Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(6):458-462

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antioxidant activities of an active fraction from *Athyrium multidentatum* (Doll.) Ching

Dongmei Liu, Jiwen Sheng*, Jie Mu, Wanzhong Li, Zhijian Li and Yanlong Sun

Department of Pharmacy, Weifang Medical University, Weifang, PR China

---

ABSTRACT

In this paper, an active fraction (TA) was obtained from Athyrium multidentatum (Doll.) Ching rhizome by silica gel and Sephadex LH-20 column chromatography. The total phenolic content in TA was determined by Folin-Ciocalteu colorimetric method. Antioxidant activity of TA was evaluated employing various established in vitro systems. As a result, TA showed stong reducing power, metal chelating ability and superoxide radical scavenging activity. In the reducing power assay, the absorbance of TA increased from 0.698 ± 0.008 to 2.053 ± 0.011 at concentrat ions of 4 to 12 μg/mL, which was stronger than vitamin C. These findings indicated that TA was excellent antioxidant and might be a promising prophylactic agent for free radical-related disease.

Key words: *Athyrium multidentatum* (Doll.) Ching, Active fraction, Antioxidant activity, Reducing power.

INTRODUCTION

Athyrium multidentatum (Doll.) Ching (AMC), a species of fern in the family Athyriaceae, distributes primarily in the Changbai Mountain area of China. The plant grows about one metre high, and thrives in damp grounds, thick woodlands or mountains. It has been recognized as an unparalleled resource of potherb and medicine for conditions like high blood pressure, anxiety and fervescence in the native for thousands of years. So far, eighteen compounds have been separated and identified from AMC, including palmitin, *β*-sitosterol, tetracosanoic acid, adipic acid, aspidin BB, *α*-spinasterol, aspidinol B, caffeic acid, quercetin, kaempferol, ursolic acid, gallic acid, 3-hydroxy-5,6-epoxy-*β*-ionone, chlorogenic acid, kaempferol-3-*β*-D-glucopyranoside, kaempferol-7-*β*-D-glucopy-ranoside, quercetin-3

-*β*-D-glucopyranoside, and quercetin-7-*β*-D-glucopyranoside [1-3]. However, regardless of these characteristic compounds, the exact active components of AMC have not yet been reported. In our previous study, we investigated the antioxidant activity of AMC and found that polyphenolic constituents might contribute to the antioxidative actions of the herb [4].

Polyphenols are important plant secondary metabolites and rich in fruits and vegetables. There are increasing evidences that polyphenols may protect cells against oxidative damage and, therefore, limit the risk of various degenerative diseases associated with oxidative stress by auto-oxidation or reduction of reactive oxygen species (ROS) [5-7]. It was reported that *Maytenus royleanus* leaves with high concentration of phenolic compounds and tannins could be used as therapeutic agent against free-radical associated damages [8]. Resveratrol, a polyphenolic phytoalexin, could extend the lifespan and delay age-related diseases through its robust ROS scavenging activity [9]. In this study, an active fraction was obtained from AMC rhizome and had its antioxidant activity evaluated by *in vitro* assay. The mechanisms on the antioxidant action were explored and interpreted preliminarily.

EXPERIMENTAL SECTION

Materials: *Athyrium multidentatum* (Doll.) Ching rhizome was harvested in Changbai Mountain area of China in September 2012 and identified by Professor Chongmei Xu (Department of Pharmacy, Weifang Medical University). The dried rhizome was crushed and stored at cool, dry and ventilated room. Gallic acid, pyrogallol, trichloroacetic acid (TCA), hydrogen peroxide (H_2O_2), ferrozine and vitamin C (Vc) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sephadex LH-20 was purchased from China National Pharmaceutical Group Co.. All chemicals and reagents, unless otherwise specified, were not purified, dried or pretreated.

Preparation of TA: 8 kg of AMC rhizome was immersed in methanol at room temperature for a month. The resultant methanolic solution was evaporated under reduced pressure and 145 g of brown extract was obtained. Subsequently, the extract was suspended in distilled water and partitioned successively with petroleum ether and aqua-saturated *n*-butanol. 45.8 g of *n*-butanol extract was acquired and chromatographed on a silica gel column (8 × 60 cm). Fraction eluted with ethyl acetate and methanol (10:1, v/v) was chromatographed on a sephadex LH-20 column (4 × 60 cm) and eluted stepwise with dichloromethane and methanol. The active fraction (TA) was prepared from fraction eluted with methanol and stored in vacuum desiccator for further analysis.

