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Essential Oil Composition and Antioxidant Activity of *Panax ginseng* of Different Regions in Korea

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The composition of the roots of *Panax ginseng* essential oils of three different regions of Korea namely Po-chen (I), Gang-won (II) and Gang-hwa (III) were examined by gas chromatography-mass spectrometry (GC-MS). The essential oil yields (mL/100g DW) were for the Po-chen (I) 0.1 %, Gang-won (II) 0.12 % and Gang-hwa (III) 0.09 %. Thirty six compounds comprising 96.9 % of the total peak area were identified in Pochen (I). Thirty eight compounds comprising 99.01 % of the total peak area were identified in Gang won (II). Thirty seven compounds comprising 99.89 % of total peak area were identified in Gang-hwa (III). The common major components in these three regions were α-panasinene (7.12, 5.92 and 3.31 %), α-elemene (5.81, 2.26 and 2.50 %), neoclovene (7.05, 9.69 and 11.88 %), 1,6,10-dodecatriene-7,11-dimethyl-3-methylene (7.44, 5.93 and 12.31 %), bicyclogermacrene (12.58, 22.61 and 25.41 %), naphthalene 1,2,3,4,5,6,7,8α-octahydro-1,8α-dimethyl-7-(1-methylethenyl)-(4.41, 3.28 and 3.28 %), selina-6-en-4-ol (3.06, 2.09 and 1.57), respectively. Water extract of three different regions ginseng roots were investigated for their antioxidant activity. Two different bioassays were used, namely scavenging of the diphenylpicrylhydrazyl (DPPH) radical method and the other reducing power of Fe^{3+} method. The total phenolic content was quantified as well. All the three different ginseng root water extracts contributed to the strongest antioxidant activity. The root water extracts of Ginseng has potential as a natural antioxidant and thus inhibit unwanted oxidation process.

Key Words: *Panax ginseng*, Araliaceae, Essential oil constituents, Different regions, Antioxidant activity.

INTRODUCTION

Panax ginseng (Korean Ginseng) is any one of eleven distinct species of slow-growing perennial plants with fleshy roots, belonging to the *Panax* genus in the family Araliaceae. It grows in the northern hemisphere in eastern Asia (mostly northern China, Korea and eastern Siberia), typically in cold climates. *Panax vietnamensis*, discovered in Vietnam, is the southernmost ginseng found. This article focuses on the series *Panax ginseng*s, which are the adaptogenic herbs, principally *Panax ginseng* and *Panax quinquefolius*. Ginseng is characterized by the presence of ginsenosides. The *Panax ginseng* has been used in traditional medicine as Asian or oriental ginseng since ancient times. *Panax ginseng*, known as Korean Ginseng, has been valued as a medicinal plant in traditional oriental medicine[1,2]. The biological and pharmacological efficacy of Korean Ginseng has been reported[3-7]. Earlier publications have been reported from ginseng roots essential oils[8-14]. To the best of our knowledge, this is the first comparative study of ginseng roots. No comparative study so far reported on ginseng roots. Two different bioassays were used, namely scavenging of the diphenylpicrylhydrazyl (DPPH) radical method and the other reducing power of Fe^{3+} method. The total phenolic content was quantified as well. All the three different ginseng root water extracts contributed to the strongest antioxidant activity. In conclusion, the root water extracts of ginseng has potential as a natural antioxidant and thus inhibit unwanted oxidation process.

EXPERIMENTAL

The fresh roots of *Panax ginseng* of three regions of Korea were collected from the Po-chen, Gang- won and Gang-hwa regions campus in Korea, August 2010.

Isolation of volatile oil: The roots of *Panax ginseng* (500 g) were subjected to hydro-distillation in Clevenger-type apparatus for a minimum of 5 h each. The resulting essential oil was obtained in a yield of 0.10, 0.12 and 0.09 % w/w, respectively from different regions of Korea (I-III). The oil after drying over anhydrous sodium sulphate and stored at 4 ºC until use.

