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Chemical composition and antioxidant, bactericidal, and matrix metalloproteinase inhibition activity of food-related plant

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ABSTRACT

The present study aimed to investigate bio-function, focusing the on antioxidant, bactericidal, and matrix metalloproteinase (MMP) regulatory activity, of food-related plants. Bioassay-coupled chromatography and bioassay guiding, the rapid and simple screening method, was used to identify potential antioxidant and bactericidal components from fifty-five food-related plants. *Lonicera japonica* exhibited moderate xanthine oxidase (XO) inhibition and antioxidant activity. *Rhodiola rosea*, provoked strongly inhibition for tyrosinase, lipoxygenase (LOX), XO and MMP-2 activity. The organic fractions of *Ligusticum sinense* had a significant antimicrobial effect against two Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and two Gram-positive species (*Staphylococcus aureus*, *and Bacillus subtilis*). The chemical structures of MMP-2-inhibiting and antimicrobial components was further identified as 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranoside and ferulic acid, respectively, which were determined by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and mass spectrometry (MS). The results suggest that *R. rosea* and *L. sinense* could be a useful natural food preservatives and potential nutraceutical ingredients.

1. Introduction

Bacterial pathogens can trigger apoptosis in infected host cells (Krzyminska, Tanska, & Kaznowski, 2011). by phagocyte-derived reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Circu & Aw, 2010). Nitric oxide (NO) and other RNS produced mainly by macrophages are among the major microbicidal agents released during inflammation to fight microorganisms as well as tumour cells. Although ROS and RNS are transiently beneficial, their chronic release impairs health. Therefore, antioxidants may play important roles in controlling disease-related oxidative stress (Halliwell, 2000) by regulating anti-inflammatory and antioxidant processes (Suzuki et al., 2012). Although food-related plants have been used in numerous areas, including nutrition, health, and cosmetics since ancient times, they are still poorly explored sources of natural antioxidants and antimicrobial agents. However, research into food plant-derived anti-inflammatory and antioxidant agents has attracted more attention in recent years.

With the emergence of multiple strains of antibiotic-resistant microorganisms, the interest in the search for potential plant-derived therapeutic compounds has greatly increased (Negi, 2012). In a recent study, Dholvitayakhun, et al. using the disc diffusion assay to investigate the antibacterial activity of Tai plants against six species of foodborne pathogen. The study demonstrates that the plant extracts possess antibacterial activity against gram-negative *Campylobacter jejuni* and gram-positive species such as *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus* known to cause food poisoning and more severe illnesses was evaluated (Dholvitayakhun, Cushnie, & Trachoo,

Abbreviations: MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; DAD, diode array detection.

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2012). The report encourage us to explore bactericidal agent from food plate.

There are many oxidative enzyme, such as tyrosinase and 15-lipoxygenase (15-LOX) catalyzes reaction coupled with ROS production, inhibition of this enzyme can be an index for antioxdative capacity of natural product. Tyrosinase inhibitors have attracted strong interest not only in the food but also in the health sciences (Yanagihara et al., 2012). Another oxidative enzyme mentioned, 15-LOX, oxidizes low-density lipoprotein (LDL), which might involve in the development of atherosclerosis. Indeed, 15-LOX inhibitors have shown anti-atherosclerotic effects *in vivo* (Malterud & Rydland, 2000; Steinberg, 1999). Furthermore, overexpression of xanthine oxidase (XO), which catalyzes the oxidation of xanthine to uric acid, causes hyperuricaemia, and the subsequent excessive production of uric acid may be controlled by the inhibiting of XO activity (Ernst & Fravel, 2009).

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases in the extracellular matrix. (Wang, Zhang, Wang, & Pei, 2007). MMP-2/9, which are type IV collagenases, are the main MMP family members and play an important role in cancer invasion and metastasis. Hence, the inhibition of MMPs is considereda promising approach to metastatic disease therapy (Singh, Singh, & Shukla, 2012). A link exists between MMPs, cancer, and oxidative stress in that the superoxide anion, which can generate different types of ROS, activates MMPs in certain cancers.

This article focuses was to evaluate the antioxidant, antimicrobial, and MMP expression-regulation activity from 55 different food plants extracts. And the active compounds were purified using the bioassays-coupled chromatography and bioassay guidelines method. The bioactive compounds found in the selected plants may be useful as natural food preservatives and potential therapeutic agents.

2. Materials and methods

2.1. Plant material and chemicals

Fifty-five food plants from 32 families (Supplementary Table 1) were freshly collected from the Taiwan Endemic Species Research Institute from 2012 to 2014, and were identified by Ms. Hsiu-Wen Huang of the Taiwan Endemic Species Research Institute, Chi-Chi township, Taiwan. Among these plants, only *Anoectochilus formosanus* is native to Taiwan. All voucher specimens (TMU-LCK-1-55) (Table 1) were deposited at the School of Pharmacy, Taipei Medical University, Taipei, Taiwan. HPLC-grade acetonitrile and methanol were purchased from JT Baker (Phillipsburg, NJ, USA). Kojic acid, L-tyrosine, and other chemicals were purchased from Sigma-Aldrich (St. Louis, USA). Ultra-pure water with a resistivity not exceeding 18 MΩ was obtained by purification of demineralized water in a Millipore mini-Q system (Bedford, MA, USA).

