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ORIGINAL ARTICLE

Preparation, Characteristics, and Antioxidant Activity of the Selenium Nanoparticles Stabilized by Polysaccharides Isolated from *Grateloupia filicina*

Bilang Cao[1], Yueling Yang[1], Chunhua Yue[1], Yuanyuan Wang[2], Pengcheng Fu[2], Yongguang Bi[1,2]

[1]Department of Pharmaceutical Engineering, College of Pharmacy, Guangdong Pharmaceutical University, Guangzhou, Guangdong, [2]Department of Biochemical Engineering, State Key Laboratory of Marine Resource Utilization in South China Sea, Hainan University, Hainan, Haikou, China

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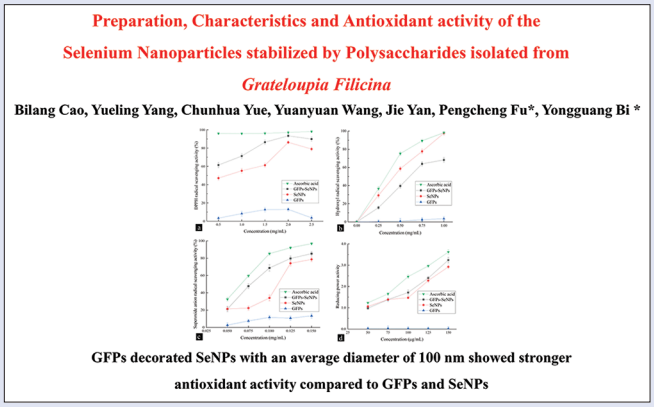
ABSTRACT

Background: Selenium is an essential trace element which is of fundamental importance to human health. Compared to organic and inorganic selenium, selenium nanoparticles (SeNPs) display unique biological and physicochemical properties. **Objectives:** In this study, we aimed to optimize the extraction parameters of polysaccharides of *Grateloupia filicina* (GFPs) and investigate the antioxidant activity of SeNPs stabilized by GFPs. **Materials and Methods:** GFPs were extracted using hot water. The extraction parameters were optimized by performing an orthogonal experiment. SeNPs were prepared under mild conditions using GFP as the modifier and the stabilizer. A scanning electron microscope (SEM) was used to characterize the prepared GFPs-SeNPs. The antioxidant activities of GFPs, SeNPs, and GFPs-SeNPs were compared by measuring 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, hydroxyl radical scavenging activity, superoxide anion radical scavenging activity, and via reducing power assay. **Results:** The optimal conditions for the extraction were determined as follows: extraction time 5 h, extraction temperature 90°C, and the ratio of water to raw material 100 mL/g. Under these conditions, the yield of GFPs was 50.34% ±0.27%. GFP-decorated SeNPs with an average diameter of 100 nm were synthesized. GFPs-SeNPs showed a higher antioxidant activity compared to GFPs and SeNPs alone. **Conclusion:** Hot water extraction was found to be efficient in the process of extraction of polysaccharides of *G. filicina*. GFPs can significantly enhance the antioxidant activity of SeNPs as modifier and stabilizer. GFPs-SeNPs is an efficient radical scavenger and may be explored as a novel antioxidant agent for use in the fields of foodstuff and medicine.

Key words: Antioxidant activity, *Grateloupia filicina*, hot water extraction, polysaccharides, selenium nanoparticles

SUMMARY

- An orthogonal experiment was investigated to optimize the extraction parameters of polysaccharides of *Grateloupia filicina*, and the antioxidant activities of polysaccharides of *G. filicina* (GFPs), selenium nanoparticles (SeNPs), and GFP-stabilized SeNPs were compared. The optimal extraction parameters were determined to be as follows: extraction time, 5 h; extraction temperature, 90°C; and the ratio of water to raw material, 100 mL/g. Under these conditions, the yield of GFPs reached 50.34% ±0.27%. GFPs-SeNPs with an average diameter of 100 nm were synthesized. They showed higher antioxidant activities compared to GFPs and SeNPs.



Abbreviations used: DPPH: 2,2-diphenyl-1-picrylhydrazyl; SeNPs: Selenium nanoparticles; GFPs: Polysaccharides of *Grateloupia filicina*; SEM: Scanning electron microscope; HO•: Hydroxyl radical; O_2•⁻: Superoxide anion radical; ANOVA: Analysis of variance.

