Supplementary data for

The noncovalent conjugations of human serum albumin (HSA) with MS/AK and the effect on anti-oxidant capacity as well as anti-glycative activity of *Monascus* yellow pigments

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1. Experimental Section

1.1 *Superoxide anion radical scavenging ability*

Superoxide anion was generated by the pyrogallic acid system and detected on a TU-1900 spectrophotometer (Puxi Analytic Instrument Ltd. of Beijing, China). The system contained 4.5 mL of Tris-HCl buffer (0.1 M，pH8.2), 1.0 mL of ddH_2O, 0.01 mL of pyrogallic acid (9 mM) and 1.0 mL of sample solution. The samples of Mps (10, 25, 50 μg/mL) were prepared and added separately into the above reaction mixture. After incubated for 1 h at 25°C，the absorbance was recorded at 320 nm. As a control group, sample solution was substituted with Tris-HCl buffer. The antioxidant activity of each sample was calculated as: *Scavenging rate* (%) = [1-($A_{control,230}$/$A_{sample,230}$)] ×100%.

1.2 *Hydroxyl radical scavenging activity*

Mps soulutions (33, 66 and 130 μg/mL) were prepared, and then were incubated with a solution containing phenanthroline (9 mM, 100 μL), phosphate buffer (10 mM, pH 7.4), $FeSO_4$ (9 mM, 100 μL) and H_2O_2 (0.1%, 100 μL) at 37°C for 30 min. The absorbance was read at 510 nm. As a control group, sample solution was substituted with distilled water. The antioxidant activity of each sample was calculated as: *Scavenging rate* (%) = [1-($A_{control,\ 510} - A_{sample,\ 510}$)] ×100%.

1.3 *$ABTS^{+}$• radical scavenging activity*

ABTS solution (7.4 mM, dissolved in deionized water) and 2.6 mM potassium persulfate were prepared, and then mixed to react at 25°C for 16-17h in the dark. The $ABTS^{+}$• solution was diluted in ethanol to an absorbance of 0.7 (± 0.02) at 734 nm before use. 100 μL of Mps (2.5, 5 and 10 μg/mL) and 3 mL of $ABTS^{+}$• solution were mixed and incubated at 25 °C in the dark for 6 min, and the absorbance was read at 734 nm. The antioxidant activity of each sample was calculated as: *Scavenging rate* (%) = [1-($A_{control,\ 734} - A_{sample,\ 734}$)] ×100%.

1.4 *Metal chelation ability*

Solutions of Mps (50 μL, 0.05, 0.10, 0.30, 0.50, 0.70, 0.90, 1.10 mg/mL) or ethylenediami-netetraacetic acid disodium salt (EDTA-Na_2) (50 μL) that servrs as positive control, was mixed with ultrapure water (160 μL) and 0.30 mM $FeSO_4$ solution (20 μL) at 25°C for 5 min. And then 0.8 mM Ferrozine solution (30 μL) was

added to the above mixture. As the sample blanks, the Ferrozine was replaced by ultrapure water. After 15 min, the absorbance was read at 562 nm. The Fe^{2+} ion chelating activity was calculated as: Chelating effect (%) = [1-($A_{sample,562} - A_{control,562}$)] ×100%.

