JOURNAL OF FOOD AND DRUG ANALYSIS XXX (2017) 1–8

Available online at www.sciencedirect.com

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Original Article

Composition of phenolic compounds and antioxidant attributes of *Cyclea gracillima* Diels extracts

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ARTICLE INFO

Article history:
Received 21 December 2016
Received in revised form
6 March 2017
Accepted 13 March 2017
Available online xxx

Keywords:
antioxidant
Cyclea gracillima Diels
flavonoid
phenol
phenolic acid

ABSTRACT

Cyclea gracillima Diels is a Taiwanese native medicinal herb. However, there are currently few relevant reports on its biochemical activity. In this study, the antioxidant attributes of the ethanol and hot water extracts of this herb were assayed using *in vitro* models, including the following: 2,2-diphenyl-1-(2,4,6-trinitrophenyl)-hydrazyl radical scavenging, Trolox equivalent antioxidant capacity, reducing power, and chelating ferrous ions. The following biochemical models were also assayed: inhibition of human low density lipoprotein oxidation, inhibition of human erythrocyte hemolysis, and scavenging oxygen radicals in human blood. The composition and content of flavonoids and phenolic acids in these extracts were also analyzed. The results showed that these extracts with high polyphenol levels presented remarkable antioxidant effects in all assays, especially when extracted with ethanol. Six phenolic acids (mainly ferulic acid, sinapic acid, and syringic acid) and 12 flavonoids (mainly narigenin, myricetin, naringin, and apigenin) were found in these extracts.

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1. Introduction

Cyclea gracillima Diels, which belongs to the family Menispermaceae, is a Taiwanese native medicinal herb that usually inhabits the margins of thickets at the low altitudes of central and southern Taiwan [1]. Many menispermaceous plants are used in folk medicine for the treatment of cough, fever, lumbago, headache, edema, wind-dampness, diabetes, asthma, and snakebite [2,3].

Recent reports have indicated that *Cyclea peltata* Hook. F. & Thomson (Menispermaceae) grown in India contains flavonoids, tannins, alkaloids, diterpenes, and saponins that have multiple properties, including antioxidant, antidiabetic, gastric antisecretory, antiulcer, anticancer, diuretic, and hepatoprotective activities [4–10]. Although *C. gracillima* Diels

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http://dx.doi.org/10.1016/j.jfda.2017.03.004

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Please cite this article in press as: Lin J-T, et al., Composition of phenolic compounds and antioxidant attributes of *Cyclea gracillima* Diels extracts, Journal of Food and Drug Analysis (2017), http://dx.doi.org/10.1016/j.jfda.2017.03.004

is used to treat inflammation, edema, and throes in Taiwan [1], there have been almost no related investigations regarding its biochemical properties.

In this work, we first determined the total phenol, flavonoid, and condensed tannin content, as well as the composition and content of flavonoids and phenolic acids in the hot water and ethanol (EtOH) extracts of *C. gracillima* Diels by high performance liquid chromatography (HPLC). The antioxidant attributes of these extracts were also evaluated by *in vitro* models including 2,2-diphenyl-1-(2,4,6-trinitrophenyl)-hydrazyl (DPPH) radical scavenging capacity, Trolox equivalent antioxidant capacity (TEAC), ferric ion (Fe^{3+}) reducing power, and ferrous ion (Fe^{2+}) chelating capacity, as well as biochemical models, including inhibition of cupric ion (Cu^{2+})-induced human low density lipoprotein (LDL) oxidation, inhibition of peroxyl radical-induced human erythrocyte hemolysis, and scavenging of oxygen radicals in human blood.

2. Materials and methods

2.1. Samples

C. gracillima Diels were gathered from the low altitude forest zone of central Taiwan, and then lyophilized using a freeze-drying system (Vastech Scientific Co., Ltd., Taipei, Taiwan) before use.

