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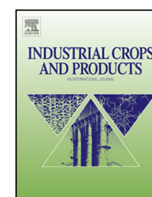

Chromatographic analysis, antioxidant, anti-inflammatory, and xanthine oxidase inhibitory activities of ginger extracts and its reference compounds

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ABSTRACT

Ginger, *Zingiber officinale* Roscoe, is a spice used as a medicinal plant in many countries. We are the first to report the HPTLC analysis of ginger extract and analysis of their active principles with comparative antioxidant, anti-inflammatory, and xanthine oxidase inhibitory activities. The five fractions were obtained by using different polarity solvents with selective extraction procedure from ginger rhizomes and found that they revealed the difference in bioactivity against studied parameters. The ethyl acetate extract (EAE) showed significant antioxidant activity studied by DPPH, FRAP, and H_2O_2 assay ($IC_{50} \pm$ SEM [μg/mL]: 6.8 ± 0.6, 12 ± 0.2, and 20 ± 2.5, respectively). In the xanthine/xanthine oxidase system, the antioxidant potentials of EAE and the water extract (WE) (% inhibition: 76% and 74%, respectively) were higher than those of the ethanol extract (EE), diethyl ether extract (DEE), and *n*-butanol extract (NBE). Regarding anti-inflammatory activity, EAE exhibited greater inhibition of lipoxidase (80%), and β-glucuronidase (78%) compared to hyaluronidase (46%) and diene-conjugates (37%). Chromatographic analysis revealed that several principal substances including 6-gingerol, 6-shogaol, and 6-paradol were responsible for the biological activities for ginger. Compound 6-gingerol revealed high FRAP-reducing activity ($IC_{50} \pm$ SEM [μM]: 5 ± 0.4). 6-Gingerol also significantly inhibited the activities of xanthine oxidase (85%), lipoxidase (87%), β-glucuronidase (85%), and hyaluronidase (56%), respectively. These results indicated that ginger rhizome fractions and its active constituents having promising antioxidant, anti-inflammatory, and anti-gout properties and might be used as potential natural drug against oxidative stress and inflammatory related diseases after successful in vivo study and clinical trials.

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1. Introduction

Ginger (*Zingiber officinale*, Zingiberacae) is commonly used as spice food and dietary supplement and has been considered as an important ingredient in Ayurvedic, Unani and Chinese herbal medicines for the treatment of various diseases and disorders such as asthma, gingivitis, catarrh, toothache, stroke, constipation, diabetes, and rheumatism (Wang and Wang, 2005; Tapsell et al., 2006). Several studies have examined and reflected that the ginger is commonly used as medicinal spice as it reflects various medicinal properties (Chrubasik et al., 2005; Badreldin et al., 2008). Ginger was reported to have medicinal properties like antimicrobial, antifungal, antiviral, antioxidant, anti-inflammatory, and anticancer activities (Bartley and Jacobs, 2000; Dugasani et al., 2009), and exhibits characteristic odors and flavors with a pungent taste (Jolad et al., 2005). As ginger is known to be having antioxidant and anti-inflammatory agent; it also exhibits cancer prevention properties, and is used as a postoperative antiemetic (Grzanna et al., 2005; Chaiyakunapruk et al., 2006; Shukla and Singh, 2007). The odor of ginger depends mainly on its volatile oil, the yield of which varies from 1% to 3%. Over 50 components of the oil have been characterized, including monoterpenoids and sesquiterpenoids (Janick, 2012). The homologous series of phenols called gingerols responsible for pungency and odor in fresh ginger. Ginger rhizome extracts contain specific phenolic compounds gingerol and its derivatives with various biological activities specifically; antioxidant and anticancer (Yeh et al., 2014). Curcumin, another active component present in ginger, has wide range of activities like antimicrobial, anticancer, antioxidant and an anti-inflammatory activity, also activate the heme oxygenase-1 activity, and protects endothelial cells against oxidative stress occurred due to free radicals (Motterlini et al., 2000). In ginger, the widely distributed compounds is 6-gingerol and its derivatives, although smaller quantities of other compounds are also present like shogaol, paradol and other phe-

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nolic acids with different chain (Badreldin et al., 2008). As the odor and pungency of the fresh and dry ginger mainly results from dehydrated forms of gingerols but in many preparations using ginger the thermal processing can produce shogaols which may lead to production of odor and pungency in fresh and dry ginger (Wohlmuth et al., 2005). The antioxidant compounds or phytochemicals from natural sources like plants, fruits, crops and spices are important in the food industry because of their usefulness in various food preparations and health promoting effects (Ibañez et al., 2003). Thus, the demand for natural antioxidants has increased due to the growing interest in the food and pharmaceutical industries for development of drug which has less side effects and potent against various diseases (Yeh et al., 2014). From literature survey, it was found that the ginger contains a number of bioactive phenolic and non phenolic constituents, which in pure form or its derivatives might be potentially useful in the treatment of various diseases like oxidative stress, diabetes, cancer, arthritis, gout, gastric ulcer, hypercholesterolemia, pain, microbial or viral infection (Chrubasik et al., 2005; Badreldin et al., 2008), here we presented many benefits of ginger from reviewed literature and formulated this study. Therefore, in this study, we investigated the HPTLC and HPLC analysis of ginger extract for bioactive constituents with antioxidant, anti-inflammatory, and xanthine oxidase inhibitory properties. Antioxidant activities of the ginger extracts with its chemical constituents like 6-gingerol, 6-shogaol, and 6-paradol were evaluated by assaying their DPPH (2,2-diphenyl-1-picryl hydrazine)- and OH-radical-scavenging activities, reducing abilities, and inhibition of xanthine oxidase (XO). Anti-inflammatory effects of ginger extracts, 6-gingerol, 6-shogaol, and 6-paradol were assessed using in vitro diene-conjugate, β-glucuronidase, and hyaluronidase lipoxidase inhibition assays. The compositions of the most active sub-fractions of 6-gingerol, 6-shogaol, and 6-paradol were determined by HPLC and HPTLC.

