Study on the Antioxidant Activities of Crude Extracts from the Roots of *Arnebia euchroma* and *Lithospermum erythrorhizon*

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**Background/Purpose.** To investigate the antioxidant activities and total polyphenol content in two Zicao species, *Arnebia euchroma* (AE) and *Lithospermum erythrorhizon* (LE).

**Methods.** The antioxidant activities of aqueous and methanolic extracts of the roots of AE and LE were evaluated by Trolox equivalent antioxidant capacity (TEAC) assay, DPPH staining, free radical scavenging capacity and reducing power methods. We also analyzed the total content of phenolic compounds, flavonols, and flavonoids to investigate whether there is a correlation between content and antioxidant activity.

**Results.** The Trolox equivalent antioxidant capacity and reducing power assays revealed that the methanol and water extracts of AE exhibited better antioxidant activity than those of LE. Methanol extracts of both species exhibited better antioxidant activity than water extracts. The order of antioxidant activity of the samples was AEM > AEW ≥ LEM > LEW. The DPPH radical scavenging test showed that AE exhibited greater capacity than LE to scavenge DPPH radicals. The order of scavenging ability was AEW > AEM > LEM > LEW. The content of total polyphenols, flavonoids and flavonols was higher in the methanol extract than in the water extract of both species, although the content was higher in AE than in LE.

**Conclusion.** This study provides evidence that the antioxidant activities of AE are greater than those of LE. Furthermore, the antioxidant activities of AE and LE are closely related to the total content of polyphenols, flavonoids and flavonols. ([Mid Taiwan J Med 2008;13:113-21](https://doi.org/10.3345/mtr.2008.07.012))

**Key words**

antioxidant activities, *Arnebia euchroma*, *Lithospermum erythrorhizon*, total polyphenols

**INTRODUCTION**

Zicao is a medicinal herb commonly used in traditional Chinese medicine. According to Shen Nung Ben Cao Jin, it is classified as a middle-grade drug [1]. The principal sources of Zicao include the roots of *Arnebia euchroma* (AE) and *Lithospermum erythrorhizon* (LE). By the nature of their texture, AE is usually referred to as “Hard Zicao” and LE as “Soft Zicao”. The roots of both plants are used in Traditional Chinese Medicine. AE and LE are bitter tasting, and their cold properties are beneficial for blood...
stasis and detoxication. Extracts of the roots are also effective in treating skin rash, pimples, eczema, measles, ulcers, burns and constipation. Although Zicao can be derived from the roots of AE and LE, AE is the most common source of the drug in Taiwan. [2-4]

Reactive oxygen species (ROS) and free radicals have been extensively studied in recent years. Research has shown that the effect of these oxygen species on cholesterol, protein and DNA might contribute to the progression of diseases and their complications [5]. More than 50 diseases are known to be caused by oxygen free radicals [6]. Some of these include cancers [7], fibrosis of the arteries [8], degenerative diseases [9], inflammation reactions [10], immune disorders [11], and diabetes [12]. ROS can cause imbalance in biological systems, and is significantly related to the intracellular reduction-oxidation state (redox).

The chemical components, tissue culture, pharmacology, toxicology and clinical efficacy of Zicao have been extensively studied [13,14]. It has been reported that Zicao has anti-bacterial, anti-HIV, anti-inflammatory, anti-reproductive, and anti-cancer properties and that it can increase immunity. However, no studies have focused on the antioxidant effect of Zicao. Therefore, this study will investigate the antioxidant activity of the methanolic and aqueous extracts from the roots of AE and LE, and compare the differences in content of total polyphenols, flavonoids and flavonols between AE and LE.

**MATERIALS AND METHODS**

**Specimens**

AE was purchased from Hsin-Long, a Chinese medicine pharmacy in Taichung. LE was purchased from the Chi-Feng-Rong-Hsin-Tang drug company, Niu-Ying-Zi, Chi-Feng City, Inner Mongolia, PRC on July 13th, 2007 by Professor Yuan-Shiun Chang, China Medical University. These two samples were identified and authenticated by Dr. Chao-Lin Kuo, Associate Professor and Chairman, School of Chinese Medicine Resources, College of Pharmacy, China Medical University. Details of these sources, including the species and genus, are listed in Table 1. The specimens were stored in the specimen room of our department.