Correspondence:

Prof. Pengcheng Fu,
State Key Laboratory of Marine Resource Utilization in South China Sea, Hainan University, Haikou 570228, Hainan, China.
E-mail: pcfu@hainanu.edu.cn
Prof. Yongguang Bi,
College of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, Guangdong, China.
E-mail: biyongguang2002@163.com
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INTRODUCTION

Literature shows that free radicals induce cell damage, which can lead to many degenerative diseases, including aging,[1] cancer,[2] poor immunity,[3] and heart diseases.[4] Therefore, it is essential to explore novel and effective antioxidants that can protect organisms from the effects of free radicals and slow down the initiation and progress of chronic diseases. Polysaccharides are ubiquitously found in micro-organisms, animals, and plants effective in preventing living organisms from oxidative damage; they do this by increasing the activities of antioxidant enzymes[5] or by scavenging the

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intracellular free radicals.[6] Polysaccharides of marine origin have extensively been used in food and pharma industries due to their potent biological activities.[7] The red seaweed genus *Grateloupia*, an intertidal red alga belongs to *Halymeniaceae*, which is mainly distributed in Indian, Pacific, and warmer parts of the Atlantic ocean.[8] *Grateloupia filicina*, originally known as *Delesseria filicina*, was later transferred to the genus *Grateloupia* after the genus was established.[9] In China, it is widespread along the southern coastlines of the Zhejiang, Fujian, and Guangdong provinces.[10] The polysaccharides of *Grateloupia filicina* (GFPs) are mainly composed of sulfate 3,6-anhydro-α-L-galactose, 1,3-linked β-D-galactose, and 1,4-linked α-L-galactose.[11] GFPs exhibit antibacterial, antioxidant, and antischistosomal activities.[12] In general, polysaccharides possess active hydroxyl groups and complex branched structures, which reduce the aggregation of the nanoparticles, modify the interface of the nanoparticles, and improve the stability of the solution containing the nanoparticles.[13]

Selenium is an essential trace element, which plays an important role in maintaining the antioxidant defense system,[14] redox homeostasis,[15] and immune regulation[16] and shows antitumor activity.[17] It is reported that selenium can effectively protect the kidneys, heart, and liver from oxidative damage.[18] However, the bioavailability and biological activity of selenium are greatly limited because of the narrow margin between the toxic and functional dosage.[19] Selenium nanoparticles (SeNPs) exhibit much lower toxicity and higher bioactivity and bioavailability compared with other selenium species.[20] SeNPs have attracted considerable attention due to their unique physicochemical properties and diverse functions. Similar to other nanomaterials, SeNPs display nanosize effect, which means that smaller nanoparticles show higher activities.[21] SeNPs have been widely applied in therapeutic,[22] medicinal,[23] environmental remediation,[24] and in biosensors.[25] However, SeNPs are poor in stability and tend to form clusters in aqueous solution, resulting in lower bioavailability and bioactivity.[26] Therefore, it is important to maintain the stability and functionality of SeNPs by preparing them with appropriate chemical reagents that both stabilize their structure and retain their bioactivity. To date, different approaches have been used to synthesize SeNPs such as physical, biological, and chemical techniques. There are many methods that have been successfully applied, such as microwave synthesis,[27] chemical reduction,[28] biosynthesis, and so forth.[29] The polysaccharides can form an efficient polymer template for the synthesis of SeNPs due to its distinctive structure and high bioactivity.

In this study, based on the results of single-factor experiment, an orthogonal experiment design was investigated to optimize the extraction conditions of GFPs to obtain high yield of active polysaccharides. Moreover, SeNPs were synthesized using GFPs as modifiers and stabilizers, and antioxidant activity of GFPs-SeNPs was also investigated. We aimed to optimize the extraction parameters of GFPs, investigate the antioxidant activity of GFPs-SeNPs, and provide a scientific basis for the medicinal use of GFPs-SeNPs.

