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Antioxidant and Hypoglycemic Ability of *Ardisia gigantifolia* Stapf Parts

Chaohao GU[1△], Shihao LIANG[1△], Yuexuan WU[1], Zhiren YAO[1], Jiaojiao LI[2*], Kaimei ZHU[1*]

1. Guilin Medical University, Guilin 541000, China; 2. Second Affiliated Hospital of Guilin Medical University, Guilin 541000, China

Abstract [**Objectives**] To study the antioxidant and hypoglycemic effects of different parts of *Ardisia gigantifolia* Stapf. [**Methods**] The hydroxyl radical scavenging activity, DPPH radical scavenging activity and total antioxidant capacity of ABTS of 75% ethanol extract of *A. gigantifolia* Stapf and the petroleum ether, ethyl acetate, n-butanol, chloroform and aqueous extract were measured with Vc as positive control. At the same time, acarbose was used as reference substance to determine the inhibitory effect of each polar site on α-glucosidase. [**Results**] All parts of *A. gigantifolia* Stapf had antioxidant activity, among which ethyl acetate had the strongest antioxidant activity, and the scavenging rate of hydroxyl radical and DPPH radical was higher than that of positive control. The results showed that petroleum ether, ethyl acetate and chloroform had a good inhibitory effect on α-glucosidase (better than acarbose). [**Conclusions**] The ethyl acetate part of *A. gigantifolia* Stapf had the best antioxidant activity and inhibitory effect on α-glucosidase. It provides a basis for further research and development of *A. gigantifolia* Stapf.

Key words *Ardisia gigantifolia* Stapf, Antioxidants, Free radicals, Hypoglycemic effect

1 Introduction

Ardisia gigantifolia Stapf is a medicinal plant in the Myrsinaceae family, and can be harvested all year round. After washing, removing fibrous roots, and drying in the sun, it can be used for medical purposes. *A. gigantifolia* Stapf is pungent and warm in nature, and has the effects of dispelling wind and dampness, strengthening bones and muscles, promoting blood circulation and removing blood stasis. It is used for the treatment of rheumatic pain, traumatic injury, postpartum blood stasis, carbuncle and ulcer[1–2]. In modern clinic, it is used to treat gouty arthritis, rheumatoid arthritis, hyperosteogeny, bone injury and fracture. Studies on chemical constituents show that *A. gigantifolia* Stapf contains phenols, quinones, sterols, triterpenes and volatile oils, etc., and has good pharmacological effects in antithrombotic, antioxidant, anti-tumor and anti-rheumatoid aspects[3]. However, there is no report on the antioxidant capacity and hypoglycemic effect of some specific parts. In this study, five different polar solvents were used to extract 75% ethanol from *A. gigantifolia* Stapf, and three different antioxidant activity methods were used to evaluate its antioxidant activity, and the inhibitory effect on α-glucosidase was used to evaluate whether it had hypoglycemic effect, which provided a scientific basis for further research and development of *A. gigantifolia* Stapf.

2 Materials and methods

2.1 Medicinal materials and reagents The sample was provided by Tang Hui, a researcher from Guangxi Institute of Botany, Chinese Academy of Sciences, and was identified as the genuine *A. gigantifolia* Stapf. Anhydrous ethanol for analysis, analytically pure petroleum ether, analytically pure ethyl ether, analytically pure ethyl acetate, analytically pure n-butanol, ABTS kit (Beyotime Biotechnology Co., Ltd.), 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH) (TCI Shanghai Co., Ltd.); ascorbic acid (Vc) (Tianjin Fuchen Chemical Reagent Co., Ltd.), α-glucosidase, 4-nitrobenzene-α-D-glucopyranoside (PNPG) (Sigma).

2.2 Systematic solvent extraction The dry product of *A. gigantifolia* Stapf (800 g) was crushed, soaked in 75% ethanol for one week according to the ratio of solid to liquid 1 : 10, and extracted for three times. 66 g of dry extract was obtained by rotating evaporation and concentration, dispersed with distilled water at 60 ℃, and extracted with petroleum ether, chloroform, ethyl acetate and water-saturated n-butanol for three times in turn. The combined extracts were concentrated under reduced pressure at 50 ℃ and dried.

