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섬쑥 추출물의 항산화 활성 및 페놀성 성분

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Antioxidant Activities and Phenolic Compounds of Extract from Hallasan Wormwood

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ABSTRACT

Background: Many *Artemisia* species are used in traditional medicine to treat gastrointestinal disorders and various other ailments. However, the antioxidant and phenolic compounds present in Hallasan wormwood (*Artemisia japonica* var. *hallaisanensis*) have not been investigated yet.

Methods and Results: Extracts of Hallasan wormwood (aerial parts and roots) were extracted using methanol and sequentially treated with solvent-solvent fractionation using *n*-hexane, ethyl acetate, and water-saturated butanol, to yield fractions with variations in polarity. The phenolic compounds present in the extracts and fractions were determined using high performance liquid chromatography (HPLC) and colorimetry. The antioxidant activities were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP), and oxygen radical antioxidant capacity (ORAC) methods. The results revealed that the ethyl acetate fraction had high total phenolic and total flavonoid contents. Furthermore, the ethyl acetate fraction of roots showed the highest antioxidant activity.

Conclusions: Metanolic extracts from the roots and aerial parts of Hallasan wormwood showed different antioxidant activities; nine phenolic acids and one flavonoid were detected in Hallasan wormwood. Thus, the finding of this study provide valuable data for research on functional substances from plants.

Key Words: *Artemisia japonica* var. *hallaisanensis*, Antioxidant, Hallasan Wormwood, Phenolic Compound

INTRODUCTION

Free radicals and other reactive oxygen species are persistently formed in the human body. The oxidation reaction is significant in human health. Oxidative stress is closely associated with several human diseases, including cancer, atherosclerosis, malaria, rheumatoid arthritis, and neurodegenerative diseases (Aruoma, 1998).

Phenolic compounds are one way to prevent oxidative stress. Phenolic compounds have an aromatic ring containing one or more hydroxyl substituent. Structurally, there are a range from simple phenol molecules to highly polymerized compounds. Representative groups are phenolic acids and flavonoids. Phenolic acid is distinguished by hydroxybenzoic acid and hydroxycinnamic acid (Bravo, 1998).

On the other, flavonoids are a general term for more than 4,000 aromatic and plant compounds including anthocyanins (Hertog *et al*., 1992). Ingestion of phenolic acids and flavonoids has been reported to have beneficial health effects.

The biological activity of phenolic compounds may be

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related to their antioxidant behaviour, which is attributed to their ability to free radical scavenging activity and metal chelating activity (Balasundram *et al*., 2006). Actually there is an increased interest in these compounds.

The genus *Artemisia* is a perennial plant and taxonomically belongs to the Asteraceae family. They grow in the warm climates of the northern and southern hemispheres, but they also adapt to a variety of ecological environments, such as mountainous and coastal areas.

Many species of *Artemisia* genus are known as aromatic plants and are frequently used medicinally in folk remedies. For instance, *A. annua* provides Artemisinin and derivatives and is used in the treatment of malaria (White, 1997). It is reported that there are various species of *Artemisia* that grow naturally all over the world, and that there are more than 300 species in Korea alone.

Carvalho *et al*. (2011) analyzed six *Artemisia* species and confirmed differences in phenolic compound content and composition. It was also reported that environmental conditions such as temperature and humidity greatly affect the antioxidant substances and activity produced in plants even in *Artemisia* spp. (Choi *et al*., 2006).

Studies of the bioactivity of *A. japonica* reported antimalarial activity, anti-cancer, anti-obesity, anti-inflammatory, and anti-arthritis (Valecha *et al*., 1994; Choi *et al*., 2013; Li *et al*., 2017). Various studies, such as the number of somatic cell chromosomes (Park *et al*., 2009) and the nucleotide sequence of ITS (Lee *et al*., 2010), confirmed that Hallasan wormwood (*A. japonica* var. *hallaisanensis*) is this taxonomically other variety. However, limited studies were carried out on phenolic compounds and antioxidants of Hallasan wormwood.

