



Original Article

The antioxidant effect of *Asparagus cochinchinensis* (Lour.) Merr. shoot in D-galactose induced mice aging model and *in vitro*

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Abstract

Background: An increasing number of plant components and their extracts have been shown to have beneficial health effects in humans. We aimed to explore the antioxidant effects of the aqueous extract of *Asparagus cochinchinensis* (Lour.) Merr. shoot *in vivo* and *in vitro*.

Methods: A total of 80 Kun Ming mice were randomly divided into four groups (20/group). The mice in the control group received a daily subcutaneous injection of saline. A daily injection of D-galactose was administered to the aging model group, the vitamin C (Vc) group (positive control group), and the extract treatment group. Regular measurement of blood cells, nitric oxide synthase (NOS), catalase (CAT) activities, superoxide dismutase (SOD) activities, nitric oxide (NO), and malondialdehyde (MDA) concentration, and the expressions of *NOS*, *SOD*, and glutathione peroxidase (*GPX*) in serum levels were obtained. Furthermore, the microstructure of mice viscera was observed using hematoxylin and eosin staining.

Results: The aqueous extract of *A. cochinchinensis* (Lour.) Merr. had similar 1,1-diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH·) [or 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+)] and higher hydroxyl radicals (or superoxide anion; $p < 0.05$) radical scavenging capabilities to Vc. Moreover, compared with the aging model group, the aqueous extract of *A. cochinchinensis* (Lour.) Merr. shoot could obviously increase NOS, CAT, and SOD activities and the NO content, and reduce the MDA content ($p < 0.05$). Additionally, the microstructure of mice viscera was obviously improved and the expressions of *NOS*, *SOD* and *GPX* were also manifestly increased in the treatment group ($p < 0.05$).

Conclusion: The aqueous extract of *A. cochinchinensis* (Lour.) Merr. shoot had a strong radical scavenging capability *in vivo* and *in vitro*, and might be used to diminish radicals in the body and consequently prevent aging.

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Keywords: antioxidant; *Asparagus cochinchinensis* (Lour.) Merr.; enzyme activity; gene expression; shoot

1. Introduction

Aging is the combined result of physiological and pathological activities. The body increasingly shows the effects of enhanced oxidation and reduced antioxidant activity during the process of aging.¹ An antioxidant is a molecule that inhibits the oxidation of other molecules. Antioxidants terminate oxidation chain reactions by removing free radical intermediates, and inhibit other oxidation reactions.² Although the accumulation of oxygen radicals plays a negative biological role in aging,³ natural antioxidants have been proven to be

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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successfully used as *in vivo* scavengers of oxygen radicals to protect the cardiovascular and cerebrovascular systems, resist cancer, and delay aging.^{4,5}

Various traditional Chinese medicines, such as wolfberry flower,⁶ *Rubus alceaefolius* Poir,⁷ and Okra leaf⁸ are demonstrated potential resources for natural antioxidants. *Asparagus cochinchinensis* (Lour.) Merr. is a genus in the plant family Liliaceae. Previous studies indicate that *A. cochinchinensis* (Lour.) Merr. not only has antibacterial and anticancer effects,^{9,10} but also has an antioxidant effect *in vivo*.^{11–13} However, the impacts of *A. cochinchinensis* (Lour.) Merr. on the expression of antioxidant enzymes, as well as on histological and pathological changes, remain unclear. The D-galactose model has been successfully used for screening antiaging drugs and health products, because a large amount of D-galactose administration can result in a series of pathological and physiological changes related to oxidative stress.¹⁴ In the present study, the effects of *A. cochinchinensis* (Lour.) Merr. shoot on radical scavenging were investigated. The effects of aqueous extract on the nitric oxide synthase (NOS), catalase (CAT), and superoxide dismutase (SOD) activities, as well as the nitric oxide (NO) and malondialdehyde (MDA) content in organs, were examined based on the D-galactose-induced aging mouse model. This study aimed to systematically elucidate the antioxidant mechanism of shoot of *A. cochinchinensis* (Lour.) Merr. aqueous extract, and provide scientific evidence for further applications of *A. cochinchinensis* (Lour.) Merr.

