

A STUDY ON THE UTILIZATION OF THE EXOTIC INVASIVE SPECIES *HYPOCHAERIS RADICATA* L. AS MANAGEMENT PERSPECTIVE

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Abstract. Although *Hypochaeris radicata* L. is a noxious alien species in Korea, there is no effective way to prevent it from spreading. Therefore, this study aims to study the possibility of using *H. radicata* as an effective natural compound source which will contribute to the utilization of the invasive alien species and some extent to prevent invasion. Hot water and ethanol extracts of *H. radicata* showed high efficiency as a natural antioxidant indicating the species may be used as a natural antioxidant material. Extracts of *H. radicata* has shown no toxic effects on cells. Rather, *H. radicata* extract has an function of inhibiting the generation of NO in high concentrations of extracts which means that *H. radicata* extract has a function of anti-inflammatory effect. When the research on the effect of *H. radicata* finds out more, the utilization of this species will be increased and the environmentally friendly public participation management will be possible due to an increase in its collection. Also, it is meaningful that we have attempted to find an ecological engineering approach through utilization methods of alien species that has been spread widely in Korea.

Keywords: *cat's-ear, natural compounds, antioxidant, anti-inflammatory, alien species*

Introduction

Invasive alien plants are species that are dispersed outside their original habitats through intentional or unintentional means, which can harm the environment. This destruction will result in loss of ecosystem function and extinction of native species, eventually reducing biodiversity, ultimately resulting in socioeconomic decline (Samways et al., 1996). However, these exotic species can be used as sources of natural compound (medicine, cosmetic etc.), that such development of these uses may help to manage such exotic species through increase the harvest of this species in the wild (Maema et al., 2016), as a kind of ecological engineering. The free radical and properties containing natural antioxidants that metabolize the endogenous products of several oxidizing species are facilitated by ecological stress (Grassmann et al., 2002) for most plants, and studies of these natural products are useful for confirming the availability of plants. Recently, there has been an increasing interest in natural antioxidants, which can protect humans from damage caused by oxidative stress (Scalbert et al., 2005). This study attempts to approach *Hypochaeris radicata* L., which causes many problems in Jeju Island and some inland regions (Kim et al., 2006), as a new perspective. *H. radicata* is managed because it is designated as a harmful species in the Ministry of Environment. However, in Jeju Island, *H. radicata* is already the most dominant species. Thus, realistic removal and management of this species are difficult

and showed no results so far (Ryu et al., 2017). From the discovery of *H. radicata* in Chungcheong and Jeolla, it shows that diffusion and settlement have already occurred inland (Hyun et al., 2018). Therefore, the case of *H. radicata* shows that there is a limitation in the existing management methods for general harmful species. Therefore, we investigated the medicinal efficacy of *H. radicata* through the view as a resource. Through this, the research applying the idea that increasing the use of these alien species is a positive management of alien species (Li et al., 2015). For this purpose, we conducted a study on the efficacy of *H. radicata*.

Materials and methods

Plant sampling

In October 2018, 10 habitats from the northern part of Jeju island, Korea were randomly selected and the *H. radicata* used for the experiment were collected. Every sampling sites were unmanaged areas inside of agricultural fields, mostly close to roads. Sampling sites were relatively flat areas and dominated by *H. radicata*. The geographic coordinates of the center of the sampling sites are 33°27'49"N, 126°29'07"E. Ten plant samples were collected from each site and used as one large sample. Harvested plants were washed and then dried for 24 h. The stems and leaves except roots were cut into the crusher and used as experimental material.