Therefore, in this study, Hallasan wormwood was divided into the above-ground part and the root to produce an extract, and fractions were prepared according to the polarity. We conducted a phenolic compound analysis and an antioxidant activity test to provide basic data for promoting its use as a functional material.

MATERIALS AND METHODS

1. Plant material

Hallasan wormwood (*A. japonica* var. *Hallaisanensis*) was collected more than individual 10 plants in the Jeju Island area of South Korea from May to June. The plant was identified and authenticated by Dr. Hyoun-Chol Kim. After air-drying the aerial parts (leaves and stems) and roots of the plant material at room temperature, the dried material was powdered (< 0.5 ㎜).

2. Reagents

Folin Ciocalteu's phenol reagent was purchased from Hayashi Pure Chemical Inc., Ltd. (Osaka, Japan). Flavonoid standard compound was obtained from ChemFaces Bio-chemical Co., Ltd. (Wuhan, China). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), phenolic acid standard compound were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Aluminium nitrate, sodium carbonate and ferric chloride were purchased from Wako Pure Chemical Inc., Ltd. (Kyoto, Japan). Methanol and the other solvents used in the extraction were purchased from Dae-Jung Chemicals and Metals Co., Ltd. (Siheung, Korea). The other chemicals used in experiment were of ACS grade, obtained from Sigma Aldrich Co., Ltd. (St. Louis, MO, USA).

3. Preparation of extraction

Hallasan wormwood extracts of aerial parts and roots were obtained by extraction with a 99.5% methanol three times each 3 hours at solid-liquid ratio of 20 ㎖/g (ratio of the solvent volume per gram of raw material). After filtering (5A filter paper, Advantech Co., Ltd., Tokyo, Japan), Hallasan wormwood extracts (aerial parts and roots) were concentrated by evaporation to remove methanol at 45℃ under reduced pressure using a rotary evaporator (Hei-VAP Precision, Heidolph, Schwabach, Germany). Methanol was removed from a portion of the Hallasan wormwood extract and it was sequentially fractionated by solvent-solvent fractionation to yield *n*-hexane (Hex), ethyl acetate (EtOAc), water-saturated butanol (BuOH) and residual water (Water) fractions respectively.

4. Total phenolic and total flavonoid content assay

Total phenolic content was measured using the Folin-Ciocalteu method (Li *et al*., 2008). After diluting the extract with 700 ㎕ of distilled water, 100 ㎕ of 50% (v/v) Folin-Ciocalteu reagent was mixed and treated for 2 hours. After that, 100 ㎕ of 20% sodium carbonate was added to develop the color, and then the absorbance at 750 ㎚ was measured using an i-Mark microplate reader (168-1135, Bio-Rad Inc., Hercules, CA, USA). Gallic acid was used as the standard compound for expressing the total phenolic content (㎎·GAE/g).

Total flavonoid content measured by reaction with aluminum nitrate. One hundred ㎕ of extract and 300 ㎕ of ethanol were mixed, and 20 ㎕ of 10% aluminum nitrate and 20 ㎕ of 1 M potassium acetate were added. The next distilled water was added with 560 ㎕ and reacted for 1 hour. The reaction solution was measured for absorbance at 415 ㎚ with a microplate reader. Quercetin was used as the standard compound for expressing the total flavonoid content (㎎·QE/g).

5. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity was measured by applying the method of Blois (1958). 40 ㎕ of extract and 160 ㎕ of 0.15 mM DPPH solution reacted in a dark room. After 30 minutes, the absorbance at 490 ㎚ was measured and the DPPH radical scavenging activity was calculated.

The concentration IC_{50} (inhibitory concentration, ㎍/㎖) required to eliminate the 50% DPPH radical was calculated using the inhibition rate measured for each concentration.

6. Trolox equivalent antioxidant capacity (TEAC) assay

TEAC measurements were performed by modifying the method of Gliszczyńska-Świgło (2006). In the ABTS solution, 2,2-azino-bis-(3-ethylbenzo-thiazoline-6-sulphonic acid) and 2.45 mM potassium persulfate were mixed and reacted for 12 hours to obtain an absorbance value of 0.70 ± 0.02 at 734 ㎚. After, 950 ㎕ of ABTS solution and 50 ㎕ of extract were mixed, reacted for 5 minutes, and the absorbance at 734 ㎚ was measured. Trolox was used as the standard compound for indicating TEAC (mM·TE/g).

