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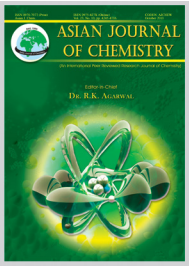

High Efficient Antioxidant Activity of Extracts from *Lepidium meyenii* Walp.

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The antioxidant activities of petroleum ether extract (E1), chloroform extract (E2), ethyl acetate extract (E3), *n*-butanol extract (E4), ethanol extract (E5) and deionized water extract (E6) obtained from *Lepidium meyenii* Walp. were investigated using various *in vitro* methods, which were reducing power and scavenging activities towards 1,1-diphenyl-2-picryhydrazyl (DPPH) radical, superoxide radical and hydroxyl radical. The amounts of total phenolic in the extracts were analyzed and determined by spectrophotometric methods. The *n*-butanol extract was found to have high levels of phenolic content (44.78 mg GAE/g) and higher reducing power. The *n*-butanol extract showed the most potent radical-scavenging activity on DPPH radicals and superoxide anion radicals, with IC_{50} values of 1.06 mg/mL and 0.30 mg/mL, respectively. There was a significant correlation between total phonolic content and total antioxidant activity (r^2 = 0.986). The *n*-butanol extracts of *Lepidium meyenii* Walp. might be valuable antioxidant natural sources and seemed to be applicable in both healthy medicine and food industry.

Key Words: Antioxidant activity, Radical-scavenging activity, Total phenolic content, *Lepidium meyenii* Walp.

INTRODUCTION

Oxidation is essential to many organisms for the production of energy to fuel biological processes. However, many human diseases are due to the uncontrolled production of reactive oxygen species. Reactive oxygen species, such as superoxide radical, hydroxyl radical and H_2O_2, can nick DNA, damage essential enzymes and proteins, or provoke uncontrolled lipid peroxidation and auto-oxidation reactions leading to cancer[1]. Besides, reactive oxygen species are also involved in the pathogenesis of several chronic diseases such as neurodegenerative diseases, ageing, rheumatoid arthritis and metabolic diseases like atherosclerosis, diabetes, hypertension, *etc.*[2].

Due to several side effects and toxic properties of many synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT)[3], increasing attention has been paied to the development of safe and effective functional foods and antioxidative agents from natural sources, so that they can protect the human body from free radicals and retard the progress of many chronic diseases[4,5].

Maca (*Lepidium meyenii* Walp.) is an herbaceous plant belonging to the family of Brassicaceae. It is mainly domesticated at altitudes between 3,500 and 5,000 m of the Peruvian Andes. In recent years *Lepidium meyenii* Walp. has gained popularity due to the functional properties of improving fertility, sexual performance, growth rate, learning and memory, antipostmenopausal osteoporosis[6], protection against UV exposure[7] and antioxidant activity[8,9]. However, no literature reported radical-scavenging activities of different extracts of *Lepidium meyenii* Walp. and the correlation between antioxidant activity and the phenolic content.

Among the natural antioxidants, phenolic antioxidants are in the forefront. In addition, the relationships between the antioxidant activities and the phenolic contents are examined in order to search for industry-scale antioxidant compounds. This study was designed to evaluate the antioxidant activities of different extracts from *Lepidium meyenii* Walp. by using four *in vitro* methods including reducing power assay, 1,1-diphenyl-2-picryhydrazyl radical-scavenging assay, superoxide anion radical-scavenging assay and hydroxyl radical-scavenging assay. Because of the important roles of the total phenolic as antioxidants, the amounts of total phenolics in the extracts were also determined. Thus, the aim of the present study is to evaluate and identify a potential new source of safe natural

antioxidants from different extracts of *Lepidium meyenii* Walp., which will develop a nutraceutical agent rich in natural antioxidants, prove beneficial for maintenance of health and may increase the demand of these bioactive substances in food, cosmetic and pharmaceutical industries.

