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Preparation, characterisation, and antioxidant activity of honeysuckle polysaccharide nano-selenium

[1]*Sun, J. R., [1]Li, J. L., [1]*Yuan, J. F., [1]Yao, L. L., [2]You, F. F., [1]Wang, D. H. and [1]Gu, S. B.

[1]College of Food and Bioengineering, Henan Engineering Research Center of Food Microbiology, Henan University of Science and Technology, No.263, Kaiyuan Ave., Luoyang 471023, Henan, China
[2]Luoyang Customs, No. 22, Leshan Ave., Luoyang 471000, Henan, China

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Abstract

Using honeysuckle as raw material, honeysuckle polysaccharide (HP) was obtained by water extraction and alcohol precipitation, and polysaccharide nano-selenium (HP-SeNPs) was prepared by HNO_3-Na_2SeO_4 method. The structure of HP, before and after selenisation, was characterised by gas chromatography-mass spectrometry, ultraviolet (UV), Fourier-transform infrared (FT-IR), scanning electron microscopy (SEM), and X-ray diffraction (XRD), and the antioxidant activity was studied. The results showed that the content of Se in HP-SeNPs could reach 10.45%. The HP-SeNPs primarily consisted of glucose and mannose, with a molecular weight of 73.8 kDa. The characterisation results revealed that HP-SeNPs exhibited a novel absorption peak at approximately 334 nm in the UV spectrum; distinct absorption peaks were observed at 1079.66 and 619.21 cm^{-1} in the FT-IR spectrum, while a broad diffraction peak appeared between 19° and 25° in the XRD pattern. SEM further demonstrated that HP-SeNPs formed particle-like surface structures, altering the surface morphology of polysaccharides. These findings collectively indicated the successful preparation of HP-SeNPs. The scavenging ability of HP-SeNPs to DPPH, ·OH, and ABTS free radicals was concentration-dependent, and the highest scavenging rates were 53.85, 62.55, and 49.37%, which were significantly higher than HP. This indicated that selenium modification could outstandingly improve the antioxidant activity of HP.

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Introduction

Honeysuckle (*Lonicera japonica* Thunb.) is a kind of traditional Chinese medicine often used for heat-clearing and detoxification, cooling, and dispersing wind/heat (Tang *et al*., 2021). Studies have shown that there are many substances beneficial to the human body in honeysuckle. At present, flavonoids, organic acids, volatile oils, polysaccharides, iridoid terpenoids, and triterpenoid saponins have been isolated from honeysuckle, which have the functions of antioxidation, lowering blood sugar, and regulating immunity. Therefore, honeysuckle is widely used in medicine, healthy food, and daily necessities (Bai *et al*., 2019).

Polysaccharide is one of the main active ingredients in honeysuckle (Zhu *et al*., 2022). It was found that honeysuckle polysaccharide had antibacterial, antiviral, anti-tumour, antioxidant, and immune-enhancing biological activities, which confirmed that polysaccharide was indeed an important pharmacodynamic material basis for its bioactivities (Fan *et al*., 2017). Zhang *et al*. (2019) extracted honeysuckle polysaccharide by water extraction and alcohol precipitation, and found that it had a strong antioxidant capacity. Su *et al*. (2017) found that honeysuckle polysaccharide had strong antioxidant ability on DPPH free radicals *in vitro*. Honeysuckle polysaccharide could restore the vitality of mice cardiac organ cells damaged by hydrogen peroxide, and significantly reduce the activities of aspartate aminotransferase, creatine phosphokinase, lactate dehydrogenase, the content of active oxygen in cells, and the content of malondialdehyde; it also

*Corresponding author.
Email: dasheng@haust.edu.cn ; jiangfengyuan@163.com

significantly increased the activities of SOD, CAT, and GSH in cardiomyocytes in a dose-dependent manner, which indicated that honeysuckle polysaccharide had good antioxidant activities (Zhou *et al*., 2020).

Selenium (Se) is one of the necessary micronutrient elements for animals and humans (Cui *et al*., 2019). It is a key element constituting a variety of selenoproteins or enzymes, which is crucial for maintaining normal life activities of the body, and has functions such as delaying aging (Zeng *et al*., 2020), preventing tumours (Ali *et al*., 2021), regulating immunity (Liu *et al*., 2020), and ameliorating cardiovascular diseases (Angelica *et al*., 2021), cancers, and diabetes (Holger *et al*., 2022). Inadequate intake of Se can lead to dysfunction of vital organs in animals and humans, and affect health. Organic Se can also significantly reduce the risk of poisoning caused by inorganic Se; so, the research on organic Se has become one of the hot spots in the medicine, food, and healthcare industries (Rayman, 2008). As a new food functional factor, nano-selenium (SeNPs) has good bioavailability and better safety, and exhibits various effects such as antioxidant (Bai *et al*., 2020a), anti-tumour (Gao *et al*., 2020a), immune regulation (Wang *et al*., 2014), antibacterial (Lesnichaya *et al*., 2022), gastric mucosa protection (Bai *et al*., 2020c), and liver protection (Wang *et al*., 2005), showing broad development prospects.