7. Ferric reducing antioxidant power (FRAP) assay

The FRAP measurements were performed by modifying the method of Gliszczyńska-Świgło (2006). After adding 150 ㎕ of FRAP working solution (1 : 1 : 10, 10 mM 2,4,6-tripyridyl-s-triazine, 20 mM $FeCl_3$, 0.3 M sodium acetate buffer) and 5 ㎕ of extract, the reaction was carried out at 37℃, for 30 minutes. The absorbance at 595 ㎚ was measured using a microplate reader, and the FRAP (mM·FE/g) of the extract was calculated as the absorbance value of $FeSO_4$ used as a standard substance.

8. Oxygen radical antioxidant capacity (ORAC) assay

ORAC was performed by modifying the method of Zulueta *et al.* (2009). Two hundred ㎕ of the extract diluted with 78 nM fluorescein solution was treated at 37℃, for 10 minutes. Next, 50 ㎕ of 221 mM 2,2'-azobis (2-aminopropane) dihydrochloride was added, and fluorescence was measured at 1-minute intervals for 1 hour under the conditions of excitation 485 ㎚ and emission 535 ㎚. At this time, the value of the decreasing fluorescence was calculated by the area under the curve, and the ORAC value was calculated using the decreasing value of the trolox-specific fluorescence.

9. High-performance liquid chromatography (HPLC) analysis

The phenolic compound analysis of the extracts and fractions was divided into phenolic acid and flavonoid, and the retention time and photodiode array (PDA) were analyzed under the same conditions by HPLC under the conditions shown in next using the respective standard compounds.

Phenolic acid chromatographic separation was performed on a Shimadzu 20A system (Shimadzu Co., Kyoto, Japan) equipped with a UV-DAD detector, using a YMC-Triart C18 column (250 ㎜ × 4.6 ㎜, 5 ㎛ hybrid silica based ODS, YMC Co., Seongnam, Korea). The mobile phase consisted of 0.1% trifluoroacetic acid containing water (A) and methanol (B). A gradient program was used as follows: 10% B in the first 5 min, 20 - 25% B during 10 - 25 min, 25 - 40% B during 30 - 45 min, 60 - 100% B during 50 - 60 min, then B held at 10% for 10 min. The flow rate was set at 1 ㎖/min and the injection volume was 10 ㎕. Column temperature was controlled at 40 ℃. Spectral data from 190 to 360 ㎚ were recorded and the phenolic acid chromatograms (6.25 - 200.00 ㎍/㎖) were monitored at 245 ㎚.

Flavonoid was performed on a Shimadzu 20A system using a YMC-Triart C18 column. The mobile phase consisted of 0.1% formic acid containing water (A) and acetonitrile (B). A gradient program was used as follows: 10 - 20% B in the first 5 min, B held at 20% for 15 min, 25 - 30% B during 30 - 45 min, 30 - 50% B during 55 - 60 min, 50 - 60% B during 60 - 70 min, 60 - 80% B during 70 - 75 min, B held at 80% for 5 min, then B held at 10% for 5 min. The flow rate was set at 1 ㎖/min and the injection volume was 10 ㎕. Column temperature was controlled at 40℃. Spectral data from 190 to 360 ㎚ were recorded and the flavonoid chromatograms (3.125 - 100.000 ㎍/㎖) were monitored at 280 ㎚.

10. Statistical Analysis

All data were analyzed in triplicate and the data were expressed as mean ± standard deviation. One-way Analysis of Variance (ANOVA) was used and Duncan`s Multiple Range

Test (DMRT) was used to confirm the significance of the differences between groups ($^{*}p < 0.05$). All statistical analysis were performed on SPSS (Ver. 20.0, SPSS Inc., Chicago, IL, USA)

RESULTS AND DISCUSSION

1. Total phenolic and total flavonoid content in Hallasan wormwood extract and fractions

The total phenolic content of the aerial parts extract was 74.94 ± 1.58 ㎎·GAE/g. Hex (11.67 ± 0.44 ㎎·GAE/g) and water fraction (24.28 ± 1.19 ㎎·GAE/g) showed lower content than the extract in the total phenolic content of fraction, but EtOAc (303.92 ± 19.04 ㎎·GAE/g) and the BuOH fraction (157.40 ± 12.55 ㎎·GAE/g) showed more than twice as high total phenolic content as the extract (Table 1).