2. Figures

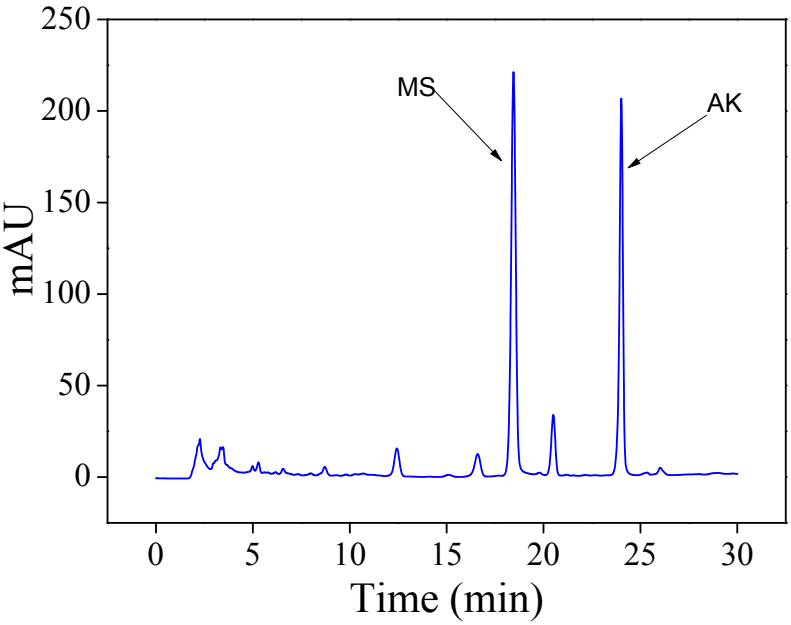

Fig. S1 HPLC profile of Mps rich in MS and AK **[1]**.

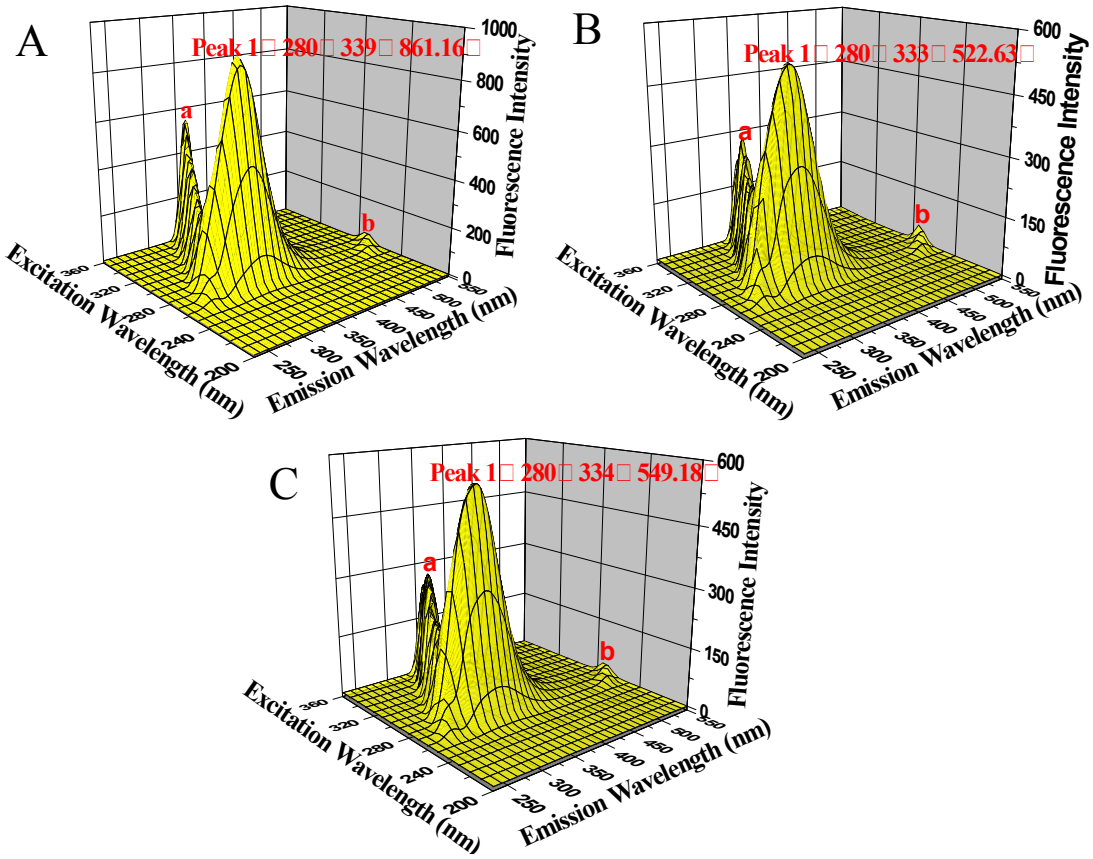

Fig. S2 Three-dimensional fluorescence spectra of HSA alone (A), MS-HSA system (B), and AK-HSA system (C) at 298 K.

