

Green Synthesis of Gold Nanoparticles Using Polyphenols from Pomelo Leaves and its Antibacterial Properties

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Abstract

Gold nanoparticles (AuNPs) were synthesized by one-step reduction of chloroauric acid using pomelo leaf polyphenol extract as reducing and protective agent. The AuNPs were characterized by UV-Vis absorption spectroscopy. Compared with AuNPs prepared with sodium citrate as reducing agent, AuNPs prepared with pomelo leaf polyphenol extract had better stability. The antioxidant properties of green-synthesized AuNPs were investigated by Bland-Williams method. The antibacterial activity of the green synthesized AuNPs was investigated by the transparent inhibition zone method and the minimum inhibitory concentration method. The results showed that the AuNPs prepared with pomelo leaf polyphenol extract as biological template had good antioxidant activity. The scavenging rate of 1, 1-diphenyl-2-trinitrophenylhydrazide free radical (DPPH•) was 95.65% when the 1.0 g/L AuNPs solution was used. The antibacterial activity of AuNPs synthesized from pomelo leaf polyphenol extract against *Staphylococcus aureus* was higher than that of AuNPs synthesized chemically. The minimum inhibitory concentration of green synthesized AuNPs was 0.85 g/L, while the minimum inhibitory concentration of chemically synthesized AuNPs was 1.58 g/L.

Keywords

Polyphenol Extract; Pomelo Leaf; Gold Nanoparticles; Antioxidant Activity; Antibacterial Activity.

1. Introduction

Nanomaterials refer to materials with 1-100 nm scale characteristics in structure, among which metal nanomaterials mainly include pure noble metals such as gold nanoparticles, silver nanoparticles, platinum nanoparticles, metal alloys, and organic and inorganic composite metal nanomaterials. Gold nanoparticles (AuNPs) have gradually become the focus of research due to their outstanding surface plasmon resonance, catalytic, electrochemical and molecular recognition properties, and have been widely used in catalysis, optoelectronics and biomedicine [1-3].

With the research of green synthesis becoming more and more active, the green preparation of gold nanoparticles has attracted people's attention. At present, the preparation methods of nano-metal materials mainly include physical method, chemical method and biosynthesis method [4-6]. Physical method refers to the physical method of crushing raw materials into ultra-fine materials, so that they reach the nanometer scale in size. This method has the advantages of low cost, high yield and simple process, but its energy consumption is high, the particle size uniformity is different, and the storage stability is not high. Chemical method refers to the formation of nanoparticles through chemical reactions between substances. At present, it mainly includes aqueous redox method, hydrothermal method, microwave synthesis method, etc. The nanomaterials synthesized by chemical method have the characteristics of high particle

size uniformity and clear chemical composition. But the surface of the material is easy to have impurities, and it is difficult to remove. The synthesized AuNPs are stable

Its qualitative is not strong, and organic solvents are needed in its synthesis, which has high toxicity and low conversion rate, so it is difficult to meet the requirements of green chemistry. The biosynthesis method is the reduction of gold nanoparticles using microorganisms or plants as reducing and protective agents. The biosynthesis method has the advantages of simple operation, non-toxic and environmental protection, and the synthesized gold nanoparticles have good stability and biocompatibility, which is conducive to its application in the field of biomedicine [7-10].

Pomelo is a rutaceae plant. Pomelo leaves are rich in polyphenols, flavonoids and other active ingredients, which have anti-inflammatory and anti-cancer effects. The essential oils in the extracts of pomelo leaves, such as thymol, perillyl alcohol and linalool acetate, have certain antibacterial effects. Pomelo leaves were extracted with different solvents, and the results of the study showed that the ethyl acetate fraction had the best antibacterial effect, while the extracts extracted with ethanol, petroleum ether, acetone and diethyl ether had poor antibacterial effect [11-13].

In this study, AuNPs were prepared in a one-step green method using pomelo leaf polyphenol extract as a biological template. The AuNPs were characterized by UV-Vis absorption spectroscopy. The antioxidant properties of AuNPs were investigated. The antibacterial effects of AuNPs synthesized from pomelo leaf polyphenol extract and chemically synthesized AuNPs against *Staphylococcus aureus* in the same particle size range were investigated using the transparent inhibition zone method and the minimum inhibitory concentration method.

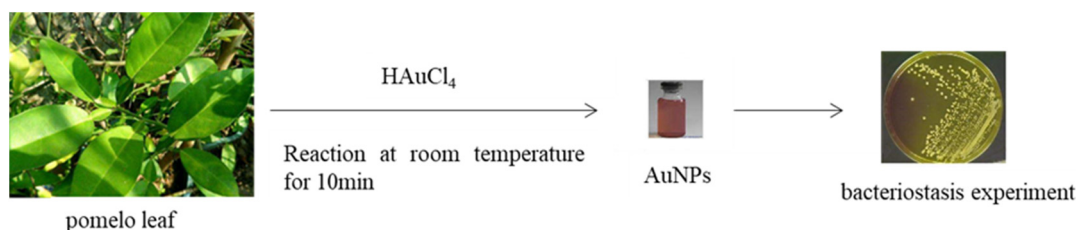


Fig 1. Study flow chart

2. Materials and Methods

2.1. Preparation of Pomelo Leaf Polyphenol Extract

Pick fresh pomelo leaves, dry them for 6 hours and grind them into powder. 50 g of pomelo leaf powder was weighed, and 95 mL pure water was added. After stirring at room temperature for 30 min, the filtrate was filtered with 0.22 μm water filter membrane, and the water extract was collected. The volume was fixed to 100 mL to obtain the water extract, and the mass concentration of the water extract was 0.5 g/mL.

