Supporting information

Synthesis of a new Ag^{+}-decorated Prussian blue analog with high peroxidase-like activity and its application in measuring the content of the antioxidant substances in *Lycium ruthenicum* Murr.

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Experimental Section.

Chemicals and materials

$Cu(CH_3COO)_2$, poly(vinylpyrrolidone) (PVP, K29–32), ethanol, ethylene glycol, $AgNO_3$, $FeSO_4 \cdot 7H_2O$, KOH, KH_2PO_4, 3,3',5,5'-tetramethylbenzidine (TMB), and dimethyl sulfoxide (DMSO) were obtained from International Aladdin Reagent Inc. (Shanghai, China). $C_6H_5Na_3O_7 \cdot 2H_2O$ (TSCD, trisodium citrate dihydrate), $CoCl_6 \cdot 2H_2O$, $K_3[Fe(CN)_6]$, and DPPH were purchased from Sinopharm Chemical Reagent Beijing Co. Ltd. (Beijing, China). H_2O_2 solution (30%) was purchased from Foshan Yuhua instrument technology Co. Ltd. (Guangzhou, China). Ascorbic acid (AA) was purchased from Alfa Aesar Chemical Co. Ltd. (China). K_2CrO_4 was purchased from Shanghai Macklin Biochemical Co. Ltd. (China). The clean, dry fruit of *Lycium ruthenicum* Murr. was obtained from Xingjiang, China.

Instrumentation

Transmission electron microscopy (TEM) images of Ag-PBA were obtained by a transmission electron microscope (FEITecnai G2 20 S-TWIN) operating at an accelerating voltage of 200 kV. The absorbance was measured on a 96-well plate

using a Molecular Devices Spectramax M5 microplate reader. X-ray diffraction (XRD) was performed on a PANalytical B.V. Empyrean with CuKα radiation at room temperature. The scanning electron microscope (SEM) images were obtained using a Zeiss Merlin Field Emission Scanning Electron Microscope (ZEISS MERLIN FE-SEM) with an accelerating voltage of 5.0 kV. A Zeiss Merlin Field Emission Scanning Electron Microscope with an Energy Dispersive Spectrometer (EDS) attachment was used to characterize the surface composition and the elemental composition of Ag-PBA and PBA. X-ray photoelectron spectroscopy (XPS) measurements were performed on a Thermo Fisher Scientific K-Alpha with an AlKα excitation source (1486.8 eV). EPR spectrum of the Ag-PBA-H_2O_2-TMB system was obtained using a Bruker A300 Electron Paramagnetic Resonance (EPR) spectrometer. The following EPR conditions were used: central magnetic field (CF), 3500.00 G; scanning width (SW), 150.00 G; scanning time (ST), 30.00 s; microwave power (MP), 3.99 mW; modulation amplitude (MA), 1.000 G; transfer time, 40.0 ms.

Optimization of the Ag^+ concentration added in the process of the synthesis of Ag-PBA

First, PBA was synthesized and then dissolved in 3 mL ethylene glycol. After dissolution, 10 mL of different concentrations of $AgNO_3$ (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mmol/L) was added slowly, dropwise, under stirring, at room temperature for 3 h. The stirred solution was centrifuged at 9500 rpm for 8 min and washed with water and ethanol twice, respectively. The precipitate was dried, weighed, and dissolved in 3 mL ethylene glycol to obtain the Ag-PBA solution. Subsequently, 15 μL Ag-PBA

containing different Ag^+ concentrations, 50 μL TMB (20 mmol/L), 50 μL H_2O_2 (50 mmol/L), and 1 mL phosphate buffer (pH 5.0) were mixed. The absorbance spectrum of the resulting solution was measured at 650 nm at 25 °C for 10 min. The absorbance values of the solutions were compared.

Stability of Ag-PBA and PBA

(1) Acid resistance of the peroxidase-like activity of Ag-PBA and PBA

First, 15 μL Ag-PBA (57.0 mg/mL) or PBA (56.7 mg/mL), 50 μL TMB (20 mmol/L), 50 μL H2O2 (50 mmol/L), and 1 mL phosphate buffer with different pH values (pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0) were mixed. The absorbance spectrum of the resulting solution was measured at 650 nm after incubation at 25 °C for 10 min.

