

Inhibition of *Porphyromonas Gingivalis* by Gold Nanoparticles Green Synthesized from *Artemisia Selengensis* Turcz Polyphenol

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Abstract

At present, many chemical methods have been proposed for the synthesis of gold nanoparticles, but the green method for the synthesis of gold nanoparticles has received more attention due to its low cost and environmental friendliness. In this study, gold nanoparticles (AuNPs) were synthesized from *Artemisia selengensis* Turcz polyphenol extract and their inhibitory effects on *Porphyromonas gingivalis* were investigated. The formation of gold nanoparticles was determined by the appearance of a peak at 540 nm in the UV-visible spectrum. The minimum inhibitory concentration (MIC) of gold nanoparticles against *P. gingivalis* was determined by concentration gradient dilution method. The minimum bactericidal concentration (MBC) of gold nanoparticles against *P. gingivalis* was determined by blood plate test. The MIC and MBC of gold nanoparticles were 12.3 µg/mL and 32.8 µg/mL against *P. gingivalis*, respectively. In summary, gold nanoparticles can be prepared from *Artemisia selengensis* Turcz polyphenol extract and may be used for the treatment of *P. gingivalis* related oral diseases.

Keywords

Green Synthesis; Gold Nanoparticles; *Artemisia Selengensis* Turcz Polyphenol; *Porphyromonas Gingivalis*; Antibacterial Effect.

1. Introduction

In the field of stomatology, bacterial infection is one of the main causes of various oral diseases. *Porphyromonas gingivalis* is a common pathogenic bacteria, which is closely related to the development of periodontitis and periodontal diseases. Inhibition of the growth of *P. gingivalis* has become one of the important strategies for the prevention and treatment of oral diseases[1].

In recent years, the application of nanotechnology in the medical field has attracted increasing attention. Gold nanoparticles are fabricated nanomaterials with many unique physical and chemical properties[2]. It is composed of nano-sized gold particles with high specific surface area, excellent biocompatibility, and tunable surface properties. These properties make gold nanoparticles a potential antibacterial material that can be used to inhibit the growth of oral pathogens[3-5].

The aim of this study was to investigate the potential of gold nanoparticles prepared from artemisinin polyphenols to inhibit the growth of *gingivalis* SPP. To prepare gold nanoparticles (AuNPs) by green synthesis of *Artemisia selengensis* Turcz polyphenols and characterize their physical and chemical properties. Subsequently, antibacterial tests were performed to evaluate the inhibitory effect of gold nanoparticles against *P. gingivalis*.

The results of this study are expected to provide new solutions for the prevention and treatment of oral diseases in the field of stomatology. A deeper understanding of the properties of gold

nanoparticles and their mechanism of action against *P. gingivalis* can provide a theoretical basis for the development of more effective oral antibacterial materials and make a positive contribution to the maintenance of oral health. At the same time, this study also provides a new case for the application of nanotechnology in the medical field and opens up new research directions for the development and application of nanomaterials.

2. Materials and Methods

2.1 Green Synthesis of Gold Nanoparticles by Artemisia Quinoa Polyphenols

Fresh *Artemisia selengensis* Turcz leaves were washed and mashed, then 95ml pure water was added, boiled for 30min, and centrifuged for separation. The supernatant was collected, constant volume to 100ml, stored at -20 °C, and thawed before use. In a 50ml conical flask, 1ml of 1.0g/ml of *Artemisia selengensis* Turcz polyphenol extract and 0.3ml of 0.01g/ml of chloroauric acid solution were added in a constant volume of water to 10 mL, slowly heated to 75 °C for 15 minutes with constant stirring during the reaction. The precipitate was removed, an appropriate amount of distilled water was added, the centrifugation was repeated twice, the supernatant was removed, and the precipitate was dried in an oven at 50 °C to obtain gold nanoparticles.

2.2 Gold Nanoparticles Characterization

For ultraviolet visible (UV-Vis) absorption spectra, uncentrifuged AuNPs were diluted 50 times with distilled water, and the distilled water was used as the blank group. The UV-vis absorption spectra were measured in the wavelength range of 280-750 nm.

2.3 Antibacterial Test

2.3.1 Resuscitation Cultures of *P. Gingivalis*

P. gingivalis was inoculated onto Columbia Blood Agar plates and cultured in an anaerobic tank containing anaerobic gas producing bag at 37 °C for 5-7 days. The size, shape, and color of the colonies growing on the surface of the medium were observed. After dark gray colonies grew on the surface of the medium, colonies with a diameter of about 1 mm were picked and inoculated in BHI medium, and cultured anaerobically for 2-3 days to logarithmic phase for subsequent experiments.