EXPERIMENTAL

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from TCI Shanghai Chemical Industry Development Co. Ltd., Brilliant green and $FeCl_3$ were bought from Tianjin Fu Chen Chemical Reagent Factory. Folin-Ciocalteau reagent was from Beijing Qisong Biotechnology Co. Ltd., Gallic acid was from Beijing Xingjin Chemical Plant. Pyrogallol, $FeSO_4$, potassium ferricyanide and trichloroacetic acid were supplied by Beijing Chemical Reagent Company. Sodium carbonate, petroleum ether, chloroform, ethyl acetate, *n*-butanol, ethanol, methanol and H_2O_2 were purchased from Beijing chemical plant. All chemicals were of analytical grade. Maca ecotypes were kindly supplied by Lijiang Gelin Hengxin Biotechnology Development Co. Ltd. The material was identified and authenticated by Zhirong Yang, Institute of Botany, Chinese Academy of Sciences.

Extraction procedure: The antioxidant compounds of *Lepidium meyenii* Walp. could be of very different polarity and as a result the materials were extracted by ultrasonication with different solvents in the order of increasing polarity[10]. Briefly, 250 g of *Lepidium meyenii* Walp. powder was ultrasonicated in 2.5 L petroleum ether with solid to solvent ratio of 1:10 (w/v) at room temperature for 0.5 h and then the extract was centrifugated to make a petroleum ether extract (E1). After extraction, the residue was extracted in sequence with chloroform, ethyl acetate, *n*-butanol, ethanol and deionized water to get E2, E3, E4, E5 and E6, respectively. The extracts were concentrated under reduced pressure by rotary evaporator at 40 ºC and the yields of solvent dried extracts were calculated. The six extracts were redissolved in their respective solvents accordingly and stored in the dark at 4 ºC for further test[11].

Determination of reducing power: The reducing power was evaluated by the potassium ferricyanide reduction methods. The reaction mixtures contained 2.5 mL phosphate buffer (0.2 M, pH 6.6), 2.5 mL potassium ferricyanide (1 %, w/v) and the extracts (0-5 mg/mL). After incubating at 50 ºC for 20 min, 2.5 mL of trichloroacetic acid (10 %, w/v) was added to terminate the reaction. A liquor of 2.5 mL was mixed with 2.5 mL deionized water and 0.5 mL $FeCl_3$ (0.1 %, w/v) and then incubated at room temperature for 15 min. The absorbance of the six extracts was measured at 700 nm[12].

In the reducing power assay, the antioxidants presented in the test extracts could reduce Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}) by donating an electron. The colour changed from yellow to different shades of green and blue, which depended on the reducing power of each extract of *Lepidium meyenii* Walp. The reducing power of the extracts could be monitored by measuring the formation of Perl's Prussian blue colour at 700 nm[13]. Increasing absorbance of the reaction mixture at 700 nm indicated an increase in the reducing power[14].

1,1-Diphenyl-2-picryl-hydrazyl radical scavenging assay: 1 mL of the extracts of different concentrations was thoroughly mixed with 2 mL of freshly prepared DPPH (0.2 mM in methanol) and 2 mL of methanol. The mixture was shaken and left for 0.5 h in the dark. Then, the absorbance was measured at 517 nm with a blank control[15]. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [1-(A-A_b)/A_0] \times 100\ \%$$

where, A_0 is the absorbance of DPPH solution without the extracts; A is the absorbance of the test extract mixed with DPPH solution and A_b is the absorbance of the sample without DPPH solution.

On reaction with antioxidants, the purple colour of DPPH fades or disappears due to its conversion to 2,2-diphenyl-1-picryl hydrazine. The more decrease in absorption of the reaction mixture, the higher free radical-scavenging activity of the extract[16].

Superoxide radical scavenging assay: The scavenging superoxide radical activities of the six extracts were measured according to the method of pyrogallol autoxidation[17,18] with some modification. 4.5 mL *Tris*-HCl buffer (0.1 M, pH 8.2), 2.4 mL deionized water and 1 mL sample were mixed and incubated for 10 min at 25 ºC. 0.1 mL pyrogallol (6 mM) was added to react for 3 min. Then 0.1 mL HCl (10 M) was added to terminate the reaction and the absorbance was measured at 325 nm[19]. The capability to scavenge the hydroxyl radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [1-(A-A_b)/A_0] \times 100\ \%$$

where, A_0 is the absorbance without extract; A is the absorbance of the test extract and A_b is the absorbance without pyrogallol.

