for naturally occurring bioactive phytochemicals for bone disease therapies, chemical constituents isolated from *P. mume* fruit were evaluated in murine pre-osteoblastic MC3T3-E1 cells and pre-osteoclastic RAW 264.7 cells in terms of their ability to affect the differentiation of osteoblasts and osteoclasts, respectively.

In the present study, twenty-three compounds, including three new compounds (**1**–**3**), were isolated from the fruits of *P. mume*. We describe herein the isolation, structural elucidation, and evaluation of the antioxidant and anti-osteoporosis activities of these isolates.

2. Materials and methods

2.1. Materials and chemicals

Fresh fruits of *P. mume* were purchased from local markets (Yusong, Daejeon, Korea) in June 2012, and authenticated by one of the authors, Prof. Young-Ho Kim (Chungnam National University, Daejeon, Korea). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH), neocuproine, fetal bovine serum (FBS), β-glycerophosphate, Triton X-100, direct red 80, alizarin red S, leukocyte acid phosphatase assay kit, sodium tartrate, *p*-nitrophenylphosphate (PNPP), phosphate-buffered saline (PBS, pH 7.4), cetylpyridinium chloride, *p*-formaldehyde, ascorbic acid, picric aicd, citric acid monohydrate, *p*-coumaric acid, D-glucose, L-glucose, D-fructose, hexamethyldisilazane/trimethylchlorosilan/pyridine (HMDS/TMCS/Pyridine, 3:1:9, v/v/v), receptor activator for NF-kB ligand (RANKL), and dimethylsulfoxide (DMSO) were purchased from Sigma–Aldrich (St. Louis, MO, USA). MC3T3-E1 cells and RAW 264.7 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). All solvents used were of analytical grade and supplied by SK Chemicals (Ulsan, Korea).

2.2. General experiment procedures

NMR spectra were recorded on a JEOL ECA 600 spectrometer (JEOL, Tokyo, Japan). ESI-MS spectra were measured using an Agilent 1100 LC-MSD trap spectrometer (Agilent, USA). HR-MS spectra were acquired using an LC/MS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan). GC analysis was carried out on an Agilent 6890 N gas chromatograph (Agilent, USA). UV spectra were measured using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). FT-IR spectra were recorded on a Nicolet 380 FT-IR spectrometer (Thermo Fisher Scientific Inc., USA). Optical rotations were measured with a JASCO P-2000 digital polarimeter (JASCO, Tokyo, Japan). Column chromatography was performed on silica gel (40–75 and 75–200 μm particle sizes, Fuji Silysia Chemical Ltd., Japan) and YMC RP-C18 resins (30–50 μm particle size, Fuji Silysia Chemical Ltd., Japan). Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} and RP-18 F_{254S} plates (Merck, Darmstadt, Germany).

2.3. Extraction and isolation

The fruits of *P. mume* (20 kg) were washed thoroughly, cored, and cut into small pieces. These were extracted three times (each for 8 h) with 30 l of MeOH under reflux conditions (60 °C). After removing the solvent *in vacuo*, the obtained residue (1150 g) was suspended in distilled water (5 l) and partitioned successively with CH_2Cl_2 (5 l × 3) and EtOAc (5 l × 3) to yield a CH_2Cl_2-soluble fraction (38 g) and an EtOAc-soluble fraction (170 g). The aqueous solution was separated by column chromatography (CC) on adsorptive macroporous resin HP-20 and eluted successively with water, 25% aqueous MeOH, 50% aqueous MeOH, 75% aqueous MeOH, and 100% MeOH.

The CH_2Cl_2 extract was subjected to silica gel CC and eluted with a gradient of n-hexane/EtOAc (12:1–1:1, v/v) to yield ten fractions (Frs. C1–C10). Fr. C1 was further subjected to silica gel CC (n-hexane/EtOAc, 60:1–12:1, v/v) to give compound **23** (108 mg). Fr. C7 was subjected to silica gel CC ($CHCl_3$/MeOH, 50:1–6:1, v/v) to afford eleven subfractions (Frs. C7–1–C7–11). Compounds **17** (5 mg), **18** (7 mg), **19** (44 mg) were obtained by the separation of Fr. C7–3 on a YMC RP-C18 column (MeOH/H_2O, 1:1.3, v/v) and a silica gel column (n-hexane/acetone, 2:1, v/v). Compound **16** (27 mg) was obtained by the separation of Fr. C7–9 on a YMC RP-C18 column (MeOH/H_2O, 1:3, v/v) and a silica gel column ($CHCl_3$/94%EtOH, 16:1, v/v).