Total antioxidant capacity (TAC) the superoxide dismutase activity (SOD) using raw plant

From plant leaves, crude extracts were prepared by re-suspending the frozen cells in 50 mM phosphate buffer (pH 7.2). The plant samples (0.1 g) were washed and then the cells were quick-frozen in liquid N₂ and milled subsequently. Phosphate buffer (1 ml; Sigma-Aldrich Corp.) was added and the suspension was stirred using Vortex mixer. Waited more than 30 min for protein extraction. After centrifuging at 14,000 rpm at 4 °C for 20 min, 400 µl of the supernatant was collected for the experiments. Absorption was measured using SpectraMax (Molecular Devices, USA). Protein quantities were determined by using Bradford assay (Bradford, 1976) to obtain a standard curve. Total Antioxidant capacity was measured by the antioxidant activity of an organic liquid using bathocuproine (Jaramillo-Flores et al., 2003) with a slight modification of the protocol. Our method comprises the following steps. First, mix a sample of the liquid (15 µl) with bathocuproine (200 µM; 585 µl) and stir using Vortex mixer and Pour a predetermined quantity (200 µl) of each of the samples into the wells of a multi-well plate. Then, Perform spectrophotometric measurements of the samples at 490 nm (S1). Add a predetermined quantity (50 µl) of copper sulfate solution to each well and incubate it at room temperature for 5 min; terminate the reaction using ethylenediaminetetraacetic acid (EDTA). Then again, Perform the second round of spectrophotometric measurements at 490 nm (S2). Total Antioxidant Capacity is calculated as $TAC = S2 - S1$ (µM/ml). Finally, convert units using the Bradford assay results (µM/mg). The superoxide dismutase activity was measured using the methods established by Peskin and Winterbourn (2000) and protocols of the WST assay by Donindo, Japan.. Using a WST-1 (2-(4-Iodophenyl)- 3-(4-nitrophenyl)-5-(2,4-disulfophenyl)- 2H-tetrazolium, monosodium salt) solution (Dojindo, Japan) and an

Enzyme Working Solution (Dojindo, Japan), SOD activities were calculated under the Dojindo's protocols.

Extract preparation

The dried aboveground plant parts (i.e., leaves and branches) were coarsely powdered by using an electric blender. 32.6 g of dried plant powder was then, a) extracted with 500 ml of 70% (v/v) ethanol for 24 h (EtOH) or b) extracted with 500 ml of 90 °C distilled water (HW: Hot Water) for 2 h. Then extracted solutions were filtered (Whatman No.1), and then concentrated with a rotary evaporator. Then the solution was freeze-dried to make the final product as powder.

Radical scavenging assay for testing antioxidant effects of extracts

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of the *H. radicata* were measured by a method suggested by Blois (1958). 180 µL of DPPH solution in 0.4 mM in ethanol was mixed with 20 µL of the extract (DW and ethanol) and incubated in the dark at 25 °C for 10 min. Then, the absorbance of the sample (A sample) and negative control (A control) consisting of only ethanol alone was measured at 517 nm. The DPPH radical scavenging activity of the sample was calculated by using *Equation 1*.

$$\text{Scavenging activity (\%)} = 100 \times \frac{(\text{A control} - \text{A sample})}{\text{A control}} \quad (\text{Eq.1})$$

ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonicacid)) radical scavenging activity of the subtropical plant extracts was measured as a method by Zhu et al. (2011). ABTS and potassium persulfate solutions (7 and 2.45 mM, respectively) were mixed and then incubated in the dark at 25 °C for 16 h. The ABTS solution was diluted with ethanol to obtain the working solution with an absorbance at 745 nm. Then, 20-µL aliquots of the plant extract samples were dissolved in ethanol at different concentrations (10–500 µg/mL), mixed with 180 µL of the working solution, incubated in the dark at 25 °C for 10 min, and then the Abs sample and Abs control (ethanol only) were measured. The ABTS radical scavenging activity was calculated by using *Equation 1*.

High performance liquid chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) was analyzed at NICEM (Seoul National University, South Korea) using Ultimate 3000 model, (Thermo Fisher Scientific, Waltham, MA USA). The flow rate was 0.8 mL/min and the gradient was given using Buffer A [0.3% trifluoroacetic acid (TFA)] and Buffer B (Acetonitrile). C-18 column (4.6*250,5 µm) was used and wavelength of 280 nm [190-800 nm diode array detection (DAD) scanning] was used for analysis. 12 standards that often used for phenolic compounds were used to detect natural compounds of *H. radicata* (syringic acid, salicylic acid, *p*-coumaric acid 4, ferulic acid, naringin, myricetin, hesperidin, *trans*-cinnamic acid, quercetin, naringenin, confertin, scopoletin).

