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Original Article

Antioxidant Activities of Ethanolic and Aqueous Extracts of *Asparagus racemosus* Roots

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ABSTRACT

Background: *Asparagus racemosus* (AR) is commonly known as shatavari, satawar or satmuli in India and in Thailand it is call sam-sib or rak-sam-sib. The dried root of AR is used in Ayurveda as an antiulcerous and antiinflammatory and has medicinal/pharmacological value. **Objective:** To investigate the antioxidant activities of *Asparagus racemosus* root extracts via total phenolic and total flavonoid contents of ethanolic and aqueous extracts. **Methods:** Antioxidant capacity measurements were carried out by DPPH, ABTS and FRAP methods. Total phenolic and flavonoid contents were determined by the Folin-Ciocalteu method and the aluminum chloride colorimetric method, respectively. **Results:** The ethanolic extract possessed higher antioxidant capacities than the aqueous extract in the three antioxidant assays ($p<0.05$).These results have shown high phenolic and flavonoid contents. The ethanolic extract of AR root possessed higher amounts of phenolic and flavonoid contents than the aqueous extract. **Conclusion:** The antioxidant capacity of the ethanolic extract was higher than that in the aqueous extract.

Key words: *Asparagus racemosus*, Antioxidant activity, Phenolic compound, Flavonoid.

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INTRODUCTION

Asparagus racemosus (AR) wild., (Family: Liliaceae), commonly known as shatavari, satawar or satmuli, is found in all over India, and is commonly known in Thailand as sam-sib or rak-sam-sib. In Ayurveda, the dried root of AR is used as a tonic, galactogogue, aphrodisiac, rejuvenator, antispasmodic, antiulcerous and anti-inflammatory agent. Steroidal saponins and sapogeninsare the pharmacological value of the AR root.[1-2] Antioxytocic activities, antihepatotoxic, hepatopathy, dyspepsia and dysentery are the uses of the root of AR for treatment.[1] Recent reports on AR indicate that the root extracts show antioxidant and antidiarrheal activities in animal models.[3-4]

Phytoanalysis of the AR root composition by Visavadiya *et al*.[5] found phytosterols 0.79%, saponins 8.83%, polyphenols 1.69%, flavonoids 0.47% and total ascorbic acid 0.76%. AR contains alkaloids, flavonoids, tannins, saponins, phenols, terpenes, polysaccharides and steroids.[5] AR root extract was found to contain flavonoids, polyphenols and vitamin C, which were found to exhibit the greatest antioxidant activity. A phytoestrogenic effect of AR root extracts has recently been found as well as antidiarrhoeal, antidyspepsia, adaptogenic, cardioprotective, antibacterial, immune adjuvant and antitussive effects. The methanolic and aqueous extracts of AR roots have been produced in tablet form, root powder in tablet form and root extract in syrup form.[6]

The objective of this research is to determine the antioxidant properties of ethanolic and aqueous extracts of the roots of AR. The ethanolic and aqueous extracts were investigated for the antioxidant properties, including DPPH, ABTS, FRAP, total flavonoid and total phenol.

MATERIALS AND METHODS

Plant Material

The plant samples (roots) of AR were collected from Khonkaen province, Thailand and identified by the authors. The voucher specimens were deposited at the Pharmaceutical Chemistry and Natural Product Research Unit, Faculty of Pharmacy, Mahasarakham University, Thailand (MSU.PH-LIL-AR1). The roots were washed thoroughly with tap water and dried at 37°C in an incubator, then cut into small pieces and powdered finely before extraction.

Preparation of Plant Extraction

About 100 g of the dried powder of the roots of AR was extracted with either 1000 mL of 95 % ethanol using a Soxhlet apparatus or boiled with water at

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100°C for 10 min. The extracts were concentrated to dryness under a reduce pressure and controlled temperature using an evaporator and a freeze-dryer, respectively.