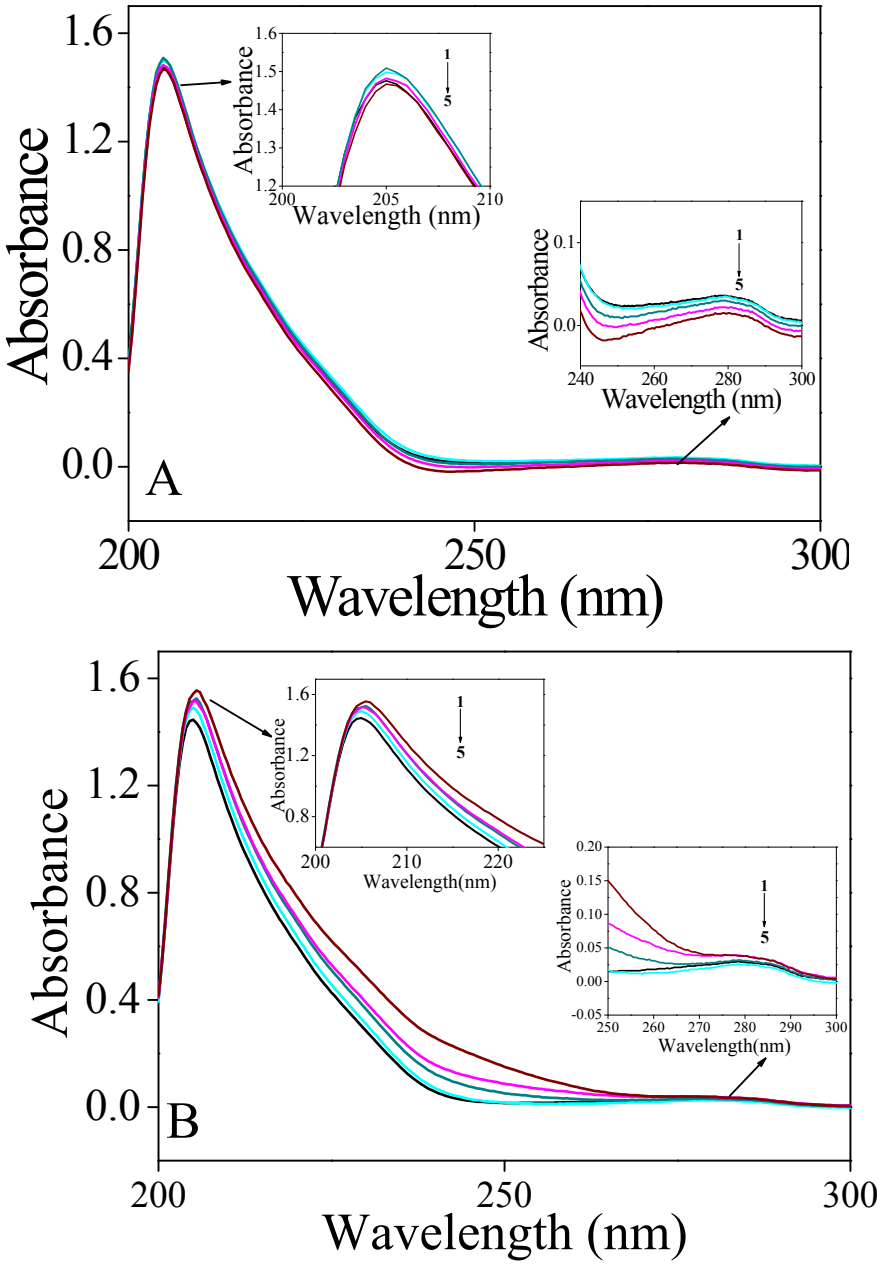

Fig. S3 UV-vis absorption spectra of MS-HSA system (A) and AK-HSA system (B). C_{HSA} = 1.0 × 10^{-6} M, C_{MS} or C_{AK}/(×10^{-6} M) (1-5): 0, 1.0, 2.0, 3.0, 5.0, respectively, pH = 7.4.