MATERIALS AND METHODS

Materials

G. filicina was purchased from Shanwei (Guangdong, China), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Aladdin Bio-Chem Technology Co. Ltd. (Shanghai, China). Glucose was purchased from Jinsui Biotechnology Co. Ltd. (Shanghai, China). Phenol, ferric chloride, and potassium ferricyanide were obtained from Zhiyuan Chemical Reagent Co. Ltd. (Tianjin, China). Polyvinyl alcohol was purchased from Jinshan Chemical Reagent Co. Ltd. (Chengdu, China). Sodium selenite was acquired from Nanjing Chemical Reagent Co. Ltd. (Nanjing, China). All other chemicals used were of analytical grade.

Isolation of *Grateloupia filicina* polysaccharides

G. filicina was ground using a high-speed pulverizer (DFY-C, LinDa Machinery Co. Ltd., Wenling, China). The dry powder was extracted in a water bath filled with distilled water which was kept at a constant temperature. The extraction time ranged from 1 to 5 h, extraction temperature ranged from 60°C to 100°C, and water to raw material ranged from 70 to 110 mL/g. Centrifugation was performed at 6000 rpm for 10 min in a centrifuge (TDL80-2B, Anting Scientific Instrument Co. Ltd., Shanghai, China) to remove precipitates. Then, the supernatants were concentrated by rotary evaporation (RE-52AA, Yarong Biochemical Instrument Factory, Shanghai, China). The concentrated solution was treated with thrice the volume of 95% (v: V) ethanol and then kept at 4°C overnight. The precipitates were collected by centrifugation for 20 min at 6000 rpm and dried to obtain polysaccharides.

Determination of the content of *Grateloupia filicina* polysaccharides

The phenol–sulfuric acid method was used to analyze the total content of polysaccharides.[30] The supernatant (1.0 mL) of each extract was added into a 10 mL test tube, and then, 1.0 mL of 5% phenol was added to it, followed by the addition of 5.0 mL of concentrated sulfuric acid. The reaction mixture was shaken vigorously and heated in a boiling water bath for 10 min. After cooling, the absorbance was recorded at 485 nm by ultraviolet-visible (UV-Vis) spectrophotometer (UV-9100, Ruili Analytical Instruments Co. Ltd., Beijing, China). Glucose was used to prepare the calibration curve. The regression equation between glucose concentration and absorbance was obtained as follows: A = 9.9812C–0.0095, R^2 = 0.9973 (linearity ranging: 0–0.12 mg/mL, A: 485 nm OD, C: Mg/mL).

Optimization of extraction conditions of *Grateloupia filicina* polysaccharides

The extraction conditions of GFPs were investigated by employing an orthogonal test design. As shown in Table 1, on the basis of the results of single-factor experiments, the extraction experiment was implemented with three factors and three levels, i.e., extraction time (3, 4, and 5 h), extraction temperature (80°C, 90°C, and 100°C), and ratio of water to raw material (90, 100, and 110 mL/g). The extraction yield (%) of GFPs was the dependent variable.

Synthesis of selenium nanoparticles stabilized by *Grateloupia filicina* polysaccharides

The method of synthesis of GFPs-SeNPs was conducted following the process reported previously with some modifications.[11] First, 10.0 mL of GFP solution (0.5%) and 10.0 mL of sodium selenite (0.1 M) were mixed uniformly; then, an equal volume of ascorbic acid solution (0.1 M) was added dropwise to the mixture under constant stirring on a magnetic stirrer at 40°C for 2 h. Furthermore, SeNPs were synthesized by the same method as described above, but the aqueous solution of GFPs was replaced by an equal volume of polyvinyl alcohol.

Characterization and measurements

The morphology and size of the SeNPs and GFPs-SeNPs were characterized using a scanning electron microscope (SEM) (MERLIN, ZEISS, Germany) at an acceleration voltage of 5 kV.

Table 1: Factors and levels of orthogonal experiment

Level	A Extraction temperature (°C)	B Ratio of water to raw material (mL/g)	C ExtractioTime (h)
1	100	110	3
2	90	100	4
3	80	90	5

Antioxidant activity assay

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

This assay was performed as previously reported with some modifications.[31] Sample (5.0 mL) was mixed with 5.0 mL of 5% DPPH (dissolved in ethanol). The reaction mixture was stirred and incubated for 30 min at room temperature in the dark. Then, the absorbance was recorded at 517 nm using a spectrophotometer (UV1101, Tian Mei Scientific Instrument Co. Ltd., Shanghai, China) against ethanol used as the blank. The percentage scavenging of DPPH free radical was expressed as follows:

Scavenging percentage (%) = $(1-[A_c - A_i]/A0) \times 100$

Where A_c is the absorbance of the sample solution in the reaction mixture, A_i is the absorbance of sample solution diluted with an equal volume of anhydrous ethanol, and A_0 is the absorbance of the control reaction (without the addition of the sample).