Escherichia coli (ATCC10536), *Pseudomonas aeruginosa* (ATCC9027), *Staphylococcus aureus* (ATCC6538) and *Bacillus subtilis* (ATCC6051) were purchased from the Bioresource Collection and Research Center referred to as the BCRC of Food Industry Research Institute in Hsinchu, Taiwan.

2.2. Sample preparation

The dried plant material (500 g) was powdered in a laboratory mill. Fifty grams of the powder were transferred to extraction bottles, mixed with 500 mL 95% EtOH, and the bottles were incubated at 32 °C for one day. Subsequently, the residue was extracted three times and the obtained extracts were concentrated *in vacuo*, freeze-dried, and stored at 4 °C until use.

2.3. High-performance liquid chromatography, diode array detection, mass spectrometry and NMR spectra analysis

The high-performance liquid chromatography(HPLC) system consisted of an Agilent 1260 series liquid chromatography system (Agilent Technologies, Lexington, MA, USA) with a diode array detection (DAD) set at 280 nm. The separation was performed at 28 °C on a Macherey-Nagel NUCLEOSHELL™ C_{18} HPLC column (250 mm × 4.6 mm, 5 μm; Macherey-Nagel GmbH, Düren, Germany). The mobile phase consisted of A(water) and B(methanol). The following gradient program was used at a flow rate of 0.5 mL/min:0%–50% B for 0–80 min, 50%–100% B for 80–90 min, and 100% B for 90–100 min. Twenty microliters of 10-mg/mL extract solution were injected. The tandem mass spectrometry(MS/MS) detection was performed on a Sciex API 2000 LC–MS/MS System (SCIEX, Framingham, MA, USA) equipped with a Turbo ionspray source with the electrospray needle voltage of −3800 V set in negative ion mode. Nitrogen (nebulizer) and turbo heater gas (490 °C) was set at a pressure of 40 and 70 psi, respectively. The curtain gas was set at 30 psi. The collision gas (nitrogen) used for the MS/MS mode at quadrupole Q2 was set to a flow rate of 3 (instrument units). Data acquisition and processing were performed using the Sciex Analyst® software (version 1.6.1; SCIEX, Framingham, MA, USA).

^{1}H and ^{13}C NMR spectra were recorded with a Bruker DRX-500 spectrometer (^{1}H, 500 MHz; ^{13}C, 125 MHz; Bruker, German), and the chemical shifts were expressed as a δ (ppm) scale with tetramethylsilane (TMS) used as an internal standard. Silica gel Kieselgel (40–63 μm; Merck) was used for column chromatography (CC). The HPLC separation was performedon a Hitachi L-7000 series instrument. A Phenomenex®Luna column (250 mm × 10 mm, 5 μm) was used for semi-preparative HPLC column chromatography.

2.4. Tyrosinase activity assay

The tyrosinase inhibitory activity was measured using tyrosine as the substrate. An aqueous solution of mushroom tyrosinase(40 μL, 50 IU/mL), phosphate buffer (160 μL, pH 6.8) (A), or phosphate buffer (120 μL, pH 6.8) was mixed with the test samples (40 μL) (B). The extracts were tested at a concentration of 500 μg/mL to assess the inhibitory effect on tyrosinase*in vitro*. Kojic acid (0.1 mg/mL in dimethyl sulfoxide (DMSO)) was used as a positive control. The mixture was preincubated at 37 °C for 5 min and L-tyrosine (80 μL, 0.1 mg/mL) was subsequently added. The mixture was incubated for 30 min at 37 °C. The amount of dopachrome was measured at 475 nm using amicroplate spectrophotometer (Molecular Devices Spectra MAX 340 PC, TekkenScientific Corp., Tokyo, Japan). The percent inhibition of the tyrosinase activity was calculated as follows:

$$\%\ \text{inhibition} = (A - B)/A \times 100 \quad (1)$$

where A = the absorbance at 475 nm without a test sample, and B = the absorbance at 475 nm with a test sample.

2.5. Lipoxygenaseactivity assay

The LOX activity was measured in borate buffer (0.2 M, pH 9.0) at 234 nm using linoleic acid (134 μM) as a substrate and after the addition of 15-LOX. The final enzyme concentration was 167 U/mL. The test substances were added as DMSO solutions at a final concentration of 1.6% DMSO. The enzyme solution was stored on ice and the controls were measured at intervals throughout the experimental period to verify that the enzyme activity was constant. Quercetin, a 15-LOX inhibitor, was used as