Anti-inflammatory Effects of 4 Medicinal Plant Extracts in Lipopolysaccharide-induced RAW 264.7 Cells

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Abstract

The anti-inflammatory activity of 4 plant extracts [guava (Psidium guajava) leaf, capillary wormwood (Artemisia capillaris Thunb.), Chinese goldthread (Coptis chinensis), and dandelion (Taraxacum platycarpum)] was investigated in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. Six phenolic compounds (gallic acid, caffeic acid, chlorogenic acid, catechin, quercetin, and baicalin) were analyzed using LC-MS/MS. Guava leaf extracts showed the highest inhibitory effects on LPS-induced nitric oxide (NO, 52.58%) and prostaglandin E\(_2\) (PGE\(_2\), 43.45%) production. The total phenolic contents (TPC) in guava leaf, capillary wormwood, Chinese goldthread, and dandelion were 426.84, 154.42, 41.73, and 122.04 mg of gallic acid equivalent (GAE)/g of extract, respectively. TPC was positively correlated with the NO-inhibitory effect (r=0.963, p<0.05) and the PGE\(_2\)-inhibitory effect (r=0.971, p<0.05) at 30 µg/mL of treatment. The guava leaf extracts contained the highest levels of gallic acid and catechin, while the capillary wormwood extracts contained the highest levels of chlorogenic acid and quercetin.

Keywords: Psidium guajava, Artemisia capillaris Thunb., Coptis chinensis, Taraxacum platycarpum, anti-inflammation

Introduction

Inflammation is a type of protective response caused by a variety of factors such as physical and chemical factors, immunological reactions, microbial infections, and tissue damage (1). Its main functions are to protect the body against a wide variety of harmful agents and to promote the renewal of normal tissue. One important role of the macrophages is the release of pro-inflammatory mediators, such as nitric oxide (NO), prostaglandin E\(_2\) (PGE\(_2\)), and various cytokines, in response to activation signals, including chemical mediators, cytokines, and bacterial lipopolysaccharide (LPS) (2). The inflammatory response in the host is important for interruption and resolution of the infectious diseases, but it is also often responsible for the signs and symptoms of the disease (3). The persistence of a process for the remission of diseases may lead to various diseases that are associated with chronic inflammation, including arthritis (4), atherosclerosis (5), and even cancer (6). There is a need to develop new anti-inflammatory drugs because the currently available anti-inflammatory drugs cause adverse effects and many patients are resistant to them. Natural products are a valuable source of novel bioactive secondary metabolites. Therefore, it is important to identify natural products that have a pharmacological or biological activity for use in pharmaceutical drug discovery and design (7,8).

The 4 medicinal plants [guava (Psidium guajava) leaf, capillary wormwood (Artemisia capillaris Thunb.), Chinese goldthread (Coptis chinensis), and dandelion (Taraxacum platycarpum)] have long been used in traditional medicine in Asian countries such as Korea, China, and Japan (9-12). A review of the available literature indicated that there has been considerable pharmacological research on these medicinal herbs. Medicinal plants typically contain various chemical compounds that may act individually, additively,
or synergistically to improve health.

Guava contains several chemical constituents that possess antibacterial, antidiarrheal, antihyperglycemic, antimarial, and antioxidant activities (13). Extracts of dandelion and Chinese goldthread have been used as anti-inflammatory agents to improve immune function (11,12). The dandelion extract has also been used as an anti-inflammatory agent to treat colitis and ulcers (14); has been used in combination with other herbs to enhance the immune response to upper respiratory tract infections, bronchitis, or pneumonia; and has been used as a compress for its antimastopathy activity (12). The rhizome of Chinese goldthread is known to contain alkaloids such as berberine, palmatine, epiberberine, coptisine, jatrorrhizine, and columbamine (10). These alkaloids have been shown to protect against peroxynitrite-induced damage and improve β-amyloid-induced memory dysfunction (15). Capillary wormwood has been utilized in the treatment of diuresis and as an anti-inflammatory agent in China and Japan. Aqueous extracts of the capillary wormwood inhibit the expression of inflammatory proteins, including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and tumor necrosis factor-α (TNF-α) (11).