Cell culture

Murine RAW 264.7 macrophages were obtained from the Korean Cell Line Bank (Seoul, South Korea). Cells were cultured in Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum, penicillin, and streptomycin sulfate (all from

GIBCO, Grand Island, NY, USA), in an incubator at 37 °C in a humidified atmosphere of 95% air and 5% CO₂. For this study the cells were mechanically passaged by dissociation every other day; they underwent fewer than 25 passages.

Toxicity of plant extract (MTT assay)

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was undertaken as described previously by Yang et al. (2013). Cells were counted with a haemocytometer, and the number of viable cells was assessed by trypan blue dye exclusion method. The RAW 264.7 macrophages were seeded in 24-well plates for 18 h and then stimulated with various treatments (250, 500, 1000, 1500 µg/mL of HW and 50, 100, 200, 500 µg/mL of 70% EtOH) or LPS (lipopolysaccharide) (1 µg/mL) for 1 h. On the day of collection, cells were incubated with MTT solution for 24 h at 37 °C in a humidified atmosphere of 95% air and 5% CO₂. The MTT-containing supernatant was removed and the formazan crystals were solubilized in dimethylsulfoxide. The absorbance of each well at 540 nm was measured using an automatic microplate reader (PowerWave X340, Bio-tech Instruments, Inc., Winooski, VT, USA).

NO (nitric oxide) concentration as indicator of anti-inflammatory effects

The NO concentration in treatments was determined based on the method of Yang et al. (2013). RAW 264.7 cells (5×10^5 cells/well) were cultured on 24-well dishes with 1 µg/mL of LPS and various treatments (250, 500, 1000, 1500 µg/mL of HW and 50, 100, 200, 500 µg/mL of 70% EtOH). After 18 h, culture supernatants were collected and nitrite, a stable oxidized product of NO, was measured by a modified Griess (1879) method. Equal volumes (100 µL) of the Griess (1879). Reagent [1% sulfanilamide and 0.1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride in 5% phosphoric acid] and *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, or *G. filicina* were incubated together at room temperature for 10 min. The absorbance of each well at 540 nm was measured using an automatic microplate reader (PowerWave X340, Bio-tech Instruments).

Statistical analysis

Differences between multiple groups were evaluated using oneway PROC analysis of variance (ANOVA). When a significant treatment effect was detected, post hoc comparisons of the means were performed using Tukey's honest significant difference test (SAS v. 9.1, SAS Institute Inc., USA). Differences were considered significant if $p < 0.05$.

Results and discussion

Total antioxidant capacity (TAC) and superoxide dismutase activity (SOD)

TAC and SOD were measured using fresh plant leaves instead of extracts (*Table 1*). To see the functions of the plant itself as a medicinal herb not the effects of extracts, TAC and SOD were selected. However, most other TAC studies of leaves of plants, results are re-directed to extracts by calculation, so it is difficult to make a direct comparison because the unit is L (liter). Rarely, among the existing studies using the same method as we did, there are results of *Brassica campestris* ssp. *napus* var. *nippo-oleifera* Makina (oilseed rape) and *Lactuca sativa* L. (lettuce), a leaf-eating crop (Song

et al., 2013). *H. radicata* is expected to have higher efficacy than conventional plants because it shows a high value of three times higher than that of this crop. In addition, the result can have the meaning of the archive showing the antioxidant value of the fresh leaf of *H. radicata*. In the case of SOD, it was possible to make a direct comparison with other papers since it came out as a unit. In the case of overseas studies using the same Dojindo SOD kit for medicinal herbs (Rafat et al., 2010), five herbicides showed an average value of about 60 unit (SOD unit). On the other hand, *H. radicata* has a value of 90 or more. Therefore, the antioxidant effect of *H. radicata* is expected to be high and gave us expectation for noticeable efficacy.