Hydroxyl radical scavenging assay

The analysis of hydroxyl radical (HO•) scavenging activity was conducted based on the previously reported method with slight modifications.[32] First, an equal volume of (2.0 mL) of $FeSO_4$ (6 mM), salicylic acid–ethanol solution (6 mM), and sample solution were sequentially added into the test tube. Then, 2.0 mL of 30% H_2O_2 was added and incubated for 30 min at 37°C. After cooling the reaction mixture, the absorbance of the mixture was recorded at 510 nm by UV-Vis spectrophotometer. The scavenging activity was assessed as the inhibition percentage of salicylic acid oxidation by HO• and calculated as follows:

Inhibition percentage (%) = $(1-[A_x - A_{x0}]/A_0) \times 100$

Where A_x is the absorbance of the sample solution in the reaction mixture, A_0 is the absorbance of the blank control, and A_{x0} is the absorbance of the solution without the developer H_2O_2.

Superoxide anion radical scavenging assay

The superoxide anion ($O_2^{\bullet-}$) radical scavenging activity of test compounds was determined using the method reported previously.[33] Briefly, 0.3 mL of 3 mM pyrogallic acid and 4.2 mL of sample solution at different concentrations were added into 4.5 mL of Tris-HCl buffer (pH 8.2, 0.1 M), which was preincubated for 20 min at 25°C. After mixing thoroughly, the absorbance at 325 nm was read immediately after every 30 s up to 3 min at 25°C against 10 mM HCl solution used as a blank. As a control, 4.2 mL distilled water was replaced with the sample solution. The superoxide radical activity to scavenging was estimated as follows:

Radical scavenging (%) = $(K_0 - K_1)/K_0 \times 100$

Where K_0 was the autoxidation rate of pyrogallol in the control group (ΔOA/min) and K_1 was the autoxidation rate of pyrogallol in the sample (ΔOA/min).

Reducing power assay

Reducing power of the sample was determined following the method previously reported[34] with some modifications. Briefly, the reaction mixture consisted of 2.5 mL of potassium phosphate buffer (0.2 M, pH 6.6), 2.5 mL of 1% potassium ferricyanide (w/v), and 2.5 mL of sample solution of various concentrations. After preincubation for 20 min at 50°C, 10% trichloroacetic acid (w/v, 1.0 mL) was added into the mixture and then centrifugated at 3000 rpm for 10 min. Then, 2.5 mL of the supernatant was combined with 0.5 mL of 0.1% ferric chloride (w/v) and 2.0 mL of distilled water. The absorbance was read at 700 nm by UV-Vis spectrophotometer against distilled water used as the blank.

Statistical analysis

All the assays were performed in triplicates, and data analysis was performed using SPSS 23.0 software (SPSS Inc., Chicago, USA). Analysis of variance was used to analyze differences among groups. Differences resulting in $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Effect of extraction time on the yield of *Grateloupia filicina* polysaccharides

The yield of GFPs was affected by the different extraction times [Figure 1a], when other extraction conditions were kept constant (temperature = 90°C and water-to-raw material ratio = 70 mL/g). According to the results, the yield of GFPs increased rapidly from 1 to 3 h, reaching a maximum at 5 h. Longer extraction time allowed for the greater extraction of GFPs; however, excessive time may result in the degradation of GFPs. Thus, 3–5 h was chosen as the optimum extraction time for further analysis.

Effect of extraction temperature on the yield of *Grateloupia filicina* polysaccharides

The yield of GFPs was affected by various temperatures of extraction [Figure 1b]. First, extraction temperature was set at 60°C, 70°C, 80°C, 90°C, and 100°C and the other extraction conditions were constant (time of extraction = 1 h and water-to-raw material ratio = 70 mL/g). Our results showed that the yield of GFPs continued to increase till at 90°C to a maximum and then decreased after the temperature exceeded 90°C. Excessive temperature may lead to changes in the structure of the polysaccharides, thereby decreasing the yield. Thus, we chose 80°C–100°C as the optimum temperature for further experiments.