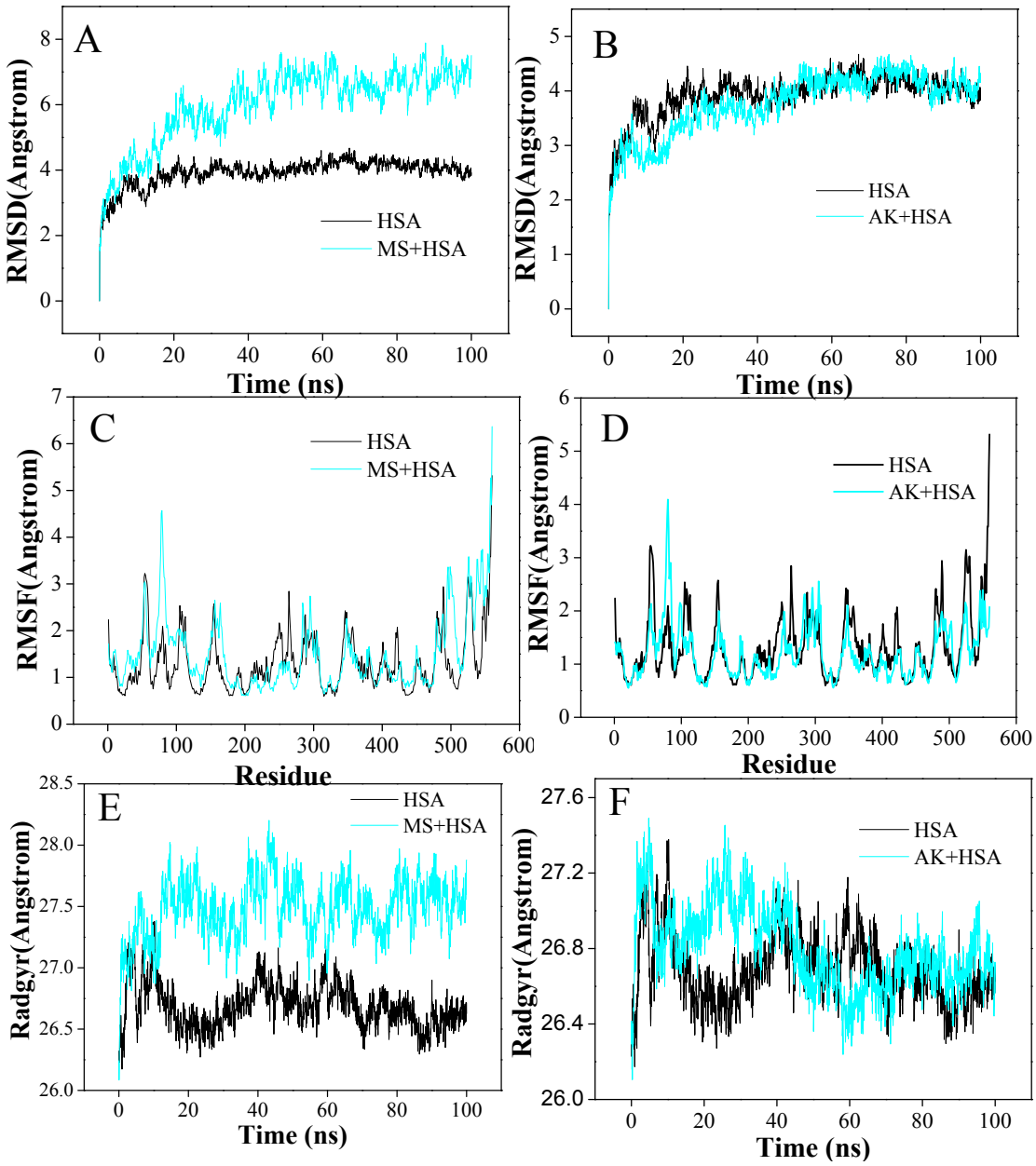

Fig. S4 Results of molecular simulation. (A) Plot of RMSD values vs simulation time for free HSA and MS-HSA system during a 100 ns MD simulation. (B) Plot of RMSD values vs simulation time for free HSA and AK-HSA system during a 100 ns MD simulation. (C and D) The RMSF values as