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*Full Length Research Paper*

# *In vitro* antioxidant activity of polysaccharide from *Gardenia jasminoides* Ellis

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**A water-soluble polysaccharide, GP, was isolated from *Gardenia jasminoides* Ellis through hot water extraction followed by ethanol precipitation. The *in vitro* free radicals scavenging tests exhibited that GP has significant scavenging abilities especially for ABTS, DPPH, and hydroxyl radicals, which suggests that the polysaccharide GP is a novel antioxidant.**

**Key words:** *Gardenia jasminoides* Ellis, polysaccharide, antioxidant activity, free radicals.

## INTRODUCTION

*Gardenia jasminoides* Ellis (Chinese name "Zhi Zi"), *Rubiaceae*, a traditional Chinese herbal medicine, has been widely recognized in many Asian countries as a diuretic, a laxative, and a choleretic, as well as a cure for hepatic pains due to cirrhosis and for abdominal pains due to dysentery (Tang and Eisenbrand, 1992). It has also been used as an analgesic, an antipyretic (Lee et al., 2005), and an anti-inflammatory medicine (Wang et al., 2004). A number of effective constituents of *G. jasminoides* Ellis have been reported, such as geniposide, gardenoside, shanzhiside, scandoside methyl ester, deacetyl-asperulosidic acid methyl ester (Machida et al., 2003), crocin (Chen et al., 2010), and polysaccharide (Li et al., 1993). Since many polysaccharides play an important role in cell-to-cell communication, cell adhesion, and molecular recognition in the immune system (Tong et al., 2009), some activated polysaccharides isolated from natural sources have lately attracted much attention in the field of biochemistry and pharmacology (Fan et al., 2009; Dwek, 1996; Jing and Yin, 2010; Ma et al., 2009). Information, however, is still lacking about the polysaccharide from *G. jasminoides* Ellis. The objective of present study was to isolate polysaccharide from *G. jasminoides* Ellis and to evaluate the associated antioxidant activity *in vitro*.

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## MATERIALS AND METHODS

### Plant materials

Plant materials of *G. jasminoides* Ellis were purchased from the traditional Chinese medicinal materials market in Chengdu, China and identified by the standards of the Pharmacopoeia of the People's Republic of China (PPRC).

### Drugs and reagents

Vitamin C (VC), BTH, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), and potassium ferricyanide [$K_3Fe(CN)_6$] were purchased from Sigma Co. (USA). Thiobarbituric acid (TBA), sodium dodecyl sulphate, nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH), and phenazine methosulphate (PMS) were purchased from Applichem (Germany). ABTS radical was purchased from Merck (Germany). All other chemicals were analytical grade made in China.

### Preparation of the polysaccharide

The crude polysaccharide was prepared according to the methods described in Luo et al. (2009) with slight modifications. Specifically, the powder of *G. jasminoides* Ellis was first extracted with petroleum ether at 70°C for 1.5 h and subsequently with 80%

ethanol at 90°C for 1.5 h. After each extraction, the solution was separated from the residue by filtration, after which the residue was dried. The residue was further extracted thrice with double-distilled water at 100°C for 2 h. All extracts were combined, concentrated, and filtrated. The resulting extract was deproteinized 5 times using the Sevag reagent (Navarini et al., 1999), and the polysaccharide was free of proteins as scanned by UV Spectra in 260 and 280 nm. Upon the removal of the Sevag reagent, the extract was precipitated by adding ethanol (4 times the volume of the aqueous extract). The final mixture was kept at 4°C overnight for further processing. The precipitate was collected afterwards by centrifugation at 4000 rpm for 20 min, and was washed successively with petroleum ether, acetone, and ethanol. The procedure of precipitation was performed iteratively, before the resulting material was dissolved in water, dialyzed against deionized water for 72 h, and freeze-dried to yield the crude polysaccharide named GP. The extracted rate of the polysaccharide (GP) was 4.27%.