Chemical analysis: Total phenolic content in TA was determined with gallic acid as standard. Briefly, 4.6 mL of reaction mixture, containing different concentrations of gallic acid (368 to 1104 μg/mL), was added 0.2 mL of Folin-phenol reagent and shaken for 1 min. Afterwards, 0.2 mL freshly prepared Na_2CO_3 (10%, w/v) was added and allowed to react for 20 min at room temperature. Finally, the absorbance was measured at 760 nm and the standard curve was established. Meanwhile, the absorbance of the sample solution (109 μg/mL) was measured according to the same method and the total phenolic content in TA was calculated by the established standard curve. All samples were tested in three times.

Reducing power assay: The reducing power of all samples was determined by the method of potassium ferricyanide reduction. All solutions were used on the day of preparation. Briefly, 3.5 mL of reaction mixture, containing different concentrations of samples (40 to 120 μg/mL) in phosphate buffer (0.2 mol/L, pH 6.8), was incubated with potassium ferricyanide (1%, w/v) for 20 min at 50℃. Then 1 mL of TCA (10%, w/v) was added to the mixture to terminate the reaction. Finally, the solution was mixed with 0.5 mL ferric chloride (1%, w/v) and the absorbance was measured at 700 nm. Measurements were performed at least in triplicate. Increased absorbance of reaction mixture indicated increased reducing power. Vc was used as positive control.

Superoxide radical scavenging assay: Measurement of superoxide radical scavenging activity was based on a modified method of Zhang *et al*. [10]. Superoxide radicals were generated in the pyrogallol autoxidantion system containing 4 mL Tris–HCl buffer (50 mmol/L, pH 8.2), 0.5 mL pyrogallol (3.5 mmol/L), and varying concentrations of samples (40–120 μg/mL). The reaction mixture was incubated at room temperature for 6 min, then terminated by 0.5 mL HCl (8 mol/L). Absorbance was measured at 320 nm against the blank. In the control, sample was substituted with Tris–HCl buffer. Measurements were performed at least in triplicate. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The scavenging capacities of the samples were compared with Vc. The capability of scavenging to superoxide radical was calculated by the following equation:

$$\text{Scavenging effect (\%)} = (1 - A_{\text{sample 320}}/A_{\text{control 320}}) \times 100$$

Metal chelating assay：The ferrous ion-chelating ability of sample was investigated with slightly modified method described by Zhang *et al*. [10]. Samples in different concentrations (5.4–16.2 μg/mL) were mixed with 1 mL ferrous chloride (2 mmol/L) and 1 mL ferrozine (5 mmol/L), then shook well and stayed still for 10 min at room temperature. The absorbance of the mixture was determined at 562 nm. Measurements were performed at least in triplicate. In the control, sample was substituted with distilled water. The ferrous ion-chelating activity was given by the following equation:

$$\text{Chelating ability (\%)} = (1 - A_{\text{sample 562}}/A_{\text{control 562}}) \times 100$$

Statistical analysis：All data listed in the figures or the tables are shown in means ± S.D. and processed with Excel software.

RESULTS AND DISCUSSION

Chemical analysis. An active fraction (TA) was obtained from AMC and the linear regression equation was Y = 0.065 X − 0.0084 with a regression coefficient of 0.9945 and a linearity range of 4−28 μg/mL. The total phenolic content in TA was 96.6% according the standard curve. TA was ample with phenolic compounds which might play an important role in the strong antioxidant activity of TA.

Reducing power assay. In the reducing power assay, the presence of reductants (antioxidants) in the tested samples would result in reducing Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}) [11], and the yellow test solution changes into various shades of green and blue colors depending on the reducing power of antioxidant samples. The reducing capacity of a compound may serve as a significant indicator of its antioxidant activity. As depicted in Figure 1, the reducing power of all samples was concentration dependent. With increased concentrations of TA, the absorption augmented from 0.698 ± 0.008 to 2.053 ± 0.011, however, the absorption of Vc was only raised from 0.637 ± 0.003 to 1.726 ± 0.001. It was obvious that the reducing power of TA was stronger than Vc at the same concentrations. Kajaria *et al.* [12] reported the absorption of quercetin was 0.856 at 1000 μg/mL. At the concentration 0.5 mg/mL, the absorption of fucoidan was only 0.25 [13], which was much lower than TA. It was evident that TA did exhibit strong reducing power and was promising as antioxidant in food and pharmaceutical industry. The reducing power is generally associated with reductones, a strong antioxidant, which can break free-radical chain by donating a hydrogen atom [14]. In our opinion, phenolic hydroxy groups in TA promoted its high donating hydrogen ability.