2.3 Determination of total antioxidant capacity of each extracted part by ABTS method

2.3.1 Preparation of standard curve. 200 μL of ABTS working solution and 10 mL (0.15, 0.3, 0.6, 0.9, 1.2 and 1.5 mM) of Trolox solution were added into the 96-well plate. After evenly mixing and incubating at room temperature for 2 – 6 min, the absorbance was measured at 405 nm wavelength.

2.3.2 Preparation of ABTS working solution. According to the instructions of the kit, ABTS solution and oxidant solution with the same volume were taken, stored at room temperature and kept away from light for 12 – 16 h, and diluted to 1 : 35 – 1 : 55 with 80% ethanol.

2.3.3 Determination of total antioxidant capacity. 200 μL of ABTS working solution was added into 96-well plate, 10 μL of PBS was added to blank control group, 10 μL of samples with different concentrations were added to sample group, and 10 μL of

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△These authors contributed equally to this work.

* Corresponding author. E-mail: 370489448@qq.com

Trolox solution was added to standard curve group. The solution was shaken gently and mixed evenly. The absorbance was measured at 405 nm after incubation at room temperature for 2 – 6 min. According to the standard curve, the total antioxidant capacity of the sample was calculated.

2.4 Determination of hydroxyl radical scavenging activity According to the method of reference[4], each part of *A. gigantifolia* Stapf was diluted to 1.60, 0.80, 0.40, 0.20, 0.10, 0.05 mg/mL solution, respectively, and the same concentration of Vc solution was used as control. 1 mL of samples were mixed with 2 mL of 1.8 mmol/L $FeSO_4$ solution and 1 mL of 1.8 mmol/L salicylic acid-ethanol, respectively, and 0.1 mL of 0.03% H_2O_2 solution was added for reaction for 30 min at 37 ℃. The absorbance at 510 nm was determined by centrifugation at 8 000 r/min for 10 min. The scavenging rate of hydroxyl radicals was calculated according to the formula:

$$\text{Hydroxyl radical scavenging rate } (\%) = [1 - (A_1 - A_2)/A_0] \times 100\% \quad (1)$$

where A_1 is 1.0 mL of extracting solution + 1.0 mL of $FeSO_4$ solution + 1 mL of salicylic acid-ethanol + H_2O_2 solution; A_2 is 1.0 mL of extracting solution + 1.0 mL of $FeSO_4$ solution + 1 mL of salicylic acid-ethanol + distilled water; A_0 is 1.0 mL of distilled water + 1.0 mL of $FeSO_4$ solution + 1 mL of salicylic acid-ethanol + H_2O_2 solution.

2.5 Determination of DPPH free radical scavenging activity According to the method of reference[4]: 2 mL of ethanol extract of *A. gigantifolia* Stapf was added into 1 mL of DPPH-ethanol solution with a concentration of 500 μmol/L. After mixing evenly, the absorbance was measured at 517 nm after 30 min reaction at room temperature. The positive control was Vc, and the scavenging rate of DPPH free radicals was calculated according to the following formula.

$$\text{DPPH radical scavenging rate } (\%) = [1 - (A_1 - A_2)/A_0] \times 100\% \quad (2)$$

where A_1 is absorbance for 2.0 mL of extracting solution + 2.0 mL of DPPH working solution; A_2 is absorbance for 2.0 mL of extract + 2.0 mL of absolute ethanol; A_0 is absorbance for 2.0 mL of pure water + 2.0 mL of DPPH working solution.