#### ABTS radicals scavenging assay

The ABTS radicals scavenging activity of GP was investigated following the methods proposed in Zhao et al. (2005) with necessary modifications. ABTS was dissolved in 0.01 M PBS (pH 7.4) to attain a 7 mM concentration. The ABTS radical cation was produced through a reaction of a 7 mM ABTS solution with 2.45 mM potassium persulphate. The mixture was allowed to stand in the dark at room temperature for 16 h before further uses. The obtained ABTS radical cation solution was diluted for an absorbance of 0.70 ± 0.02) at the 734 nm and equilibrated at 30°C for 30 min. Samples (volume 0.2 ml) of various concentrations (0.001-4.0 mg/ml) were mixed with 2.0 ml of such a diluted ABTS radical cation solution. The absorbance at the 734 nm was measured immediately after a 20 min reaction of the mixture at room temperature. Using Vitamin C as standard, we calculated the ABTS radicals scavenging effect as follows: scavenging effect (%) = $[A_0 - (As - A_b)] / A_0 \times 100$, where $A_0$ denotes the $A_{734}$ of ABTS without the sample, As denotes the $A_{734}$ of the sample and ABTS, and $A_b$ is the $A_{734}$ of the sample without ABTS.

#### Hydroxyl radicals scavenging assay

The antioxidant activity was determined by hydroxyl radicals as described in Luo et al. (2010) with some modifications. Briefly, samples of various concentrations (0.001 to 4.0 mg/ml) were incubated with 2 mM EDTA–Fe (volume 0.5 ml), 3% $H_2O_2$ (volume 1.0 ml), and crocus of a concentration of 0.36 mg/ml in a 4.5 ml sodium phosphate buffer (150 mM, pH 7.4) for 30 min at 37°C. The hydroxyl radical was detected from the absorbance at 520 nm. The hydroxyl radical scavenging effect was calculated as follows: Scavenging effect (%) = $[(A_0 - As) / A_0] \times 100$, where $A_0$ and As are the $A_{520}$ of the control and of the sample, respectively.

#### Superoxide anion scavenging assay

The assessment of the superoxide anion scavenging activity of GP was based on the methods described in Zhao et al. (2003) and Wang et al. (2008) with some alterations. A 4.5-ml Tris-HCl buffer (volume 50 mmol/L, pH 8.2) and 1.0 ml test samples with various concentrations (0.001 - 4.0 mg/ml) were mixed in tubes covered with lids. The mixtures were incubated in water bath for 20 min at 25°C, after which 0.4 ml of pyrogallol (concentration 25 mmol/L), preheated to 25°C, was added immediately. The reaction lasted for 4 min and was terminated by a 0.1 ml HCl solution (concentration 8 mol/L), followed by a centrifugation of the resulting mixture at 4000 rpm for 15 min. The absorbance of the sample and the control were estimated by a UV spectrophotometer at the 325 nm wavelength. The scavenging activity was calculated using the following equation: Scavenging effect (%) = $(A_0 - As) / A_0 \times 100$, where $A_0$ and As are the absorbance without and with the sample, respectively.

#### DPPH radicals scavenging assay

The DPPH radical scavenging capacity was examined by the methods modified on those given in Braca et al. (2001) and Sun et al. (2010). Briefly, Vitamin C was used as reference. 3 ml of the sample was added to 1 ml of 0.1 mM methanol solution of DPPH. The absorbance at 517 nm was measured after the solution was kept at room temperature for 30 min. The DPPH radical scavenging effect was calculated as follows: Scavenging effect (%) = $[A_0 - (A - A_b)] / A_0 \times 100$, where $A_0$ is the $A_{517}$ of DPPH without the sample, A denotes the $A_{517}$ of the sample and DPPH, and $A_b$ is the $A_{517}$ of the sample but without DPPH.

#### Reducing power

The reducing power of GP was quantified by the methods outlined in Raza et al. (2007) and Yen et al. (1995) with some modifications. BHT was used as reference material. GP and BHT of a variety of concentrations (0.001 to 4.0 mg/ml) were tested. 1.0 ml of the sample was first mixed with a phosphate buffer (volume 2.5 ml, concentration 0.2 mol/l, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (volume 2.5 ml, 1%). The mixture was then incubated at 50°C for 20 min. The reaction was terminated by a 2.5-ml TCA solution (0.1%) and the resulting mixture was centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (concentration 6 mmol/l). The absorbance of the obtained material was measured at 700 nm. It was anticipated that increased reducing power would be associated with increased absorbance of the test mixture.

#### Statistical analysis

Data were analyzed by the ANOVA functionality of the SPSS software. Significant levels were set at $P < 0.05$. All data are expressed here as the mean ± the standard deviation (n = 3).