a function of residue numbers. (E and F) The radius of gyration (R_g) of native HSA, MS-HSA system and AK-HSA system as a function of MD simulation time.

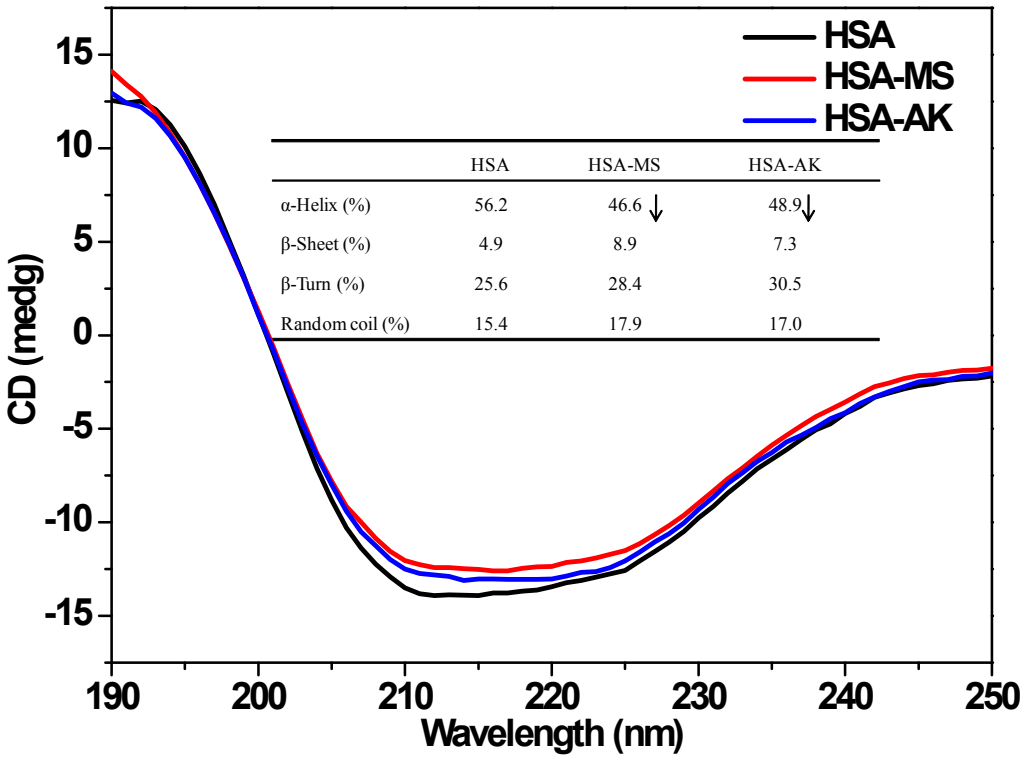

Fig. S5 Far-UV CD spectra of HSA (1.0×10^{-6} M) in the absence and presence of MS (3.0×10^{-6} M) and AK (3.0×10^{-6} M).