Due to its specific physicochemical properties and biological activities, SeNPs have been widely concerned, and have potential application value in food, medicine, agriculture, and other fields. Polysaccharide is ideal modifier and stabiliser for nanomaterials (Shi *et al*., 2021). Polysaccharides contain a lot of hydroxyl, amino, sulphuric acid, and carboxyl groups, which can provide a favourable microenvironment for the growth of Se atomic clusters, adsorb and encapsulate nano-Se, and control its agglomeration and growth. At the same time, polysaccharide molecules have numerous modifiable sites, diverse biological activities, and good biocompatibility; as a result, polysaccharide nano-Se has strong modifiability and unique biological activities, which may not only be a multifunctional Se supplement, but also have the potential to be developed into nano-drug carrier. Polysaccharide nano-Se system have shown antioxidant (Bai *et al*., 2020b), anti-tumour (Gao *et al*., 2020a), anti-inflammatory (Wang *et al*., 2014), antibacterial (Lesnichaya *et al*., 2022), immunoregulatory (Xia *et al*., 2019), and anti-diabetic (Zeng *et al*., 2018) activities. *Lycium barbarum* Se polysaccharide (Se-LBPS) was synthesised by the HAMID method, and its scavenging rate on ·OH and superoxide anion radicals was 60.51 and 47.9%, respectively, which was significantly higher than before selenisation (Hamid *et al*., 2017). Zhu *et al*. (2016) reported that the Se polysaccharide of *Cordyceps militaris* showed stronger scavenging activity on DPPH, ·OH, and superoxide free radicals than natural polysaccharide. Ye *et al*. (2016) reported that the Se polysaccharide of *Astragalus membranaceus* could observably reduce the levels of glutamic oxaloacetic transaminase, alanine aminotransferase, and malondialdehyde, and increase the activities of glutathione, glutathione peroxidase, and superoxide dismutase. Ping *et al*. (2017) reported that polysaccharide nano-Se from *Auricularia auricula* significantly increased reactive oxygen species levels in MCF-7 tumour cells, and induced MCF-7 cell apoptosis through the aspartic proteolytic enzyme pathway. Bai *et al*. (2020b) reported that chitosan nano-Se had a significant scavenging effect on ·OH-, $\cdot O^{2-}$, and DPPH, and its scavenging ability was about 1/5, 1/3, and 1/7 of vitamin C, respectively, which was significantly better than chitosan, and the scavenging ability of nano-Se played a major role. Chen *et al*. (2022) proved that *Polygonatum sibiricum* polysaccharide nano-Se had a strong scavenging ability on DPPH and ABTS, and could effectively alleviate the cytotoxicity of PC-12 cells induced by H_2O_2.

In the present work, honeysuckle polysaccharide (HP) was obtained by water extraction and alcohol precipitation, which were subsequently utilised as modifiers and stabilisers for synthesising Se polysaccharide (HP-SeNPs) using the nitric acid-sodium selenite method. The structure of HP and HP-SeNPs was characterised by gas chromatography-mass spectrometry (GC-MS), ultraviolet (UV), Fourier-transform infrared (FT-IR), scanning electron microscopy (SEM), and X-ray diffraction (XRD), and their antioxidant activity was studied. The present work would provide a theoretical basis for the comprehensive development and utilisation of honeysuckle, and the development of Se-rich functional food.

Materials and methods

Materials and reagents

Honeysuckle was purchased from Luoyang, Henan Province. Ethanol absolute, trichloroacetic acid (TCA), nitric acid, sodium sulphate, concentrated sulphuric acid, ferric trichloride, and potassium ferricyanide were purchased from Tianjin Komeo Chemical Reagent Co., Ltd. Ferrous sulphate, potassium bromide, vitamin C, and anhydrous pyridine were purchased from Luoyang Haohua Chemical Reagent Co., Ltd. Sodium selenite, barium chloride, sodium hydroxide, hydrogen peroxide, trifluoroacetic acid, methanol, DPPH, and ABTS were purchased from Tianjin Deen Chemical Reagent Co., Ltd.

Extraction of polysaccharide from honeysuckle

After mechanically crushed, 10 g of honeysuckle powder was obtained, added with distilled water at a ratio of 1:20 (g/mL), and placed in water bath for 2 h at 80°C. Next, the sample was centrifuged at 4,000 rpm for 10 min. The supernatant was condensed to 1/3 of its original volume. After cooling, three times anhydrous ethanol was added, and the solution was incubated at 4°C for 24 h. The precipitation was taken after centrifuging at 4,000 rpm for 15 min, added with 20 times of distilled water, and then an appropriate amount of trichloroacetic acid was added to make the concentration 50 g/L, followed by shaking for 20 min. After shaking, the supernatant was removed after centrifuging for 15 min, three times anhydrous ethanol was added, and the solution was incubated at 4°C for 24 h. Finally, centrifuging was performed at 4,000 rpm for 15 min, and the precipitate was obtained and subjected to vacuum freeze-drying to yield the honeysuckle polysaccharide (HP).

Preparation of HP-SeNPs

Selenium polysaccharide was prepared by the HNO_3-Na_2SeO_4 method (Sun *et al*., 2023). Briefly, 200 mg of HP was accurately weighed, added with 20 mL of 0.5% nitric acid solution, and stirred to dissolve. Then, 200 mg of Na_2SeO_3 and 200 mg of $BaCl_2$ were added, stirred to dissolve, and reacted in a water bath at 75°C for 7 h. After the mixture cooled to room temperature, the pH of the reaction solution was adjusted to neutral with 1 mol/L NaOH solution, and then 200 mg of Na_2SO_4 was added to precipitate $BaCl_2$ (Shi *et al*., 2021). Then, centrifugation at 4,000 rpm was performed for 10 min, and the supernatant was dialysed in a dialysis bag for 72 h, while changing the water every 3 h. Next, the dialysate was removed when no red colour appeared after ascorbic acid was added. Finally, HP-SeNPs were obtained after freeze-drying of dialysate.

Determination of selenium content

The Se content of HP-SeNPs was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Lian *et al*., 2022). Briefly, 100 mg of HP-SeNPs was accurately weighed and placed in the digestive tube. Then, 5 mL of HNO_3 and 1 mL of H_2O_2 were added, shaken, mixed evenly, and then digested in the microwave digestion apparatus. The instrument power of the microwave digestion instrument was 800 W, the initial temperature was 25°C, then increased to 120°C at 20°C/min, then increased to 150°C at 10°C/min for 5 min, then increased to 200°C at 10°C/min for 10 min, then decreased to 55°C at 10°C/min, and the operation was stopped. After digestion and cooling, the digestive solution was transferred into a conical bottle, added with a few glass beads, and continued to heat on the electric plate until nearly dry. Then, 2.5 mL of 6 mol/L hydrochloric acid solution was added and heated until the solution was clear and colourless with white smoke. After cooling, it was transferred to a 5 mL volumetric flask, added with 1.5 mL of 100 g/L potassium ferricyanide solution, volume-fixed with ultra-pure water, and mixed well to be tested. At the same time, the reagent blank test was done.