2.2. Green Synthesis of Aunps from Polyphenol Extracts of Pomelo Leaves

AuNPs were prepared by mixing 100 μL of pomelo leaf polyphenol extract with 25 μL of 0.01% chloroauric acid solution, adding water to 2.5 mL, heating at 70 $^{\circ}\text{C}$ for 30 min, and stirring at a constant speed.

2.3. AuNPs Synthesized by Traditional Chemical Methods

AuNPs were synthesized using sodium citrate as reducing agent. After 50 mL 0.01% chloroauric acid solution was heated to boiling, 1.5 mL 1% sodium citrate solution was quickly added, heated for 10 min with stirring, and cooled to room temperature to prepare AuNPs, whose maximum absorption wavelength was 532 nm.

2.4. Evaluation of Antioxidant Activity

The antioxidant capacity of AuNPs was determined by DPPH• using the Bland-Williams method. The 3.943 mg DPPH• powder was accurately weighed and dissolved in 100 mL absolute ethanol to obtain 1 mmol/L DPPH• solution. Solutions of AuNPs at concentrations of 0, 0.5, 1.0, 1.5, 2.0 and 2.5 g/L were used. 2 mL AuNPs with different mass concentrations were added to 2 mL DPPH• solution, mixed evenly and stored for 1 h in the dark. The absorbance of the mixed solution of AuNPs and DPPH• was measured at 524 nm with absolute ethanol set to zero. The absorbance A_j of 2 mL ethanol mixed with 2 mL AuNPs solution and the absorbance A_0 of 2 mL ethanol mixed with 2 mL DPPH• solution was also measured. Three parallel experiments were averaged.

2.5. Determination of Antimicrobial Activity

The antibacterial properties of the green AuNPs in this experiment were determined by filter paper method. 25 mL LB broth solid medium (0.5 g LB broth powder and 0.8 g agar powder were accurately weighed and dissolved in 25 mL deionized water) was placed in a conical flask. The mouth of the flask was sealed with aluminum foil, and then the conical flask was placed in a pressure steam pot for sterilization for 20 min (0.1 MPa, 0. 121.5 °C). In a sterile environment, the sterilized broth solid medium was poured into a sterile plate, cooled and solidified. Then, 100 μ L suspension of *Staphylococcus aureus* (single colonies were picked from agar medium containing *Staphylococcus aureus* using an inoculation ring in a sterile environment, inoculated into 25 mL LB liquid broth medium, sealed, placed on a shaker for 6 h at 37 °C) was injected onto the medium and evenly coated. One piece of filter paper was placed in the middle of the plate containing bacteria, and then the medium was placed in an incubator at 37 °C for 12 h. After multiple experiments, the diameter of the inhibition zone was measured by the cross method. Three parallel experiments were averaged.

2.6. Determination of the Minimum Inhibitory Concentration (MIC)

Different masses of green synthesized AuNPs powders were ultrasonically dispersed in 10 mL sterile water to obtain AuNPs solutions with mass concentrations of 0, 0.25, 0.50, 1.00, 1.50, 2.50 and 5.00 g/L. Under sterile conditions, 90 μ L of sterilized liquid broth medium (weighing 0.5 g LB broth powder dissolved in 25 mL deionized water) and 20 μ L of 1×10^7 CFU/mL *Staphylococcus aureus* suspension were added to the well of a sterile 96-well plate. After slight shaking, 90 μ L of AuNPs solution with different mass concentrations were dropped into each well, and then 96-well plates were encapsulated and placed at 37 °C for 12 hours. A total of 100 μ L bacterial suspension was aspirated and evenly spread on agar solid medium (0.8 g agar powder dissolved in 25 mL deionized water) for 24 hours to check whether there was bacterial growth on agar medium, and the minimum inhibitory concentration was the first sample concentration with single colony plate. Three parallel experiments were averaged. Similarly, the minimum inhibitory concentration of chemically synthesized AuNPs particles was determined using the method described above.

3. Results

3.1. Characteristics of Gold Nanoparticles

The AuNPs solution prepared with pomelo leaf polyphenol extract was purple red, and the UV-vis absorption spectrum showed that the absorption peaks of pomelo leaf polyphenol extract were located at 220 nm and 277 nm (polyphenolic compounds), and the UV-vis absorption spectrum of prepared AuNPs was in the UV-vis absorption spectrum. The absorption peak at 220 nm and 277 nm disappeared, and the absorption peak appeared at 536 nm, which was the unique surface plasmon resonance (SPR) characteristic of AuNPs, see [Fig. 2](#).