Table 1. Total antioxidant capacity and superoxide dismutase activity of *H. radicata*

Sample	Value
TAC ($\mu\text{M}/\text{mg}$ protein)	389.35 ± 41.34
SOD (U/mg protein)	94.03 ± 2.21

Each value represents mean \pm S. D. (n = 4)

Radical scavenging assay for testing antioxidant effects of extracts

Free radicals are known to cause various diseases by causing oxidative stress in the body. Therefore, DPPH and ABTS radical scavenging activities were measured to confirm the free radical inhibitory activity of HW extract and 70% EtOH extract on the aboveground part of *H. radicata*. As a result, the DPPH radical scavenging activity of EtOH extract ($\text{IC}_{50} = 139.63 \pm 5.30 \mu\text{g}/\text{mL}$) was higher than that of HW extract ($\text{IC}_{50} = 167.91 \pm 3.94 \mu\text{g}/\text{mL}$) (Table 2). In the ABTS radical scavenging activity, HW extract ($\text{IC}_{50} = 58393 \pm 13.31 \mu\text{g}/\text{mL}$) was higher than EtOH extract ($\text{IC}_{50} = 155.94 \pm 35.27 \mu\text{g}/\text{mL}$) (Table 2). When DDPH radical scavenging activity of *H. radicata* was compared with other popular medicinal plants such as *Trigonella foenum-graecum* and *Elettaria cardamomum* (Khalaf et al., 2008). The IC_{50} value of *H. radicata* was lower. Therefore, it can be said that *H. radicata* showed higher efficiency as a natural antioxidant than popular medicinal plants (*T. foenum-graecum* and *E. cardamomum*). This suggests that *H. radicata* may be used as a natural antioxidant material. After conducting this experiment, we've found a new reference that has investigated DPPH and ABTS of *H. radicata* extract (Ko et al., 2017). This paper also showed that *H. radicata* extract has strong antioxidant effects, which is consistent with our results. However, our DPPH value of HW extract is low compared to other paper from a research group who have studied this plant steadily (Senguttuvan et al., 2014) while the local paper (Ko et al., 2017) showed similar value (EtOH) to our results. As Korea and India are very distant counties, ecotypes of *H. radicata* in India could be different. Also as climate condition of Korea is different from India (cold winter in Korea), it would affect the chemical composition of plants.

Anti-inflammatory activity and toxicity of HW and EtOH extracts in RAW 264.7 cells

To determine the toxicity and anti-inflammatory effects of extracts, the most common method is to examine the cell viability by RAW 264.7 cells using NO production and MTT assay. In the HW extract, NO production was significantly increased in LPS only treatment (LPS +, Sample 0 $\mu\text{g}/\text{mL}$) compared to control (LPS -, Sample 0 $\mu\text{g}/\text{mL}$), but HW extracts treated with 500, 1000, and 1500 $\mu\text{g}/\text{mL}$ showed

that NO production decreased by concentration compared to LPS only treatment (Fig. 1). This shows that the higher the concentration, the stronger resistant the inflammation by NO product inhibition. Cell viability was also reduced in all treatments compared to control (LPS -, Sample 0 µg/mL) and all values are lower than the value of LPS only treatment (LPS +, Sample 0 µg/mL), but with the exception of 1500 µg/mL treatment. As the LPS itself is toxic, it is a normal response. However, when the extract is added, the toxicity is alleviated considering low values in most concentration range. In general, when the cell viability is 80% or more, it is considered that there is no problem, so that the overall extract, even including 1500 µg/mL treatment are safe to be considered as none toxic.