Effect of ratio of water to raw material on the yield of *Grateloupia filicina* polysaccharides

The yield of GFPs was affected by different ratios of water to raw material [Figure 1c]. In order to optimize this variable, other variables were kept constant (time = 1 h and temperature = 90°C). With an increasing ratio of water to raw material, the yield of GFPs also increased which reached a maximum of 42.01% at 100 mL/g and then dropped slightly. This may be attributed to the fact that an appropriate ratio of water to raw material contributes to the full expansion and rapid diffusion of the polysaccharides so that it can enhance the yield of GFPs. For efficient energy- and cost-saving extraction procedure, 90–110 mL/g was chosen as the optimum ratio for further experiments.

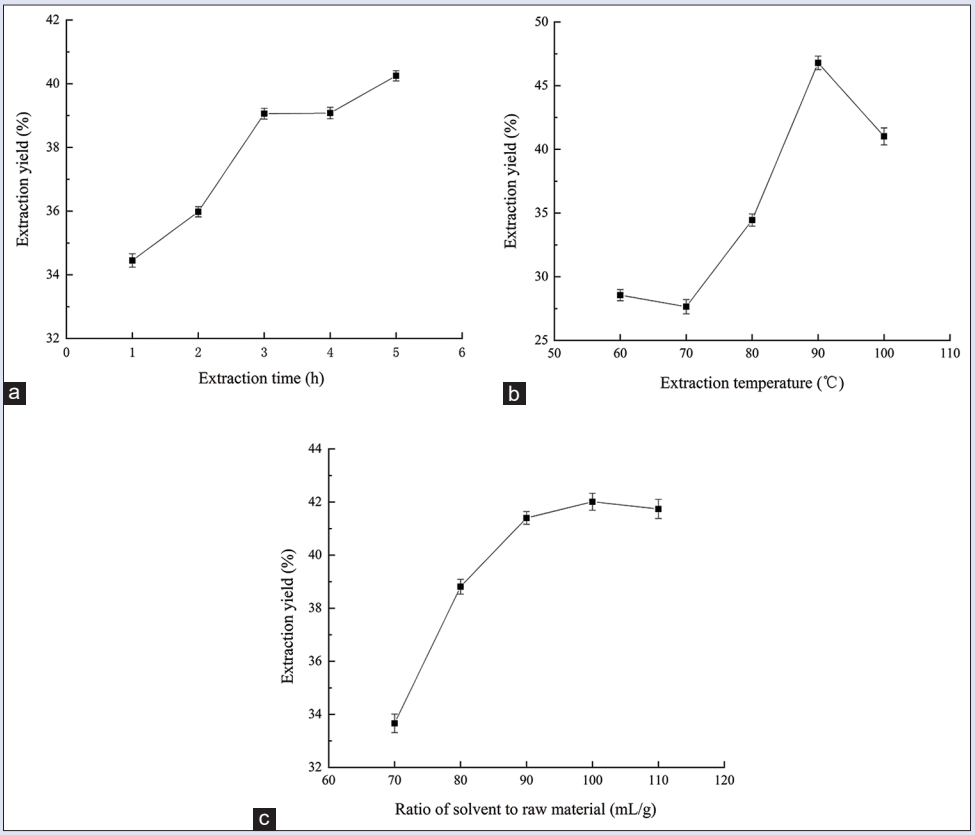

Figure 1: Effects of extraction time (a), extraction temperature (b), ratio of water to raw material (c) on the extraction yield of polysaccharides of *Grateloupia filicina*

Table 2: Analysis of the results of orthogonal experiment

Number	A Extraction temperature (°C)	B Ratio of water to raw material (mL/g)	C Extraction time (h)	Extraction yield of GFPs (%)
1	1	1	1	32.22
2	1	2	2	41.37
3	1	3	3	42.18
4	2	1	2	44.49
5	2	2	3	50.34
6	2	3	1	42.93
7	3	1	3	40.11
8	3	2	1	37.88
9	3	3	2	43.89
k_1	38.60	38.94	37.68	
k_2	45.92	43.20	43.25	
k_3	40.63	43.00	44.21	
R	7.32	4.26	6.53	