Characterisation of HP and HP-SeNPs

Monosaccharide composition analysis

Following the method described by Tang *et al*. (2017), 20 mg of sample was accurately weighed, added with 3 mL of trifluoroacetic acid, sealed with nitrogen, hydrolysed in a boiling water bath for 6 h, cooled and concentrated to dry, dissolved with 2 mL of methanol, and concentrated to dry three times. After trifluoroacetic acid was completely removed, glycolol acetate derivatives were prepared.

The monosaccharide mixture was transferred into a plugged test tube, added with appropriate amount of inositol, 10 mg of hydroxylamine hydrochloride, and 0.5 mL of anhydrous pyridine, mixed and sealed, and placed in a constant temperature water bath at 90°C with shaking for 30 min. After reaction and cooling to room temperature, 0.5 mL of acetic anhydride was added to seal, and

shaken at 90°C for 30 min. Saccharonitrile acetate derivatives were obtained after cooling to room temperature.

The GC-MS analysis conditions were chromatographic column = HP-5MS; injection port parameters = shunt mode; initial temperature = 280°C; pressure = 8.21 psi; shunt ratio = 5:1; shunt flow = 5.0 mL/min; ion source temperature = 300°C; transmission line temperature = 250°C; gas type = helium; and heating program = initial temperature 60°C, 5°C/min to 280°C, and retained for 3 min.

Determination of molecular weight

Gel permeation chromatography (GPC) was employed to determine the molecular weight (Mw) distributions of HP and HP-SeNPs (Yue *et al.*, 2022). The GPC analysis was conducted using a TSK Gel-3000XL column (7.8 × 300 mm, 5 μm) with a differential refractive index detector. Ultrapure water containing 0.1 mol/L sodium nitrate served as the mobile phase, and the sample was injected into the chromatographic column system at a flow rate of 1 mL/min.

Ultraviolet analysis

The UV analysis was performed following the method described by Sun *et al*. (2023). Briefly, 20 mg of HP and HP-SeNPs were accurately weighed and re-dissolved in distilled water, with the final concentration of polysaccharide solution of 1 mg/mL. Ultraviolet-visible spectrophotometry was used to scan in the range of 200 - 400 nm with a wavelength interval of 5 nm.

FT-IR analysis

The FT-IR analysis was performed following the method described by Sun *et al*. (2023). Briefly, 2 mg of HP and HP-SeNPs were weighed respectively, and mixed with potassium bromide (KBr) at a ratio of 1:100 for grinding. Using KBr as background, the detection was carried out in the range of 4000 - 400 cm^{-1} wave.

Scanning electron microscopy analysis

The HP and HP-SeNPs were accurately weighed and adhered to a carbon conductive dielectric plate. After spraying gold, the samples were scanned at room temperature under a scanning electron microscope to observe and compare the surface structure of honeysuckle polysaccharide, before and after selenisation.

X-ray diffraction analysis

The crystal form changes of polysaccharide, before and after selenisation, were analysed by XRD (Sun *et al*., 2023). An appropriate amount of samples were taken on a glass carrier plate, crushed and compacted, and placed on the loading platform of XRD with Cu Kα radiation, 40 kV voltage, 30 mA current, and 4(°)/min scanning rate.

Antioxidant activities of HP and HP-SeNPs in vitro

Scavenging effects on DPPH free radical

Following the method described by Sun *et al*. (2023), 10 mL of HP and HP-SeNPs solutions with mass concentration gradients of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL were configured. Next, 2 mL of HP and HP-SeNPs solutions with different concentrations were added to the test tube together with 2 mL of 0.1 mmol/L DPPH-ethanol solution, while shaking evenly and avoiding light, at room temperature, for 30 min. Absolute ethanol was used as blank zero, and ascorbic acid as a positive control. The absorbance was measured at wavelength 517 nm using a UV-vis spectrophotometer, and the scavenging rate on DPPH free radical was calculated using Eq. 1:

$$\text{DPPH radical scavenging rate (\%)} = \left[\frac{1-(A_a-A_b)}{A_c}\right] \times 100\% \quad \text{(Eq. 1)}$$

where, A_a = sample absorbance; A_b = sample background absorbance; and A_c = distilled water absorbance as blank.

Scavenging effects on hydroxyl free radical

Following the method described by Sun *et al*. (2023), five test tubes were selected as the experimental group, one test tube as the zero-adjustment group, and one test tube as the control group. Then, 10 mL of HP and HP-SeNPs solution, respectively, with mass concentration gradients of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL, were configured. Next, 1 mL of 7.5 mmol/L phosphate buffer (pH 7.4), 1 mL of 3.25 mmol/L phenanthroline solution, 1 mL of 1.5% $FeSO_4$ solution, and 1 mL of 1.5% H_2O_2 were added to the experimental group, and mixed with 2 mL of different concentrations of HP and HP-SeNPs solutions. Distilled water was used to replace 2 mL of polysaccharide solution in the zero-adjustment group, and 2 mL of sample and 1 mL of 1.5% H_2O_2 in the control group. The absorbance was measured at 510 nm after bathing at 37°C for 1 h. Ascorbic acid was

used as a positive control using the same method. The scavenging rate on hydroxyl free radical was calculated using Eq. 2:

$$\text{Hydroxyl radical scavenging rate (\%)} = \frac{A_i}{A_j} \times 100\% \quad \text{(Eq. 2)}$$

where, A_i = experimental group absorbance; and A_j = control group absorbance.

Scavenging effects on ABTS radical

Following the method described by Sun *et al.* (2023), HP and HP-SeNPs solutions with mass concentration gradients of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL were configured for 10 mL. Five test tubes were added with 4 mL of ABTS solution and 1 mL of different concentrations of HP and HP-SeNPs solution, and shaken well to make the reaction complete. Reacting at room temperature without light for 6 min, the absorbance was determined at 734 nm. Ascorbic acid was used as a positive control, and the scavenging rate on ABTS free radical was calculated using Eq. 3:

$$\text{ABTS radical scavenging rate (\%)} = \left[\frac{1-(A_1-A_2)}{A_0}\right] \times 100\% \quad \text{(Eq. 3)}$$

where, A_1 = absorbance of the sample; A_2 = absorbance of anhydrous ethanol instead of ABTS; and A_0 = absorbance of water instead of the sample.