The total phenolic content of the roots extract was 53.04 ± 2.50 ㎎·GAE/g, which was lower than that of the aerial parts extract. The total phenolic content of the roots fractions tended to be similar to that of the aerial parts fractions. Hex (7.20 ± 1.31 ㎎·GAE/g) and water fractions (29.45 ± 1.08 ㎎·GAE/g) were lower than the roots extract. EtOAc (420.00 ± 11.99 ㎎·GAE/g) and BuOH fractions (69.33 ± 9.12 ㎎·GAE/g) showed a higher phenolic content than the roots extract (Table 1).

In a study by Ahmed *et al*. (2014), the hexane fraction reported that the removal of chlorophyll and non-polar components affected improving the extraction efficiency of phenolic components. In this experiment, the total phenolic content from the Hex fraction was low, and the high phenol content was confirmed in the EtOAc and BuOH fractions. It is probable that the Hex fraction obtained a non-phenolic substance and the phenolic component was fractionated with EtOAc and BuOH.

Flavonoids are components belonging to polyphenols, which are plant pigments with 15 carbon skeletons and C3-C6-C3 as the basic structure, and were found to be contained in almost all parts such as leaves, stems, and roots of plants (Hertog *et al*., 1992). Flavonoids are substances known to have direct protective effects on cells as well as antioxidant, anti-inflammatory, tumor, antiviral and antibacterial properties due to their biological properties (Cazarolli *et al*., 2008). The total flavonoid content of the aerial parts extract and the roots extract was 31.47 ± 0.65 ㎎·QE/g and 52.09 ± 1.53 ㎎·QE/g, respectively (Table 1).

The total phenolic content of the aerial parts extract (74.94 ± 1.58 ㎎·GAE/g) was higher than that of the roots extract (53.04 ± 2.50 ㎎·GAE/g), but the total flavonoid content was found to be higher in the roots extract (52.09 ± 1.53 ㎎·QE/g). Kim *et al*. (2012) reported that no high correlation was found between the total flavonoid content with the total phenolic content of various plant extracts. However, the total flavonoid content of each fraction showed a tendency similar to the total phenolic content. This is considered as the result of the flavonoids belonging to polyphenols being displayed as being fractionated with EtOAc and BuOH solvent in the process of fractionation.

As a result, the roots EtOAc fraction showed the highest total phenolic content and total flavonoid content, and is thought to have a large amount of phenolic compounds.

2. Antioxidant activity of Hallasan wormwood extract and fractions

DPPH radical scavenging activity, TEAC, FRAP and ORAC analysis were performed to test the antioxidant activity of the Hallasan wormwood extracts and fractions.

The DPPH radical scavenging activity IC_{50} of the aerial parts

Table 1. Total phenolic and total flavonoid content of extract and fractions from aerial parts and roots of Hallasan wormwood.

Samples[1)]	Total phenolic content (㎎·GAE/g[2)])		Total flavonoid content (㎎·QE/g[3)])	
	Aerial parts	Roots	Aerial parts	Roots
Methanol extract	74.94±1.58[c]	53.04±2.50[c]	31.47±0.65[c]	52.09±1.53[c]
Hex fraction	11.67±0.44[d]	7.20±1.31[e]	12.56±0.35[d]	12.97±0.72[d]
EtOAc fraction	303.92±19.04[a]	420.00±11.99[a]	93.18±2.18[a]	145.35±2.73[a]
BuOH fraction	157.40±12.55[b]	169.33±9.12[b]	62.61±1.61[b]	116.62±5.75[b]
Water fraction	24.28±1.19[d]	29.45±1.08[d]	7.69±0.22[e]	8.65±0.42[d]