2.6 Inhibitory effect of *Ardisia gigantifolia* Stapf extracts in different solvents on α-glucosidase According to the method of reference[5]: 10 μL of *A. gigantifolia* Stapf extracts with different concentrations (1.60, 0.80, 0.40, 0.20, 0.50, 0.25 mg/mL) were added into a 96-well plate, 10 μL of PBS and 10 μL of 0.5 U/mL α-glucosidase solution were added respectively, then incubated at 37 ℃ for 15 min, 20 μL of 3 mmol/L PNPG solution was added, incubated for 10 min, and 150 μL of 0.1 mol/L Na_2CO_3 solution was added to terminate the reaction. The absorbance was measured at 405 nm by microplate reader, and blank group, control group and sample control group were set up separately. The experiment was carried out according to Table 1, with acarbose as the positive control.

Table 1 Inhibitory effect of α-glucosidase

Group	Blank	Control	Sample
PBS	30	20	10
Sample	0	0	10
PNPG	20	20	20
α-glucosidase	–	–	10
Sodium carbonate	150	150	150

The inhibitory effect of the extract on α-glucosidase is calculated according to the formula:

$$\text{Inhibition rate} = [1 - (A_1 - A_2)/A_3] \times 100\% \quad (3)$$

where A_1 is the absorbance value of sample group; A_2 is the absorbance value of blank group; A_3 is the absorbance value of control group.

2.7 Statistical processing SPSS 20.0 software was used for statistical analysis. The measurement data were expressed by mean ± standard deviation, and the difference was statistically significant when $P < 0.05$.

3 Results and analysis

3.1 Determination of total antioxidant capacity by ABTS method The total antioxidant capacity test kit (ABTS method) was used to detect the antioxidant activity of each extract of *A. gigantifolia* Stapf, and the results showed that each extract had certain antioxidant activity (Table 2).

Table 2 Antioxidant activity of the extracts of *Ardisia gigantifolia* Stapf

Solvent	Concentration mg/ mL	*OD* value	Total antioxidant capacity // mmol/L
Alcohol extract	1.60	0.042 ± 0.001	1.662 ± 0.002
	0.80	0.046 ± 0.002	1.651 ± 0.006
	0.40	0.055 ± 0.003	1.625 ± 0.009
	0.20	0.066 ± 0.004	1.596 ± 0.011
	0.10	0.088 ± 0.001	1.534 ± 0.002
	0.05	0.113 ± 0.020	1.462 ± 0.056
	1.60	0.045 ± 0.001	1.654 ± 0.002
Petroleum ether	0.80	0.049 ± 0.002	1.642 ± 0.005
	0.40	0.058 ± 0.004	1.618 ± 0.001
	0.20	0.085 ± 0.004	1.546 ± 0.011
	0.10	0.092 ± 0.001	1.523 ± 0.004
	0.05	0.128 ± 0.006	1.422 ± 0.018
Ethyl acetate	1.60	0.041 ± 0.001	1.666 ± 0.002
	0.80	0.047 ± 0.002	1.648 ± 0.007
	0.40	0.055 ± 0.004	1.627 ± 0.010
	0.20	0.064 ± 0.004	1.602 ± 0.011
	0.10	0.075 ± 0.003	1.570 ± 0.008
	0.05	0.084 ± 0.005	1.546 ± 0.001
N-butanol	1.60	0.041 ± 0.001	1.665 ± 0.001
	0.80	0.044 ± 0.001	1.658 ± 0.004
	0.40	0.007 ± 0.003	1.595 ± 0.008
	0.20	0.073 ± 0.001	1.575 ± 0.001
	0.10	0.086 ± 0.003	1.540 ± 0.008
	0.15	0.102 ± 0.008	1.493 ± 0.021
	1.60	0.041 ± 0.001	1.663 ± 0.002

(To be continued)

(Continued)