## RESULTS AND DISCUSSION

### Effect of GP on scavenging ABTS radicals

Since the ABTS radical assay is suitable for both organic and aqueous solvent systems (Wu et al., 2006), it is often used for evaluating total antioxidant power of single compounds and complex mixtures of various plants (Katalinic et al., 2006; Huang et al., 2008). The scavenging ability of GP for ABTS free radical is shown in (Figure 1). The scavenging activities of GP and of Vitamin C are well correlated especially at higher concentrations. For the positive control (Vitamin C), the scavenging activity appears to be ineffective (between 2.55 and 28.41%) at low doses from 0.001 to 0.01 mg/ml, where GP has a similar low activity. It is remarkable that GP exhibited a considerable scavenging power at higher doses (e.g.,

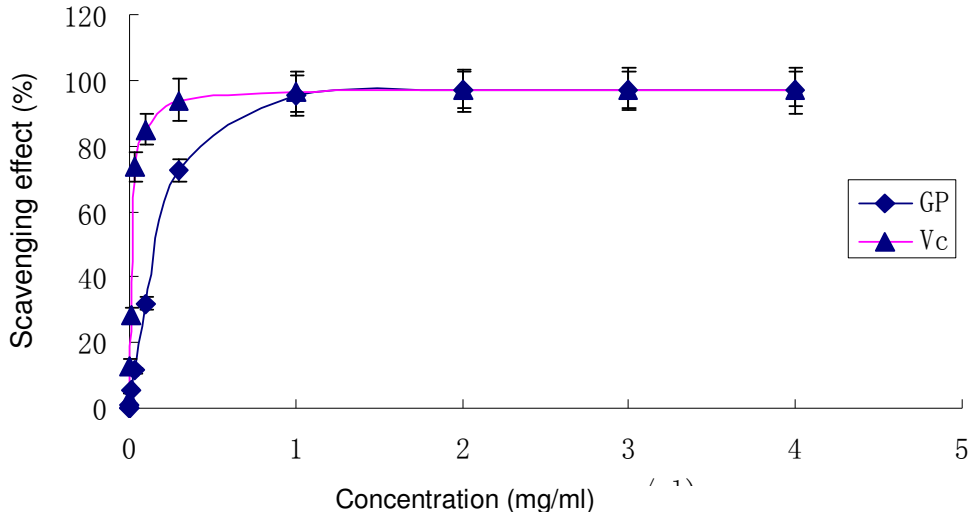


**Figure 1.** ABTS scavenging activity of GP and the reference (Vitamin C).

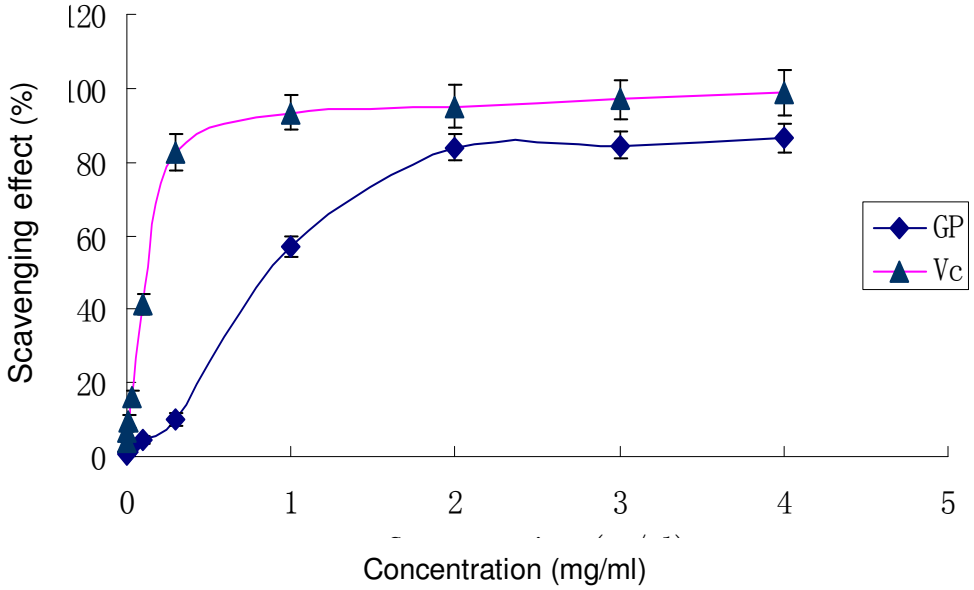


**Figure 2.** The hydroxyl radicals scavenging activity of GP and the reference (Vitamin C).

95.59% at 1.0 mg/ml and 97.10% at 4.0 mg/ml), a pattern that closely resembles that of Vitamin C (e.g., 96.45% at the concentration of 1.0 mg/ml and 97.34% at 4 mg/ml). The results thus indicate that GP has a strong scavenging power for ABTS radicals and can be explored as a potentially novel antioxidant.