DPPH radical scavenging assay

The antioxidant activities of the ethanolic and aqueous extracts of the AR root were determined using a method based on the reduction of the ethanolic solution of color-free radical 1, 1 diphenyl-1-2 picrylhydraxyl (DPPH).[7-8] An ascorbic acid solution was used as a reference standard. A varied dose of each extract (10-1000 ug/mL, dissolved in 10% DMSO) was added to a volume of 750 µL DPPH in absolute ethanol, incubated at room temperature in the dark for 20 min and measured for absorbance at 517 nm using a 96-well plate with microtiter plate reader (BMG LABTECH GmbH, Ortenberg, Germany). The inhibition percentages of the radical scavenging activity of the samples were expressed as follows:

% inhibition = [(A control-A sample)/A control] x 100

The absorbance values of the DPPH solutions without or with a sample added were the control and samples, respectively.

The inhibitory concentration of the sample requires a scavenging activity for the DPPH radical of 50% (IC_{50} value) as obtaining from a linear regression analysis of a calibration curve.

ABTS radical scavenging activity

A decolorization test was performed using the spectrophotometric method of previous studies with a slight modification.[9-10] A stock solution of ABTS was mixed, 7 mM of ABTS stock solution (pH 7.4) with 140 mM potassium persulfate ($K_2S_2O_8$). Ethanol was used to dilute the ABTS for the working solution. The test concentrations of the standard ascorbic acid and extracts were as previously described for the DPPH assay. The ABTS working solution (100 µL) with an equal volume of ethanol served as a control and was incubated for 30 min at room temperature. The absorbance of each solution was determined at 743 nm usinga 96-well plate with microtiter plate reader (BMG LABTECH GmbH, Ortenberg, Germany). The percentage of inhibition was expressed as follows:

% inhibition = [(A control-A sample)/A control] x 100

The control was the absorbance of the ABTS solution without a sample.

An effective concentration for the sample requires scavenging of the ABTS radical at 50% (EC_{50} value) and this was obtained by linear regression analysis of a calibration curve plotting % inhibition versus concentration.

FRAP assay (Ferric reducing antioxidant power)

The FRAP assay was undertaken with some modification to the method of Benzie and Strain.[11] The ratio of the 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM ferric chloride was 10:1:1 (v/v/v), respectively. A total of 30 µL of the sample extract was added to 270 µL of the FRAP reagent and mixed well. After 30 min incubation at room temperature, the absorbance values of the samples were subsequently measured at 593 nm in three replicates using a microplate reader (BMG LABTECH GmbH, Ortenberg, Germany).The antioxidant capacity of the extract was compared with a trolox calibration curve and expressed as µmol Trolox equivalent (TE) per gram of extract.

Determination of total flavonoid contents

Flavonoid contents were measured by the aluminium chloride colorimetric methodas described by Kim *et al*. with slight modification.[12] Fifty milligrams of each AR extract was suspended in 10 mL of 80% methanol, sonicated for 15 min and filtered through Whatman filter paper No.42 (125 mm). Then mixed 0.3 mL of filtrate, 3.4 mL of 30% methanol, 0.15 mL of 0.5 M $NaNO_2$ and 0.15 mL of 0.3 M $AlCl_3$-$6H_2O$ in a test tube. Add 1 mL of 1M NaOH for 5 min, and then the absorbance was measured at 510 nm. A standard calibration curve was constructed using known quercetin concentrations against absorbance. Total flavonoid contents were determined on the basis of absorbance and calculated as mg quercetin equivalent per gram of extract (mg QE/g powder).

Determination of total phenolic compound

Total soluble phenolic content in the extracts was determined using the Folin-Ciocalteu method as previously described by Thaipong *et al*. with a slight modification.[13] 10% Folin-Ciocalteu solution (400 µl) was mixed with 200 µl of the sample solution (1.0 mg/mL) in a volumetric flask and incubated at room temperature for 10 min before being mixed with 0.2 mL of 7% Na_2CO_3 solution. Finally the mixture was diluted with deionized distilled water and made up to 10 mL in a volumetric flask. The mixture was kept at room temperature for 2 hours and then had the absorbance measured at 725 nm. The total phenolic compounds in the extract were presented as grams of gallic acid equivalent (GAE) using an equation obtained from the regression line of a standard gallic acid graph: $y = 0.2177x+0.0005$, $r^2=0.999$. Where, y was the absorbance and x was the concentration. Total phenolics were then calculated as mg gallic acid equivalent per gram of extract (mg GAE/g extract).