Solvent	Concentration mg/ mL	*OD* value	Total antioxidant capacity // mmol/L
Chloroform	0.80	0.044 ±0.001	1.657 ±0.002
	0.40	0.118 ±0.001	1.623 ±0.003
	0.20	0.056 ±0.002	1.533 ±0.006
	0.10	0.088 ±0.007	1.451 ±0.004
	0.05	0.117 ±0.001	1.450 ±0.018
	1.60	0.348 ±0.001	0.804 ±0.002
Water	0.80	0.357 ±0.004	0.778 ±0.012
	0.40	0.386 ±0.005	0.698 ±0.013
	0.20	0.405 ±0.004	0.643 ±0.103
	0.10	0.418 ±0.006	0.607 ±0.018
	0.15	0.446 ±0.005	0.530 ±0.013

3.2 Scavenging ability of hydroxyl radicals The mechanism of scavenging hydroxyl radical is that hydroxyl radical can react with benzene ring on salicylic acid molecule to produce 2, 3-dihydroxybenzoic acid. The absorbance value was measured at 510 nm. If there are antioxidants in the reaction system, the absorbance value will decrease. The scavenging ability of hydroxyl radicals is shown in Fig. 1A.

3.3 Scavenging effect on DPPH radical The mechanism of DPPH scavenging ability is that under the action of antioxidants, purple DPPH radical can be reduced to yellow non-free radical DPPH-H. The absorbance value was measured at 517 nm. There is a linear relationship between the change degree of absorbance value and the scavenging degree of free radicals in a certain range. The study on antioxidant activity of the extracts of *A. gigantifolia* Stapf showed that the extracts had strong antioxidant activity. The scavenging rate (EC_{50}) of DPPH radical and hydroxyl radical by V_C was (0.14 ±0.005) and (0.182 ±0.015) mg/mL, respectively, and the scavenging rate of extracts of *A. gigantifolia* Stapf was higher than that of positive control. Ethyl acetate and n-butanol had the highest scavenging rate for the two free radicals, indicating that the active antioxidant components of *A. gigantifolia* Stapf were mainly dissolved in ethyl acetate and n-butanol (Fig. 1B).

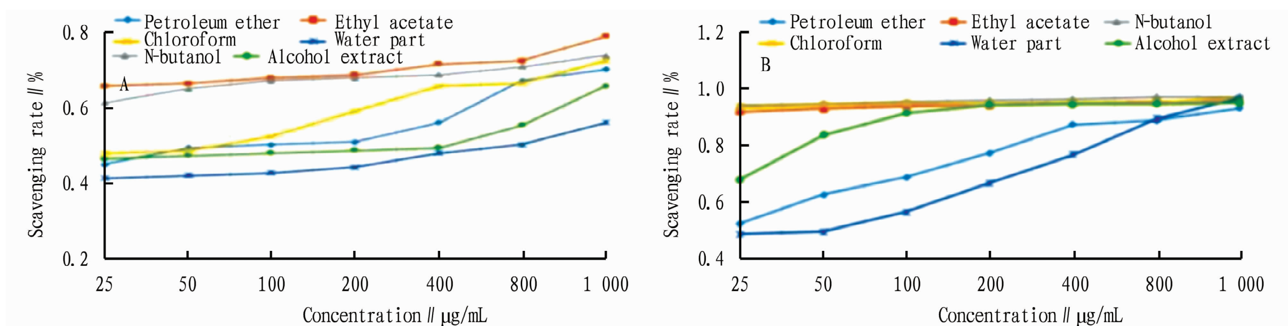

Fig. 1 Scavenging rate of hydroxyl radical (A) and DPPH radical (B) by different parts of *Ardisia gigantifolia* Stapf

3.4 Inhibitory effect of *A. gigantifolia* Stapf extracts in different solvents on α-glucosidase The inhibitory effect of each extract of *A. gigantifolia* Stapf on α-glucosidase showed that all the effective parts had certain inhibitory effect on α-glucosidase (Fig. 2A), and the inhibitory effect of all the effective parts was higher than that of the positive control of acarbose (IC_{50} = 3.2 ± 0.002 mg/mL). The inhibitory effect of ethyl acetate site was the most obvious, and it was much higher than that of the positive control of acarbose in the same concentration (Fig. 2B). This indicated that the active components of *A. gigantifolia* Stapf which exerted α-glucosidase inhibition were mainly dissolved in ethyl acetate.