The EtOAc extract was subjected to silica gel CC and eluted with a gradient of CH_2Cl_2/MeOH (25:1–4:1, v/v) to yield five fractions (Frs. E1–E5). Fr. E3 was further separated by silica gel CC ($CHCl_3$/94%EtOH, 8:1–4:1, v/v) to yield six subfractions (Frs. E3–1–E3–6). Fr. E3–1 was filtered to yield compound **21** (840 mg). Fr. E3–3 was subjected to silica gel CC ($CHCl_3$/MeOH/H_2O, 10:1:0–6:1:0.1, v/v/v) followed by repeated YMC RP-C18 CC (acetone/H_2O, 1:15–1:5, v/v) to provide compounds **10** (25 mg) and **13** (18 mg). Fr. E3–6 was repeatedly subjected to silica gel CC ($CHCl_3$/MeOH/H_2O, 4:1:0.1–1.8:1:0.1, v/v/v) and YMC RP-C18 CC (MeOH/H_2O, 1:10–1:1.3, v/v) to yield compound **15** (10 mg). Fr. E3–4 was subjected to silica gel CC (CH_2Cl_2/MeOH/H_2O, 10:1:0.1–4:1:0.1, v/v/v) to yield six fractions (Frs. E3–4–1–E3–4–6). Fr. E3–4-1 was filtered to yield compound **20** (85 mg). Compound **1** (47 mg), and a mixture of **2** and **3** (62 mg) were obtained by repeated separations of Fr. E3–4-5 on a YMC RP-C18 column (MeOH/H_2O, 1:6–1:4, v/v).

The 25% MeOH elute (30 g) was subjected to silica gel CC eluted with CH_2Cl_2/MeOH/H_2O (9:1:0.1–4:1:0.1, v/v/v) to yield five fractions (Frs. W1–W5). Fr. W1 was further subjected to silica gel CC (n-hexane/EtOAc/94%EtOH, 4:20:1–0:2:1, v/v/v) to yield six subfractions (Frs. W1–1–W1–6). Frs. W1–3, W1–4, and W1–6 were filtered to yield compounds **5** (30 mg), **6** (445 mg), and **4** (79 mg), respectively. Fr. W3 was separated by silica gel CC (EtOAc/94%EtOH, 10:1–3:1, v/v) to yield seven subfractions (Frs. W3–1–W3–7). Fr. W3–2 was further subjected to YMC RP-C18 CC (MeOH/H_2O, 1:10–1:3, v/v) and silica gel CC (CH_2Cl_2/MeOH/H_2O, 7:1:0.1, v/v/v) to yield compound **14** (89 mg). Fr. W3–5 was purified on a silica gel column ($CHCl_3$/MeOH, 6:1, v/v) and a YMC RP-C18 column (MeOH/H_2O, 1:3, v/v) to yield compound **8** (25 mg). Fr. W3–6 was filtered to provide compound **9** (484 mg). Fr. W3–7 was purified on a silica gel column eluted with $CHCl_3$/MeOH/H_2O (4:1:0.1, v/v/v) to yield compound **7** (1767 mg). Compound **11** (245 mg) was obtained by silica gel CC (CH_2Cl_2/94%EtOH, 3.5:1–3:1, v/v) followed by recrystallization from Fr. W4. The 75% MeOH elute (16 g) was subjected to silica gel CC eluted with $CHCl_3$/MeOH in a gradient (8:1–3:1, v/v) to afford seven fractions (Frs. M1–M7). Fr. M5 was separated on a YMC RP-C18 column (MeOH/H_2O, 1:1.8, v/v) to yield compounds **12** (9 mg) and **22** (17 mg).

2.4. Acid hydrolysis of compounds *1*–*3*

Each compound (5 mg) was separately dissolved in a 5 ml mixture of 5% aqueous H_2SO_4 and 1,4-dioxane (1:1, v/v), and then heated to 90 °C in a water bath for 3 h. After neutralisation with Amberlite IRA-400 (OH^- form), the solution was evaporated to dryness under reduced pressure. A small amount of the residue was dissolved in MeOH and analysed by a TLC comparison [RP-18 TLC, MeOH/H_2O (2:1, v/v), R_f 0.45] with standard *p*-coumaric acid.