Gas chromatography-mass spectroscopy (GC-MS) analysis of essential oil: Samples of essential oil were diluted in hexane (spectroscopic grade) and analyzed in a Finnigan Focus GC/Finnigan Focus DSQ MS system (Thermo Co., Germany) apparatus equipped with VB-WAX bonded PEG

capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness). Helium (1 mL/min) was used as a carrier gas. Sample volume was injected in the split mode 10 μL (split less). The injector was kept at 150 ºC. The column was maintained at 50 ºC for 10 min and then programmed to 200 ºC at 2 ºC and held for 0.5 h at 200 ºC. Detector temperature was held at 250 ºC. The MS was operated in EI mode at 70 eV in the in the m/z range 25-350. The identification of the compounds was performed by matching their recorded mass spectra of the GC-MS data system. Quantitative data were obtained from electronic integration peak areas and comparing their retention time and mass spectra library with those found in the literature and supplemented by the Wiley (Wiley 7th Mass Spectral Library) & NIST MS Search 2.0 (National Institute of Standards and Technology) GC-MS libraries.

Total phenolic content: The total phenolic content was determined by the Folin-Ciocalteu (FC) method[15] and expressed as grammes of gallic acid equivalents per 100 g plant extract. Distilled water (3.16 mL) was mixed with a DMSO solution of the test compound (200 μL). Then, 200 μL of FC reagent was added. After 5 min, 600 μL of 20 % sodium carbonate solution was added and the solutions were mixed again. The solutions were left at room temperature for 2 h. Then the absorption of the developed blue colour was determined at 765 nm, using a Macasys Optizen 2120UV plus UV-spectrophotometer (Mecasys, Korea).

Free radical scavenging activity: The antioxidant activity of the three different ginseng root water extracts, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl ($DPPH^-$) free radical, was determined by the method described by Katerere and Eloff[16]. The different concentrations (0.25-2.5 mg) of the tested samples (0.2 mL; extracts and tocopherol) were taken in different test tubes with 4 mL of a 0.006 % MeOH solution of $DPPH^-$. Water (0.2 mL) in place of the extract was used as control. Absorbance at 517 nm was determined after 0.5 h. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula, percentage radical scavenging activity = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard.

Reducing power: The reducing power of the different ginseng root water extracts was determined according to the method of Oyaizu[17]. Different ginseng root water extracts of concentration (0.25-2.5 mg) in 1 mL of distilled water was mixed with phosphate buffer (2.5 mL, 0.2 M/L, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1 %). The mixture was incubated at 50 ºC for 20 min. A portion (2.5 mL) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 1000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1 %) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. All analysis were run in triplicate and averaged.

RESULTS AND DISCUSSION

Chemical constituents of the essential oil: Three different regions of Po-chen (I), Gang-won (II) and Gang-hwa (III), Ginseng roots were hydrodistilled and obtained the oil percentage 0.1, 0.12 and 0.09 %, respectively. The identification of the compounds was performed by matching their recorded mass spectra of the GC-MS data system. Quantitative data were obtained from electronic integration peak areas and comparing their retention time and mass spectra library with those found in the literature and supplemented library. Other methods of identification and took help of by comparing their mass data with data of libaray[18,19]. Major components were the sesquiterpene hydrocarbons in these three Ginseng roots, cedrene (2.79, 2.07 and 0.87 %), α-gurjunene (1.29, 0.95 and 0.0 %), α-panasinene (7.12, 5.92 and 3.31 %), α-elemene (5.81, 2.26 and 2.50 %), neoclovene (7.05, 9.69 and 11.88 %), 1,6,10-dodecatriene-7,11-dimethyl-3-methylene (7.44, 5.93 and 12.31 %), bicyclogermacrene (12.58, 22.61 and 25.41 %), naphthalene 1,2,3,4,5,6,7,8α-octahydro-1,8α-dimethyl-7-(1-methylethenyl)-(4.41, 3.28 and 3.28 %), selina-6-en-4-ol (3.06, 2.09 and 1.57 %), longifolene (1.99, 1.23 and 0.81 %), in addition, the oxygenated compounds (–)-spathulenol (2.45, 2.64 and 3.01 %), patchouli alcohol (0.95, 0.40 and 0.58 %), veridiflorol (0.56, 0.40 and 0.58 %), ledol (0.63, 0.49 and 0.42 %), globulol (2.88, 1.90, 2.17 %), falcarinol (1.05, 1.81 and 3.04) and other could be identified in (Table-1). Table-1 showing current chemical composition on the analysis of the volatile oils summarizes from three Ginseng roots. However, the comparison of present results with literature shows qualitative and quantitative difference in the composition of *Panax ginseng* roots oil.