2.2. Chemicals

Acetonitrile (ACN), EtOH (95%), methanol (MeOH), acetic acid (CH_3COOH), and Folin-Ciocalteu's phenol reagent were obtained from Merck (Darmstadt, Germany). An Ultrapure water purification system (Lotun Co., Ltd., Taipei, Taiwan) was used to prepare distilled deionized water (dd H_2O). Phenolic acid standards: *p*-anisic acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, gallic acid, gentisic acid, *p*-hydroxybezoic acid, rosmarinic acid, sinapic acid, syringic acid, and vanillic acid; flavonoid standards: apigenin, catechin, daidzein, diosmin, epicatechin, eriodictyol, genistein, glycitein, hesperidin, hesperetin, isorhamnetin, kaempferol, luteolin, naringin, narigenin, myricetin, quercitrin, rutin, neohesperidin, morin, and quercetin; aluminum chloride; ascorbic acid; 2-2′-azino-bis-(3-ethylbenz-thiazoline-6-sulphonic acid) (ABTS); ferrozine; EDTA; luminol; heparin; 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox); vanillin; DPPH; horseradish peroxidase; trichloroacetic acid (TCA); aluminum chloride ($AlCl_3$); ferrous chloride ($FeCl_3$); potassium ferricyanide ($K_3Fe(CN)_6$); ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$); copper sulfate ($CuSO_4$) and sodium dihydrogen phosphate were purchased from Sigma (St. Louis, MO, USA). Hydrochloric acid (HCl), sodium carbonate, sodium hydroxide (NaOH), sodium nitrite ($NaNO_2$), disodium hydrogen phosphate, and hydrogen peroxide (H_2O_2) were obtained from Wako Co. (Osaka, Japan).

2.3. Preparation of hot water and ethanolic extracts of C. gracillima Diels

Twenty grams of *C. gracillima* Diels was extracted with 500 mL of EtOH for 24 hours. After filtrating using Whatman No. 1 filter paper, the extract was concentrated to dryness by a rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan). For hot water extraction, 20 g *C. gracillima* Diels was refluxed with distilled water (500 mL) at 98 ± 2°C for 1 hour followed by filtrating and cooling. The extract was then lyophilized in the freeze-dryer system. Each extractive procedure was carried out in triplicate. These extracts were stored under nitrogen at −80°C until use.

2.4. Determination of phytochemicals in the extracts of C. gracillima Diels

Folin–Ciocalteu's phenol reagent was used to measure the content of total phenols as described by Julkunen-Titto [11]; gallic acid was used as a standard and the results were expressed as mg gallic acid equivalent (GAE)/g of dried extract. The total flavonoid content was detected using 5% $NaNO_2$, 10% $AlCl_3$, and 1M NaOH solutions according to the method of Zhishen et al [12]; (+)-catechin was used as a standard and the results were expressed as mg catechin equivalent (CE)/g of dried extract. The content of condensed tannin was measured with 4% vanillin (prepared in MeOH) and HCl as described by Liu et al [13]; (+)-catechin was used as a standard and the results were expressed as mg CE/g of dried extract. The conditions in the report of Chen et al [14] were used to analyze the compositions of phenolic acid and flavonoids in the *C. gracillima* Diels extracts as follows: stationary phase, Inspire C18 column (250 × 4.6 mm, 5 μm; Dikma Technologies Inc., Lake Forest, CA, USA); mobile phase, ACN (Solvent A) and H_2O with 2% CH_3COOH (Solvent B) (2–4% A from 0 to 25 minutes and kept at 4% A from 25 to 40 minutes; 4–10% A from 40 to 50 minutes; 10–15% A from 50 to 60 minutes; 15–18% A from 60 to 110 minutes; 18–20% A from 110 to 115 minutes; 20–22% A from 115 to 135 minutes; and 22–25% A from 135 to 150 minutes); flow rate, 0.8 mL/min. An HPLC system with a Shimadzu LC-10AT HPLC pump system, a Shimadzu SCL-10A system controller module (Kyoto, Japan), and an S-3210 photodiode-array detector (Schambeck SFD GmbH, Bad Honnef, Germany) were used in this work. Electrospray ionization mass spectrometers (ESI-MS) data were recorded on a Thermo Finnigan LCQ classic quadrupole ion trap HPLC-Mass (MS) system (Gentech, Arcade, NY, USA) under the following conditions: nebulizer pressure, 70 psi; capillary temperature, 200°C; dry gas flow, 11 L/min; electrospray voltage of the ion source, 3000 V; capillary exit, −159 V; and skimmer, 20 V. Identification of compounds was carried out by comparing their retention times, UV-Vis, and mass spectral data to those of the authentic reference standards.

2.5. Antioxidant assays in vitro

Each extract was dissolved in MeOH, and then different concentrations of extract solutions were prepared by serial dilution.

DPPH is a powerful reagent to evaluate the scavenging capacity of extracts for free radicals [11]. The scavenging effect on DPPH radicals was evaluated as described by Epsin et al [15]. Each solution (200 μL) was mixed with 50 μL of 1mM DPPH (prepared in MeOH). After a 30-minute reaction, the absorbance (Abs) was measured at 517 nm (Multiskan Spectrum;

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