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Antioxidant and Nitrite Scavenging Ability of Mugwort Extracted with Different Solvents

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Abstract. This study was conducted to investigate the physiological activity of mugwort (*Artemisia princeps* Pamp.) extracts from five different solvents (ethanol, ethyl acetate, methanol, water, and hot water). The highest total phenolic contents (82.75 mg/g) and total flavonoid contents (22.53 mg/g) found in water extracts. Also, the water extracts had the higher ferrous chelating activity (63.63%), and nitrite scavenging ability (80.06%) and the smaller IC_{50} values in ferrous iron metal chelating activity, nitrite scavenging ability, and DPPH radical scavenging activity (0.89 mg/mL, 0.39 mg/mL, and 0.14 mg/mL, respectively) than other solvent extracts. As the above results, physiological activity in water extracts of mugwort is promising source of functional food ingredients.

Keywords: mugwort, antioxidant activity, nitrite scavenging ability

1. Introduction

Recently, consumer demands for functional and safe food, if possible free of conventional chemical substances. Because of concerns about the toxicological safety of artificial additives, the use of natural preservatives has increased considerably in the last few decades. The application of the natural ingredients are a promising technology since many plants, herb, spice and vegetables substances have antioxidant and antimicrobial properties and low toxicity compared with those of synthetic phenolics antioxidant such as BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole), and propyl gallate [1]. Among natural antioxidants, mugwort (*Artemisia princeps* Pamp.), commonly known as medicinal herb, is a perennial plant widely distributed in Japan, Korea, China and Europe. In Oriental countries, including Korea, mugwort is widely used as a food or food additive. This plant contains bioactive compounds, such as phenolics, vitamins A, B_1, B_2 and C as well as various minerals [1]. In recent scientific literature, mugwort has been reported to have anti-atherosclerotic, anti-inflammatory, anti-scratching behavioral, anti-bacterial, and radical scavenging [2]. However, no previous study has examined the effect of the different solvents from mugwort on antioxidant and nitrite scavenging ability. Therefore, the aim of this study was to evaluate the physiological activity of different solvents (ethanol, ethyl acetate, methanol, water and hot water) from mugwort on total phenolic contents, total flavonoid contents, ferrous iron chelating activity, nitrite scavenging ability, and DPPH radical scavenging activity.

2. Material and Methods

2.1. Preparation of extracts

Commercial samples of dried mugwort were purchased from a local market. After separating the leaves from the dried mugwort, they were ground using a blender for 1 min. Ground mugwort leaves (10 g) were extracted with 200 mL of ethanol, ethyl acetate, methanol and distilled water overnight (24 h) in a shaker at room temperature and the hot water extraction of mugwort was prepared ground leaves (10 g) extracted in 20

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volumes of distilled water at 100 ℃ for 4 h. The each extracts were filtered through filter paper No. 1 and evaporated with a rotary evaporator at < 50 ℃. After evaporation, the each extracts from mugwort were dissolved in dimethyl sulfoxide (DMSO).

2.2. Determination of physiological activity

2.2.1. Total phenolic content (TPC)

The Folin-Ciocalteu reagent assay was used to determine the TPC [3]. The TPC was expressed as milligram gallic acid equivalents (GAE) per gram of sample (mg/g).

2.2.2. Total flavonoid content (TFC)

The TFC of samples was determined by modified colorimetric method described by Sakanaka et al. [4]. The results were expressed as milligram hesperidin equivalents (HE) per gram of sample (mg/g).

2.2.3. Ferrous iron chelating activity (FCA)

FCA of samples was measured according to the method of Yen and Chung [5]. The activity of the extracts to chelate ferrous iron was calculated using the following equation: clelating activity (%) = [1 − (absorbance $_{sample}$ / absorbance $_{control}$)] × 100. The results were compared with EDTA which was used as a positive control.

2.2.4. The DPPH scavenging activity (DPPH)

The effect of different solvents from mugwort were studied using the modified method of Brand-Williams et al. [6]. The inhibitory percentage of DPPH was calculated according to the following equation: scavenging activity (%) = [1 − (absorbance $_{sample}$ / absorbance $_{control}$)] × 100. The results were compared with ascorbic acid which was used as a positive control.

2.2.5. Nitrite scavenging ability (NSA)

NSA of each extracts was determined according to the method of Gray and Dugan [7]. The ability of the samples was calculated with the equation: NSA (%) = [1 − (absorbance $_{sample}$ / absorbance $_{control}$)] × 100. The results were compared with ascorbic acid which was used as a positive control.

2.2.6. IC_{50} value

The IC_{50} value (mg/mL) was calculated as effective concentration as which the physiological activity was 50%.