Total phenolic content: The distribution of phenolic compounds in ginseng root water extracts demonstrated that the Gang-hwa water extract contained highest amount 633.9 mg gallic acid equivalents (GAE) per 100 g extract, followed by Gang-won (492.8 mg) and Po-chen (432.8 mg) GAE per 100 g extract. The FC method is actually not an antioxidant test but instead an assay for the quantity of oxidizable substances, *i.e*., phenolic compounds. Correlations between the content of phenolic compounds and antioxidant activity are described[20].

$DPPH^-$ radical-scavenging activity: The free radical-scavenging activity of the different ginseng roots water extract was tested through $DPPH^-$ method[20] and the results were compared with tocopherol. DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. The method is based on the reduction of methanolic $DPPH^-$ solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. The extract was able to reduce the stable radical $DPPH^-$ to the yellow-coloured diphenylpicrylhydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (*e.g*., hydroquinone, pyrogallol, gallic acid) and aromatic amines (*e.g*., *p*-phenylene diamine, *p*-aminophenol), reduce and decolorize 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability[21]. The content of total phenolic compounds in the extracts might explain their high antioxidant activities. In this study, three different ginseng water extracts showed a remarkable antioxidant activity, one of the possible mechanisms is polyphenolic-associated compounds (formation of non-extractable complex between high molecular weight phenolics and polysaccharides).

TABLE-1
COMPOSITION OF KOREAN *Panax ginseng* ROOTS
ESSENTIAL OIL FROM THREE DIFFERENT REGIONS

Retention time	Compounds	Po-chen region (%)	Gang-won (%)	Gang-hwa (%)
20.95	10,10-Dimethyl-1, 11-dedihydrotricyclo[6.3.0]undecanone-3	1.20	0.93	0.44
22.79	Cedrene	2.79	2.07	0.87
25.75	Valencene	–	–	0.62
25.80	α-Gurjunene	1.29	0.95	–
27.82	α-Panasinene	7.12	5.92	3.31
28.06	Bicyclo[5.3.0]decane-2-methylene-5-(1-methylvinyl)-8-methyl	–	–	0.84
28.22	Guaia-3, 9-diene	0.87	–	–
28.34	Valencene	–	0.90	0.62
29.08	Longifolene	1.99	1.23	0.81
29.31	2-(3-isopropyl-4-methyl-pent-3-en-1-ynyl)-2-methylcyclobutanone	–	–	0.68
31.17	Caryophyllene	1.09	0.84	0.47
31.83	Calarene	0.64	0.84	0.87
32.19	Bicyclo[5.2.0]nonane 2-methylene-4,8,8-trimethyl-4-vinyl	–	–	0.26
32.25	1*H*-Cyclopropanaphthalene, 1α,2,3,5,6,7,7α-7β-octahydro-1,1,7,7-α-tetramethyl	2.32	6.10	3.27
32.77	α-Elemene	5.81	2.26	2.50
33.27	Carvacrol methyl ether	–	0.27	–
34.64	(–)-α-Neoclovene	7.05	8.26	5.00
35.28	1*H*-Cyclopropazulene, 1α,2,3,4,4α,5,6β-octahydro-1,1,4,7-tetramethyl	7.76	9.69	11.88
36.81	α-Humulene	2.28	2.06	2.60
37.12	Isocaryophillene	1.92	1.75	1.97
37.33	α-Neoclovene	1.92	1.76	1.47
38.04	1,6,10-dodecatriene-7,11-dimethyl-3-methylene	7.44	5.93	12.31
39.18	α-Selinene	0.89	0.51	np
40.59	Bicyclogermacrene	12.58	22.61	25.41
40.99	*trans*-α-Bisabolene	0.34	np	np
41.46	Cyclohexane 1-ethenyl-1-methyl-2,4-*bis*(1-methylethenyl)	np	np	2.77
41.79	Naphthalene 1,2,3,4,5,6,7,8α-octahydro-1,8α-dimethyl-7-(1-methylethenyl)-	4.41	3.28	–
47.52	1,4-Methanobenzocyclodecene,1,2,3,4,4α-5,8,9,12,12α-decahydro	0.47	1.30	0.67
48.77	Neoisolongifolane hydroxy	–	0.26	np
51.37	Bicyclo[4.1.0]heptane 7-(phenylmethylene)-	np	0.37	np
52.62	Patchouli alcohol	0.95	0.96	1.24
53.63	Veridiflorol	0.56	0.40	0.58
54.93	Ledol	0.63	0.49	0.42
55.44	Tricyclo[7.2.0.0(2,6)]undecan-5-ol 2,6, 10,10-tetramethyl	2.82	2.02	1.45
56.45	Guaiol	0.54	0.44	0.59
57.44	(–)-Globulol	2.88	1.90	2.17
58.94	Rosifoliol	0.54	0.39	0.54
59.30	2-Napthalenemethanol decahydro-α,α,4α-trimethyl-8-methylene	np	np	0.40
59.59	3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)]undecan-1-ol	1.75	1.21	0.99
59.88	(–)-Spathulenol	2.45	2.64	3.01
60.41	Selina-6-en-4-ol	3.06	2.09	1.57
63.46	2-Methoxy-4-vinylphenol	0.48	np	0.44
64.57	Isospathulenol	1.29	1.27	1.18
67.30	10,12-Octadecadiynoic acid	1.17	0.48	0.38
70.07	Oxacyclotetradeca-4,11-diyne	5.16	2.56	1.07
71.21	Fenuron	np	0.26	np
103.70	Falcarinol	1.05	1.81	3.04