Hydroxyl radical scavenging assay: The hydroxyl radical system was generated by the Fenton reaction, which contained 1 mL brilliant green (0.435 mM), 0.5 mL $FeSO_4$ (2 mM), 1.5 mL H_2O_2 (3.0 %) and 1 mL samples. After incubation at room temperature for 20 min, the absorbance of the mixture was measured at 624 nm. The hydroxyl radical-scavenging rate was calculated as the following equation:

$$\text{Scavenging effect (\%)} = (1-A/A_0) \times 100\ \%$$

where, A is the absorbance of the test extract and A_0 is the absorbance of control. Hydroxyl radicals gave a brilliant green colour, so the absorbance change of the reaction mixture indicated the scavenging ability for hydroxyl radicals[20].

Determination of total phenolic content: The total phenolic content in the extracts was determined according to the colourimetric Folin-Ciocalteu method[21] with gallic acid as a standard compound. It was based on the oxidation of polyphenols to a blue coloured complex with an absorbance maximum of 765 nm. 1 mL of extracts and 5 mL of Folin-Ciocalteau reagents were mixed and shaken. After 1 min, 4 mL of aqueous sodium carbonate (7.5 %) was added. The mixture was vortexed and allowed to stand at room temperature without light for 30 min. The absorbance was read at 765 nm using a 2802 UV/VIS spectrophotometer. Total phenolic contents of extracts were calculated from the calibration curve and expressed as mg gallic acid equivalents (GAE)/g extract[22].

All experiments were carried out in triplicate and the results were presented as mean values ± SD (standard deviations). Statistical analysis was done using ORIGIN 8.0. Linear regressions were performed to indicate the relationship between the total phenolic contents and the data from the antioxidant assays.

RESULTS AND DISCUSSION

Preparation of different extracts: After removal of the solvents using a vacuum rotary evaporator, the final yields of the E1, E2, E3, E4, E5 and E6 were 0.63, 0.95, 0.60, 0.36, 5.13 and 32.91 %, respectively. According to the result, the different solvents had different abilities to extract substances from *Lepidium meyenii* Walp. In general, the amounts of total extractable compounds decreased with decreasing polarity of the solvent in order.

Reducing power: The reducing power of a sample is an important parameter reflecting one aspect of its antioxidation property. Earlier studies have indicated that reducing power of the plant is one of the key mechanisms for the possible antioxidant activity and may serve as a significant indicator of potential antioxidants[23]. In this assay, all the six extracts of *Lepidium meyenii* Walp. showed a good concentration-dependent manner with a perfect reducing power (Fig. 1). The reducing power of all extracts from *Lepidium meyenii* Walp. was presented in the following order: E4 > E5 > E6 > E3 > E2 > E1. Reducing power of six different extracts from *Lepidium meyenii* Walp. indicated that reducing power played a role in the antioxidation.

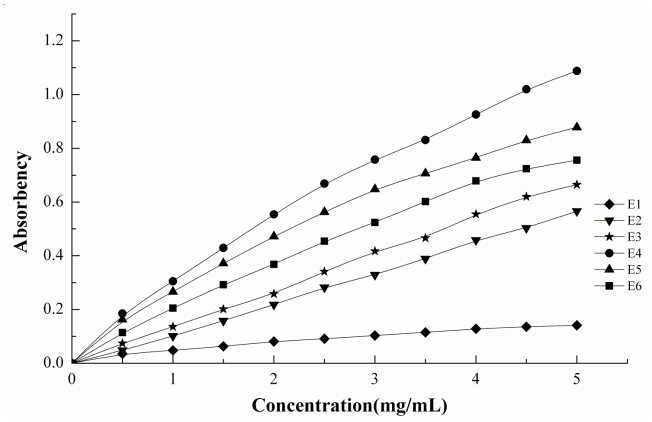

Fig. 1. Reducing power of different *Lepidium meyenii* Walp. extracts *in vitro*

DPPH radical-scavenging activity: Fig. 2 illustrated that the potent antioxidant capacities were found in all tested extracts in a concentration-dependent manner. At 5 mg/mL, the DPPH radical-scavenging activity in E4 was 98.0 %, higher than that in E5 (94.33 %), E2 (69.52 %), E6 (65.13 %), E3 (54.90 %) and E1 (21.32 %). The IC_{50} values (the concentration with scavenging activity of 50 %) of scavenging activities on DPPH radical was found 1.06 mg/mL for E4.