[1)]Sample; *n*-hexane (Hex), ethyl acetate (EtOAc), water-saturated butanol (BuOH) and residual water (Water) fractions. [2)]㎎·GAE/g; total phenolic content analyzed as gallic acid equivalent ㎎/g of sample. [3)]㎎·QE/g; total flavonoid content analyzed as quercetin equivalent ㎎/g of sample. [*]Different letters (a - e) in each sample are significantly different at by Duncan`s Multiple Range Test (DMRT $p < 0.05$).

extract was 469.78 ± 3.67 ㎍/㎖, and the EtOAc fraction was 121.69 ± 0.37 ㎍/㎖, which was the highest activity (Table 2). The IC_{50} of the roots extract was 108.66 ± 1.44 ㎍/㎖, which was higher than that of the aerial parts EtOAc fraction, and the roots EtOAc fraction was 7.58 ± 1.14 ㎍/㎖, which was equivalent to that of BHA (8.09 ± 0.17 ㎍/㎖).

As a result of TEAC, the aerial parts extract (369.75 ± 10.34 mM·TE/g) and the root extract (381.67 ± 13.26 mM·TE/g) showed similar activities (Table 2). The TEAC values of the aerial parts EtOAc fraction and roots EtOAc fraction were 1167.38 ± 40.95 and 1283.42 ± 54.49 mM·TE/g, which were the highest among the fractions.

The FRAP method is an experiment to measure the ability to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), and the reducing power can be compared and analyzed. The ability to donate electrons to reactive oxygen species and free radicals by various mechanisms of antioxidant activity is called the reducing power, and it is known that this is closely related to the antioxidant activity (Siddhuraju *et al*., 2002).

The FRAP of the aerial parts extract was 1131.29 ± 13.56 mM·FE/g. The highest aerial part fractions were the BuOH fraction (1592.68 ± 25.81 mM·FE/g). The roots extract showed a FRAP value of 473.98 ± 5.22 mM·FE/g, which was lower than that of the aerial parts extract. However, the roots EtOAc fraction was 5046.91 ± 88.39 mM·FE/g, the highest of all samples (Table 3).

The ORAC values are aerial part EtOAc fraction (25.69 ± 1.18 mM·TE/g), BuOH fraction (22.88 ± 0.55 mM·TE/g), extract (20.04 ± 0.54 mM·TE/g), Hex fraction (11.89 ± 0.30 mM·TE/g), and water fraction (5.02 ± 1.46 mM·TE/g) had the highest content in that order (Table 3).

The roots extract showed 12.82 ± 0.48 mM·TE/g, which was lower than that of the aerial parts extract. In addition, the EtOAc fraction (28.57 ± 1.01 mM·TE/g) except for the Hex (15.67 ± 0.29 mM·TE/g), BuOH (21.13 ± 1.18 mM·TE/g), and water (4.37 ± 0.10 mM·TE/g) fractions showed higher activity than the roots extract and fractions (Table 3).

von Gadow *et al*. (1997) reported that caffeic acid and rutin had higher DPPH radical scavenging activity than BHA. From the results of this study, it was impossible to confirm a direct

Table 2. DPPH radical scavenging activity and TEAC of extract and fractions from aerial parts and roots of Hallasan wormwood.

Samples[1)]	DPPH IC_{50}[2)] (㎍/㎖)		TEAC (mM·TE/g[3)])	
	Aerial parts	Roots	Aerial parts	Roots
Methanol extract	469.78±3.67[e]	108.66±1.44[c]	369.75±10.34[c]	381.67±13.26[c]
Hex fraction	N/A	442.81±18.51[e]	29.08±9.29[e]	72.24±3.34[d]
EtOAc fraction	121.69±0.37[b]	7.58±1.14[a]	1167.38±40.95[a]	1283.42±54.49[a]
BuOH fraction	367.99±6.94[c]	46.69±1.86[b]	519.38±23.29[b]	663.60±18.65[b]
Water fraction	246.86±3.70[c]	233.97±1.48[d]	129.56±6.82[d]	118.52±1.76[d]
BHA	8.09±0.17[a]		-	

[1)]Samples; *n*-hexane (Hex), ethyl acetate (EtOAc), water-saturated butanol (BuOH) and residual water (Water) fractions, butylated hydroxyanisole (BHA). [2)]DPPH IC_{50}; Amount required for a 50% inhibition of 0.15 mM DPPH radical after 30 minutes. [3)]mM·TE/g; TEAC analyzed as trolox equivalent mM/g of sample. [4)]N/A; no value was obtained. [*]Different letters (a - e) in each sample are significantly different at by Duncan`s Multiple Range Test (DMRT $p < 0.05$).