(2) Thermal stability of the peroxidase-like activity of Ag-PBA and PBA

Ag-PBA (57.0 mg/mL) or PBA (56.7 mg/mL) was incubated at 10, 20, 30, 40, and 50 °C for 30 min, added into the reaction solution of 50 μL TMB (20 mmol/L) and 50 μL H_2O_2 (50 mmol/L) at 25 °C, and the enzyme activity was measured.

(3) Storage stability of the peroxidase-like activity of Ag-PBA and PBA

To measure the stability of Ag-PBA and PBA to storage, Ag-PBA (57.0 mg/mL) and PBA (56.7 mg/mL) were stored at room temperature. The peroxidase mimetic activity of Ag-PBA and PBA was measured once per day for 4 weeks.

(4) Reusability of the peroxidase-like activity of Ag-PBA and PBA

The reusability of Ag-PBA and PBA was evaluated by assay of the peroxidase-like activity. After each reaction, Ag-PBA or PBA was recovered, washed with

deionized water, and then placed into a new reaction system to measure the peroxidase-like activity.

Optimization of the reaction conditions for the Ag-PBA method

(1) Optimization the reaction temperature

15 μL Ag-PBA (57.0 mg/mL), 50 μL H_2O_2 (50 mmol/L), 50 μL TMB (20 mmol/L), and 1 mL phosphate buffer (pH 5.0) were mixed into a 1.5 mL centrifuge tube. Then, the reaction was performed at 0, 5, 15, 20, 25, 30, 35, 40, 45, and 50 °C for 10 min to measure the enzyme activity.

(2) Optimization of TMB concentration

15 μL Ag-PBA (57.0 mg/mL), 50 μL H_2O_2 (50 mmol/L), 1 mL phosphate buffer (pH 5.0), and different volumes of TMB (20 mmol/L) (5.5, 11, 16.5, 22, 27.5, 33, 38.5, 44, and 49.5 μL) were mixed into 1.5 mL centrifuge tubes. Each tube was filled with the deionized water until the total volume in each tube was 1100 μL, producing final concentrations of TMB, respectively, of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 mmol/L. The absorbance spectrum of the resulting solution was measured at 650 nm after incubation at 25 °C for 10 min.

(3) Optimization of the H_2O_2 concentration

15 μL Ag-PBA (57.0 mg/mL), TMB (20 mmol/L) with the optimum volume, 1 mL phosphate buffer (pH 5.0), and different volumes of H_2O_2 (50 mmol/L) (10, 15, 20, 25, 30, 35, 40, 45, and 50 μL) were mixed into a 1.5 mL centrifuge tube. Each tube was filled with the deionized water until the total volume in each tube was 1100 μL, producing final concentrations of H_2O_2, respectively, of 0.45, 0.68, 0.91, 1.14, 1.36,

1.59, 1.82, 2.05, 2.27 mmol/L. The absorbance spectrum of the resulting solution was measured at 650 nm after incubation at 25 °C for 10 min.

(4) Optimization of the reaction time

15 μL Ag-PBA (57.0 mg/mL), 38.5 TMB (20 mmol/L), 40μL H_2O_2 (50 mmol/L), and 1 mL phosphate buffer (pH 5.0) were mixed into a 1.5 mL centrifuge tube. Then, the centrifuge tube was filled with the deionized water until the total volume in the tube was 1100 μL. The absorbance was measured at 650 nm at 25 °C once a minute for 20 min.

Potential Interference with the Ag-PBA method

(1) Interference of reducing substances with the Ag-PBA method

15 μL Ag-PBA (57 mg/mL), 38.5 μL TMB (20 mmol/L), and 40 μL H_2O_2 (50 mmol/L) were mixed into a 1.5 mL centrifuge tube. Then, the centrifuge tube was filled with phosphate buffer (pH 5.0) until the total volume in the tube was 1100 μL; this was named group 1.