It was generally observed that the DPPH radical-scavenging effect increased as the concentration of the extract increased to a certain extent and then leveled off, even with further increases in the extract concentration. For example, E4 at a concentration of 1-3 mg/mL exhibited 48.67-92.29 % scavenging activity of DPPH radical, whilst its scavenging activity was 98.0 % at the concentration of 5 mg/mL and no significant increase in the DPPH scavenging effect was observed with further increase in dosage.

Superoxide radical-scavenging activity: The scavenging effects of different extracts of *Lepidium meyenii* Walp. on superoxide radicals were shown in Fig. 3. They exhibited

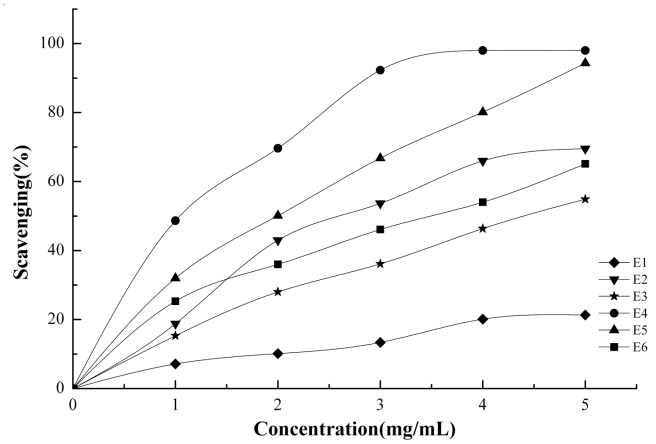

Fig. 2. Scavenging effect of different *Lepidium meyenii* Walp. extracts on DPPH radical *in vitro*

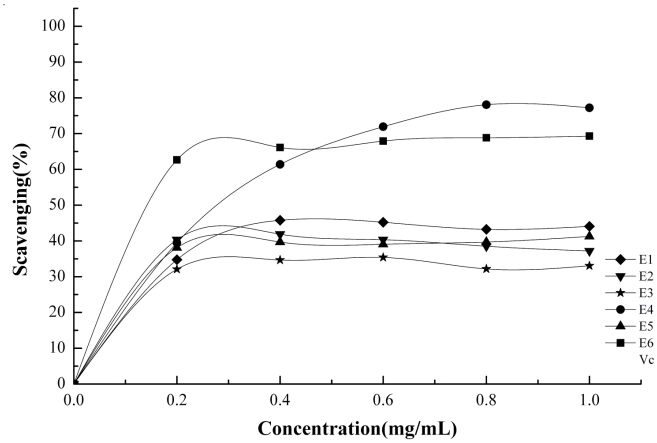

Fig. 3. Scavenging effect of different *Lepidium meyenii* Walp. extracts on superoxide radical *in vitro*

concentration-dependent superoxide radical-scavenging activities. At a concentration of 5 mg/mL, their superoxide radical-scavenging activities were in the following order: E4 > E6 > E1 > E5 > E2 > E3. E4 showed the highest scavenging effects on superoxide radicals (IC_{50}: 0.30 mg/mL), indicating that the compounds with strongest superoxide radical scavenging activity in *Lepidium meyenii* Walp. were of medium polarity and could be explored as a novel potential antioxidant.