GFPs: Polysaccharides of *Grateloupia filicina*

Optimization of extraction conditions of *Grateloupia filicina* polysaccharides

The extraction parameters may affect the yield of GFPs and might cause it to degrade, thereby making them lose their bioactivity and pharmacological activity. Thus, based on the results of single-factor experiments, optimization of the suitable parameters to obtain the maximum yield of bioactive polysaccharides can be performed by employing an orthogonal experiment. The extracts obtained from various tests in the extraction of GFPs were quantitatively analyzed, and the extraction yields of different extracts were calculated. Owing to various combinations of extraction parameters, it is obvious from Table 2 that there were certain differences in yields based on various orthogonal tests. Based on the results (R_A [7.32] > R_C [6.53] > R_B [4.26]), the temperature of extraction was found to be the most important determinant of the yield of GFPs. The optimum combination of variables was $A_2B_2C_3$, that is, extraction temperature as 90°C, extraction time of 5 h, and the ratio of water to raw material of 100 mL/g. Through the confirmatory test, the extraction yield of GFPs was 50.34% ±0.27% of the dry material.

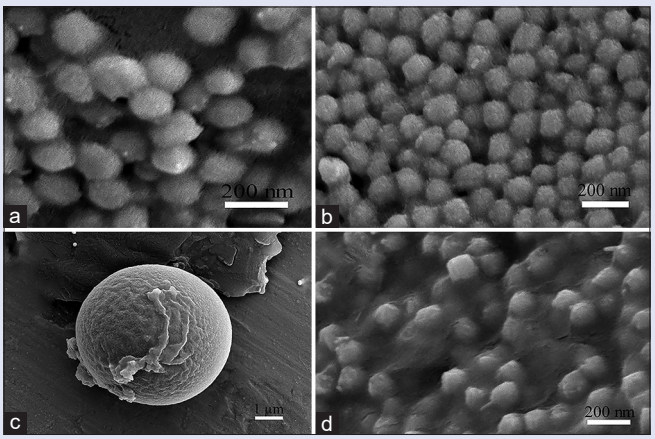

Figure 2: Scanning electron microscope images of selenium nanoparticles in the presence of polysaccharides of *Grateloupia filicina* aqueous solutions in (a and b); scanning electron microscope images of selenium nanoparticles in the absence of polysaccharides of *Grateloupia filicina* aqueous solutions in (c and d)

Morphology and size of selenium nanoparticles stabilized by *Grateloupia filicina* polysaccharides

In the formulation process of GFPs-SeNPs, the polysaccharides and sodium selenite solution were first mixed well. Next, we added ascorbic acid into the reaction mixture to reduce the SeO_3^{2-} to selenium atoms. As the concentration of selenium atoms increases, the atomic selenium immediately aggregates into SeNPs under the stabilization of GFPs. By monitoring color changes in solution, this aggregation caused by the reduction of sodium selenite by ascorbic acid can be clearly observed. The color of the solution gradually changed from colorless to yellow and finally showed orange-red. SEM was used to measure the morphology and size of the GFPs-SeNPs. As shown in Figure 2, uniform spherical SeNPs were obtained in the presence of GFPs [Figure 2a and b], whereas selenium particles agglomerated in the absence of GFPs [Figure 2c and d]. The average particle size of GFPs-SeNPs was about 100 nm, which is significantly smaller than that of SeNPs. It confirmed that elemental selenium could be stabilized by GFPs. Indeed, there are reactive carboxyl and hydroxyl groups in the chemical structure of polysaccharides, which have a great effect on the formation, growth, and stabilization of SeNPs.[35] These results demonstrated that GFPs play an important role in the formation of uniform spherical nanoparticles and prevent the formation of aggregates of SeNPs. According to the previous reports, the morphology of gum arabic-stabilized SeNPs was spherical in shape,[36] whereas the SeNPs prepared with 0.1% chitosan solution were rod-like and elliptical in shape.[37] Thus, it could be concluded that polysaccharides exert different effects on the formation and growth of SeNPs.

