



Antioxidant Activity and Antioxidant Mechanism of Brazilin and Neoprotosappanin from *Caesalpinia sappan* Lignum

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The compounds, brazilin and neoprotosappanin, have been isolated from *Caesalpinia sappan* Lignum. The structures were characterized by NMR spectra. The antioxidant activity of brazilin and neoprotosappanin were evaluated by using free radical scavenging assays, namely 1,1'-diphenyl-2-picrylhydrazyl. The IC₅₀ values of brazilin and neoprotosappanin are 0.067 and 0.375 μg/mL, respectively, much higher than the IC₅₀ of vitamin C, 0.542 μg/mL. The result shows that brazilin and neoprotosappanin possess powerful antioxidant activity, which can be used as function-rich antioxidants and whose application promising are very extensive.

Keywords: *Caesalpinia sappan* Lignum, Brazilin, Neoprotosappanin, Antioxidant activity, Antioxidant mechanism.

INTRODUCTION

Caesalpinia sappan L is distributed mainly in southeast Asia, especially around the southwest of China. The stems of *Caesalpinia sappan* L can be used as herbal medicine, which has been used as a remedy for blood stasis, detumescence analgesic efficacy, indications of amenorrhoea dysmenorrhoea, postpartum blood stasis, thoracic and abdominal pain, traumatic swelling and pain and so on¹. In previous research, some compounds were isolation and identification from *Caesalpinia sappan* L and they have a broad range of demonstrated pharmacological properties *e.g.*, antihepatotoxicity, antiplatelet activity and antiinflammatory activities, *etc*²⁻⁷. To our best of knowledge, no previous work has been reported on the antioxidant activity of neoprotosappanin, while the antioxidant activity of brazilin were rarely reported, too.

In the present study, we evaluated the antioxidant activity of brazilin and neoprotosappanin from *Caesalpinia sappan* Lignum by using free radical scavenging assays, namely 1,1'-diphenyl-2-picrylhydrazyl and proposed their antioxidant mechanism.

EXPERIMENTAL

Caesalpinia sappan Lignum was collected in Chengdu Chinese herbal medicine market, Sichuan province of China in April 2011 and authenticated by Professor Yue-Hua Wang of the Chengdu University, where a voucher specimen has

deposited. 1,1'-Diphenyl-2-picrylhydrazyl was purchased from Sigma Chemicals Co. (St. Louis, Mo, USA). All chemicals and solvents used were of analytical grade.

NMR spectra were measured on a Bruker DRX-400 instrument with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh; Qingdao Marine Chemical Inc.). Thin-layer chromatography (TLC) was carried out on silica gel 60 F254 on glass plates (Qingdao Marine Chemical Inc.) using various solvent systems.

Extraction and isolation: Dry *Caesalpinia sappan* Lignum (2 kg) were extracted with ethanol (3 × 5 L) for 24 h. The extract was suspended in water (1000 mL) to form a suspension and degreased with petroleum ether and then extracted with EtOAc. The EtOAc extract (15.8 g) was chromatographed on a silica gel column employing cyclohexane-acetone (3:1 → 1:3) as eluent to provided five fractions. After repeatedly column chromatography of five fractions over a silica gel column using CHCl₃-CH₃OH (20:1 → 4:1) as eluent afforded brazilin (260 mg) and neoprotosappanin (25 mg).

1,1'-Diphenyl-2-picrylhydrazyl radical scavenging activity: Free radical scavenging activity of brazilin and neoprotosappanin were measured by (1,1'-diphenyl-2-picrylhydrazyl) using vitamin C as positive control. In brief, a solution of 1,1'-diphenyl-2-picrylhydrazyl in methanol (0.507 mM) was prepared. Then, 100 mL of this solution was added to 4.9 mL of brazilin or neoprotosappanin or vitamin C solution at different doses. The mixture was then shaken and allowed

to stand at room temperature for 0.5 h, with the absorbance measured at 517 nm in a spectrophotometer against blank samples. Radical scavenging capacity was expressed as percentage effect (E %) or inhibition percentage (%) and calculated using the following equation:

$$\text{Percentage effect (E \%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

$$\text{Inhibition percentage (IC \%)} = 1 - \text{percentage effect (E \%)}$$

Different sample concentrations were used to obtain anti-radical curves for calculating the EC₅₀ or IC₅₀ values. Antiradical curves were plotted referring to concentration on the x axis and their relative scavenging capacity on the y axis. The EC₅₀ or IC₅₀ values were processed using five different statistical programs⁸.