Hydroxyl radical-scavenging activity: All the six extracts of *Lepidium meyenii* Walp. were found to have the ability to scavenge hydroxyl radicals at the concentrations between 0.04 and 0.2 mg/mL. The results showed hydroxyl radicals scavenging activities of the six extracts were in the order of E4 > E5 > E6 > E2 > E1 > E3. All the extracts exhibited potent or moderate activity in a concentration-dependent manner. As shown in Fig. 4, the highest hydroxyl radical scavenging activity was found in E4, showing 7.94 %, while those of E1, E2, E3, E5 and E6 were 1.66, 3.59, 1.22, 6.49 and 4.21 %, respectively.

Total phenolics content: The majority of natural antioxidants were phenolic compounds or polyphenols and the antioxidant activity of many natural extracts was due to such phenolic compounds. The increasing evidence showed that the consumption of a variety of phenolic compounds present in natural foods might lower the risk of serious health disorders.

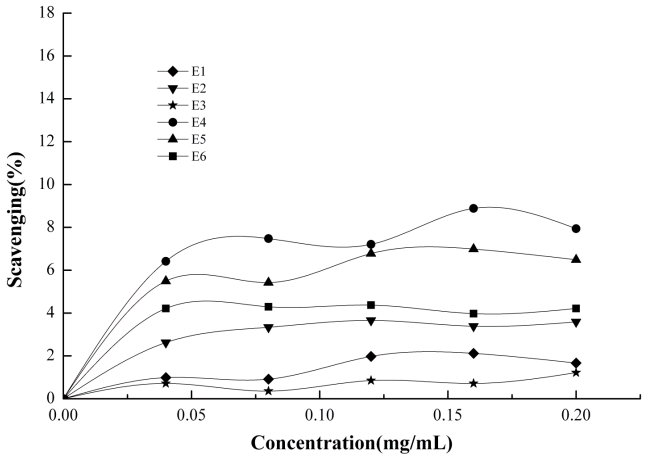

Fig. 4. Scavenging effect of different *Lepidium meyenii* Walp. extracts on hydroxyl radical *in vitro*

From Table-1, it was observed that *n*-butanol extract had a highest phenolic content (44.78 mg) compared with the other extracts expressed as gallic acid equivalents (GAE)/g of extract, which might explain the reason for its strong and efficient *in vitro* antioxidant properties. A wide range of total phenolics content was found in the six extracts, which ranged from 2.65 to 44.78 mg/g sample. The difference in the antioxidant activity of the six extracts may be ascribed to the difference in the total phenolic content as well as the phenolic compositions.

TABLE-1
CONTENTS OF TOTAL PHENOLIC IN DIFFERENT FRACTIONS OF *Lepidium meyenii* Walp.

Fraction	Total phenolic content (mg GAE/g)
E1	2.65
E2	14.60
E3	12.12
E4	44.78
E5	18.76
E6	16.19

Correlation between total phenolic content and antioxidant activity: In this work, the correlation between the radical scavenging activity and phenolic content of all the six extracts was studied using a linear regression analysis. A low correlation was found between total phenolic content and superoxide radical-scavenging activity, while a higher correlation was found between total phenolic content and hydroxyl radical-scavenging activity. Statistical analysis also indicated a significant association between total phenolic content and reducing power ($R^2 > 0.9678$) as well as between total phenolic content and DPPH radical-scavenging activity ($R^2 > 0.9436$).

Conclusion

All the six different solvent extracts of *Lepidium meyenii* Walp. with antioxidant activity, as seen in the DPPH radical-scavenging activity assay, superoxide radical-scavenging activity assay, hydroxyl radical-scavenging activity and reducing power assay were investigated for the first time. The extracts exhibited different levels of antioxidant activity in the systems tested. Antioxidant extract from *n*-butanol had the highest amounts of phenolic content and antioxidant activity as well as free radical-scavenging activity, indicating their potential application in the food industry as nutritional supplements, functional food components or food antioxidants. This evaluation may shed light on a better understanding of *Lepidium meyenii* Walp. as a potential functional and valuable bioactive source of natural antioxidant for its high antioxidant activity. The results provide a valuable basis for developing *Lepidium meyenii* Walp. as valuable food additives to enhance human nutrition via their phytochemicals and antioxidant activity.

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