15 μL Ag-PBA (57 mg/mL), 38.5 μL TMB (20 mmol/L), 40 μL H_2O_2 (50 mmol/L), and 20 μL $FeSO_4$ (50 mmol/L) were mixed into a 1.5 mL centrifuge tube. Then, the centrifuge tube was filled with phosphate buffer (pH 5.0) until the total volume in the tube was 1100 μL; this was named group 2.

15 μL Ag-PBA (57 mg/mL), 38.5 μL TMB (20 mmol/L), 40 μL H_2O_2 (50 mmol/L), and 60 μL ascorbic acid (90 mmol/L) were mixed into a 1.5 mL centrifuge tube. Then, the centrifuge tube was filled with phosphate buffer (pH 5.0) until the total volume in the tube was 1100 μL; this was named group 3.

The absorbance of each of three groups was measured at 650 nm after the solution was incubated at 25 °C for 10 min. The absorbance values of the three groups were compared.

(2) Interference of Ag^+ dissolution in the phosphate buffer solution with the Ag-PBA method

K_2CrO_4 powder was added to the phosphate buffer solution (pH 5.0) until the concentration of K_2CrO_4 reached 1 mol/L. Then, 5 mL phosphate buffer (pH 5.0) containing 1 mol/L K_2CrO_4 was added to a 50 mL beaker, and one drop of $AgNO_3$ solution at different concentrations was added, in order, to determine the concentration at which Ag^+ formed a precipitate.

Then, fresh Ag-PBA was prepared, and 60 mg Ag-PBA powder was added into 5 mL phosphate buffer solution (pH 5.0) containing 1 mol/L K_2CrO_4. The solution was left to stand for 1 h and observed to see whether brick-red precipitate formed.

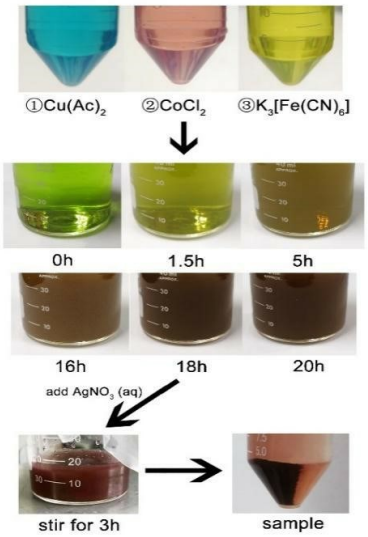

Figure S1. Color changes in the solution during the synthesis of Ag-PBA.

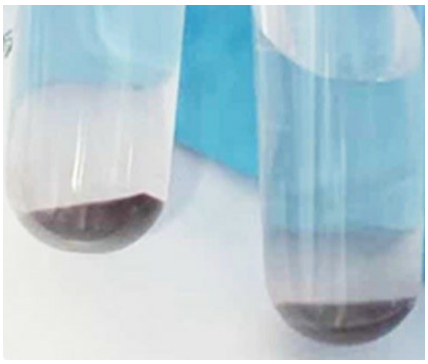

Figure S2. Photographs of Ag-PBA.

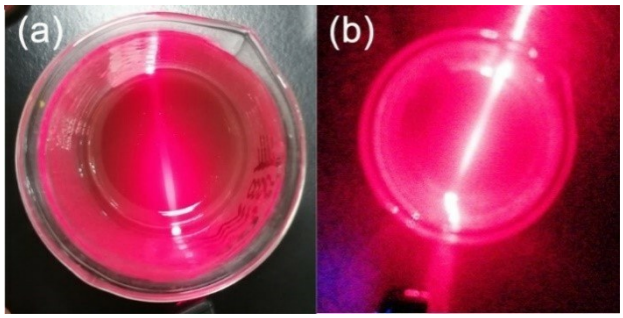

Figure S3. Images of the Tyndall effect on the solution (a) in a bright environment (b) in the dark.