Statistical analysis

The main experiments were performed and repeated at least three times unless stated otherwise. The experimental results were presented as mean ± SD (standard deviation). The SPSS ver. 18.0 software was used for statistical analysis *via* ANOVA. *Post hoc* ANOVA analysis was conducted with the Duncan test, and $p < 0.05$ was taken as statistically significant.

Results and discussion

Physicochemical properties of HP-SeNPs

The HP was selenised to obtain HP-SeNPs, and the Se content was 10.45%. The HP-SeNPs were flocculent, light brown in colour, and soluble in water.

Characterisation of HP and HP-SeNPs

Monosaccharide composition of HP-SeNPs

The monosaccharide composition of HP-SeNPs by GC-MS is shown in Figure 1. The HP-SeNPs were mainly composed of mannose and glucose, and the molar mass ratio was 1:2.63.

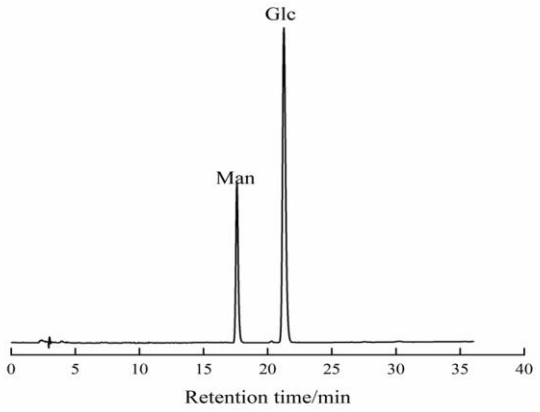

Figure 1. GC-MS chromatogram of HP-SeNPs.

Molecular weight of HP and HP-SeNPs

The Mw of HP-SeNPs was 73.8 kDa, whereas the Mw of HP was 152.4 kDa (Table 1). Interestingly, selenylation modification led to a significant decrease in the Mw of HP-SeNPs compared to the original HP, consistent with previous findings (Wang *et al.*, 2021). The decrease in Mw could potentially be attributed to polysaccharide hydrolysis, which might occur at an accelerated rate under conditions involving high acid concentration and elevated temperature (Zhan *et al.*, 2021).

Table 1. Molecular mass distribution of HP and HP-SeNPs.

Sample	Time range (min)	Molecular weight (kDa)
HP	12.45 - 15.28	152.4
HP-SeNPs	14.53 - 16.67	73.8

UV analysis of HP and HP-SeNPs

The UV spectra of HP and HP-SeNPs are shown in Figure 2. No obvious absorption peak of HP existed between 200 - 400 nm, and a downward trend was observed with the increase in wavelength, which was consistent with the general features of polysaccharide. In addition, no absorption peak existed at 260 and 280 nm, indicating no proteins and nucleic acids in HP. However, HP-SeNPs had a wider absorption band between 300 - 380 nm, and the

strongest absorption peak at 334 nm, which was the same as the absorption peak of Se at 334 nm (Zhu *et al*., 2020). The results thus confirmed that Se was present in HP-SeNPs, and that HP was successfully selenised.

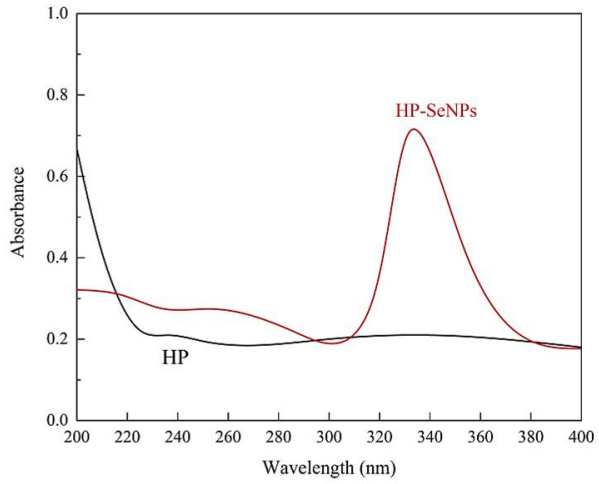

Figure 2. UV-Vis absorption spectrum of HP and HP-SeNPs.

FT-IR analysis of HP and HP-SeNPs

The FT-IR spectra of HP and HP-SeNPs are shown in Figure 3. Typical characteristic absorption peaks of polysaccharide were observed in HP at 3301.57, 2996.00, 1617.64, 1075.21, and 535.69 cm^{-1}. Polysaccharide is a polyhydroxy compound, and there are many hydrogen bonds between polysaccharide molecules; so, a strong and wide absorption peak at 3301 cm^{-1} existed, which indicated the stretching vibration of O-H. The stretching vibration peak of C-H was observed at 2996.00 cm^{-1}. The peak at 1617.64 cm^{-1} indicated that there was a C=O functional group in the polysaccharide molecule. The stretching vibration peak of C-O was observed at 1075.21 cm^{-1}. Compared with HP, some characteristic absorption peaks of polysaccharide in HP-SeNPs were still obvious, indicating that the main structure of HP had not been destroyed, but the peak shape of some characteristic peaks had changed, and the absorption wavelength had a certain red or blue shift (Figure 3). The absorption peak of HP at 1617.64 cm^{-1} was red-shifted to 1612.72 cm^{-1} after selenisation. The possible reason was that selenite and C-O produced nucleophilic addition in the process of selenisation, and the covalent binding led to the shift of the absorption peak. In addition to the characteristic absorption peak of polysaccharide, there was a symmetrical stretching vibration absorption peak of O-Se-O observed near 1079.66 cm^{-1}, and an asymmetric stretching vibration absorption peak of Se=O observed at 619.21 cm^{-1}. Compared to HP, the absorption peaks of polysaccharide in HP-SeNPs remained distinct, indicating the preservation of HP's main structure. However, certain peak shapes underwent modifications, and showed noticeable shifts towards either red or blue wavelengths. These findings potentially suggested the formation of novel bonds between O-H and C-O groups of HP and the surface of SeNPs, such as O-H···Se bonds and C-O···Se bonds (Du *et al*., 2023).