2.2.7. Statistical analysis

Statistical analysis and comparisons among means were carried out using the statistical package SPSS 18.0 (SPSS Inc., Chicago, IL, USA) ($p < 0.05$). Correlations were determined by correlation analyses using Pearson's linear correlation coefficient with the above statistical software package.

3. Results and Discussion

Total phenolic content (TPC) and total flavonoid content (TFC) of the different solvents from mugwort are presented in Table 1. In this study, water extracts had the highest TPC (82.75 mg/g) and TFC (22.53 mg/g), followed by hot water, methanol, ethanol and ethyl acetate. These results are in agreement with the finding of Hong et al. [8] who proposed that polyphenol content of mugwort was higher than the total flavonoid content and phenolic compounds contribute to the overall antioxidant activities of plants mainly due to their redox properties.

Generally, the mechanisms of phenolic compounds for antioxidant effects are neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals. In addition, the correlation obtained that phenolic and flavonoid are closely related, since TPC was positively correlated to TFC (R^2 = 0.874, $p < 0.01$) (Fig. 1). Results are in accordance with those of Zhou et al. [9], who reported that the contents of flavonoids and phenolics were positively correlated to the antioxidant capacities in plant extracts.

We measured Fe^{2+} chelating activity (FCA) of the mugwort extracts from different solvents ranging from 0.1 to 1.5 mg/mL (Fig. 2a). The water extracts had the highest FCA, and differences in chelating activity of all samples were significant ($p < 0.05$). According to Rohman et al. [10], ferrozine can quantitatively form complexes with Fe^{2+}. The complex formation can be disrupted by the presence of other complexing agents which cause a reduction in the red colour intensity of complexes. Substances or samples that can decrease its

color intensity can be considered as antioxidant through the mechanism of inhibition of heavy metal. Also, these author proposed that chelating agents, which form σ-bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion.

Table 1: Comparative total phenolic contents (TPC) and total flavonoid contents (TFC) of different solvents from mugwort.

Solvents	Total phenolic contents (mg of GAE/g)	Total flavonoid contents (mg HE/g)
Ethanol	76.47 ± 2.85^{C}	17.88 ± 1.59^{C}
Ethyl acetate	50.12 ± 2.79^{A}	13.08 ± 1.84^{A}
Methanol	70.77 ± 2.64^{B}	14.75 ± 1.28^{B}
Hot water	78.16 ± 2.82^{C}	18.79 ± 1.33^{C}
Water	82.75 ± 1.51^{D}	22.53 ± 1.14^{D}

All values are mean ± SD of the three replicates.
$^{A-D}$ Values with different letters are significantly different ($p < 0.05$).
The total phenolic and flavonoid contents are expressed as gallic acid equivalents (GAE) and hesperidin equivalent, mg/g of dry weight for the samples, respectively.

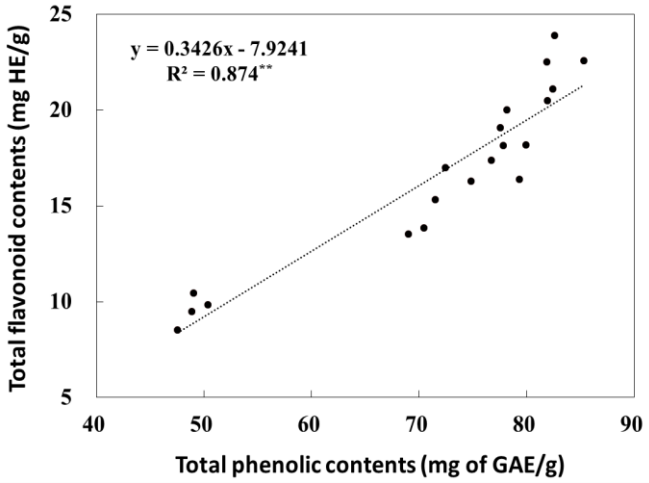

Fig. 1: Correlation between total flavonoid contents and total phenolic contents ($^{**}p < 0.01$).

