Isolation, Identification and Determination of Antioxidant in Ginger (*Zingiber officinale*) Rhizome

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The antioxidative compounds and antioxidant contents of ginger (*Zingiber officinale*) rhizomes were determined. Substances reextracted using ethyl acetate from crude methanol extract of fresh ginger rhizome were separated through thin layer chromatography. Ten phenolic antioxidative bands were visualized through color reactions using ferric chloride-potassium ferricyanide and 1,1-diphenyl-2-picrylhydrazyl (DPPH). The antioxidative compounds were purified through preparative TLC and high performance liquid chromatography (HPLC), among which, five antioxidants were identified as 4-, 6-, 8-, and 10-gingerols and 6-shogaol on the basis of their molecular weights determined through LC-MS. As shown in experiments using DPPH free radicals, 6-Gingerol and PT4-HP8 (unknown) were revealed to be more efficient than BHT (butylated hydroxy toluene). Contents of gingerols were determined through reverse phase HPLC. Total gingerol contents (sum of 6-, 8-, and 10-gingerols) in rhizomes of different ginger varieties varied significantly. The HG55 (collected at Wanju district in Korea) and the HG52 (imported from Brazil) showed the highest gingerol contents.

Key words: *antioxidant, ginger,* Zingiber officinale, *gingerol, free radical.*

Many enzymes and secondary compounds of higher plants have been demonstrated through *in vitro* experiments to protect against oxidative damages by inhibiting or quenching free radicals and reactive oxygen species.[1)] Much attention has been focused on antioxidative compounds present in edible plants due to safety concerns with synthetic antioxidants.[2)] Some spices, already known to have antiseptic activities, have been utilized in the storage of meat from earlier times. In Asia, ginger (*Zingiber officinale* Roscoe) has long been used as a flavoring agent, carminative, stimulant, and crude medicine, and is now the most important rhizomatous spice for international trade. The principal compounds responsible for the pungency of ginger are gingerols and shogaols. Gingerols are homologous series of phenolic ketones present in the rhizomes of ginger.[3-4)] They have been shown not only to exhibit a number of pharmacological effects including inhibition of prostaglandin biosynthesis,[5)] anti-hepatotoxicity,[6)] cardiotonic,[7)] and anti-platelet,[8)] but also have antioxidative effects.[9-10)] This experiment was thus carried out to find antioxidant compounds and determine their contents in ginger cultivars collected from various district of Korea and foreign countries.

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Abbreviations: BHA, butylated hydroxyanisole; DW, dry weight; MW, molecular weight.

Materials and Methods

Sample preparation. Sixteen ginger cultivars collected from local district and foreign countries were cultivated from April to October 1997 at the Honam Agricultural Experiment Station, Iksan City, Korea. Rhizomes harvested were kept in a deep freezer (Forma model 8580) at -80°C until further use.

Isolation of antioxidative compounds. The rhizomes, HG-55 collected from Wanju district in Korea, were freeze-dried and ground. The powdered ginger rhizome (120 g) were extracted using methanol (Fisher, HPLC grade), and the extracts were concentrated under a reduced pressure to remove methanol. The remaining aqueous solution was partitioned using ethyl acetate. The ethyl acetate fraction was spotted on TLC plates precoated with silica gel 60 F_{254} (particle size 250 μm, Merck) to test for the presence of antioxidants. Total ethyl acetate fraction was absorbed onto a glass column (6.5 × 25 cm) packed with Kisegel 60 (Merck) and continuously eluted with toluene : ethyl acetate (7 : 3) as a mobile phase. The antioxidative activity of each fraction was monitored through 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging activity. The fractions with DPPH radical scavenging activity were pooled and dried *in vacuo* using a rotary evaporator and dissolved in MeOH. The active fraction of silica gel column was separated through preparative TLC and subsequently scraped off with a spatula. Each scraped zone was desorbed by MeOH and concentrated *in vacuo*. Each prep-TLC fraction was separated through HPLC. These preparative TLC and HPLC were repeated several times.

Thin-layer chromatography. For antioxidant identification, the samples were applied as spots on a 5 × 20 cm plate (silica gel 60 F_{254}, Merck). For preparation purpose, the samples were applied as a strip on a 20 × 20 cm plate (layer thickness 1 mm, silica gel 60 F_{254}, Merck). Toluene : ethyl acetate (7 : 3) was used as the developing solvent. The developed plate was examined by spraying with 1 mM DPPH solution for visualizing antioxidant compounds and 5% ferric chloride-potassium ferricyanide in ethanol for phenolic compounds. DPPH scavenging zones were scraped and desorbed by methanol.

High performance liquid chromatography (HPLC). The chromatography was conducted on a Waters Associate system that included two Model M-515 pumps, Model 2487 UV detector, and Millenium-32 chromatography manager. Detection was based on UV absorption at 282 nm. Preparative HPLC was carried out using a stainless-steel column (Supelcosil LC-18, 250 × 21.2 mm) and 80% MeOH as the mobile phase. The flow rate was 8 m*l*/min. Anaytical HPLC for determining the contents of gingerols was carried out using a stainless-steel column (Whatman 10-ODS-1, 250 × 4.6 mm) and 65% MeOH as the mobile phase. The flow rate was 1 m*l*/min.