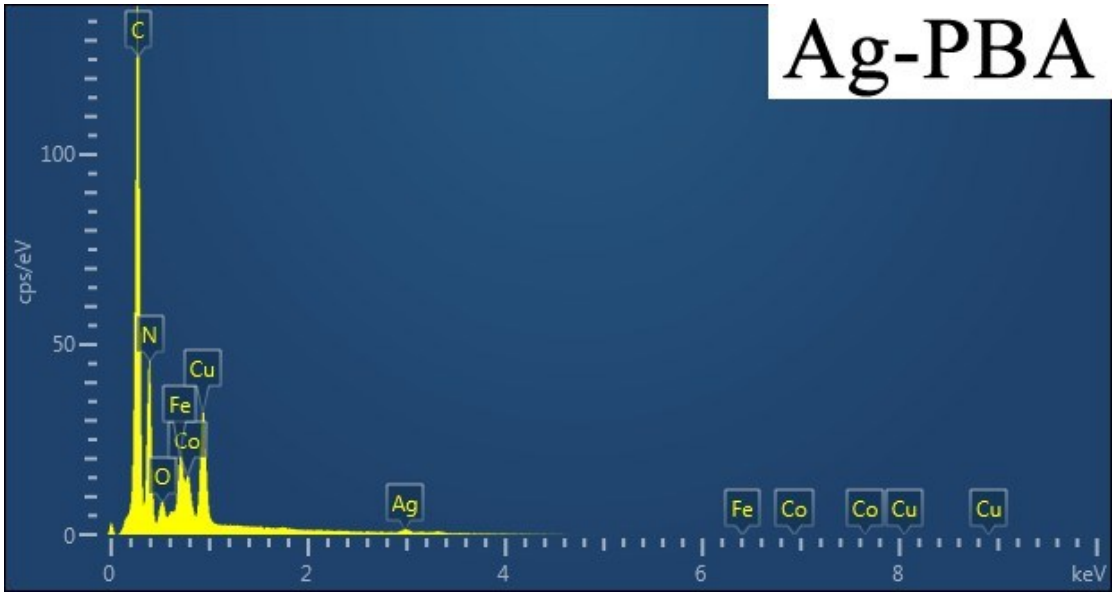

Figure S4. EDS spectrum of Ag-PBA.

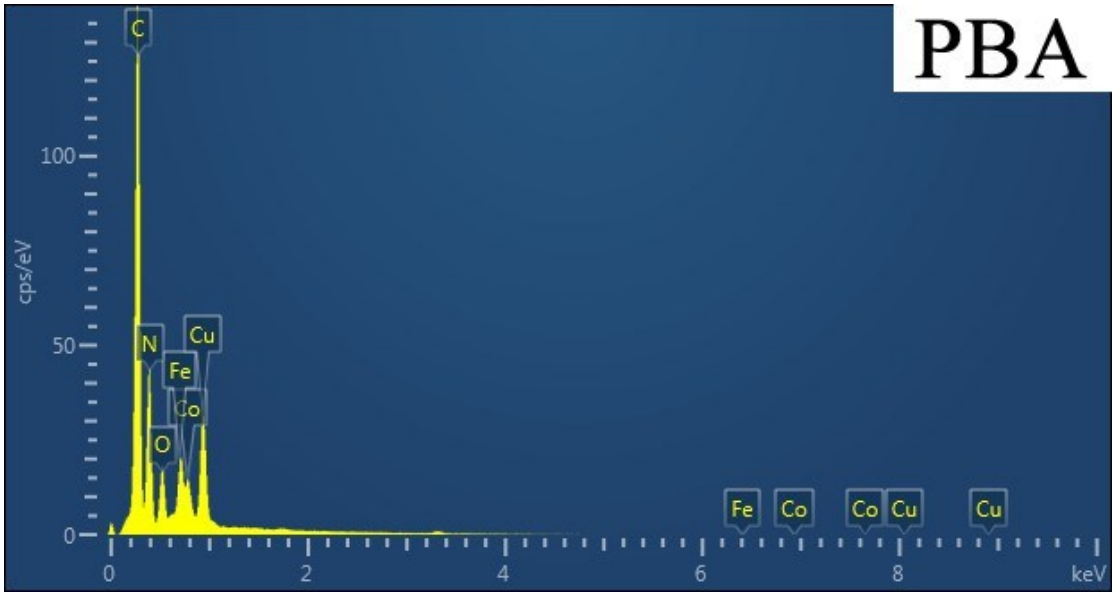

Figure S5. EDS spectrum of PBA.