Statistical analysis: Mean data values are presented with standard error of the mean (mean ± SD). Analysis of variance followed by Dunnett's test for pairwise comparison was used for statistical validation of the data, with significance defined as $p < 0.05$.

RESULTS AND DISCUSSION

The compounds (Fig. 1), brazilin and neoprotosappanin were identified by comparison of their ¹H and ¹³C NMR spectra with values reported in the literature⁴⁻⁵ and their spectra data are given below:

Brazilin: Yellow amorphous powder: ¹H NMR (400 MHz, DMSO-*d*₆) and ¹³C NMR: (100 MHz, DMSO-*d*₆), (Table-1).

Neoprotosappanin: Yellow amorphous powder: ¹H NMR (400 MHz, DMSO-*d*₆) and ¹³C NMR: (100 MHz, DMSO-*d*₆), (Table-1).

1,1'-Diphenyl-2-picrylhydrazyl radical scavenging activity: DPPH[•] assay is a reliable method to determine the antioxidant capacity of biological substrates and EC₅₀ or IC₅₀ is generally used as an indicator of antioxidant capacity for

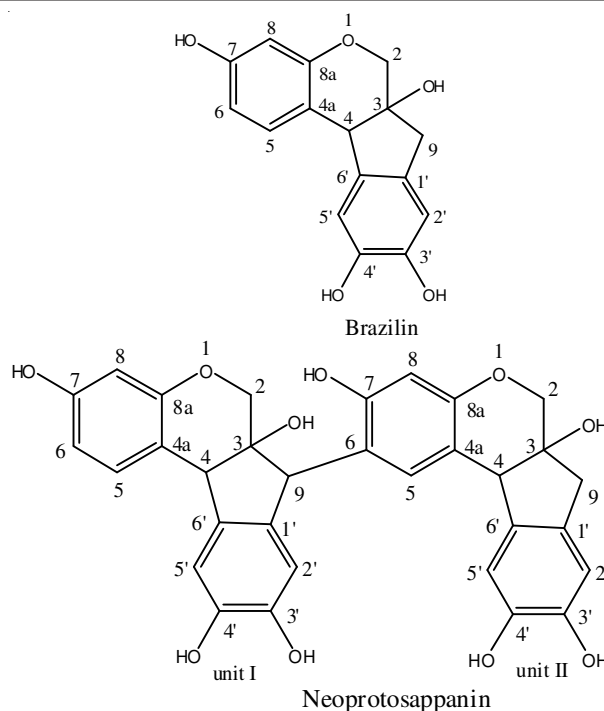


Fig. 1. Structures of brazilin and neoprotosappanin

plant extracts and pure compounds. The inhibition percentage (IC %) of samples are shown in Table-2.

Vitamin C, brazilin and neoprotosappanin exhibited appreciable scavenging properties against the radical and the inhibition percentages were proportional to the concentration of the sample. The IC₅₀ values for brazilin and neoprotosappanin, vitamin C were 0.067 µg/mL, 0.375 and 0.542 µg/mL, respectively. The experimental results indicate that brazilin and neoprotosappanin have more powerful antioxidants than vitamin C and brazilin possess most powerful antioxidants among them.