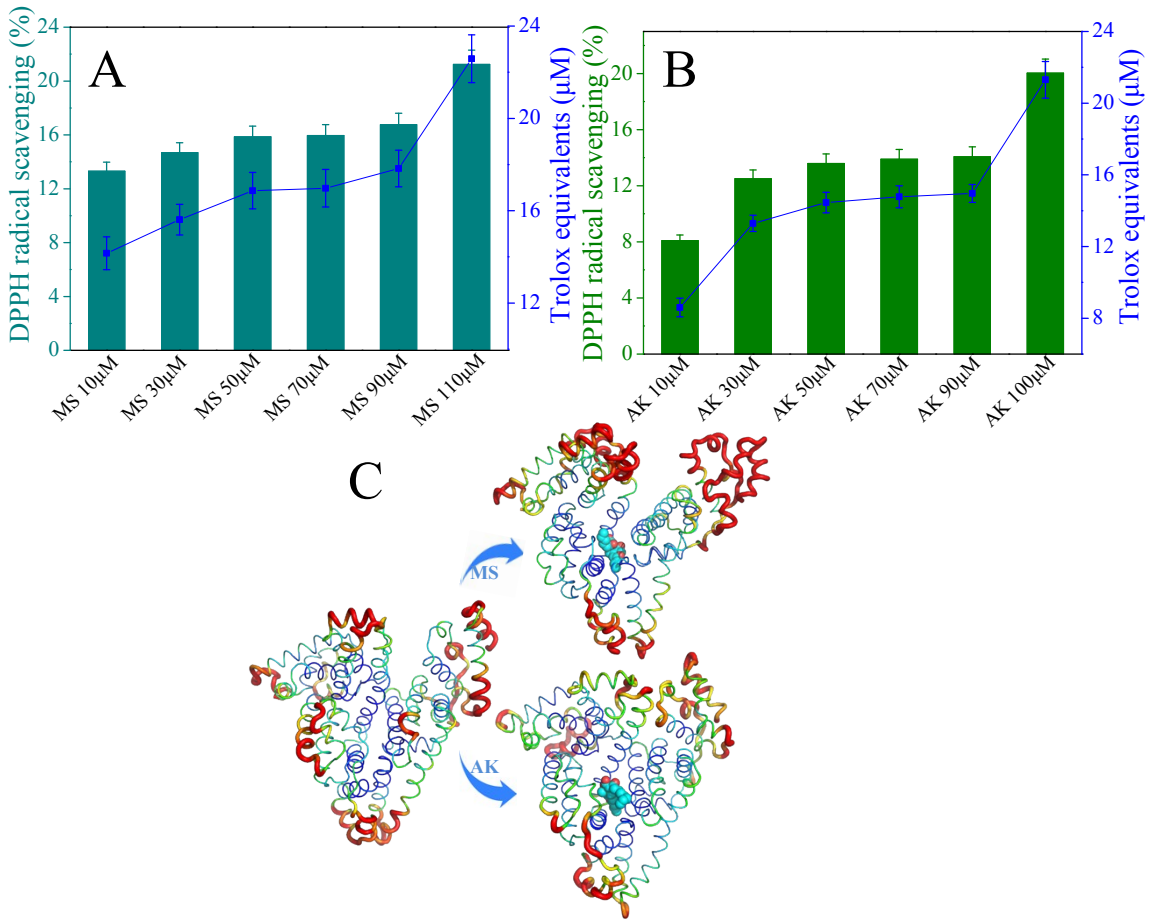

Fig. S6 DPPH antioxidant activity and the Trolox equivalent antioxidant capacity (TEAC)[2] of different concentrations of MS (A) and AK (B). (C) B-factor putty representation of structures of HSA (free and bounded with MS or AK). Red colors and a wider tube indicate regions with higher B-factors, whereas shades of blue and a narrow tube indicate regions lower B-factors.