## Effect of GP on scavenging hydroxyl radicals

The hydroxyl radical in cells can easily cross cell membranes at specific sites and react with most biomolecules, which further causes tissue damages and cell death. It is thus important to remove the hydroxyl radical for the protection of living systems (Yang et al., 2008). The hydroxyl radical scavenging assay is often used for evaluating the total antioxidant power of single compounds and complex mixtures of plants. The hydroxyl radical scavenging activities of the polysaccharide and of Vitamin C are given in (Figure 2). In this figure, a concentration-dependent manner of the detected scavenging activities for the hydroxyl radical is evident. Significantly similar to Vitamin C ($P < 0.05$), the polysaccharide GP exhibited very high radical scavenging

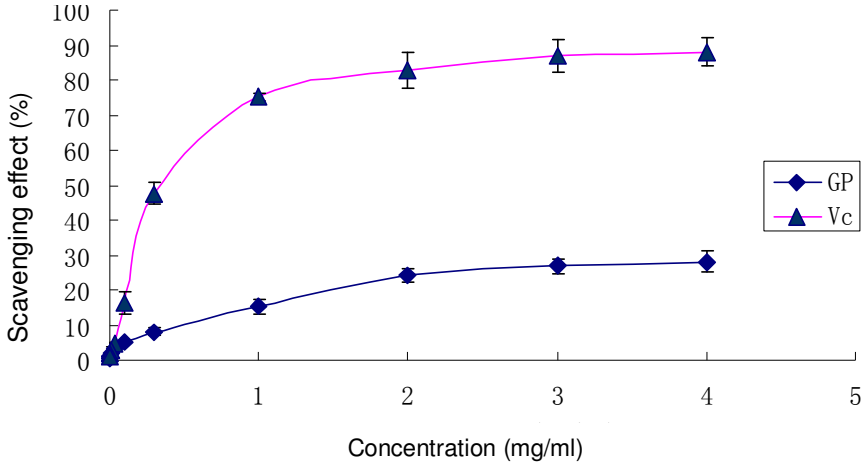


**Figure 3.** Superoxide radicals scavenging activity of GP and the reference (Vitamin C).

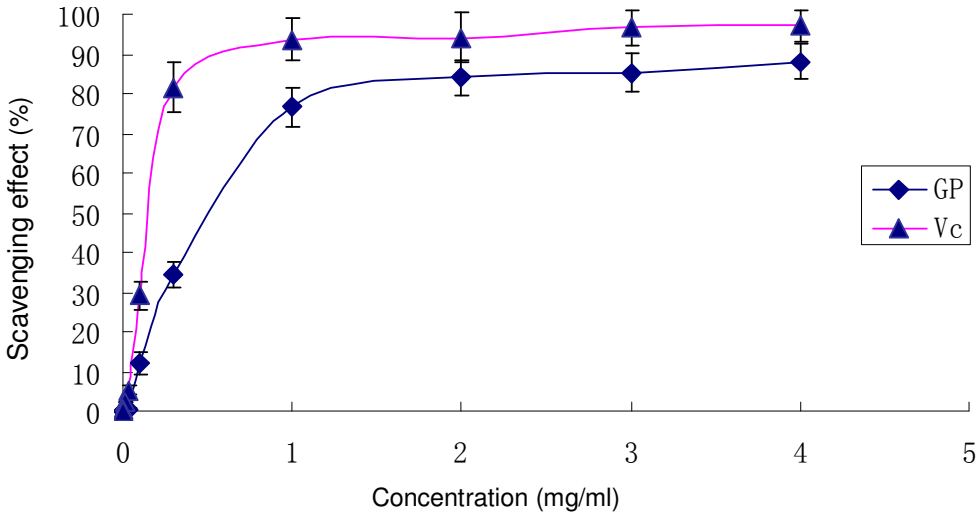


**Figure 4.** DPPH scavenging activity of GP and the reference (Vitamin C).

at high doses, e.g., in a range from 2 to 4 mg/ml. We deduced from the results that GP has significant effects on the hydroxyl radical scavenging.