**Extractions**

**Methanolic extracts.** Sliced rhizome (100 g) was macerated with methanol (1000 mL) for 7 days at room temperature. The filtrate was collected. The procedure was repeated three times. The combined methanol extracts were evaporated to dryness.

**Aqueous extracts.** Sliced rhizome (100 g each) was boiled in 1000 mL distilled water for 1h. Filtration and collection of the extracts were repeated three times. The combined decoctions were evaporated to dryness. For each sample, yields were calculated as a percentage by dividing the quantity of dry mass obtained after extraction by the dry weight of rhizome (100 g).

**Test reagents**

Folin-Ciocalteu solution was purchased from Merck. Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) and ABTS (2,2’-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid) diammonium salt were purchased from Roche. DPPH (1,1-diphenyl-2-picrylhydrazyl), Tris (hydroxymethyl) aminomethane, reduced glutathione (GSH), (++)-Catechin, Rutin and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

**Methods**

**Trolox equivalent antioxidant capacity (TEAC).** Total antioxidant capacity was measured

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Table 1. Two sources of Zicao, their generic name, common name, abbreviations and comparative yields of aqueous and methanolic extracts (%)

<table>
<thead>
<tr>
<th>Chinese name</th>
<th>Specimens</th>
<th>Common name</th>
<th>Aqueous extracts (%)</th>
<th>Methanolic extracts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xing-Jiang Zicao</td>
<td>AE</td>
<td>Soft Zicao</td>
<td>10.56</td>
<td>7.21</td>
</tr>
<tr>
<td>Zicao</td>
<td>LE</td>
<td>Hard Zicao</td>
<td>12.53</td>
<td>6.58</td>
</tr>
</tbody>
</table>

*Extraction yields (%) = (quantity of dry mass obtained after extraction) / (dry weight of rhizome)×100. AE = Arnebia euchroma; LE = Lithospermum erythrorhizon.
using the ABTS assay [15]. A standard calibration curve was constructed for Trolox at different concentrations. An aliquot (2 µL) of sample was added to 20 µL ethanol solution, and then immediately mixed with 180 µL ABTS radical cation solution. The absorbance was read at 734 nm within 1 minute.

**Antioxidant activity by DPPH staining** [16]. An aliquot (3 µL) of each sample and standard (BHT and GSH) was carefully loaded onto a 20 cm x 20 cm TLC sheet (silica gel 60 F254; Merck) and allowed to dry (3 min). Drops of each sample were loaded in order of decreasing concentration along the row. The staining of the silica plate was based on the procedure reported by Huang et al [17].

**Determination of total phenolic compounds (TPC).** Total phenolic compounds were estimated by the Folin-Ciocalteu method [18]. A total of 20 µL of each sample solution was added to 200 µL distilled water. After mixing gently, 40 µL of Folin-Ciocalteu phenol reagent was added to the mixture. The mixture was allowed to stand at room temperature for 5 minutes, after which time 40 µL of 20% sodium carbonate was added. The resulting blue complex was measured at 680 nm. The total polyphenol content was expressed as mg (+)-catechin equivalent/g dry weight. The dry weight indicated was the sample dry weight.

**Determination of flavonoids content (TFC).** An aliquot of 100 µL of each sample and standard (rutin) solution was added individually to equal volumes of solution of 2% AlCl3, mixed evenly and allowed to stand at room temperature for 10 minutes. The absorbance was then read at 430 nm. The total flavonoid content was expressed as mg rutin equivalent/g dry weight. The dry weight indicated was the sample dry weight.

**Free radical scavenging capacity.** The free radical scavenging capacity of crude extracts was estimated using the DPPH assay according to Yamaguchi et al [21]. The percentage of DPPH discoloration of the sample was calculated according to the equation: % discoloration = (ABSsample - ABScontrol/ABScontrol) x 100%. EC50 value was the effective concentration at which DPPH radicals were scavenged by 50% and was obtained by interpolation from linear regression analysis [21].

**Determination of reducing power.** The reducing power of the crude extract and standards was determined according to the method reported by Yen and Chen [22]. Increased absorbance of the reaction mixture indicated an increase in reducing power.

**Statistical analyses**

Experimental results are presented as the mean ± standard deviation (SD) of three parallel measurements. Differences were estimated by Scheffé’s multiple range tests. Probability values of less than 0.05 were regarded as significant.