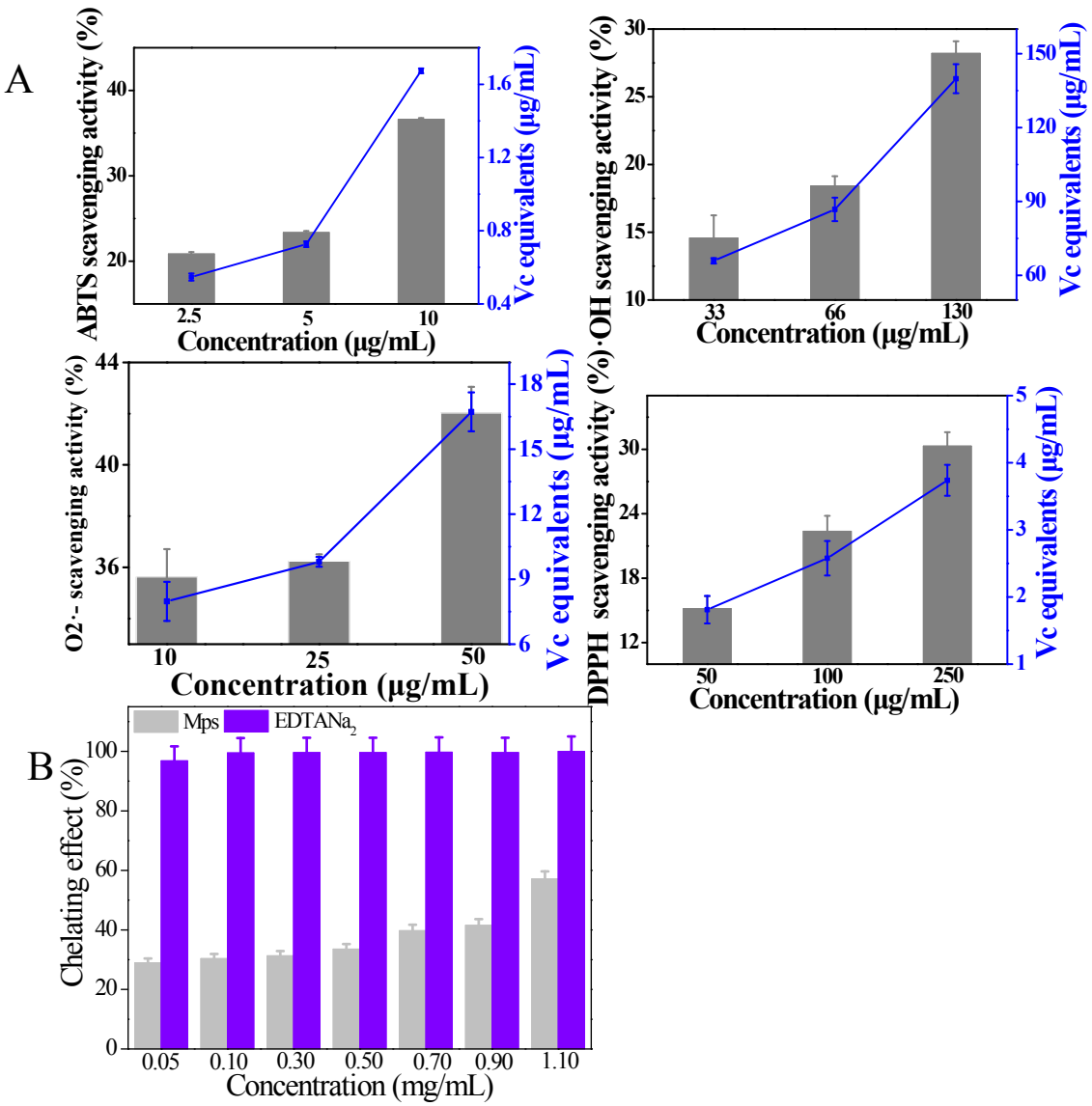

Fig. S7 (A) The scavenging abilities of Mps on ABTS•+, •OH, O_2^{-}•, and DPPH• radicals. (B) The effects of Mps on Fe^{2+}-chelating capacity.

References

[1] D. Chen, C. Xue, M. Chen, S. Wu, Z. Li, C. Wang, Effects of blue light on pigment biosynthesis of Monascus, Journal of Microbiology 54(4) (2016) 305-310.

[2] M.J.T.J. Arts, G.R.M.M. Haenen, H.P. Voss, A. Bast, Antioxidant capacity of reaction products limits the applicability of the Trolox Equivalent Antioxidant Capacity (TEAC) assay, Food and Chemical Toxicology 42(1) (2004) 45-49.