## Effect of GP on scavenging superoxide radicals

Superoxide anion radical is known as an initial radical that plays an important role in the formation of other reactive oxygen-species, such as hydrogen peroxide, or singlet oxygen in living systems (Stief et al., 2003). For the present study, the scavenging ability of GP on superoxide free radical is shown in (Figure 3). As seen from the figure, the superoxide scavenging activity of GP increased only slightly with increasing concentrations. At a high dose of 4 mg/ml, for instance, the scavenging rates of Vitamin C and GP are 87.99 and 28.07%, respectively. The results indicate that polysaccharide GP exhibited insufficient superoxide radical scavenging activity.

## Effect of GP on scavenging DPPH radicals

The DPPH free radical is a stable radical with a maximum absorption at 517 nm and can readily be scavenged by an antioxidant. For this reason, it has been widely adopted as a reference for evaluating the free radical scavenging activities of natural compounds (Amarowicz et al., 2004). In our experiment, the scavenging ability of GP on DPPH free radical was examined using the DPPH colorimetric assay. In (Figure 4), all the samples showed evident scavenging activity in a concentration-dependent manner. Furthermore, the scavenging activity of both

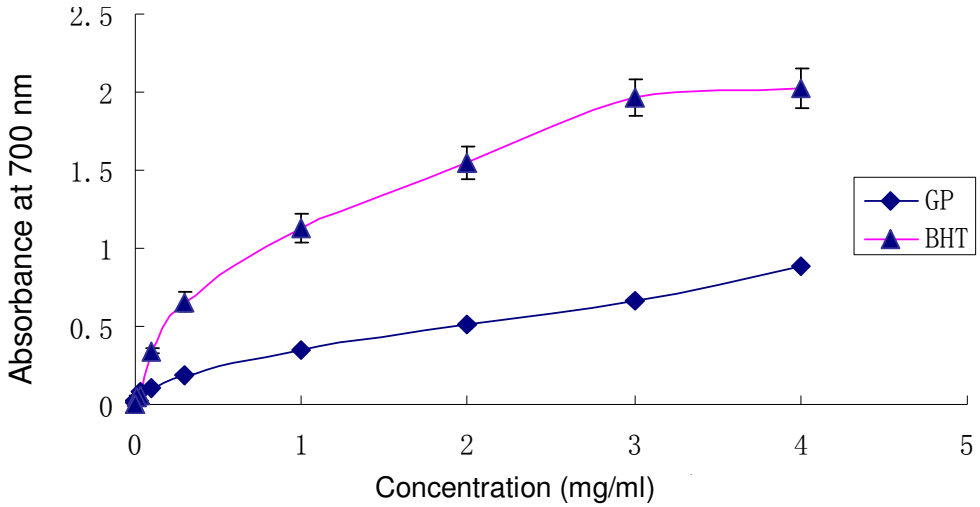


**Figure 5.** Reducing ability of GP and the reference (Vitamin C).

samples increased significantly with increasing concentrations. At a high dose such as 4.0 mg/ml, GP had a fairly high rate of scavenging activity on DDPH radical, 88.13%, although that of Vitamin C was even higher (97.07%). Therefore, the antioxidant activity of the polysaccharide GP is considered to be strong at high doses in terms of DPPH radical scavenging. Our results also suggested that the scavenging effect of GP for DPPH radical generation can help prevent or ameliorate oxidative damages.

### Effect of GP on reducing power

A direct correlation between antioxidant activities and reducing power has been previously reported (Yildirim et al., 2001). In order to measure the reducing power of GP, the $Fe^{3+}$-$Fe^{2+}$ transformation in the presence of samples of various concentrations was investigated. BHT was used as reference material. The reducing capability of GP and the reference material are presented in (Figure 5). A concentration-dependent manner is again identified of the reducing power of both samples. In the entire range of concentration, BHT has a consistently higher absorbance, implying a greater reducing power for almost all cases compared to GP.

## Conclusion

In the present investigation, the polysaccharide (GP) was isolated from *G. jasminoides* Ellis by water extraction and ethanol precipitation. An assessment of the antioxidant ability *in vitro* indicated that GP has significant radical scavenging abilities for ABTS, DPPH, and hydroxyl radicals. The scavenging effects were found to be comparable to those of the positive control, which led to our suggestion that the polysaccharide GP should be explored as a novel antioxidant. Compared to the respective references, nevertheless, GP exhibited insufficient reducing power and scavenging capabilities for superoxide anion radical. This necessitates a further scrutiny of its antioxidant activity *in vivo* in the future.

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