Table 2. Antioxidant activities of *H. radicata* extracts

Sample	DPPH radical scavenging activity	ABTS radical scavenging activity
HW	167.91 ± 3.94	187.04 ± 11.54
EtOH	139.63 ± 5.30	203.6 ± 4.68
Ascorbic acid	6.48 ± 0.25	22.34 ± 0.87

Each value represents mean ± S. D. (n = 4). IC50 (IC50 = µg/mL) values were calculated from regression lines using eight different concentrations in triplicate experiments. HW; hot water extracts. EtOH; 70% ethanol extract

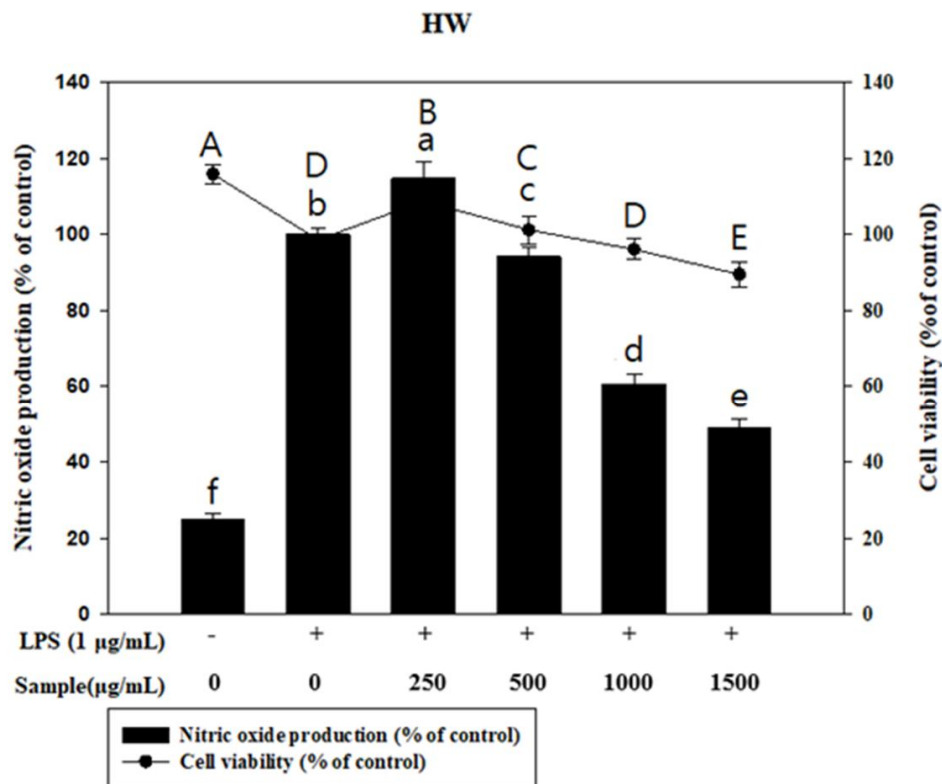


Figure 1. Effect of HW extract on cell viability and nitric oxide production in LPS-stimulated RAW 264.7 cells. Raw 264.7 cell were incubated with 250, 500, 1000 and 1500 µg/mL of HW extract for 1 h, and then incubated with LPS (1 µg/mL) for 24 h. The data expressed as a mean ± SD (n = 4). Symbols or bars having the same letter are not significantly different at the 0.05 level. Uppercase letters show difference between symbols, while lowercase letters show difference between bars

In the 70% EtOH extract, NO production was significantly increased in the LPS only treatment (LPS +, Sample 0 $\mu\text{g/mL}$) compared to the control (LPS -, Sample 0 $\mu\text{g/mL}$). However, EtOH extracts treated with 500, 1000, and 1500 $\mu\text{g/mL}$ showed that NO production decreased (Fig. 2). Therefore, EtOH extract appears to have anti-inflammatory effect like HW treatment and its effects are considered to be stronger than HW extract. The cell viability was significantly higher in the 50 $\mu\text{g/mL}$ to 200 $\mu\text{g/mL}$ treatment groups, and these results are showing alleviated toxicity at this concentration. And the highest concentration (500 $\mu\text{g/mL}$) did not show any significant difference with control. Therefore, this suggests that *H. radicata* extract induces cell proliferation by minimizing the effect of cytotoxicity. As a result, the *H. radicata* extract has not been shown to be toxic to the cell. Rather, in some treatments, it can protect cells by reducing the harmful effects on cells. And the *H. radicata* extract can be regarded as a function of inhibiting the generation of NO in high concentrations of extracts. In other words, *H. radicata* extract has a function of anti-inflammatory effect.