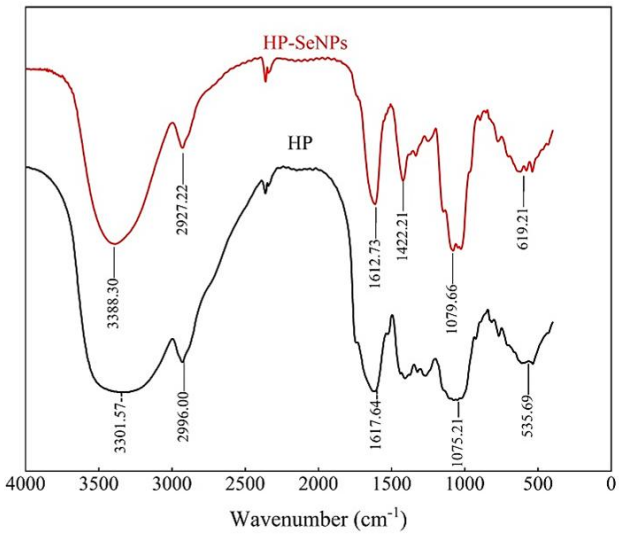

Figure 3. FT-IR of HP and HP-SeNPs.

SEM analysis of HP and HP-SeNPs

The SEM of HP and HP-SeNPs are shown in Figure 4. The surface of HP was relatively intact forming a flaky structure, and the surface was complete, smooth, and compact (Figure 4a). However, the surface of HP-SeNPs was rough, uneven, spherical, and strip morphology increased (Figure 4b). The possible reason was the combination of polysaccharide with Se which changed the original aggregation state of polysaccharide, or degraded the polysaccharide. The results showed that Se atoms combined with HP, and aggregated on the HP surface, changing the surface morphology. In the solubility experiment, it was found that the solubility of HP-SeNPs was better than HP, indicating that the aggregation state of HP was changed, and the surface dispersion was better due to the selenisation reaction.

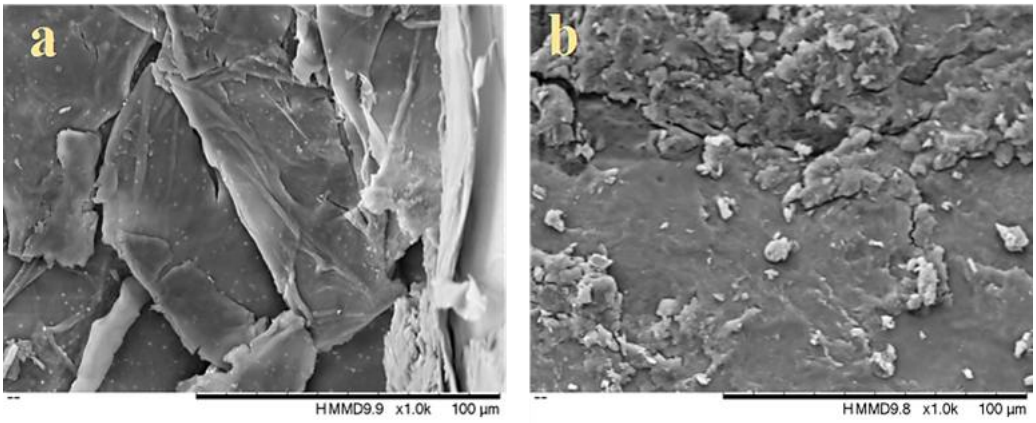

Figure 4. SEM of **(a)** HP and **(b)** HP-SeNPs.

XRD analysis of HP and HP-SeNPs

The XRD analysis results of HP and HP-SeNPs are shown in Figure 5. There was no obvious absorption peak of HP in the range of 10 - 50°, indicating that there was less crystalline polysaccharide and poor crystallinity in HP, which was consistent with the results of SEM. However, HP-SeNPs had a dispersion peak in the range of 19 - 25°, which might be due to the fact that the selenisation modification made some polysaccharides present a microcrystalline state (Sun *et al*., 2023).

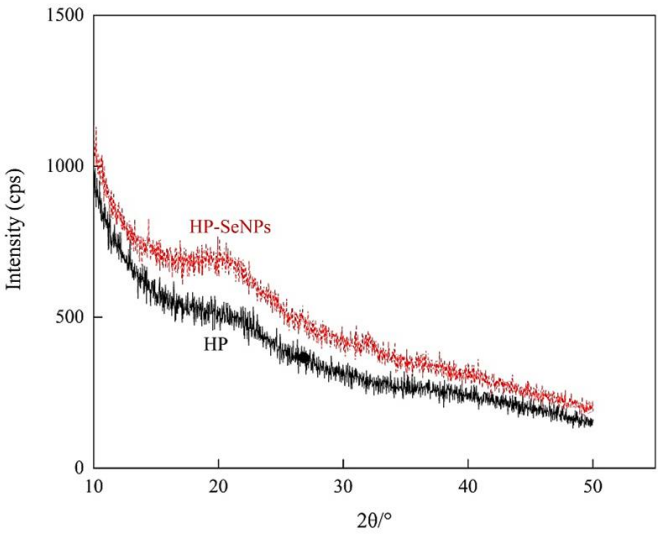

Figure 5. XRD of HP and HP-SeNPs.

The presence of Se in HP-SeNPs was indicated by a strong absorption peak at approximately 334 nm in the UV spectrogram (Figure 2). The FT-IR analysis confirmed that the main structure of HP remained intact after selenisation. The XRD analysis suggested that the modification with Se might have resulted in a microcrystalline state for the polysaccharide, as evidenced by the dispersion peak observed between 19° - 25° in HP-SeNPs (Figure 5). The surface of HP-SeNPs exhibited a comparatively rough and uneven texture, distinguishing it from HP (Figure 4). In summary, the successful synthesis of HP-SeNPs was achieved.