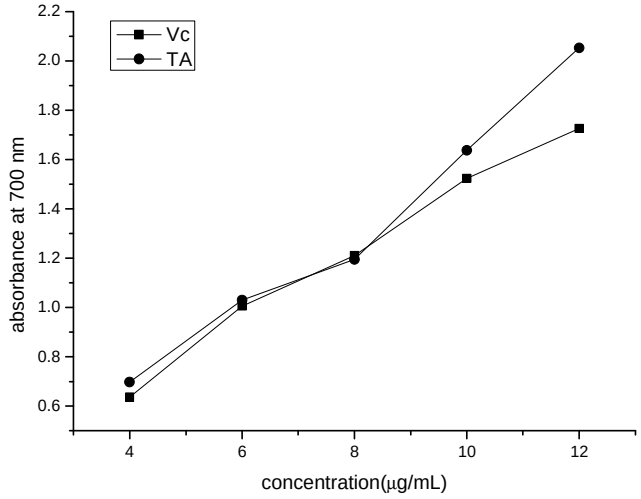

Figure 1. Reducing power of the active fraction (TA) and Vc. Values are means ± S.D. (*n* = 3)

Superoxide radical scavenging assay. Superoxide anion is the least active and harmful to the organism among ROS. However, it is one of the precursors of the singlet oxygen and hydroxyl radicals that can indirectly initiate lipid peroxidation, and induce pathological incidents such as arthritis and Alzheimer's disease [15]. Therefore, Superoxide radical scavenging capacity in the human body is the first line of defense against oxidative stress. The scavenging activity of all tested samples on superxoide radicals was described in Figure 2. Except for at 3.6 μg/mL, the scavenging ability of all tested samples was shown in a concentration-dependent fashion. TA exhibited stronger scavenging activity than Vc at the concentrations lower than 5.45 μg/mL. The scavenging ability of superoxide radical by TA were over 60% at concentrations from 3.6 to 10.8 μg/mL. Chiang *et al*. [16] reported the scavenging ability of isoflavonoid-rich *Flemingia macrophylla* extract against superoxide radical was only 68.6 ± 2.4% at 500 μg/mL. Compared with these results, TA displayed stronger scavenging activity on superoxide radical than *Flemingia macrophylla* extract. Evidence showed that high quantities of condensed tannins and catechins were responsible for the strong antioxidant capacity [17]. Our data indicated that scavenging activity on superoxide radical of TA have a direct, positive correlation with the antioxidation observed.

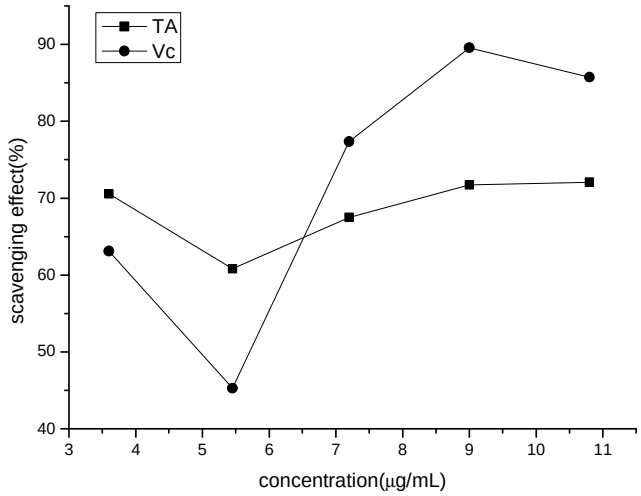

Figure 2. Scavenging effects on superoxide radical of the active fraction (TA) and Vc. Values are means ± S.D. (*n* = 3)

Metal chelating assay. Radical damage is partly due to the formation of hydroxyl radical by reactions similar to the Fenton reaction. Compounds that can chelate metal ions may minimize the production of hydroxyl radical by inhibiting the Fenton reaction [18]. The metal chelating capacity is significant since it reduces the concentration of transition metal ions affecting lipid peroxidation and impedes the formation of other secondary free radicals [19]. As displayed in Figure 3, ferrous ion-chelating effect of all tested samples was independent of the concentrations. The relationship between chelating effect and concentration was complicated. At low concentration below 7 μg/mL, TA exhibited a descending curve, however, the chelating ability increased again at 8.75 and 10.5 μg/mL. The highest chelating value of TA was 14.36% at concentration of 3.5 μg/mL. The chelation efficiency to Fe^{2+} depended on the number of hydroxyl and the hydroxyl substitution in the ortho position was desirable [20]. *A. lanata* extract with high amount of gallic acid showed notable metal chelating activity [21]. Furthermore, the biological activities of polyphenols depend on several structural parameters such as the type of polyphenol, molecular weight, number/position and conformation of phenolic hydroxyl. Lin and Lin found the galloyl group and phenolic hydroxyl groups at the 3' position were responsible for the anti-inflammatory activity of epigallocatechin gallate [22]. Proanthocyanidins with lower molecular size were more effective as superoxide anion and hydroxyl radical scavengers and xanthine oxidase inhibitors [23]. We supposed that the phenolic hydroxy groups might contribute to the chelating ability of TA.