Statistical analysis

Results were expressed as means ± SEM. Statistical analysis was determined using a one-way ANOVA followed by Student's t-tests. P-values <0.05 were considered to be statistically significant.

RESULTS

The percentage yields of the aqueous and the ethanolic extracts of AR were 27.41 and 20.88, respectively.

DPPH radical scavenging assay

The antioxidant activity of ascorbic acid by DPPH method was found to be greater than those of the ethanolic and the aqueous AR root extracts (Table 1). There was a significant decrease in the concentration of the DPPH radical due to the scavenging ability of both extracts. The ethanolic extract had a scavenging activity that was better than that of the aqueous extract.

Table 1: Antioxidant activities of AR root extracts

Herbal extracts	Antioxidant activities		
	DPPH (IC50; µg/mL)	ABTS (IC50; µg/mL)	FRAP (µmol trolox /100 g extract)
Water extract	1121.24±121.80*	923.31±8.46*	252.46±0.44*
95% EtOH extract	950.34±83.02*	247.42±31.89*	299.74±5.43*
L-ascorbic acid	3.37±0.02*	7.63±0.13*	-

* in the same column showed significant differences ($p<0.05$)

ABTS RADICAL SCAVENGING ACTIVITY

There was a decrease in the concentration of the ABTS radical due to the scavenging abilities of both extracts (Table 1). The ethanolic extract of the AR roots had a scavenging activity that was better than that of the aqueous extract.

FRAP assay (Ferric reducing antioxidant power)

The trolox equivalents in the ethanolic and aqueous extracts of AR roots are shown in Table 1. The trolox equivalent scavenging ability of the ethanolic extract of the AR roots was significantly greater than that of the aqueous extract of the AR roots.

Determination of total phenolic compounds

The total phenolic contents in the ethanolic and the aqueous extracts of AR roots are shown in Table 2. The ethanolic extract had significantly higher total phenolic contents than the aqueous extract.

Table 2: Phenolic and flavonoid contents of AR root extractions.

Herbal extracts	Total phenolic contents (mg gallic acid/100 g extract)	Total flavonoids (mg Quercetin/100 g extract)
Water extract	0.10±0.01*	0.08±0.01*
95% EtOH extract	4.82±0.38*	0.48±0.02*

*in the same column showed significant differences ($p<0.05$)

Determination of total flavonoid contents

The total flavonoids in the ethanolic and the aqueous extracts of AR roots are shown in Table 2. The ethanolic extract had significantly higher total flavonoids than the aqueous extract of AR roots.

DISCUSSION

The results of this present study indicated that the plant extracts were moderately free radical scavengers, which could reduce or reverse damage caused by free radicals in the human body. The antioxidant activity of the ethanolic extract of AR roots was better than that from the aqueous extract. The total phenolic contents and total flavonoids in the ethanolic extract were also higher than those in the aqueous extract. This study showed that the scavenging ability, total phenolic compounds and total flavonoid contents of the ethanolic extract were higher than those from the aqueous extract but were four times lower than those reported from a methanolic extract.[2] The scavenging ability of the aqueous extract in this study was better than the study of Kongkaneramit *et al.*[1] The previous study reported that the main components were saponins such as shatavarin, sarsapogenin and kaempferol.