Antioxidant activities

In vitro experiments have shown that SeNPs with the size of 5–200 nm can scavenge free radicals in a size-dependent manner.[18] In this study, the size of SeNPs can be reduced remarkably by adding GFPs as the modifier and the stabilizer. Hence, the GFPs-SeNPs were proved to possess stronger antioxidant activity compared to GFPs and SeNPs.

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

The stable lipophilic free- and nitrogen-centered DPPH radical has been conventionally used to investigate the scavenging activity of antioxidants.[38] The mechanism of DPPH radical scavenging activity is based on the reduction of ethanolic DPPH solution in the presence of hydrogen-donating antioxidants as a result of the formation of the nonradical form of DPPH-H by the reaction.[39] The DPPH radical scavenging activities of GFPs, SeNPs, and GFPs-SeNPs all enhanced with the increase of concentration [Figure 3a]. GFPs-SeNPs exhibited less scavenging activity than that of ascorbic acid (reference compound), but it showed stronger DPPH radical scavenging effect than GFPs and SeNPs. At a concentration of 2.0 mg/mL, the scavenging activity of GFPs-SeNPs on DPPH radical was around 93.4%. The values of 50% inhibitory concentration (IC_{50}) (mg/mL) for SeNPs and GFPs-SeNPs were 0.662 and 0.364, respectively. These results indicated that GFPs-SeNPs are more effective DPPH radical scavengers than GFPs and SeNPs. This might be due to the difference in hydrogen-donating abilities. The introduction of hydroxyl groups enhances the interaction between the radicals and SeNPs.

Hydroxyl radical scavenging activity

HO•s are generated during the process of oxidative metabolism and attacking most biological matrices, thereby destabilizing them.[40] Compared with other free radicals, HO•s are the most reactive under *in vivo* conditions. It can easily react with biomolecules such as proteins, DNA, lipids, and carbohydrates and may cause cell death or tissue damage.[41] Therefore, scavenging HO•s is very important to protect cellular structures and function. In this experiment, the HO• was generated by a Fenton reaction which reacts with salicylic acid to form a colored product. The formation of the colored product can be reduced by adding a HO• scavenger. SeNPs and GFPs-SeNPs were effective in scavenging HO• in a dose-dependent manner (0.00–1.00 mg/mL) [Figure 3b]. The ability of GFPs-SeNPs to scavenge HO•s was significantly stronger than that of GFPs. These results show that the polysaccharides stabilized SeNPs and significantly improved the scavenging activities of HO•s compared with the polysaccharides.

Superoxide anion radical scavenging activity

$O_2^{\bullet-}$, precursors of various reactive oxygen species, may damage the DNA leading to various diseases.[42] They are known to cause ischemia–reperfusion injury[43] and form other detrimental free radicals such as hydrogen peroxide and HO•, which trigger free radical chain reactions. Figure 3c shows the scavenging abilities of GFPs, SeNPs, and GFPs-SeNPs against $O_2^{\bullet-}$. The scavenging activity of GFPs-SeNPs against $O_2^{\bullet-}$ was found to be weaker than that of the positive control ascorbic acid but was stronger than GFPs and SeNPs. Moreover, GFPs-SeNPs effectively scavenged $O_2^{\bullet-}$ in a dose-dependent manner (0.05–0.15 mg/mL). At a concentration of 0.15 mg/mL, the scavenging ability of GFPs, SeNPs, and GFPs-SeNPs was found to be 13.39%, 78.67%, and 85.33%, respectively. The IC_{50} values (mg/mL) for SeNPs and GFPs-SeNPs were 0.113 and 0.083, respectively. These results suggested that GFPs-SeNPs are more effective scavengers of $O_2^{\bullet-}$ than that of GFPs and SeNPs. A positive correlation was observed between the antioxidant activity (IC_{50} value) obtained by DPPH and HO• (r = 0.9996), DPPH and $O_2^{\bullet-}$(r = 0.9877), and HO• and $O_2^{\bullet-}$ (r = 0.9874) for test compounds. These results clearly show that GFPs-SeNPs possess strong hydrogen-donating capacity, which also explains its antioxidant activity.