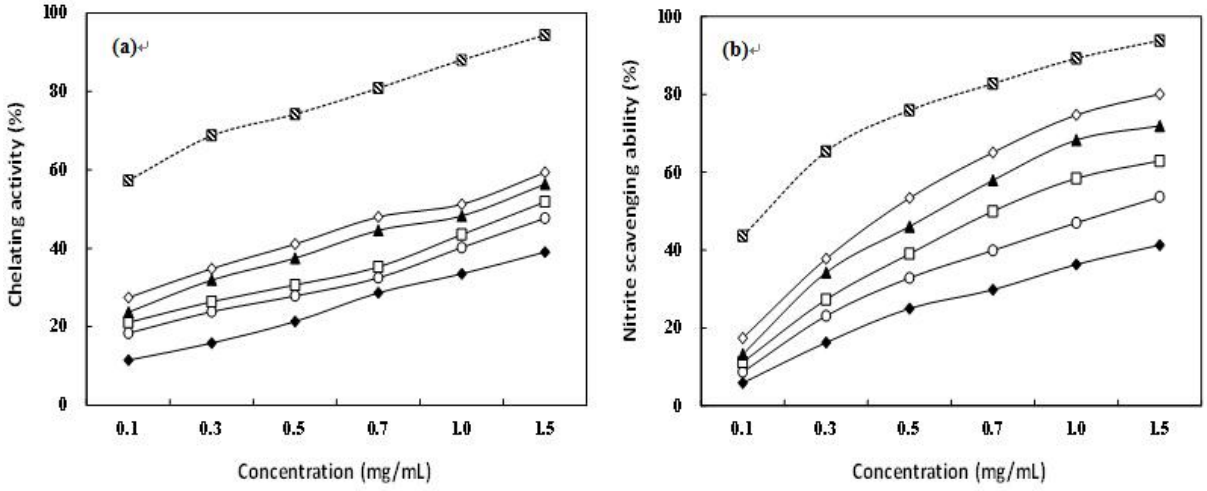

Fig. 2: Ferrous iron chelating activity (a) and nitrite scavenging ability (pH 1.2) (b) of different solvents from mugwort. All values are mean ± SD of the three replicates. (▨) EDTA (a) and ascorbic acid (b): positive control, (○) Ethanol: ethanol extracts from mugwort, (◆) Ethyl acetate: ethyl acetate extracts from mugwort, (□) Methanol: Methanol extracts from mugwort, (▲) Hot water: hot water extracts from mugwort at 100 ℃ for 4h, (◇) Water: water extracts from mugwort.

Nitrite scavenging ability (NSA) of different solvent from mugwort was dependent on concentration and the results of NSA were very similar to those of TPC, TFC, FCA, and DPPH. In this study, water extracts of mugwort was more active ($p < 0.05$) than the other solvent extracts (Fig 2b). The NSA of the treatments was ranked as follows: ascorbic acid (positive control: 93.82%) > water (80.06%) > hot water (71.91%) > methanol (62.92 %) > ethanol (53.65%) > ethyl acetate (41.29%).

The DPPH assay is most commonly used to evaluate the antioxidant activity of extracts, because this method is fast and easily available [11]. The IC_{50} values of water extract from mugwort were 0.14±0.02 mg/mL (DPPH), 0.89±0.02 mg/mL (FCA), and 0.39±0.01 mg/mL (NSA), respectively (Table 2). The water extract was most active when their IC_{50} values were measured by each solvents extracts, indicating that the water extract more responsive to low concentration. Rohman et al. [10] suggested that the smaller IC_{50} value, the higher antioxidant activity of the plant extract.

Table 2: IC50[1)] **(**mg/mL) values for physiological activity of different solvents from mugwort.

Solvents	DPPH scavenging activity	Ferrous iron chelating activity	Nitrite scavenging ability
Ethanol	1.03 ± 0.02^{D}	1.76 ± 0.02^{E}	0.66 ± 0.02^{E}
Ethyl acetate	4.19 ± 0.07^{E}	2.84 ± 0.03^{F}	1.62 ± 0.03^{F}
Methanol	0.32 ± 0.02^{C}	1.39 ± 0.04^{D}	0.85 ± 0.02^{D}
Hot water	0.33 ± 0.03^{C}	1.11 ± 0.03^{C}	0.43 ± 0.01^{C}
Water	0.14 ± 0.02^{B}	0.89 ± 0.02^{B}	0.39 ± 0.01^{B}
Ascorbic acid[2)]	0.02 ± 0.01^{A}	–	0.08 ± 0.01^{A}
EDTA	–	0.09 ± 0.01^{A}	–

All values are mean ± SD of the three replicates.
[1)] IC_{50}: amount required for 50% reduction.
[2)] Ascorbic acid and EDTA: positive control.
[A-F] Values with different letters are significantly different ($p < 0.05$).

In conclusion, the water extracts had the highest total phenolic contents, total flavonoid contents, ferrous iron chelating activity, DPPH radical scavenging activity, and nitrite scavenging ability. Taken together, these finding proposed that water extracts of mugwort can be used as functional food material with antioxidant and nitrite scavenging activities.

4. Acknowledgement

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