However, until now, the differences in the bioactive components and pharmacological activities of these 4 medical plants remained obscure. In addition, because it has been demonstrated that the phenolic content of plants correlates with anti-inflammatory activities, it was assumed that the extracts and their differing phenolic contents would have distinctive anti-inflammatory activities. Phenolic compounds are one of the most important groups of compounds that occur in plants. These compounds exhibit anticarcinogenic, anti-inflammatory, antitherogenic, antithrombotic, immune-modulating, and analgesic activities, and they exert these functions as antioxidants (16,17).

This study investigated the anti-inflammatory effects of the 4 different plant extracts (guava leaf, capillary wormwood, Chinese goldthread, and dandelion) in vitro and then identified and quantified 6 selected components, most of which are bioactive polyphenolics. LC-MS/MS was used to identify and quantify these compounds.

**Materials and Methods**

**Chemicals and reagents** Gallic acid, caffeic acid, (+)-catechin hydrate, chlorogenic acid, quercetin, and baicalin were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol and water were purchased from J.T. Baker (Phillipsburg, NJ, USA). RPMI 1640 medium and phosphate-buffered saline (PBS) were purchased from WelGENE Inc. (Daegu, Korea). Lipopolysaccharides from *Escherichia coli* serotype 0111:B4 (LPS), NaN3, Griess reagent, dimethyl sulfoxide (DMSO), and MTT reagent were obtained from Sigma-Aldrich. Fetal bovine serum and penicillin-streptomycin were obtained from Gibco Life Technologies (Rockville, MD, USA). Prostaglandin E2 (PGE2) enzyme immune assay (EIA) kit was purchased from R&D Systems (Minneapolis, MN, USA).

**Plant materials and extract preparation** In March 2011, whole plants of the capillary wormwood (*Artemisia capillaris* Thunb.) and dandelion (*Taraxacum platycarpum*) and rhizomes of Chinese goldthread (*Coptis chinensis*) were obtained from the Department of Oriental Pharmacy, Kyung Hee Medical Center, Seoul, Korea. The guava (*Psidium guajava* L.) leaves were obtained from the College of Applied Life Science, Jeju National University, Jeju, Korea. The plant was taxonomically identified and authenticated by Dr. SK Cho, Faculty of Biotechnology, College of Applied Life Science, Jeju National University, Jeju, Korea. Voucher specimens of the plant materials are kept in our laboratory (Korea Food Research Institute, Seongnam, Korea) for further reference. The dried powder (20 g) of these medicinal plants (guava, capillary wormwood, Chinese goldthread, and dandelion) was extracted in 400 mL of 70% ethanol at 30°C for 6 h. Each ethanol extract was filtered using filter paper (Whatman no. 4). The ethanol was removed under reduced pressure by rotary evaporation, and the water residue was removed by lyophilization. For testing, the extracts were dissolved in PBS and diluted to the desired concentrations.

**Assay for total phenolic content (TPC)** Total TPC of the filtered extracts was determined using the Folin-Ciocalteu reagent (18), with slight modifications. Around 0.5 mL of the filtered extract (1:10 dilution) was added to 0.5 mL of 50% Folin-Ciocalteu reagent. After 3 min, 10 mL of 2% Na2CO3 solution was added, and the solution was incubated for 1 h at room temperature. TPC was measured at 750 nm by using a spectrometer (Milton Roy, New York, NY, USA), and gallic acid was used as a standard at concentrations of 0.0625, 0.125, 0.25, 0.50, and 1.0 mg/mL. From the calibration curve, the mean absorbance was used to determine the TPC of the filtered extracts in gallic acid equivalents (GAE; R2=0.9964).

**Cell culture** RAW 264.7 mouse macrophage cells were purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea) and were maintained in RPMI 1640 medium that contained 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin G, and 100 mg/mL streptomycin at 37°C in a humidified incubator (5% CO2 and 95% air).

**Cell viability** Cytotoxicity was measured using colorimetric