238 **Table S1.** Elemental composition of PBA from the EDS spectrum

Element	Wt%	Wt% σ	At%
C	33.78	0.27	54.99
N	18.09	0.21	25.26
O	4.28	0.09	5.23
Fe	18.60	0.41	6.51
Co	9.41	0.35	3.12
Cu	15.84	0.20	4.87

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Table S2. Elemental composition of Ag-PBA from the EDS spectrum

Element	Wt%	Wt% σ	At%
C	30.72	0.30	54.51
N	17.45	0.23	26.55
O	1.40	0.07	1.87
Fe	18.60	0.42	7.10
Co	9.78	0.35	3.54
Cu	15.09	0.21	5.06
Ag	6.94	0.53	1.37

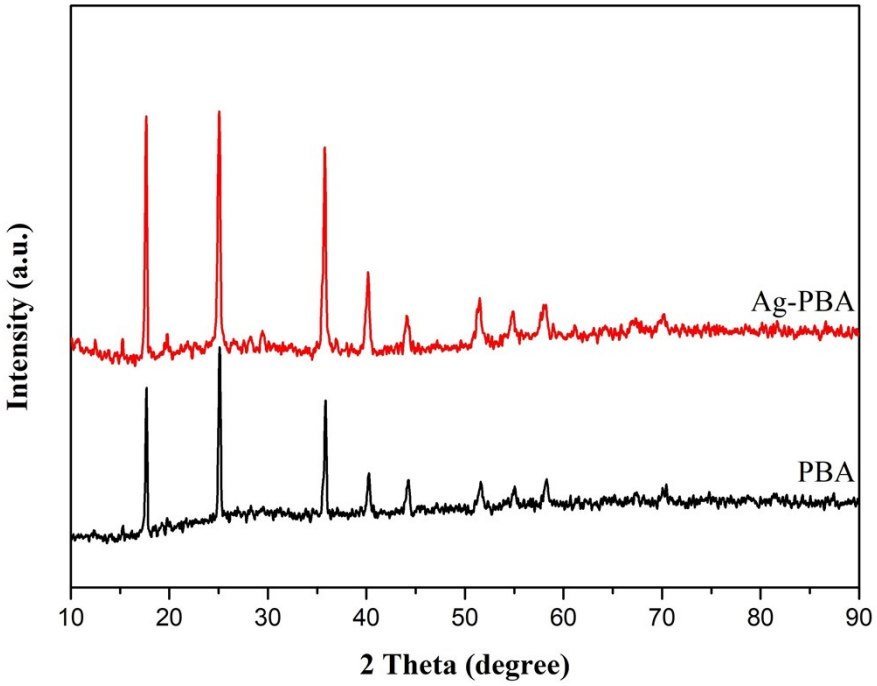

Figure S6. XRD patterns of Ag-PBA and PBA.

290 **Table S3.** Absorbance of the sample solutions measured by the Ag-PBA method and

291 the DPPH method

Sample solution	Method	A_1	A_2	A_3	A_4	A_5	Average value	RAD%	Concentration (mg/mL)
1	Ag-PBA	0.164	0.182	0.153	0.168	0.164	0.166	2.4	13.72
	DPPH	0.671	0.664	0.687	0.665	0.679	0.673	1.2	13.96
2	Ag-PBA	0.351	0.342	0.347	0.364	0.368	0.354	2.6	11.17
	DPPH	0.914	0.892	0.928	0.945	0.901	0.916	1.8	10.97
3	Ag-PBA	0.628	0.635	0.611	0.624	0.619	0.623	1.2	7.40
	DPPH	1.206	1.215	1.237	1.227	1.222	1.221	0.8	7.21
4	Ag-PBA	0.780	0.812	0.798	0.801	0.786	0.795	1.3	5.02
	DPPH	1.405	1.417	1.436	1.392	1.388	1.408	1.1	4.91
5	Ag-PBA	1.022	1.012	1.045	1.025	1.013	1.023	0.9	1.86
	DPPH	1.658	1.677	1.662	1.682	1.671	1.670	0.5	1.69