Mass spectrometry. Mass Spectrometry was carried out using an electrospray ionization/mass spectrometer (VG Biotech platform) at the Korea Basic Science Institute.

Determination of antioxidative activity through DPPH radical scavenging method. The DPPH radical scavenging method of Duh and Yen[11] was modified as follows. The soluble compounds isolated from ginger was standardized to give stock solutions containing 100 μg soluble solid per 1 m*l* methanol. The stock solutions were diluted to 5, 10, 20, 40, 60, and 100 μg/m*l* in MeOH and 100 μ*l* of each concentration was added to the methanolic solution (900 μ*l*) of DPPH radical; final concentration of DPPH was 0.1 mM. The mixture was shaken vigorously and kept standing at room temperature for 30 min. The absorbency of the resulting solution was measured using a Beckman spectrophotometer DU 70 at 517 nm.

Determination of gengerol compounds. Ginger rhizomes (5 g, fresh weight) were chopped and loaded in the cellulose thimble tube in the Soxhlet extractor. They were then continuously extracted with acetone (100 m*l*, Fisher HPLC grade) for 8 h. One milliter eugenol (1 mg/m*l*) was added as an internal standard. The acetone extract was concentrated in vacuo, dissolved with MeOH, filtered through a membrane syringe filter (0.45 μm PTFE, Cole-Parmer), and then analyzed through HPLC. Each determination was repeated three times.

Results and Discussion

Isolation and identification of antioxidative compounds from ginger. The freeze-dried ginger powder was extracted with methanol and fractionated with ethyl acetate. The ethyl acetate fraction was separated on TLC. Antioxidative spots were visualized as yellow to purple by spraying DPPH solution, and phenolic compounds resulted in deep-blue to green by spraying 5% ferric chloride-potassium ferricyanide in ethanol (Fig. 1). The antioxidative compounds in ginger were tentatively identified as phenolic compounds as demonstrated through the above methods. The ethyl acetate fraction was further separated through silica-gel column chromatography, and the fractions with DPPH scavenging activity were pooled. The column fraction was separated into five fractions (PT1-PT5) through preparative-TLC (Fig. 1), and each fraction was separated through preparative-HPLC. Ten antioxidative compounds were isolated, and Rf values were measured through TLC, molecular weight through LC-MS, and the amount of compound to scavenge 50% of 100 μM DPPH through DPPH radical scavening method (Table 1). Among them, five compounds were confirmed as 4-, 6-, 8-, and 10-gingerols and 6-shogaol based upon the published data.[1,3-4,12] Other compounds with amounts less than the identified compounds were not identified. The antioxidative activities of most compounds isolated from ginger were inferior to BHA, but the 6-gingerol, PT4-HP8 (MW 308), PT1-HP3 (MW 296), and 4-gingerol were superior to BHT. According to Lee and Ahn,[9] the mixture of 6-gingerol and 10-gingerol showed lower antioxidative activity than BHA and BHT in beta-carotene-linoleic acid-water emulsion system. Aeschbach *et al.*[10] reported that 6-gingerol decreased the peroxidation of phospholipid liposomes in the presense of iron(III) and ascorbate. Kawai *et al.*[13] isolated 6-, 8, and 10-gingerols from the methanolic extract of ginger as anti-emetic principles. Gingerols were also revealed the main pungency compounds in ginger,[12,14-15] and showed various pharmacological effects as anti-hepatotoxicity,[6] anti-platelet,[8] and cardiotonic.[7] The 6-gingerol showed highest

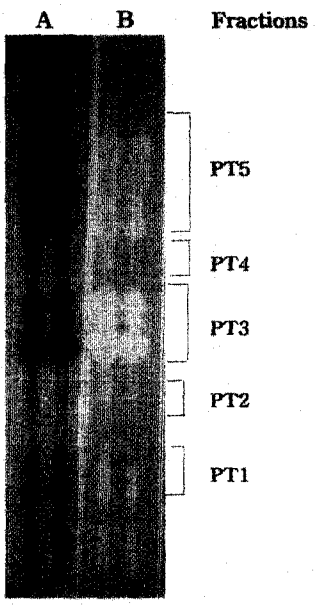

Fig. 1. Thin-layer chromatographic separation of ethyl acetate fraction of ginger rhizome methanol extract. (A) phenolic compounds were visualized by spraying 5% Ferric chloride-potassium ferricyanide in ethanol. (B) Antioxidative compounds were visualized by spraying 1 mM DPPH.

Table 1. The molecular weight, Rf value in TLC, and free radical scavenging activity of antioxidative compounds isolated from ginger rhizome.