Reducing power assay

Antioxidants quench the free radicals by transferring their electrons to the free radicals. The electron-donating property of antioxidants is usually evaluated by the reducing power assay. The reducing power activity of test compounds was detected by the potassium ferricyanide reduction method. The Fe^{3+}/ferric cyanide complex is reduced to ferrous form and subsequently changed from yellow to blue-green.[44] An increase in the absorbance at 700 nm indicates increasing reducing

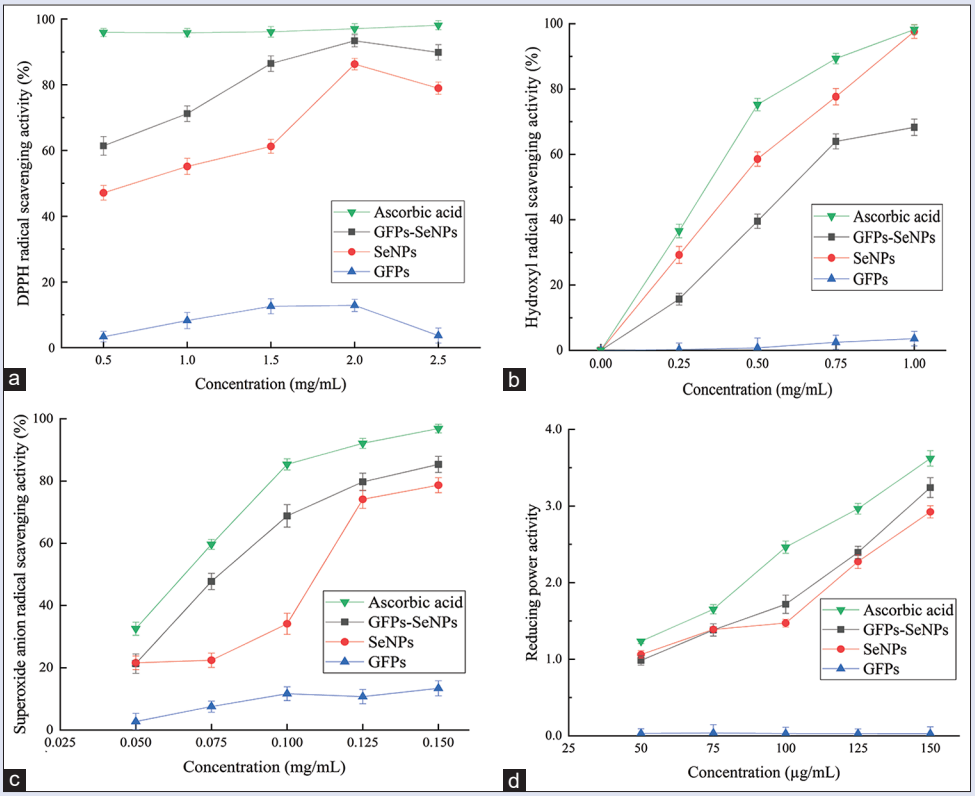

Figure 3: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (a), hydroxyl radical scavenging activity (b), superoxide anion radical scavenging activity (c), and reducing power (d) of samples

power. The intensity of the color formed by the reaction mixture shows the reducing power of the test compounds, which is highly correlated with its antioxidant activity. GFPs-SeNPs displayed stronger reducing power than that of GFPs and SeNPs [Figure 3d]. These results show that GFPs can significantly enhance the stability of the nanoparticles by donating its electron to the free radical.

CONCLUSION

In this study, an efficient method of extraction of GFPs has been developed. The optimum conditions for the extraction of GFPs were as follows: time, 5 h; extraction temperature, 90°C; and the ratio of water to raw material 100 mL/g. Under these optimal conditions, the yield was 50.34% ±0.27%. SeNPs were prepared using GFPs as the modifier and stabilizer. The morphology and size of GFPs-SeNPs were characterized by SEM. The SEM images revealed that the structure and shape of GFPs-SeNPs were uniform and they have the ability to prevent the formation of aggregates of SeNPs. The antioxidant activity of GFPs-SeNPs *in vitro* was evaluated by DPPH radical scavenging assay, HO• scavenging assay, $O_2^{\bullet-}$ scavenging assay, and via reducing power assay. According to our results, GFPs-SeNPs possess the powerful antioxidant capacity and could be further explored as a dietary supplement or has medicinal applications. However, a further pharmacological and chemical investigation should be performed to investigate the mechanism of pharmacological activities of GFPs-SeNPs.

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Conflicts of interest

There are no conflicts of interest.

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