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

Ladachart Taeponsorat1,*, Surapong Rattana2,3

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. **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.

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, aphyrodissiac, rejuvenator, antispasmodic, antiulcerous and anti-inflammatory agent. Steroidal saponins and sapogenins are 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.2-4 Phytoanalysis of the AR root composition by Visa-vadiya *et al*. 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.3 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 antioxidheal, antiulcer, 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
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-2-picrylhydrazyl (DPPH). 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 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 antioxidant activities of AR root extracts 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. A 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 µl of 7% NaCO₃ solution. 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, r²=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).

ABTS radical scavenging activity

A decolorization test was performed using the spectrophotometric method of previous studies with a slight modification. A stock solution of ABTS was mixed, 7 mM of ABTS stock solution (pH 7.4) with 140 mM potassium persulfate (K₂S₂O₈). 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 734 nm using 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 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.

FRAP assay (Ferric reducing antioxidant power)

The FRAP assay was undertaken with some modification of the method of Benzie and Strain. 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.

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.

Table 1: Antioxidant activities of AR root extracts

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

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

<table>
<thead>
<tr>
<th>Herbal extracts</th>
<th>Total phenolic contents (mg gallic acid/100 g extract)</th>
<th>Total flavonoids (mg Quercetin/100 g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract</td>
<td>0.10±0.01</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td>95% EtOH extract</td>
<td>4.82±0.38</td>
<td>0.48±0.02</td>
</tr>
</tbody>
</table>

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. The scavenging ability of the aqueous extract in this study was better than the study of Kongkaneramit et al. 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 activity. Phenolic and flavonoid acids, ethanolic and aqueous extracts were found to be 3.37, 950.34 and 21.24 µg /mL, respectively, when using the DPPH method. The effectiveness of the antioxidant enzymes, superoxide dismutase and ascorbic acid were increased by the effects of AR aqueous and methanolic extracts.

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. 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. 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. Polyphenolic compounds and phenolic acids, found in plants, have multiple biological effects, including antioxidant activity. Ascorbic acid shows very strong antioxidative activities. 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.

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.

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