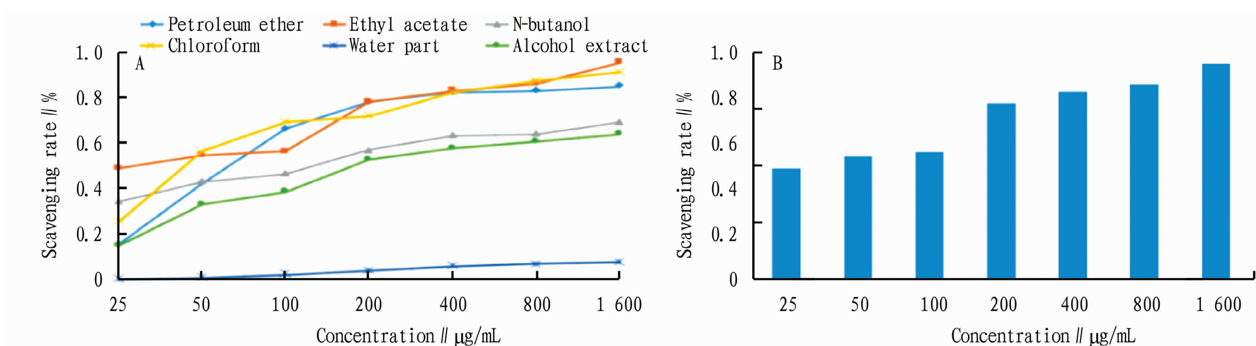

Fig. 2 Inhibitory effect on α-glucosidase by different parts (A) and ethyl acetate (B) of *Ardisia gigantifolia* Stapf

4 Discussion

The antioxidant activity of extracts from *A. gigantifolia* Stapf in different solvents was compared, and the results showed that ethyl acetate was superior to other solvents. In this study, the antioxidant capacity of each part was evaluated comprehensively by three methods: ATPS total antioxidant capacity, DPPH free radical and

hydroxyl free radical. The antioxidant sequences obtained by the three evaluation systems were consistent, which showed that the ethyl acetate extract had the strongest antioxidant activity and the water extract had the worst antioxidant activity. Studies have shown that phenols are the main components of antioxidant activity in plants[6]. Phenols are mainly represented by flavonoids, which play an antioxidant role by complexing metal ions and inhibiting the generation of free radicals[7-8]. There are many compounds in *A. gigantifolia* Stapf, such as phenols, quinones, sterols, coumarins, triterpenes and volatile oils. At present, nine phenolic components such as phenolic acids and phenolic glycosides were isolated from *A. gigantifolia* Stapf, and flavonoids such as quercetin, kaempferol and catechin were detected from *A. gigantifolia* Stapf by Liang Wei *et al*, which indicated that catechin and quercetin were the main material basis of antioxidant activity[9].

By comparing the inhibitory effects of different parts of *A. gigantifolia* Stapf on α-glucosidase, the results showed that petroleum ether, ethyl acetate and chloroform parts were superior to other parts, indicating that the effective components may be mainly dissolved in these three parts. Diabetes mellitus is a disease that seriously affects human health. The occurrence of diabetes mellitus is closely related to oxidation, and glucose in starch is linked in the form of α-1,4 glycosidic bonds and α-1,6 glycosidic bonds. α-glucosidase inhibitors generally have the chemical structure of monosaccharides or oligosaccharides, and can effectively inhibit the activity of glycohydrolase and effectively control the postprandial blood sugar level of diabetic patients. α-glucosidase inhibitors also have anti-cancer, anti-HIV and anti-atherosclerosis effects[5].

To sum up, *A. gigantifolia* Stapf has good antioxidant and hypoglycemic effects. Nowadays, people pay more and more attention to health. *A. gigantifolia* Stapf, as a kind of Chinese herbal medicine which can be used as medicine and food, has high development value. This study provides a theoretical basis for the further development and utilization of *A. gigantifolia* Stapf.

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