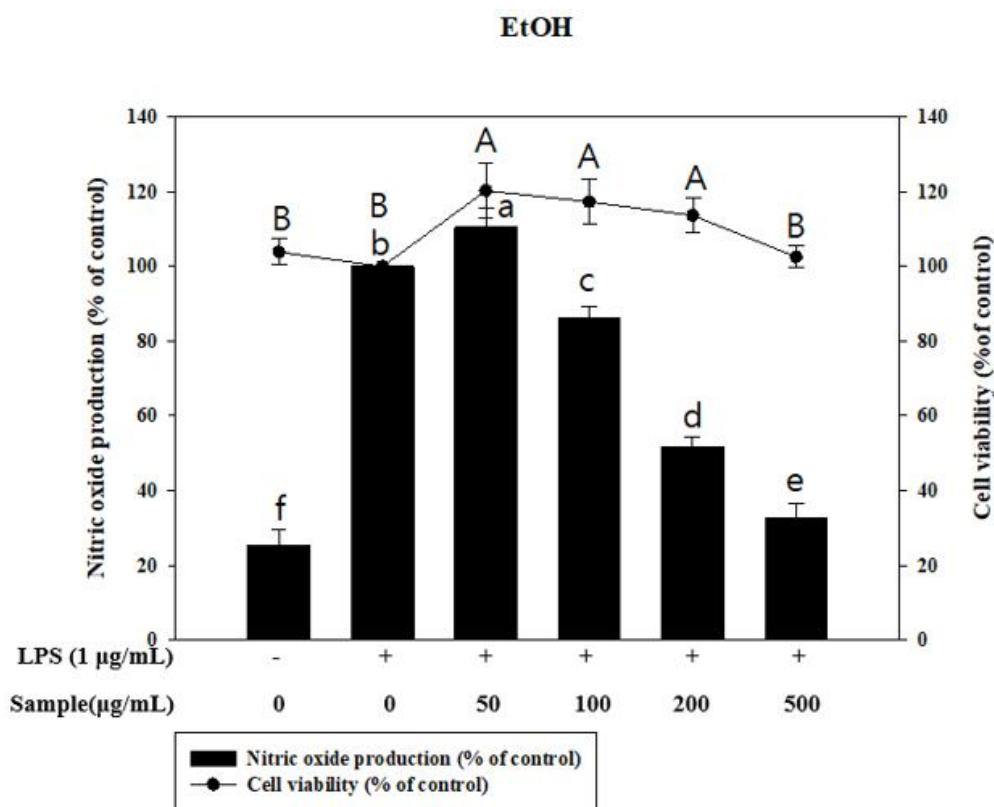


Figure 2. Effect of EtOH extract on cell viability and nitric oxide production in LPS-stimulated RAW 264.7 cells. Raw 264.7 cell were incubated with 50, 100, 200 and 500 $\mu\text{g/mL}$ of EtOH extract for 1 h, and then incubated with LPS (1 $\mu\text{g/mL}$) for 24 h. The data expressed as a mean \pm SD ($n = 4$). Symbols or bars having the same letter are not significantly different at the 0.05 level. Uppercase letters show difference between symbols, while lowercase letters show difference between bars

HPLC

Since the extracts of *H. radicata* have shown some effects, HPLC analysis was expected to detect a certain level of natural products. However, only 1-Trans-Cinnamic

acid was detected in the detection of phenolic compounds using 12 commonly used standards (Fig. 3). This suggests that the substances showing the function of the above results are slightly different from those of other plants, and *H. radicata* extracts are likely to contain new natural products rather than general ones. This shows that it is possible to obtain unusual natural products through a detailed analysis of this species in the future.