np: Not present.

These kinds of phenolic compounds show antioxidant activity due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triple oxygen or decomposing peroxide. The different water extracts exhibited a concentration-dependent antiradical activity by inhibiting $DPPH^{-}$ radical. Three of the selected extracts from the ginseng root exhibited good free radical scavenging activities *i.e.*, above 70 %, the activity increased with increasing concentration. Of the different ginseng root water extracts Gang-hwa exhibited the highest activity of 79.3 %, followed by Gang-won (74.13 %) and Po-chen (72.2 %). The DPPH activity of tocopherol showed higher degree of free radical-scavenging activity than that of the extracts at each concentration points. Wangsteen *et al.*[22] reported that ethyl acetate

extract from the coriander leaves exhibited highest antioxidant activity when compared with other extracts. Whereas Li *et al*.[23] reported that the polysaccharide fraction from the fruits of *Lycium barbarum* exhibited a weak DPPH activity. This is similar to other studies wherein they have reported that only 0.3 mg/mL tocopherol, 0.23 mg/mL BHT and 0.1 mg BHA exhibited a free radical scavenging activity equivalent to 3.9 mg/mL of red bean and 10 mg/mL of sesame coat extract[24,25].

Reducing power: Antioxidant effect exponentially increases as a function of the development of the reducing power, indicating that the antioxidant properties are concomitant with the development of reducing power[26]. Okuda *et al*.[27] have reported that the reducing power of tannins from medicinal plants prevents liver injury by inhibiting formation of lipid peroxides. Reductones are believed not only to react directly with peroxides but also prevent peroxide formation by reacting with certain precursors. The reducing power of the different ginseng root water extracts increased with increasing concentration from 0.25-2.5 mg. Reducing power of the ginseng root water extracts followed the order-Ganghwa < Gangwon < Pohon. The activity of tocopherol was pronouncedly higher than the test samples. This is in line with the observations of several other workers wherein the reducing power of BHT and tocopherol[25] was higher than the extracts. In the present study, though the ginseng root water extracts exhibited a moderate reducing power they did have an activity that reveals that the ginseng root water extracts are electron donors and can react with free radicals and convert them to stable products thus terminating the free radical chain reactions.

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