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Functional components in *Scutellaria barbata* D. Don with anti-inflammatory activity on RAW 264.7 cells

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ABSTRACT

The objectives of this study were to determine the variety and amount of various functional components in *Scutellaria barbata* D. Don as well as study their anti-inflammatory activity on RAW 264.7 cells. Both ethanol and ethyl acetate extracts were shown to contain the functional components including phenolics, flavonoids, chlorophylls, and carotenoids, with the former mainly composed of phenolics and flavonoids, and the latter of carotenoids and chlorophylls. Both extracts could significantly inhibit ($p < 0.05$) the production of lipopolysaccharide-induced nitric oxide, prostaglandin E_2, interlukin-6, and interlukin-1β, as well as the expressions of phosphor extracellular signal-regulated kinase and phosphor-c-Jun N-terminal kinase (p-JNK), but failed to retard tumor necrosis factor-α expression. Both ethanol and ethyl acetate extracts had a dose-dependent anti-inflammatory activity on RAW 264.7 cells. Furthermore, the anti-inflammatory efficiency can be varied for both ethanol and ethyl acetate extracts, which can be attributed to the presence of different varieties and amounts of functional components, as mentioned above. This finding suggested that S. *Barbata* extract may be used as an anti-inflammatory agent for possible future biomedical application.

1. Introduction

Ban-Zhi-Lian, a traditional Chinese herb with the scientific name *Scutellaria barbata* D. Don or *Scutellria rivularis* Wall, belongs to the Labiatae species [1]. *S. barbata* is widely grown in Asian countries such as Taiwan, China, Korea, and Japan, and has been reported to possess vital biological activities, including antibacterial [2,3], anticancer [1], and anti-inflammatory activities [4].

Inflammation, a kind of innate immunity, represents an important protective function of humans against invasion of foreign substances such as bacteria and virus. After invasion, the body immunity function can be induced for subsequent

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activation of macrophages to resist foreign pathogens and ameliorate damage to human health [5]. However, prolonged inflammation can elevate oxidative stress *in vivo*, leading to an increase in the incidence of chronic diseases such as cancer and cardiovascular disease. In addition, during inflammation, many proinflammatory factors such as nitric oxide (NO), tumor necrosis factor-α (TNF-α), prostaglandin E_2 (PGE_2), and interleukins (IL) can be released by macrophages and neutrocytes, after which both can migrate to the inflammatory site to kill microorganisms and endotoxins. However, the excessive secretion can cause damage to tissues [5]. Among the various proinflammatory factors, NO plays a key role in the various forms of physiology and pathology. Physiology can regulate nerve conduction, immune function, inflammation, and artery smooth muscle relaxation [6,7], while pathology can be associated with incidence of atherosclerosis, chronic inflammation and cancer [8].

In several earlier studies, *S. barbata* was shown to exhibit anti-inflammatory activity; however, the functional components and underlying mechanism remained uncertain [9,10]. *S. barbata* has been reported to contain flavonoids, phenolic acids, terpenoids, carotenoids, chlorophylls, polysaccharides, essential oils, alkaloids, organic acids, and trace elements [1,11]. However, only the compositions of flavonoids, phenolic acids, carotenoids, terpenoids, polysaccharides, and essential oils were determined [1,12]. Thus, some other vital functional components such as chlorophylls need to be determined as well. In a previous study, we developed a high-performance liquid chromatography–photodiode array detector–mass spectrometry–atmospheric pressure chemical ionization (HPLC–DAD–MS–APCI) method for separation and quantitation of various carotenoids in *S. barbata*, and a total of 18 carotenoids were identified with all-*trans*-lutein being present in the most abundant amount [13].

The objectives of this study were to determine total flavonoids, total phenolics, carotenoids, and chlorophylls in both ethanol and ethyl acetate extracts of *S. barbata*. In addition, the anti-inflammatory activity of these functional components toward RAW 264.7 cells was investigated.

2. Methods

2.1. Materials

A total amount of 3 kg *S. barbata* was procured from a local Chinese drug store and subjected to cleaning, freeze drying (−40°C, 60 mTorr), grounding into powder, pouring into several separate bags, sealing under vacuum, and storing at −20°C until use.