TABLE-1
¹H NMR AND ¹³C NMR DATA OF COMPOUND 1 (IN CDCl₃), δ IN PPM, J IN HZ

Position	Brazilin		Neoprotosappanin		Neoprotosappanin			
	δ	δ _H	Unit I	δ	δ _H	Unit II		
1	—	—	1	—	—	1	—	
2	69.9	3.85 (d, 1H, J = 11.2 Hz); 3.57 (d, 1H, J = 11.2 Hz)	2	71.0	3.96 (d, 1H, J = 11.2 Hz); 4.01 (d, 1H, J = 11.2 Hz)	2	70.8	3.88 (d, 1H, J = 11.2 Hz); 3.65 (d, 1H, J = 11.2 Hz)
3	76.6	—	3	80.4	—	3	78.0	—
4	49.8	3.84 (s, 1H)	4	50.2	4.01 (s, 1H)	4	51.0	4.01 (s, 1H)
5	131.2	7.14 (d, 1H, J = 8.4 Hz)	5	131.8	7.19 (d, 1H, J = 8.4 Hz)	5	133.6	6.72 (s, 1H)
6	109.0	6.42 (dd, 1H, J = 2.4, 8.4 Hz)	6	109.8	6.42 (dd, 1H, J = 2.4, 8.4 Hz)	6	120.1	—
7	156.8	—	7	157.6	—	7	156.2	—
8	103.0	6.21 (d, 1H, J = 2.4 Hz)	8	104.0	6.29 (d, 1H, J = 2.4 Hz)	8	104.0	6.37 (s, 1H)
9	42.2	2.70 (d, 1H, J = 15.6 Hz); 2.87 (d, 1H, J = 15.6 Hz)	9	49.1	4.49 (s, 1H)	9	42.8	2.71 (d, 1H, J = 15.6 Hz); 2.97 (d, 1H, J = 15.6 Hz)
1'	130.0	—	1'	137.8	—	1'	137.3	—
2'	112.3	6.64 (s, 1H)	2'	114.0	6.57 (s, 1H)	2'	112.5	6.55 (s, 1H)
3'	144.6	—	3'	145.4	—	3'	145.3	—
4'	144.2	—	4'	145.7	—	4'	145.4	—
5'	111.9	6.55 (s, 1H)	5'	112.6	6.87 (s, 1H)	5'	112.6	6.43 (s, 1H)
6'	135.8	—	6'	135.1	—	6'	135.3	—
4a	114.6	—	4a	115.8	—	4a	115.2	—
8a	154.3	—	8a	155.6	—	8a	154.4	—

TABLE-2
INHIBITION PERCENTAGE (IC %) OF SAMPLES

Sample	Final concentration (µg/mL)	Inhibition percentage (IC %)	IC ₅₀ (µg/mL)
Brazilin	0.50000	76.83	0.067
	0.25000	70.12	
	0.12500	57.30	
	0.06250	49.08	
	0.03125	41.76	
Neoprotosappanin	1.0000	85.97	0.375
	0.5000	70.12	
	0.2500	46.34	
	0.1250	30.49	
	0.0625	21.26	
Vitamin C	1.0000	79.88	0.542
	0.5000	48.17	
	0.2500	27.44	
	0.1250	24.22	
	0.0625	19.90	

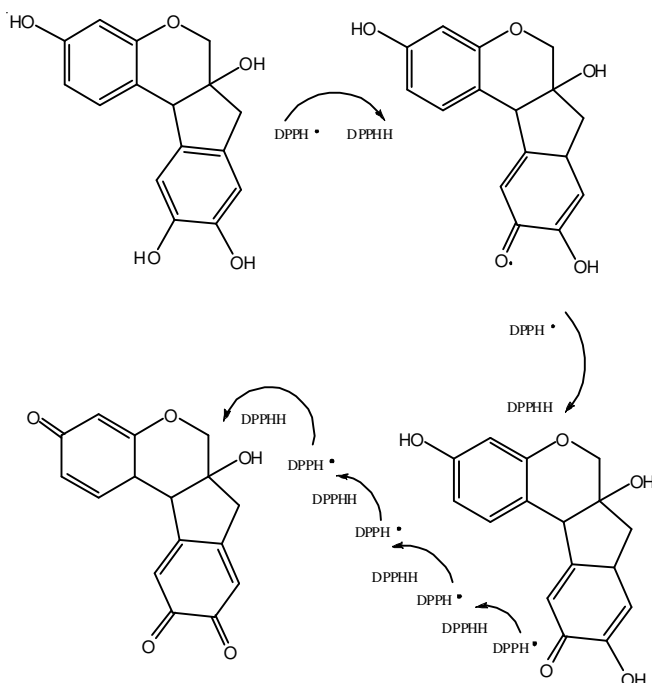


Fig. 2. Possible antioxidant mechanism of brazilin scavenging 1,1'-diphenyl-2-picrylhydrazyl radical

Free radical is a major factor causing many serious diseases. Brazilin and neoprotosappanin have powerful antioxidant activity, so they can clear away free radical in human body, guard against diseases and postpone senility. The experiment results indicate that brazilin and neoprotosappanin have more powerful antioxidants than vitamin C and brazilin is a potential source of antioxidant compound.

Antioxidant mechanism: The antioxidant activity of brazilin and neoprotosappanin may be due to the phenolic hydroxyl groups which have very strong effects on antioxidation and elimination of free radical. Due to a high degree of structural similarity, the antioxidant mechanism of brazilin and neoprotosappanin may be similar. The antioxidant mechanism of brazilin is shown in Fig. 2.

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