292 RAD, relative average deviation

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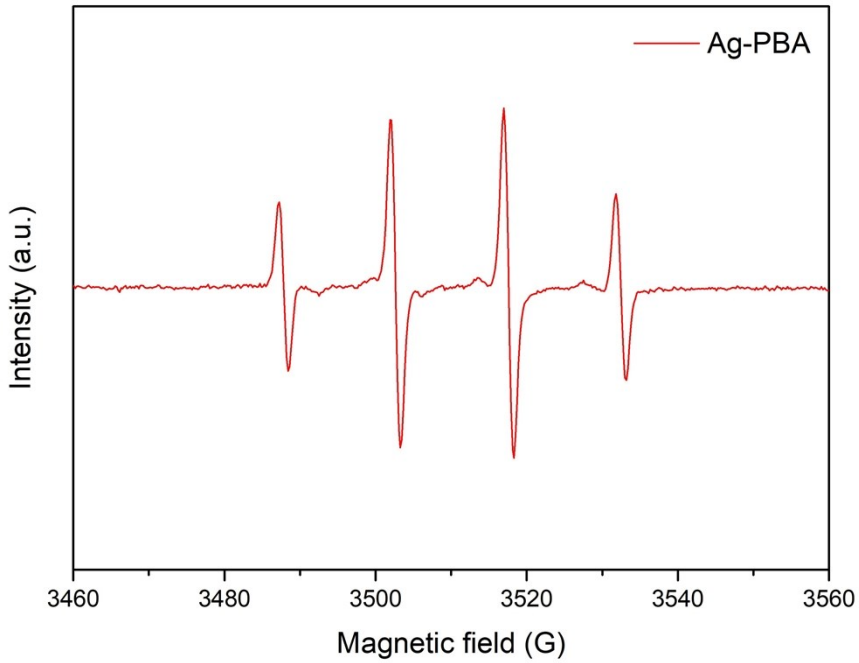
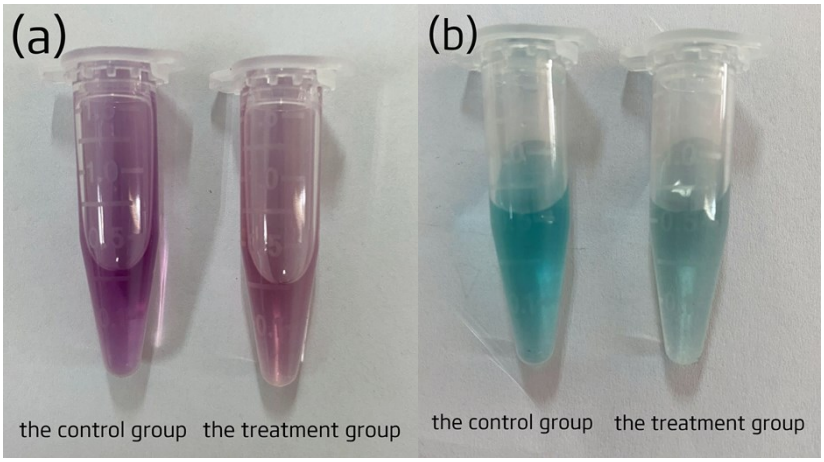


Figure S7. EPR spectrum of the Ag-PBA-H_2O_2-TMB system.

316 

317 **Figure S8.** Antioxidant activity of anthocyanidins measured by the DPPH method (a)

318 and the Ag-PBA method (b).

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