The presence of phenolic compounds is shown mostly via the antioxidant activities of plant sources. Due to their hydroxyl groups, phenols are very important plant constituents with a good scavenging ability. The presence of large quantities of phenolic compounds does not necessarily always correlate with antioxidant effects.[6] AR has been reported to have antioxidant activity. Methanolic root extracts were found to markedly increase superoxide dismutase, while decreasing lipid peroxidation in rats.[14-15] In addition, AR has recently been shown to contain saponins, steroidal[16] and racemofuran.[17] The aqueous extract has also been shown to exhibit anantioxidant effect in rat liver and inactivation of superoxide dismutase[18] and the amelioration of oxidative stress.[19] The antioxidant activity in terms of mean inhibitory concentrations (IC_{50}) of ascorbic acid, ethanolic and aqueous extracts were found to be 3.37, 950.34 and 1121.24 µg /mL, respectively, when using the DPPH method. The effective concentration (EC_{50}) of a crude ethanol extract of AR was 381.91 µg/mL,[20]600 µg /mL.[1] The methanolic extract of the root of AR was 4158.8 µg/mL when using butylated hydroxyl toluene as a standard.[2] The present study showed that both the ethanolic and the aqueous extracts of the roots have moderate antioxidant activities.

Although AR extracts exhibited far less antioxidant activity than ascorbic acid when tested *in vitro* using the DPPH method,[15-16] when considering the antioxidant enzyme: superoxide dismutase and ascorbic acid were increased by the effects of AR aqueous and methanolic extracts.[1]

AR extracts contained saponins as the main constituents (8.83%), followed by polyphenols (1.69%), phytosterols (0.79%), ascorbic acid (0.76%) and flavonoids (0.47%), respectively.[5] These compounds could be responsible for decreased cholesterol levels and increased fecal sterol excretion in hypercholesteremic rats. Phytosterols have been reported to complete and displace cholesterol from intestinal bile acid micelles and decrease cholesterol circulation.[21-22] Saponins reduced the plasma cholesterol level by interfere with the enterohepatic circulation of bile acids and so making it unavailable for intestinal absorption of cholesterol.[23] Polyphenolic compounds and phenolic acids, found in plants, have multiple biological effects, including antioxidant activity.[24] Ascorbic acid shows very strong antioxidative activities.[25]

Our *in vitro* studies showed that AR has moderate free radical scavenging action. The antioxidant properties of both the ethanolic and the aqueous extracts of AR can be attributed to the presence of ascorbic acid, flavonoids and polyphenols, which in turn may be responsible for its antioxidative effects.[2]

CONCLUSION

These results have shown high phenolic and flavonoid contents. The ethanolic extract of AR root possessed higher amounts of phenolic and flavonoid contents than the aqueous extract. In conclusion, the antioxidant capacity of the ethanolic extract was higher than the aqueous extract.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Kongkaneramit L, Witoonsardsilp W, Peeungvicha P, Ingkaninan K, Waranuch N, Sarisuta N. Antioxidant activity and antiapoptotic effect of *Asparagus racemosus* root extracts in human lung epithelial H460 cells. Exp Ther Med. 2011;2(1):143-8.
2. Naquvi KJ, Dohare S, Shuaib M. *In vitro* antioxidant activity *Asparagus racemosus* root. Int J Biomed Res. 2011;2(4):228-35.
3. Joshi JDS. Chemistry of Ayurvedic crude drugs: Part VIII: Shatavari 2. Structure elucidation of bioactive shatavarin I and other glycosides. Indian J Chem Section B Organ Chem. 1988;27(1):12-6.
4. Hayes PY, Jahidin AH, Lehmann R, Penman K, Kitching W, De Voss JJ. Structure revision of shatavarins I and IV, the major component from the roots of *Asparagus racemosus*. Terahedron Letter. 2006;47:6965-9.
5. Visavadiya N, Narasimhacharya RL. *Asparagus* Root regulates cholesterol metabolism and improves antioxidant status in hypercholesteremic rats. eCAM. 2009;6(2):219-26.
6. Bopana N, Saxena S. *Asparagus racemosus*-Ethnopharmacological evaluation and conservation needs. J Ethnopharmacol. 2007;110(1):1-15.
7. Rattna S, Katisart T, Sungthong B, Butiman C. Acute and sub-acute toxicities of Thai Silkworm Powder (*Bombyx mori* Linn.) from three races in male Wistar rats and *in vitro* antioxidant activities. Pharmacog J. 2017;9(4):541-5.
8. Zhang WW, Duan XJ, Huang HL, Zhang Y, Wang BG. Evaluation of 28 marine algae from the Qingdao coat for antioxidative capacity and determination of antioxidant efficiency and total phenolic content of fractions derived from *Symphyocladia latiuscula* (Rhodomelaceae). J Appl Phycol. 2007;19(2):97-108.
9. Joshi MB, Saatarkar A. Phytochemical investigation of the roots of *Grewia microcos* Linn. J Chem Pharm Res. 2013;5(7):80-7.
10. Rahim AA, Rocca E, Steinmetz J, Kassim MJ, Ibrahim MS, Osman H. Antioxidant activities of mangrove *Rhizophora* apiculate bark extract. Food Chem.