**RESULTS**

**Extraction yield**

Methanolic and aqueous extract yields of AE and LE are shown in Table 1. The percentages of the aqueous extract (W) yields in descending order were as follows: LEW (12.53%) > AEW (10.56%). The methanolic extract (M) yields in decreasing order were as follows: AEM (7.21 %) > LEM (6.58 %).

**Trolox equivalent antioxidant capacity (TEAC)**

The ABTS assay is a method to evaluate total antioxidant power of single compounds and complex mixtures of various plants [23]. The results of ABTS assays are expressed as TEAC values. Higher TEAC values indicate that a sample has stronger antioxidant activity. TEAC
values determined from the calibration curve in this study are shown in Fig. 1. Antioxidant activities of the water and methanolic extracts were in the following decreasing order: AEM (0.48 ± 0.03 mM) > AEW (0.36 ± 0.02 mM) > LEM (0.34 ± 0.01 mM) > LEW (0.06 ± 0.01 mM). Among the 4 extracts, AEM had the strongest total antioxidant capacity.

**Antioxidant activity by DPPH staining**

DPPH staining allows for semi-quantitative visualization and rapid screening of antioxidant activity. Each diluted sample was applied as a dot on a TLC plate, and then stained with DPPH solution (Fig. 2). White spots with strong intensity appeared quickly at a concentration of 125 µg/mL for AEW (the final amount in the spot: 0.375 µg dry matter), and 250 µg/mL for AEM and LEM (final amount: 0.75 µg dry matter); the lowest intensity of LEW was at a concentration of 1000 µg/mL (final amount: 3 µg dry matter) [17]. Moreover, significant numbers of white spots appeared in antioxidants (BHT and GSH) at the concentration of 125 µg/mL.

**Free radical scavenging effect by DPPH**

The relatively stable organic radical DPPH is widely used to investigate the scavenging activities of natural compounds, such as phenolics and anthocyanins, or crude mixtures, such as the ethanol or water extract of plants. DPPH radical is scavenged by antioxidants through the donation of a proton, which forms the reduced DPPH. The color change from purple to yellow after the reduction can be quantified by its decrease in absorbance at a wavelength of 517 nm. Radical scavenging activity increases with increasing percentage of free radical inhibition [24]. The reaction is as follows:

\[
\text{DPPH} \cdot + \text{AH} \rightarrow \text{DPPH} : \text{H} + \text{A} \cdot
\]

Table 2 shows the EC_{50} values for the radical-scavenging activity of the different extracts in two Zicao species, GSH, and BHT using the DPPH colorimetric method. It was found that AEW had the lowest IC_{50} value (97.66 ± 0.69 µg/mL), followed by AEM (201.95 ± 3.39 µg/mL), LEM (228.00 ± 0.77 µg/mL) and LEM (926.34 ± 0.72 µg/mL). The two extract fractions differed significantly in radical-scavenging activity (p < 0.05). The most active sample was AEW; however, its capacity was still higher than the two positive controls (BHT and GSH) in the DPPH assay.
Determination of the reducing power

We investigated the reducing capacity of extracts of two Zicao species by measuring the Fe$^{3+}$-Fe$^{2+}$ conversion. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [22]. The antioxidant activities of putative antioxidants have been attributed to various mechanisms, such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued proton abstraction, and radical scavenging [17]. The reducing power of the different extract fractions from the two Zicao species is shown in Fig. 3. Reduced GSH and reduced BHT were used as the positive controls. The different extract fractions from the two Zicao species exhibited a dose-dependent reducing power activity within the concentration range 0, 250, 500, 1000, and 2000 µg/mL. AEM had the highest reducing power, followed by AEW, LEM, and LEW. The methanol extracts of the two Zicao species had higher reducing power than the water extracts. The reducing power differed significantly between different extract fractions ($p < 0.05$). However, at the same concentration, the reducing power of all the sample products was less than the standard. Therefore, we conclude that AEM had the strongest reducing power.