Antioxidant activity of HP and HP-SeNPs in vitro

DPPH free radical scavenging ability

The scavenging ability of HP and HP-SeNPs on DPPH free radical is shown in Figure 6a. Within the concentration range of 0.2 - 1.0 mg/mL, both HP and HP-SeNPs had strong scavenging effects on DPPH free radical, and the scavenging rate was positively correlated with the concentration of HP and HP-SeNPs. At the concentration of 1.0 mg/mL, the scavenging rates of HP and HP-SeNPs on DPPH free radicals were the highest, 44.19 and 53.85%, respectively. The scavenging rate of HP-SeNPs on DPPH free radical was 21.86% higher than HP. In the tested concentration range, the scavenging ability of HP-SeNPs on DPPH free radical was significantly stronger than HP ($p < 0.05$). The results showed that selenisation could observably enhance the scavenging ability of HP on DPPH free radical.

Hydroxyl free radical scavenging ability

Hydroxyl free radical is a kind of free radical with high reactivity in the human body, leading to oxidative damage to the human body. Therefore, the scavenging ability of hydroxyl free radical is also one of the very important indexes to detect and evaluate antioxidant activity *in vitro*. The scavenging ability of HP and HP-SeNPs on hydroxyl free radical is shown in Figure 6b. Both HP and HP-SeNPs had strong scavenging effects on hydroxyl free radical within the

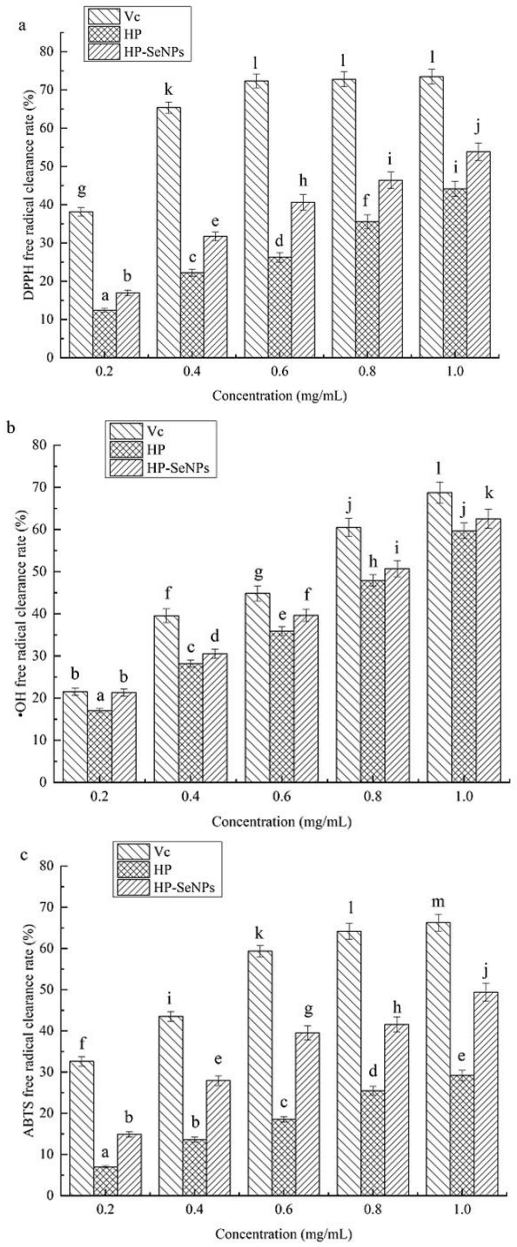

Figure 6. Antioxidant activities of HP and HP-SeNPs. **(a)** DPPH free radical; **(b)** ·OH free radical; and **(c)** ABTS free radical. Values with different lowercase letters are significantly different ($p < 0.05$).

concentration range of 0.2 - 1.0 mg/mL, and the scavenging rate on hydroxyl free radical was positively correlated with the concentration of HP and HP-SeNPs. When the concentration was 1.0 mg/mL, both HP and HP-SeNPs had the highest scavenging rates on hydroxyl free radical, 59.70 and 62.55%, respectively. Within the tested concentration range, HP-SeNPs had a significantly stronger scavenging ability on hydroxyl free radical than HP ($p < 0.05$). The results showed that selenisation could observably enhance the scavenging ability of HP on hydroxyl free radical. The possible reason was that the Se moiety in HP-SeNPs could form complex with free metal ions, thus exerting a stronger antioxidant effect on hydroxyl radicals.

ABTS free radical scavenging ability

The ABTS can form blue-green cationic ABTS free radical by reacting with potassium persulphate, and antioxidants can react with ABTS free radical to discolour the system, reflecting the antioxidant capacity (Xie *et al*., 2020). The scavenging ability of HP and HP-SeNPs on ABTS free radicals is shown in Figure 6c. Within the concentration range of 0.2 - 1.0 mg/mL, both HP and HP-SeNPs had certain scavenging effects on ABTS free radical, and the scavenging rate on ABTS free radical was positively correlated with the concentrations of HP and HP-SeNPs. At the concentration of 1.0 mg/mL, the scavenging rates of HP and HP-SeNPs on ABTS free radical were the highest, 29.14 and 49.37%, respectively, and the scavenging rate of HP-SeNPs on ABTS free radical was 69.42% higher than HP. In the tested concentration range, the scavenging ability of HP-SeNPs on ABTS was significantly stronger than HP ($p < 0.05$). The results showed that selenisation could observably enhance the scavenging ability of HP on ABTS free radical, and this might have been due to the better solubility of SeNPs, increasing the probability of reaction with free radicals.

In summary, the results of scavenging experiments on DPPH, hydroxyl, and ABTS free radicals showed that selenisation was an effective way to improve the antioxidant activity of HP. The Se group in HP could activate the H atoms of isomerised carbon, thus improving the scavenging ability on free radicals (Zhu *et al*., 2016; Gao *et al*., 2020b). In addition, Se, as a cofactor of Se-dependent antioxidant enzymes, such as GSH-Px, TRx, and SOD, could be converted into active centres of these enzymes (Cheng *et al*., 2018). Therefore, HP-SeNPs might increase the activity of Se-dependent antioxidant enzyme, and further exhibit strong antioxidant activity *in vivo*.