Fractions	Compound name(MW)	Rf in TLC[1)]	Purity(%)	Antioxidative activity[2)] SC_{50}(μg/m*l*)
PT1-HP1	UK[3)] (129)	0.22	91.5	17.5
PT1-HP2	UK (261)	0.24	87.5	85
PT1-HP3	UK (296)	0.31	84.9	17.5
PT2-HP4	4-gingerol (266)	0.42	76.4	19
PT3-HP5	6-gingerol (294)	0.50	98.1	13
PT3-HP6	8-gingerol (322)	0.54	90.5	53
PT3-HP7	10-gingerol (340)	0.57	94.5	46
PT4-HP8	UK (308)	0.70	95	12
PT5-HP10	6-shogaol (276)	0.78	80.6	37
PT5-HP11	UK (UK)	0.81	60.9	26
BHA				3.3
BHT				31
Eugenol				3.4

[1)]Migration distance of the compounds divided by migration distance of the solvent front in TLC plate (Silica gel 60 F_{254})
[2)]The concentraction of compounds required to scavenge 50% of 100 μM DPPH.
[3)]UK: Unknown

Table 2. Contents of gingerol compounds in various varieties of ginger collected from various countries.

Variety[1)]	Collection country	6-gingerol	8-gingerol	10-gingerol	Total gingerol
		mg/g DW			
HG-55	Korea (Wanju)	24.66	4.44	4.73	33.83
HG-51	Brazil	27.89	2.53	3.09	33.51
HG-40	India	7.64	1.28	1.34	10.26
HG-52	Korea (Hongsung)	8.81	3.34	2.06	14.21
HG-39	Thailand	13.24	2.45	2.03	17.72
HG-11	Korea (Nonsan)	12.39	1.95	1.91	16.25
HG-13	Korea (Yeongarm)	14.80	3.69	3.57	22.06
HG-15	Korea (Bosung)	14.09	2.55	2.23	18.87
HG-38	Japan	8.28	2.16	1.66	12.10
HG-50	USA	10.01	2.64	5.04	17.69
HG-53	China	20.86	3.05	1.77	25.68
HG-54	Korea (Jumuk)	18.41	2.87	3.62	24.90
HG-43	Korea (Seosan)	12.68	0.93	1.56	15.17
HG-36	Philippines	11.54	3.81	2.96	18.31
HG-1	Korea (Wanju 1)	7.81	1.88	1.64	11.33
HG-37	Philippines	12.93	2.42	2.76	18.11
CV(%)		42.6	35.1	43.1	36.5

[1)]Names of breeding lines designated by Honam Agricultural Experiment Station.

pungency.[4)]

Determination of gingerols in ginger. The HPLC separation of acetone extract of fresh ginger rhizome are shown in Fig. 2. Identification of gingerol compounds in the extract was confirmed by comparing the retention times with the above isolated gingerols. The chromatographic separations of gingerols are similar to previous reports,[14-15)] which were conducted using gradient elution of methanol in analytical HPLC. But 4-gingerol and PT5-HP11 could not be detected due to the presence of trace amount in the acetone extract, and PT1-HP1 was not separated well. According to Chen *et al.*,[14-15)] 6-, 8-, and 10-gingerols were the dominant compounds identified in liquid CO_2 extract, and 6-shogaol showed trace amount. Based upon the results of HPLC analysis, contents of gingerols, the main antioxidative compounds in ginger, were measured in different ginger varieties collected from various countries including Korea (Table 2). The total gingerol content (6-, 8-, and 10-gingerols) ranged from 10.26-33.83 mg/g DW, and the coefficient value was 36.5%. 6-Gingerol content was the most abundant and varied among varieties (7.64-27.89 mg/g DW). The Wanju (HG-55) and Brazil (HG-51) collection cultivars showed the highest concentrations of total gingerols.

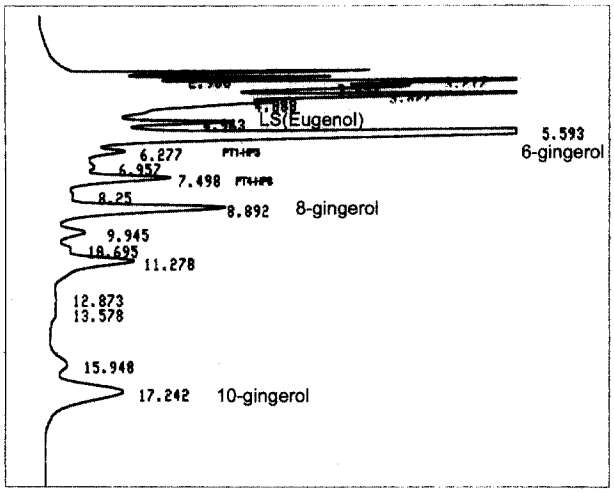

Fig. 2. HPLC separation of acetone extracts of ginger rhizome. Eugenol was added as an internal standard.

Although the methods of Chen *et al.*[14] and Bartly,[4] using liquid CO_2 and HPLC analysis, was different, the results obtained in this study was similar. The variation in gingerol contents among different varieties offers a possible index to distinguish or judge the selection of breeding line for the development of high quality ginger.

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