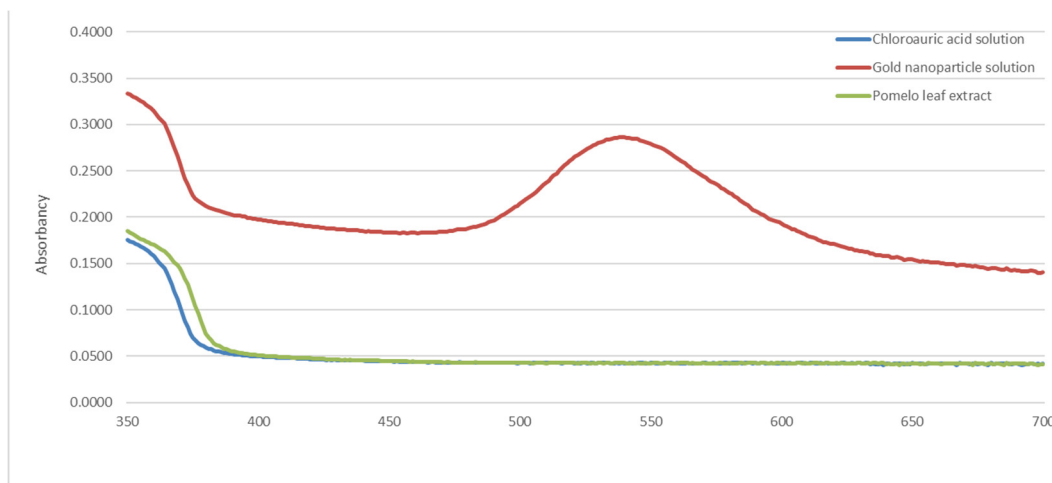


Fig 2. Uv-vis absorption spectra of polyphenol extracts from pomelo leaves and AuNPs

3.2. Analysis of Antioxidant Properties

The antioxidant capacity of the tested substance can be indirectly understood by measuring the absorbance change of DPPH• at 524 nm with a spectrophotometer. AuNPs could scavengers DPPH• and there was a significant dose-response relationship, which indicated that AuNPs prepared from pomelo leaf polyphenol extract had good antioxidant activity. When the mass the concentration of AuNPs reached 1.0 g/L, the DPPH• scavenging rate reached 95.96%. This is mainly due to the hydrogen supply capacity of residual DMY on AuNPs, where hydroxyl groups on DMY can provide hydrogen ions to neutralize free radicals and prevent the oxidation of lipids, proteins, and nucleic acids, thereby reducing cell damage caused by oxidative stress.

3.3. Antimicrobial Analysis

Transparent inhibition zone method is one of the most widely used methods to measure bacterial drug sensitivity, which has the advantages of convenient operation, low cost and accurate results. The combination of clear inhibition zone method and minimum inhibitory concentration method can determine the antibacterial activity of drugs intuitively and accurately. The antibacterial activity of green synthesized AuNPs and chemically prepared AuNPs is shown in FIG. 3. Inhibition circles appeared on the culture medium, indicating that AuNPs synthesized using pomelo leaf polyphenol extract had an inhibitory effect on *Staphylococcus aureus*. In contrast, the antibacterial effect of chemically prepared AuNPs was less pronounced. This indicates that the antibacterial effect of AuNPs prepared using the polyphenol extract of pomelo leaves is higher than that of chemically synthesized AuNPs. It has been shown that the enhanced bioactivity of small particles is mainly due to the decrease of particle size and the increase of specific surface area.

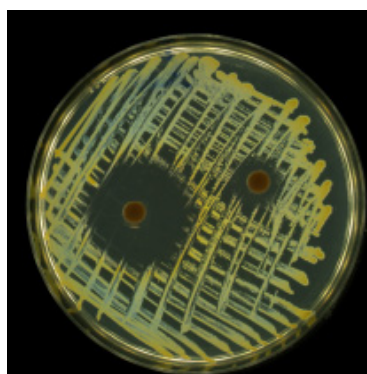


Fig 3. Pictures of the antibacterial activity of green-synthesized AuNPs (left) and chemically prepared AuNPs (right)

The MIC test showed that the MIC of chemically synthesized AuNPs was 1.58 g/L, while the MIC of AuNPs synthesized by camellia luteolin was 0.85 g/L. It was further demonstrated that AuNPs prepared from camellia cambogia flavone had higher antibacterial activity.

4. Conclusion

In this study, the AuNPs were synthesized using the polyphenol extract of naringenia leaf as a reducing and protective agent. The method is fast, simple, green and environmentally friendly. Compared with AuNPs prepared by the traditional method, AuNPs prepared by this method have better stability. AuNPs with good antioxidant and antibacterial activities can be prepared by using pomelo leaf polyphenols extract, which is conducive to promoting the application of AuNPs in agriculture, food and medical safety.

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