2. Methods

2.1. Pharmaceutical preparation

The pharmaceutical preparation was performed according to those steps described in Zhang et al.¹¹ A total of 20 g of powdered shoots of *A. cochinchinensis* (Lour.) Merr. were extracted in 160 mL water, and thereafter boiled and extracted three times (1 h/time). The three extracts were combined, filtered, and concentrated using a rotating evaporator to obtain the aqueous extract of *A. cochinchinensis* (Lour.) Merr. The aqueous extract of the shoot of *A. cochinchinensis* (Lour.) Merr. was extracted strictly in accordance with Chinese pharmacopoeia. Each preparation was undertaken by one specific person and strictly controlled. In addition, liquid chromatography was conducted to certify that the aqueous extract from each preparation had similar major compositions. Meanwhile, the dried extract yield obtained from the original materials was approximately 8%. Then, the aqueous extract was dissolved in distilled water for stocking extract solution (0.7 g/mL, frozen for use).

2.2. Measurement of radical scavenging *in vitro*

1,1-Diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH·) has been effectively used to monitor chemical reactions involving radicals, which was commonly used in antioxidant assays.¹⁵ The measurement of DPPH· scavenging was performed as described

previously.¹⁶ Briefly, 2 mL of extract solution was quickly mixed with 2 mL 1.25×10^{-4} mol/L DPPH, and placed in the dark at room temperature for 30 minutes. Subsequently, the absorbance at 517 nm was measured. Ethanol was used as the negative control, while vitamin C (Vc) was used as the positive control. The DPPH· scavenging rate was determined using $D\% = [1 - (A_i - A_j)/A_c] \times 100\%$ (A_i , the absorbance after the extracts were added; A_j , the basal absorbance; A_c , the negative control absorbance). The measurement of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS⁺) scavenging was performed as described previously.¹⁷ The ABTS⁺ scavenging rate was determined as follows: $D\% = (1 - A_i/0.7) \times 100\%$ [A_i , the absorbance at 734 nm after the *A. cochinchinensis* (Lour.) Merr. extracts were added]. Furthermore, the measurements of hydroxyl radicals (OH) and superoxide anion were performed using a kit developed by the Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) based on the Fenton reaction. The Fenton reaction is the most common chemical reaction for the generation of hydroxyl radicals, which is proportional to the amount of superoxide anion. Typically, the Griess reagent turns red when an electron acceptor is given. There was a proportional relationship between the color depth and the amount of OH. The absorbance value was detected at 510 nm by using the scientific microplate reader (Thermo Multiskan Spectrum, Vantaa, Finland).

2.3. Animals used for the antioxidant capability of *A. cochinchinensis* (Lour.) Merr

A total of 80 male Kun Ming mice (2 months old, weighing 20 ± 2 g) for laboratory use were obtained from the Xiangya Medical School of Central South University (Changsha, China). The mice were housed three per cage under constant environmental conditions (20–24°C; 12 hour light-dark cycle) and were given *ad libitum* access to standard pelleted food and water. This study was carried out in strict accordance with the recommendations in the national guidelines for the use of animals in scientific research “Regulations for the Administration of Affairs Concerning Experimental Animals”. The protocol was also approved by Xiangya Hospital, School of Medical Central South University (permit number 2008-0002). All surgeries were rapidly performed under diethyl ether anesthesia, and all efforts were made to minimize suffering.

2.4. Aging and drug treatment models' construction

The mice were randomly divided into four groups (20 mice/group) including a control group, an aging model group, a Vc group (positive control), and an extract treatment group. For the aging model group, the Vc group, and the extract treatment group, all mice received daily a subcutaneous injection of D-galactose at a dose of 100 mg/kg for aging model construction. For mice in the control group, an equal volume of physiological saline was injected. After that, mice in the Vc positive control group received daily intragastric administration of Vc, while mice in the extract treatment group received prepared aqueous extract at the dose of 200 mg/kg of body weight. In

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