2.2. Chemicals and reagents

Carotenoid standards, including all-*trans*-zeaxanthin and all-*trans*-β-carotene, were purchased from Sigma-Aldrich Co. (St Louis, MO, USA), while all-*trans*-lutein was obtained from Fluka Chemical Co. (Buchs, Switzerland) and 9- or 9′-*cis*-neoxanthin from ChromaDex (Irvine, CA, USA). Internal standard all-*trans*-β-*apo*-8′-carotenal was also from Fluka Chemical Co. Anhydrous sodium sulfate was from Nacalai Tesque Co. (Kyoto, Japan). Chlorophyll standards including chlorophyll a, chlorophyll b, and internal standard zinc-phthalocyanine were from Sigma-Aldrich Co. Silica gel 60 thin-layer chromatography plates (0.5 mm thickness) were from Merck Co. (Darmstadt, Germany); 95% ethanol was from Taiwan Tobacco and Wine Monopoly Bureau (Tainan, Taiwan). Deionized water was made using a Milli-Q water purification system from Millipore Co. (Bedford, MA, USA). Solvents including ethanol, ethyl acetate, chloroform, hexane, acetone, toluene, methanol, acetonitrile, and methylene chloride were from Merck Co. Macrophage cell RAW 264.7 was from Bioresource Collection and Research Center (Hsinchu, Taiwan). Reagents for cell culture were from Invitrogen Co. (Carlsbad, CA, USA), Hyclone Co. (Logan, UT, USA), and Sigma-Aldrich Co. Lipopolysaccharide (LPS; from *Escherichia coli* O26:B6) was also from Sigma-Aldrich Co. BCA protein assay reagent kits and NE-PER nuclear and cytoplasmic extraction reagents were from Pierce Co. (Belvidere, IL, USA). The prostaglandin E2 enzyme immunoassay (PGE_2 EIA) kit was from Bnzo Life Sciences Co. (Farmingdale, NY, USA). The DuoSet enzyme-linked immunosorbent assay (ELISA) kits for cytokine determination were from R&D Systems Co. (Minneapolis, MN, USA). Western blotting reagents were from Cell Signaling Co. (Danvers, MA, USA), BD Biosciences Co. (San Jose, CA, USA), and Anaspec Co. (Fremont, CA, USA).

2.3. Instrumentation

The HPLC instrument is composed of an Agilent G1311A pump, a G1316A column temperature controller, a G1315B photodiode array detector, and a 6130 quadrupole mass spectrometer with multimode ion source (APCI and electrospray ionization; Agilent Co., Palo Alto, CA, USA). The Beckman DU640 spectrophotometer was from Beckman Co. (Fullerton, CA, USA). The N-1000 rotary evaporator was from Eyela Co. (Tokyo, Japan). The Sorvall RC5C high-speed centrifuge was from Du Pont Co. (Wilmington, DL, USA). The DC400H sonicator was from Chuan-Hua Co. (Taipei, Taiwan). The V-U shaker was from Hsiang-Tai Co. (Taipei, Taiwan). A YMC C_{30} polymeric column (250 × 4.6 mm^2 ID, 5 μm particle size) used to separate carotenoids was from YMC Co. (Kyoto, Japan). A HyPURITY C_{18} column (150 × 4.6 mm^2 ID, 5 μm particle size) used to separate chlorophylls was from Thermo-Keystone Co. (Bellefonte, CA, USA). The freeze dryer (FD24) was from Gin-Ming Co. (Taipei, Taiwan). The VersaMax ELISA microplate reader was from Molecular Devices Co. (Sunnyvale, CA, USA).

2.4. Preparation of S. barbata extracts

S. barbata powder (96 g) was poured into 12 centrifuged flasks separately with 8 g each, followed by addition of 160 mL ethanol or ethyl acetate to six flasks each. Then the solution was sonicated at 25°C for 10 minutes, shaken for 20 minutes, and centrifuged at 15,400*g* for 20 minutes. The supernatant was then collected and filtered through a No. 1 filter paper. After evaporation to dryness under vacuum, the residue was weighed. All the residues were combined, and 2.9082 g powder was obtained from the ethanol extract, while 1.4399 g powder was obtained from the ethyl acetate extract. Next, both powder from ethanol extracts and that from ethyl acetate extracts

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