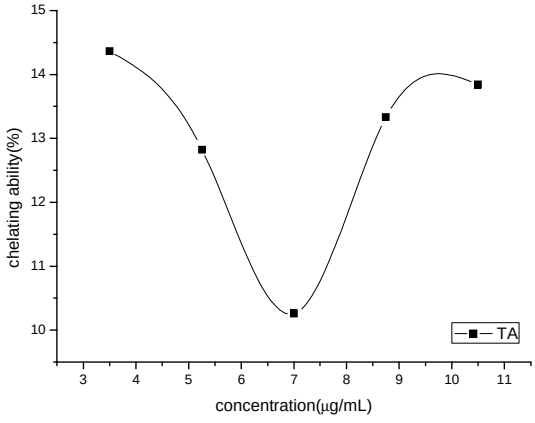

Figure3. Chelating effect of the active fraction (TA) on ferrous ions. Values are means ± S.D. (*n* = 3)

CONCLUSION

In a word, TA exhibited strong antioxidant activities *in vitro* by virtue of their strong reducing power, free radical scavenging effect, and chelating ability. The antioxidant activities of TA might attribute to its ability of scavenging ROS. TA could be beneficial for health promotion.

Acknowledgements
This study was supported by the Natural Science Foundation of Shandong province (ZR2013HQ024), the National Natural Science Funds (81274049), and Traditional Chinese medicine Science and Technology development projects of Shandong province (2015-230).

REFERENCES

[1] DM Liu; JW Sheng; YL Sun; XH Wang; XL Wang, *J. Pharm. Res*., **2013**, 32, 10, 16.
[2] C Ji; MW Zhang; SJ Zhang; M Zhao, *J. Qiqihar Univ*., **2013**, 29, 76-78.
[3] JL Wang; MW Zhang; C Ji; WZ Zhang; SJ Zhang, *Chin. Trad. Patent Medi*., **2013**, 35, 105-108.
[4] JW Sheng; DM Liu; ZJ Li; L Qi; WF Zhang, *J. Med. Plants Res*., **2011**, 5, 7000-7005.
[5] KB Pandey; SI Rizvi, *Oxid. Med. Cell. Longev*., **2009**, 2(5), 270-278.
[6] KW Lee; HJ Lee, *Mech. Ageing Dev*., **2006**, 127(5), 424-431.
[7] S Lee; I Lee; W Mar, *Arch. Pharm. Res*., **2003**, 26(10), 832-839.
[8] M Shabbir; MR Khan; N Saeed, *BMC Complement Altern. Med*., **2013**, 22, 143.
[9] M Kitada; D Koya, *Oxid. Med. Cell Longev*., **2013**, 568093.
[10] Q Zhang; LC Gong; FR Meng; FY Sun; JL Gao, *Forest By-product & Speciality China*, **2010**, 104, 16-19.
[11] TT Zhao; QB Zhang; HM Qi; H Zhang; X Niu; Z Xu; Z Li, *Int. J. Biol. Macromol*., **2006**, 38(1), 45-50.
[12] DK Kajaria; M Gangwar; AK Sharma; YB Tripathi; JS Tripathi; S Tiwari, *Anc. Sci. Life*, **2012**, 32(1), 24-28.
[13] J Wang; QB Zhang; ZS Zhang; JJ Zhang; PC Li, *Int. J. Biol. Macromol.*, **2009**, 44, 170-174.
[14] YC Chung; CT Chang; WW Chao; CF Lin; ST Chou, *J. Agric. Food Chem.*, **2002**, 50(8), 2454-2458.
[15] CR Wade; PG Jackson; J Highton; AM van Rij, *Clin. Chim. Acta*., **1987**, 164(3), 245-250.
[16] HM Chiang; HH Chiu; ST Liao; YT Chen; HC Chang; KC Wen, *Evid. Based Complement Alternat. Med*., 2013, 696879.
[17] S Weidner; A Powałka; M Karamać; R Amarowicz, *Int. J. Mol. Sci*., **2012**, 13(3), 3444-3457.
[18] AE Hagerman; KM Riedl; GA Jones; KN Sovik; NT Ritchard; PW Hartzfeld; TL Riechel, *J. Agric. Food Chem*., **1998**, 46, 1887-1892.
[19] PD Duh; YY Tu; GC Yen, *LWT-Food Sci. Technol*., **1999**, 32, 269-277.
[20] GC Yen; PD Duh; DY Chuang, *Food Chem*., **2000**, 70, 437-441.
[21] C Privat; JP Telo; V Bernardes-Genisson; A Vieira; JP Souchard; F Nepveu, *J. Agric. Food Chem*., **2002**, 50(5), 1213-1217.
[22] YL Lin; JK Lin, *Mol. Pharmacol*., **1997**, 52(3), 465-472.
[23] R Arimboor; C Arumughan, *J. Food Sci*., **2012**, 77(10), C1036-1041.