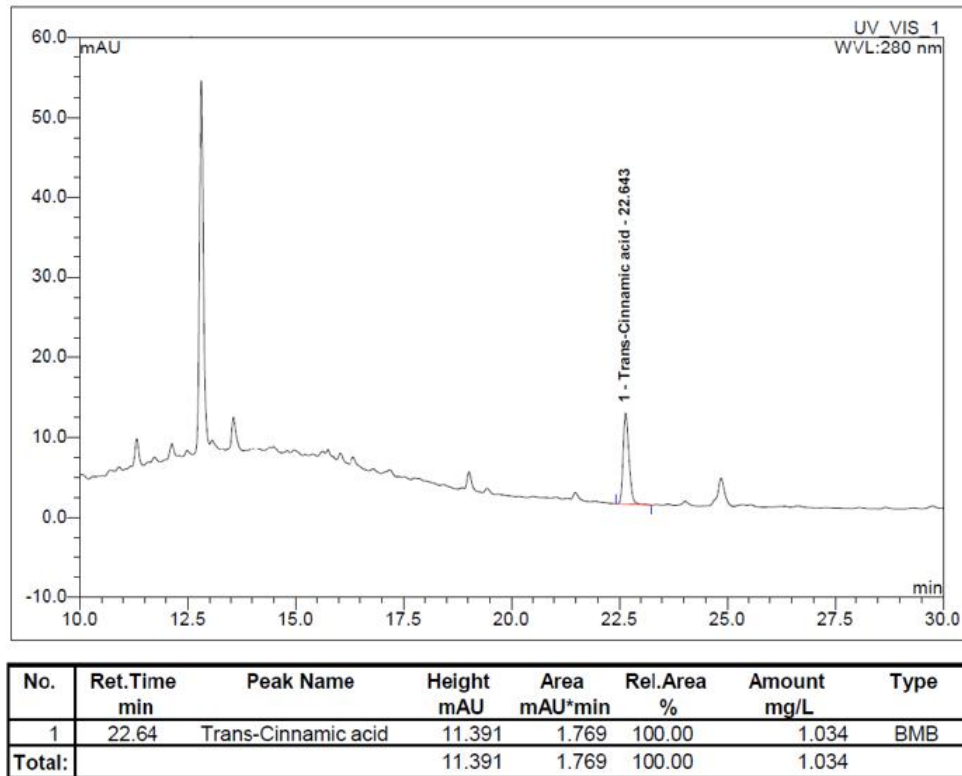


Figure 3. High performance liquid chromatography (HPLC) result of HW extract

Conclusion

H. radicata has been reported as an invasive alien species that has been degrading ecosystem functions as a result of environmental damage, resulting in the extinction of native species and ultimately ecosystem diversity. However, despite the fact that it has been designated as a dangerous species, there is no effective way to prevent it from spreading. In Jeju Island, frequent mowing is used for environmental management of *H. radicata*, but still the species are dominating the island. It implies that we need a better engineering solution of management of *H. radicata*. Therefore, this study aims to contribute to the utilization of invasive alien species and resource management, and to some extent to prevent diffusion, by exploring the possibility of using *H. radicata*, an invasive alien species already introduced in Korea. All results show that *H. radicata* can be used for natural antioxidants and anti-inflammatory effects. Further researches could find correlation between environmental factors (soil, climate, species composition etc.) and medicinal effects of *H. radicata* and molecular reactions such as expression of Nuclear factor-kappa B. When the researches on effect of *H. radicata* are discovered more, the utilization of this species will be increased and the environmentally friendly

public participation management will be possible due to the increase of the collection. As some medicinal or gardening herbs such as *Panax ginseng* (Park and Park, 2007), *Hanabusaya asiatica* (Park et al., 2013), *Saccolabium japonicum* (Jung et al., 2012) are endangered because it is being overharvested (Sangwon and Seonyeong, 2005), same effects can help to reduce *H. radicata* by increased usage. As harvesting wild vegetables are the common act in local (Hwang, 1991), this management mechanism is worthy to study. Therefore, we believed that it can be contributed to the prevention of diffusion of *H. radicata* to some degree when functions of *H. radicata* are discovered and be known to the public. In addition, it is meaningful that we have attempted to find an ecological approach (management through utilization) through utilization methods of alien species that has been spread widely in Korea.

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