Table 3. FRAP and ORAC of extract and fractions from aerial parts and roots of Hallasan wormwood.

Samples[1)]	FRAP (mM·FE/g[2)])		ORAC (mM·TE/g[3)])	
	Aerial parts	Roots	Aerial parts	Roots
Methanol extract	1131.29±13.56[b]	473.98±5.22[c]	20.04±0.54[c]	12.82±0.48[d]
Hex fraction	69.64±1.17[e]	98.34±1.57[e]	11.89±0.30[d]	15.67±0.29[c]
EtOAc fraction	976.20±17.37[c]	5046.91±88.39[a]	25.69±1.18[a]	28.57±1.01[a]
BuOH fraction	1592.68±25.81[a]	2168.27±18.57[b]	22.88±0.55[b]	21.13±1.18[b]
Water fraction	298.25±4.52[d]	382.74±3.22[d]	5.02±1.46[e]	4.37±0.10[e]

[1)]Samples; *n*-hexane (Hex), ethyl acetate (EtOAc), water-saturated butanol (BuOH) and residual water (Water) fractions. [2)]mM·FE/g; FRAP analyzed as $FeSO_4$ mM/g of sample. [3)]mM·TE/g; ORAC analyzed as trolox mM/g of sample. [*]Different letters (a - e) in each sample are significantly different at by Duncan`s Multiple Range Test (DMRT $p < 0.05$).

correlation between the antioxidant activity of the extract and fractions with these contents, but it is speculated that these phenolic compound components indirectly affected the antioxidant activity. In particular, total flavonoid content and antioxidant activity tended to be similar.

Antioxidant activity tends to be similar to the total phenolic content than individual phenolic compound content. Thus, total phenolic content and total flavonoid content were associated with the antioxidant activity of extracts and fractions.

In conclusion, the roots EtOAc fraction had the best antioxidant activity.

3. Composition and content of phenolic compound in Hallasan wormwood extract and fractions

Table 4 shows the results of phenolic compound using HPLC for the Hallasan wormwood extract and fractions.

Nine phenolic acid and 1 flavonoid were detected aerial parts from Hallasan wormwood. The main phenolic acids were chlorogenic acid, caffeic acid, and rutin were the main components of flavonoids.

The content of chlorogenic acid, caffeic acid, rutin, and taxifolin in the EtOAc fractions is higher than that of other fractions, and the components belonging to *p*-coumaric acid, ferulic acid, sinapinic acid and benzoic acid groups are also included.

Five phenolic acid and 1 flavonoid were detected roots from Hallasan wormwood. The main phenolic acids were chlorogenic acid and caffeic acid.

The EtOAc fractional extract with the highest phenolic compound content contains *p*-coumaric acid and ferrulic acid. However, it was not detected in other root fraction extracts. The components with the highest content in the analyzed phenolic compounds were chlorogenic acid and caffeic acid. Caffeic acid had the high hydrogen donating capacity toward the DPPH radical (von Gadow *et al*., 1997).

Chlorogenic acid and caffeic acid are components that are related to each other. Caffeic acid has a stronger antioxidant activity than that of chlorogenic acid and chlorogenic acid is hydrolyzed into caffeic acid in the intestine (Sato *et al*., 2011). Therefore, ingestion of Hallasan wormwood fractional extract with high chlorogenic acid content predicts its potential health benefits.