2. Materials and methods

2.1. Chemicals

The chemicals used in this study were bovine serum albumin (BSA) and quercetin which was purchased from Daejung Chemicals, Korea. 2-Diphenyl-1-picrylhydrazine radical (DPPH), thiobarbituric acid (TBA), trichloroacetic acid (TCA), bovine testis hyaluronidase (BTH), nicotinamide adenine dinucleotide phosphate (NADPH), 5,5-dithiobis-(2-nitrobenzoicacid) (DTNB), lipoxidase from glycine max (LOX), luminol, linoleic acid (LA), nitro blue tetrazolium (NBT), xanthine, allopurinol (AL), and xanthine oxidase were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). 6-Gingerol (6G), 6-shogaol (6S), and 6-paradol (6P) were procured from Chromadex (Santa Ana, CA, USA). Glutathione (GSH). The chemicals and solvents used for this study were of HPLC grade, unless stated otherwise.

2.2. Plant material

Ginger rhizomes (5 kg) (*Zingiber officinale* Roscoe) were purchased from a local supermarket, Seoul, Korea. The ginger rhizomes were stored at 4 °C and washed thoroughly prior to use. Washed ginger was sliced into small pieces and dried in an oven at 60 °C for ~1 h. Unpeeled ginger was used for solvent extraction since peeled ginger loses much of its essential oil content.

2.3. Extract and sub fractions preparation

Various solvent systems [distilled water (DWE), 70% ethanol (EE), diethyl ether (DEE), ethyl acetate (EAE) and *n*-butanol (NBE)] were used to prepare the extracts and sub-fractions. Powdered ginger (10 gm) was separately extracted with distilled water (5 × 200 mL) in an ultrasonicator bath (Wise-Clean, Korea) at 40 ± 5 °C, for 1 h. The collected supernatants were filtered through funnel using glass wool and washed with 10 mL of extraction solvent. The filtered residue was then concentrated to dryness under vacuum (Buchi System, Switzerland) at 40 ± 5 °C and subjected to subsequent cooling at −80 °C, further lyophilized using a vacuum concentrator until to get a constant weight. For subfractions preparations, the accurately weighed 5 gm of ginger rhizome was separately extracted using methanol (5 × 100 mL) and once with 100 mL of 80% (v/v) methanol using ultrasonicator bath (Wise-Clean, Korea) at controlled temperatures (40 ± 5 °C) for 1 h. To obtain subtractions, the methanolic extracts were further diluted with water and successively partitioned using ethanol, diethyl ether, ethyl acetate and *n*-butanol after solvent evaporation under reduced pressure. All extracts obtained by successive extraction and evaporation of solvent were concentrated to dryness at 40 ± 5 °C under a vacuum (Büchi System, Switzerland), further subjected to cooling at −80 °C, and lyophilized using a vacuum dryer to gain a constant weight (Tomczyk et al., 2011; Bazylko et al., 2013)

2.4. HPTLC analysis

HPTLC chromatograms for ginger rhizome extracts developed using a mobile phase (ethyl acetate:water:formic acid, 85:10:5 [v/v/v]) and the plates (Silica gel 60, F254) were subjected to spraying with anisaldehyde reagent (the reagent was prepared using ice-cooled methanol (170 mL), acetic acid (170 mL), sulfuric acid (10 mL), and 1 mL of anisaldehyde compound. The added mixture was then heated at 10 °C for 3 min and cooled to room temperature). For the qualitative analysis of reference compounds, the standards like 6-gingerol, 6-shogaol, and 6-paradol (1 mg) were individually dissolved in 10 mL methanol, and each was applied to the plates as 10 mm bands. Sample application was performed using the CAMAG-Linomat IV automated spray on band applicator equipped with a 10 mL syringe and operated with the following settings: 10 mm band length, 10 mL/s application rate, 4 mm distance between, 1.5 cm distance from the plate side edge, and 2 cm distance from the bottom of the plate. After development, the plates were air-dried for 15 min, and the chromatograph was visualized under CAMAG TLC Scanner 3 to quantify the bands of these available compounds in ginger extract using the WIN CATS software (version 4X). The scanner operating parameters were: Mode: absorption/reflection; slit dimensions; 5 × 0.1 mm; scanning rate: 20 mm/s and monochromatic band width: 20 nm at an optimized wavelength of 254 nm and in the visible range (Rai et al., 2006; Nile and Park, 2014a).

2.5. HPLC analysis of ginger extract

HPLC analysis of ginger extract, along with reference compounds 6-gingerol, 6-shogaol, and 6-paradol was performed using an Agilent® 1100 LC System (Agilent Technologies Inc., Palo Alto, CA, USA) with an auto-injector sampler programmed at 5 μL capacity per injection. HPLC chromatographic separations were performed on Zorbax Stable Bond, C18 column (4.6 mm × 50 mm, 1.8 μm). All operations, acquisitions, and data analysis were controlled using the Chemstation software (Agilent Technologies, USA). The separation was performed with a mobile phase consisting of acetonitrile and water (85:15, v/v) and a chromatographic run time of 20 min at 30 °C with a flow-rate of 1.0 mL/min, and chromatograms were monitored at 280 nm (Wang et al., 2009; Schwertner et al., 2007).

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