2008;107(1):200-7.
11. Benzie IFF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Method Enzymol. 1999;299:15-27.
12. Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem. 2003;81(3):321-6.
13. Thaipong K, Boonprokob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Comp Anal. 2006;19(6-7):669-75.
14. Bhatnagar M, Sisodia SS, Bhatnagar R. Antiulcer and antioxidant activity of *Asparagus racemosus* Willd and Withania somnifera Dunal in rats. Ann NY Acad Sci. 2005;1056(1):261-78.
15. Sairam K, Priyambada S, Aryya NC, Goel RK. Gastroduodenal ulcer protective activity of *Asparagus racemosus*: an experimental, biochemical and histological study. J Ethnopharmacol. 2003;86(1):1-10.
16. Hayes PY, Jahidin AH, Lehmann R, Penman K, Kitching W, De Voss JJ. Steroidal saponins from the roots of *Asparagus racemosus*. Phytochemistry. 2008;69(3):796-804.
17. Wiboonpun N, Phuwapraisirisan P, Tip-pyang S. Identification of antioxidant compound from *Asparagus racemosus*. Phytother Res. 2004;18(9):771-3.
18. Kamat JP, Boloor KK, Devasagayam TPA, Venkatachalam SR. Antioxidant properties of *Asparagus racemosus* against damage induced by γ-radiation in rat liver mitochondria. J Ethnopharmacol. 2000;71(3):425-35.
19. Agrawal A, Sharma M, Rai SK, Singh B, Tiwari M, Chandra R. The effect of the aqueous extract of the roots of *Asparagus racemosus* on hepatocarcinogenesis initiated by diethylnitrosamine. Phytother Res. 2008;22(9):1175-82.
20. Potduang B, Meeploy M, Giwanon R, Benmart Y, Kaewduang M, Supatanakul W. Biological activities of *Asparagus racemosus*. Afr J Tradit Complement Altern Med. 2008;5(3):230-7.
21. Quilez J, Garcia-Lorda P, Salas-Salvado J. Potential uses and benefits of phytosterols in diet: present situation and future directions. Clin Nutr. 2003;22(4):343-51.
22. Ikeda I, Sugano M. Inhibition of cholesterol absorption by plant sterols for mass intervention. Curr Opin Lipidol. 1998;9(6):527-31.
23. Harwood HJ,Chandler CE, Pellarin LD, Bangerter FW, Wilkins RW, Long CA, *et al*. Pharmacologic consequences of cholesterol absorption inhibition: alteration in cholesterol metabolism and reduction in plasma cholesterol concentration induced by the synthetic saponin β-tigogenin cellobioside (CP-88818;tiqueside). J Lipid Res. 1993;34(3):377-95.
24. Paramapojn S, Gritsanapan W. Free radical scavenging activity determination and quantitative analysis of curcuminoids in Curcumaa zedoaria rhizome extracts by HPLC method. Curr Sci. 2009;97(7):1069-73.
25. Dekkers JC, Doornen LJP, Han CG. The role of Antioxidant Vitamins and Enzymes in the Prevention of Exercise-Induced Muscle Damage. Sports Med. 1996;21(3):213-38.

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