Determination of total polyphenolic compounds, flavonoids and flavonols

Polyphenol compounds are essential for the anti-oxidation process and for bioactivities in plants [25,26]. The total polyphenol, flavonoid, and flavonol content of the two Zicao species are shown in Table 3. The total polyphenol content is expressed as µg of (+)-catechin equivalent per mg of dry weight. The total polyphenol content of the extracts of the two Zicao species ranged from 81.50 to 150.70 µg CE/mg, and decreased in the following order: AEM > LEM > AEW > LEW. The methanol extracts of the two Zicao species had higher polyphenolic content than the water extracts.

The total flavonoid content was expressed as µg of rutin equivalent per mg of dry weight. The total flavonoid content of the extracts of the two Zicao species ranged from 13.04 to 87.17 µg RE/mg, and decreased in the following order: AEM > LEM > AEW > LEW. The methanol extracts of the two Zicao species had higher flavonoid content than the water extracts.

The total flavonol content was expressed as µg of (+)-catechin equivalent per mg of dry weight. The total flavonol content of the extracts of the two Zicao species ranged from 1.50 to 51.87 µg CE/mg, and decreased in the following order: AEM > LEM > AEW > LEW.
order: AEM > LEM > AEW > LEW. The methanol extracts of AEM had higher flavonol content than the water extracts.

**DISCUSSION**

The relation between diseases and free radicals has been proved by many studies. UV light, radiation, smoking, alcohol consumption, stress and high cholesterol consumption can increase the process of cell oxidation [32]. This study aimed to establish a platform for in vitro evaluation of antioxidant capacity of herbal plants.

The results indicate that AE's methanolic and aqueous extracts were more effective than LE extracts. In terms of the contents of the extracts, the extracts of AE were shown to contain more total polyphenols, flavonoids, and flavonols than LE. There was a close correlation between the antioxidant capacity and the amount of polyphenols, flavonoids, and flavonols present in the plant. Total polyphenols play a vital role in anti-oxidization as well as in the biological functions of the plant [26,27]. Other studies have also indicated that the anti-oxidative properties of polyphenols in edible plants and plant products may help prevent diseases [33]. For example, fruits such as blueberry, cranberry and *Sambucus nigra* have been proven to be rich in flavonoids that protect endothelial cells from oxidation, a key factor in the development of cardio-vascular diseases [34]. The methanol extracts of LE have been reported to exhibit high antioxidant activities with high total phenolic content [35]. Phenolic compounds such as flavonoids, phenolic acid and tannins possess diverse biological properties such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic activities. These biological properties might be due to their antioxidant activities [36].

In conclusion, the results from our in vitro experiments, demonstrated that the phytochemicals in the Zicao species might have significant antioxidant activities, which might be related to the total amount of polyphenols and flavonoids. The additive roles of phytochemicals might contribute significantly to the potent antioxidant activity in vitro. Hence, Zicao could be used as an easy accessible source of natural antioxidants in pharmaceutical and medical industries. For this reason, further work should be performed to isolate and identify the antioxidative components of Zicao.

**REFERENCES**


新疆紫草及紫草之抗氧化活性研究

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背景/目的  本研究擬探討新疆紫草及紫草之抗氧化活性及其總多酚類成分之含量，
以期了解兩者間之藥理活性差異。

方法  本研究探討新疆紫草(Arnebia euchroma (Royle) Johnst.；AE)及紫草
(Lithospermum erythrorhizon Sieb. & Zucc.；LE)根部水及甲醇萃取物之抗氧化
活性；分析方法有Trolox 總抗氧化能力、DPPH 染色法、清除DPPH 自由基能力、還
原力等，並將這些樣品進行總多酚類、類黃酮類及黃酮醇類成分含量測定，以了解其抗
氧化作用與這些成分含量之間的關係。

結果  在Trolox 總抗氧化能力、DPPH 染色法、清除DPPH 自由基能力、還原力等
實驗評估上，新疆紫草(AE)之水及甲醇萃取物的活性皆大於紫草(LE)。成分測定方
面，新疆紫草(AE)的兩種萃取物所含總多酚類、類黃酮類和黃酮醇類皆大於紫草
(LE)。

結論  本研究證明新疆紫草之抗氧化活性大於紫草，且其活性表現與兩者所含總多酚
類、類黃酮類和黃酮醇類多少可能有密切相關。(中醫藥研究 2008;13:113-21)

關鍵詞
抗氧化活性，新疆紫草，紫草，總多酚類

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收文日期：2008 年1月16日  修訂日期：2008 年3月30日
接受日期：2008 年5月7日