There was a difference in the phenolic compound content analyzed in the aerial parts and roots of Hallasan wormwood. Jang and Kang (2019) analyzed the phenolic acid and flavonoid composition and content of *A. princeps* var *orientalis* extract. As for the phenolic acid, chlorogenic acid was detected in the leaves and stems, and *p*-coumaric acid in the roots was contained. And the flavonoid found taxifolin on the leaves and stems and quercetin on the roots. Chlorogenic acid, *p*-coumaric acid, and rutin detected in the present study results were confirmed in Hallasan wormwood. However, quercetin was not detected.

This is probably because Hallasan wormwood is taxonomically another variety. Ryu *et al*. (2011) reported the contents of 12 phenolic acids (gallic acid, protocatechuic acid, gentisic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, chlorogenic acid, salicylic acid, *p*-coumaric acid, sinapinic acid, ferulic acid, and *t*-cinnamic acid) and 3 flavonoids (rutin, quercetin and, kaempferol) contained in *A. annua* leaf and stem extracts. It had a difference from the phenolic compound of *A. annua* extract.

Seong *et al*. (2015) collected *Perilla frutescens* from China, Japan and South Korea and analyzed and compared phenolic compounds. As a result, it was found that the phenol content and composition ratio differed depending on the species collected by *Perilla frutescens*. This suggests that various *Artemisia* spp. phenolic compound studies are needed.

The phenolic compounds content analyzed by HPLC and antioxidant activity in this study did not appear to have a significant effect. However, the extract having a high total flavonoid content with the total phenolic content had a high antioxidant activity. Therefore, there will be main phenolic compounds that affect unanalyzed antioxidants, except for the detected phenolic acids and flavonoids.

From the results presented in this study, the EtOAc fractions of Hallasan wormwood root have good antioxidant activities. Total phenolic and total flavonoid contents are the predominant constituents that correlate with antioxidant activity. This study highlights the antioxidant activities of the phenolic constituents of Hallasan wormwood.

Use of Hallasan wormwood extract and fractions should take into account the toxicity. And not all phenolic compounds reported to date have been analyzed in this study. Therefore, for these plants to be useful as therapeutic agents, and toxicity studies of the extract and fractions are necessary. Also, additional investigation of unknown substances that have not been analyzed is required.

Table 4. Composition and content of phenolic compound in Hallasan wormwood extract and fractions.

Phenolic compounds	Sample (μg/mℓ)									
	Aerial parts					Roots				
	Extract	Fractions				Extract	Fractions			
		Hex	EtOAc	BuOH	Water		Hex	EtOAc	BuOH	Water
	Benzoic acid group									
PA[1]	ND[2]	ND	61.76±2.54	ND	ND	ND	ND	0.35±0.39	ND	ND
SA[2]	ND	ND	27.65±2.52	ND	ND	ND	ND	ND	ND	ND
HBA[3]	ND	28.51±1.31	31.39±2.05	ND	ND	ND	ND	ND	ND	ND
VA[4]	ND	ND	31.97±1.81	ND	ND	ND	ND	ND	ND	ND
GA[5]	ND	ND	8.80±0.39	ND	ND	ND	ND	ND	ND	ND
	Cinnamic acid group									
CA[6]	7.85±0.73	ND	328.39±10.70	1.85±0.41	1.00±0.26	23.62±2.59	ND	249.47±18.70	ND	ND
CMA[7]	ND	ND	43.14±2.71	ND	ND	ND	ND	64.67±7.83	ND	ND
FA[8]	ND	ND	86.45±3.06	ND	ND	ND	ND	8.47±0.99	ND	ND
CRA[9]	482.83±5.50	ND	2858.23±87.65	629.43±11.95	423.73±9.94	1251.38±92.73	ND	1282.29±104.68	3048.28±177.18	5.57±0.56
	Flavonoid group									
RT[10]	18.96±0.13	ND	51.55±0.37	33.19±0.02	ND	ND	ND	10.74±0.78	ND	ND

[1]PA; protocatechuic acid, [2]SA; syringic acid, [3]HBA; *p*-hydroxybenzoic acid, [4]VA; vanillic acid, [5]GA; gallic acid, [6]CA; caffeic acid, [7]CMA; *p*-coumaric acid, [8]FA; Ferulic acid, [9]CRA; chlorogenic acid, [10]RT; rutin. Values are means ± standard deviations (n = 3). ND; not detected.

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