Conclusion

The UV, FT-IR, SEM, and XRD analyses showed that honeysuckle polysaccharide nano-selenium (HP-SeNPs) was successfully synthesised. The Se content of HP-SeNPs could reach 10.45%. The HP-SeNPs were mainly composed of glucose and mannose. The scavenging ability of HP-SeNPs on

DPPH, ·OH, and ABTS free radicals was concentration-dependent, and the highest scavenging rates were 53.85, 62.55, and 49.37% for DPPH, ·OH, and ABTS radicals, significantly higher than HP. The results indicated that Se treatment could observably improve the antioxidant activity of HP. The present work aimed to establish a theoretical basis for the comprehensive development and utilisation of honeysuckle, as well as to explore its potential as a source of Se-enriched functional food. However, further investigation is necessary to examine the *in vivo* activity of HP-SeNPs, and understand the mechanisms underlying its antioxidant properties.

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References

Ali, R., Mansour, P., Dhifaf, S. and Mikael, B. 2021. Selenium stimulates the antitumour immunity: Insights to future research. European Journal of Cancer 155: 256-267.

Angelica, K., Tian, H., Li, M., Wang, Y., Olav, A., Zang, J. and Cao, Y. 2021. Selenium status in the body and cardiovascular disease: A systematic review and meta-analysis. Critical Reviews in Food Science and Nutrition 61: 3616-3625.

Bai, K., Hong, B., He, J. and Huang, W. 2020a. Antioxidant capacity and hepatoprotective role of chitosan-stabilized selenium nanoparticles in concanavalin A-induced liver injury in mice. Nutrients 12: 857.

Bai, K., Hong, B., Huang, W. and He, J. 2020b. Selenium-nanoparticles-loaded chitosan/chitooligosaccharide microparticles and their antioxidant potential: A chemical and *in vivo* investigation. Pharmaceutics 12: 43.

Bai, K., Hong, B., Tan, R., He, J. and Hong, Z. 2020c. Selenium nanoparticles-embedded chitosan microspheres and their effects upon alcohol-induced gastric mucosal injury in rats: Rapid preparation, oral delivery, and gastroprotective potential of selenium nanoparticles. International Journal of Nanomedicine 15: 1187-1203.

Bai, X., Chai, Y., Shi, W., Li, Y., Zhang, T. and Liu, P. 2019. *Lonicera japonica* polysaccharides attenuate ovalbumin-induced allergic rhinitis by regulation of Th17 cells in BALB/c mice. Journal of Functional Foods 65: 103758.

Chen, W., Cheng, H. and Xia, W. 2022. Construction of *Polygonatum sibiricum* polysaccharide functionalized selenium nanoparticles for the enhancement of stability and antioxidant activity. Antioxidants 11: 240.

Cheng, L., Wang, Y., He, X. and Wei, X. 2018. Preparation, structural characterization and bioactivities of Se-containing polysaccharide: A review. International Journal of Biological Macromolecules 120: 82-92.

Cui, D., Ma, J., Liang, T., Sun, L., Meng, L., Liang, T. and Li, S. 2019. Selenium nanoparticles fabricated in laminarin polysaccharides solutions exert their cytotoxicities in HepG2 cells by inhibiting autophagy and promoting apoptosis. International Journal of Biological Macromolecules 137: 829-835.

Du, H. X., Tan, X. Y., Li, Z. X., Dong, H., Su, L. J., He, Z. K., … and Cao, L. T. 2023. Effects of *Schisandra chinensis* polysaccharide-conjugated selenium nanoparticles on intestinal injury in mice. Animals 13: 930.

Fan, J., Feng, H., Yu, Y., Sun, M., Liu, Y., Li, T., … and Sun, M. 2017. Antioxidant activities of the polysaccharides of *Chuanminshen violaceum*. Carbohydrate Polymers 157: 629-636.

Gao, P., Bian, J., Xu, S., Liu, C., Sun, Y., Zhang, G., ... and Liu, X. 2020a. Structural features, selenization modification, antioxidant and anti-tumor effects of polysaccharides from alfalfa roots. International Journal of Biological Macromolecules 149: 207-214.

Gao, X., Li, X., Mu, J., Tang, H., Su, J., Zhang, Y., ... and Xie, Y. 2020b. Preparation, physicochemical characterization, and anti-proliferation of selenium nanoparticles stabilized by *Polyporus umbellatus* polysaccharide. International Journal of Biological Macromolecules 152: 605-615.

Hamid, M., Liu, D., Abdulrahim, Y., Khan, A., Qian, G. and Huang, K. 2017. Inactivation of Kupffer cells by selenizing astragalus polysaccharides

prevents CCl4-induced hepatocellular necrosis in the male Wistar rat. Biological Trace Element Research 179: 226-236.

Holger, S., Duntas, L. and Rayman, M. 2022. The role of selenium in type-2 diabetes mellitus and its metabolic comorbidities. Redox Biology 50: 102236.

Lesnichaya, M., Perfileva, A., Nozhkina, O., Gazizova, A. and Graskova, L. 2022. Synthesis, toxicity evaluation and determination of possible mechanisms of antimicrobial effect of arabinogalactane-capped selenium nanoparticles. Journal of Trace Elements in Medicine and Biology 69: 126904.

Lian, Y. P., Zhu, M. M., Yang, B., Wang, X. F., Zeng, J. Q., Yang, Y. J., … and Feng, L. 2022. Characterization of a novel polysaccharide from *red ginseng* and its ameliorative effect on oxidative stress injury in myocardial ischemia. Chinese Medicine 17(1): e111.

Liu, T., Xu, L., He, L., Zhao, J., Zhang, Z., Chen, Q. and Chen, T. 2020. Selenium nanoparticles regulates selenoprotein to boost cytokine-induced killer cells-based cancer immunotherapy. Nano Today 35: 100975.

Ping, Z., Liu, T., Xu, H., Meng, Y., Li, W., Xu, X. and Zhang, L. 2017. Construction of highly stable selenium nanoparticles embedded in hollow nanofibers of polysaccharide and their antitumor activities. Journal of Nanoparticle Research 10: 3775-3789.

Rayman, M. 2008. Food-chain selenium and human health: Emphasis on intake. British Journal of Nutrition 100: 254-268.

Shi, X., Yong, Y., Wu, J. and Wang, S. 2021. Synthesis, characterization, and biological activity of selenium nanoparticles conjugated with polysaccharides. Critical Reviews in Food Science and Nutrition 61: 2225-2236.

Su, D., Li, S., Zhang, W., Wang, J. and Wang, L. 2017. Manhua, structural elucidation of a polysaccharide from *Lonicera japonica* flowers, and its neuroprotective effect on cerebral ischemia-reperfusion injury in rat. International Journal of Biological Macromolecules 99: 350-357.

Sun, J. R., Li, J. L., Yao, L. L., You, F. F., Yuan, J. F., Wang, D. H. and Gu, S. B. 2023. Synthesis, characterization and antioxidant activity of selenium nanoparticle decorated with polysaccharide from hawthorn. Journal of Food Measurement and Characterization 17: 6125-6134.

Tang, W. M., Jin, L., Xie, L. H., Huang, J. Q., Wang, N., Chu, B. Q., … and Zhang, Y. 2017. Structural characterization and antifatigue effect *in vivo* of maca (*Lepidium meyenii* Walp) polysaccharide. Journal of Food Science 82(3): 757-764.

Tang, X., Liu, X., Zhong, J. and Fang, R. 2021. Potential application of *Lonicera japonica* extracts in animal production: From the perspective of intestinal health. Frontiers in Microbiology 12: 719877.

Wang, H., Wei, W., Zhang, S. Y., Shen, Y. X., Yue, L., Wang, N. P. and Xu, S. Y. 2005. Melatonin-selenium nanoparticles inhibit oxidative stress and protect against hepatic injury induced by Bacillus Calmette-Guerin/lipopolysaccharide in mice. Journal of Pineal Research 39: 156-63.

Wang, J., Zhang, Y., Yuan, Y. and Yue, T. 2014. Immunomodulatory of selenium nano-particles decorated by sulfated *Ganoderma lucidum* polysaccharides. Food and Chemical Toxicology 68: 183-189.

Wang, L. B., Li, L. Y., Gao, J. Y., Huang, J., Yang, Y., Xu, Y. Q., … and Yu, W. Q. 2021. Characterization, antioxidant and immunomodulatory effects of selenized polysaccharides from dandelion roots. Carbohydrate Polymers 260: 117796.

Xia, I., Cheng, J., Wu, M., Wong, K., Kong, H., Zheng, X., … and Kwok, K. 2019. Dietary chitosan-selenium nanoparticle (CTS-SeNP) enhance immunity and disease resistance in zebrafish. Fish and Shellfish Immunology 87: 449-459.

Xie, L., Shen, M., Wen, P., Hong, Y., Liu, X. and Xie, J. 2020. Preparation, characterization, antioxidant activity and protective effect against cellular oxidative stress of phosphorylated polysaccharide from *Cyclocarya paliurus*. Food and Chemical Toxicology 145: 111754.

Ye, S., Zhang, J., Liu, Z., Zhang, Y., Li, J. and Li, Y. 2016. Biosynthesis of selenium rich exopolysaccharide (Se-EPS) by *Pseudomonas* PT-8 and characterization of its antioxidant activities. Carbohydrate Polymers 142: 230-239.

Yue, L. Q., Song, X. X., Cui, X., Zhang, Q., Tian, X. J., Yang, X. H., … and Wang, Y. P. 2022. Synthesis, characterization, and evaluation of microwave-assisted fabricated selenylation astragalus polysaccharides. International Journal of Biological Macromolecules 221: 8-15.

Zeng, R., Farooq, M., Zhang, G., Tang, Z., Zheng, T., Su, Y., ... and Zhu, J. 2020. Dissecting the potential of selenoproteins extracted from selenium-enriched rice on physiological, biochemical and anti-ageing effects *in vivo*. Biological Trace Element Research 196: 119-130.

Zeng, S., Ke, Y., Liu, Y., Shen, Y., Zhang, L., Li, C., ... and Liu, Y. 2018. Synthesis and antidiabetic properties of chitosan-stabilized selenium nanoparticles. Colloids And Surfaces B - Biointerfaces 170: 115-121.

Zhan, Q. P., Chen, Y., Guo, Y. F., Wang, Q., Wu, H. and Zhao, L. Y. 2021. Effects of selenylation modification on the antioxidative and immunoregulatory activities of polysaccharides from the pulp of *Rose laevigata* Michx fruit. International Journal of Biological Macromolecules 206: 242-254.

Zhang, T., Liu, H., Bai, X., Liu, P., Yang, Y., Huang, J., ... and Min, X. 2019. Fractionation and antioxidant activities of the water-soluble polysaccharides from *Lonicera japonica* Thunb. International Journal of Biological Macromolecules 151: 1058-1066.

Zhou, X., He, G., Ma, J., Tang, M., Tian, G., Xun, G. .... and Kui, L. 2020. Protective effect of a novel polysaccharide from *Lonicera japonica* on cardiomyocytes of mice injured by hydrogen peroxide. Biomed Research International 52: 5279193.

Zhu Z., Liu, F., Gao, H., Sun, H., Mng, M. and Zhang, Y. 2016. Synthesis, characterization and antioxidant activity of selenium polysaccharide from *Cordyceps militaris*. International Journal of Biological Macromolecules 93: 1090-1099.

Zhu, J., Jia, Y., Wang, C., Zhou, W., Shu, Y., Zhang, K., ... and Guo, R. 2022. *Lonicera japonica* polysaccharides improve longevity and fitness of *Caenorhabditis elegans* by activating DAF-16. International Journal of Biological Macromolecules 229: 289.

Zhu, J., Yu, C., Han, Z., Chen, Y., Wei, X. and Wang, Y. 2020. Comparative analysis of existence form for selenium and structural characteristics in artificial selenium-enriched and synthetic selenized green tea polysaccharides. International Journal of Biological Macromolecules 154: 1408-1418.