2.3.2 Determination of MIC and MBC

The gold nanoparticles solution was diluted with BHI medium using a concentration gradient dilution method. *P. gingivalis* colonies were inoculated in BHI medium and cultured at 37 °C for 2-3 days to the logarithmic phase, and the concentration was adjusted to 1×10^7 CFU/mL. The *P. gingivalis* colonies were diluted 1:100 with BHI medium and inoculated into 96-well plates. The final concentration of gold nanoparticles was 0, 0.488, 0.977, 1.953, 3.906, 7.812, 15.625, 31.25, 62.5, 125, 250, 500 µg/mL. Each group was set with three compound holes, sealed and placed in an anaerobic tank, and incubated at 37°C for 48 hours. The optical density (OD) value of bacterial solution at 600nm was detected by microplate reader, and the minimum drug concentration in the hole that completely inhibited bacterial growth was the MIC.

P. gingivalis solution was adjusted to the concentration of 1×10^8 CFU/mL and inoculated on Columbia Blood Agar plate, and different concentrations of gold nanoparticles (0, 7.812, 15.625, 31.25, 62.5, 125 µg/mL) were placed in an anaerobic tank and incubated in a constant temperature incubator at 37°C for 7 days to observe the growth of bacteria. The concentration of gold nanoparticles on the blood plate without bacterial growth was defined as MBC.

2.4 Statistical Analysis

SPSS 23.0 statistical analysis software was used for data analysis. T-test was used for comparison between two groups, and One-wayANOVA was used for statistics between multiple groups. $P < 0.05$ was considered statistically significant.

3. Results

Generally speaking, different nanoparticles will have different positions of absorption peaks in UV-Vis spectra. For example, copper nanoparticles will have a strong absorption peak at 600-650 nm, gold nanoparticles will have a strong absorption peak at 500-600 nm, and silver nanoparticles will show a strong absorption peak at about 410 nm. Therefore, the type of nanoparticles prepared can be determined by UV-Vis spectroscopy. At the same time, the size and morphology of nanoparticles in the same sol can be judged by the position and shape of the peak.

In this study, *Artemisia selengensis* Turcz polyphenol extract was used as a gold ion reducing agent and stabilizer. Gold nanoparticles were prepared by adding 5, 10, 15, and 20 mL of the extract, and the spectra were scanned with UV-Vis at wavelengths from 280 to 750 nm. All the prepared samples showed a relatively strong absorption peak near 540 nm, indicating the appearance of gold nanoparticles in the solution. The highest absorbance value and obvious blue shift were observed in the gold nanoparticles prepared using 10 mL extraction solution, indicating that the gold nanoparticles prepared under this condition had the highest precipitation rate and the smallest particle size.

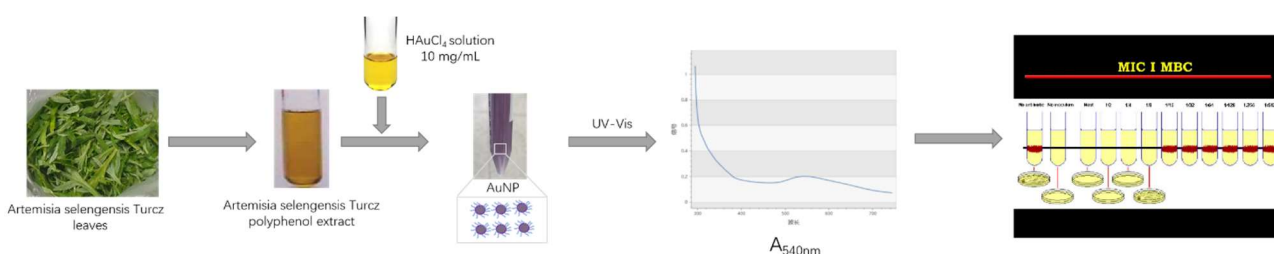


Fig. 1 Study flow chart

To quantitatively investigate the antibacterial effect, the minimum inhibitory concentrations of AuNPs were determined. The minimum inhibitory concentration of *P. gingivalis* was 12.3 µg/mL, and the minimum bactericidal concentration was 32.8 µg/mL. Compared with AuNPs reported previously, gold nanoparticles prepared from *Artemisia annua* polyphenol extract showed better antimicrobial activity, which may be due to the combined effects of multiple active substances in *Artemisia selengensis* Turcz polyphenol extract. It is known that the antibacterial ability of AuNPs is also related to their size, shape and capping agent. Large nanoparticles allow a large surface area to contact bacterial cells, implying that small particles may have higher interactions than larger particles. The small size of the nanoparticles prepared in this study may be one of the reasons for the good antibacterial activity. In addition, the antibacterial effect of AuNPs prepared in this study was better than that of most AuNPs prepared using plant extracts, which may be due to the synergistic antibacterial effect of the active substances in the *Artemisia selengensis* Turcz polyphenol extract and AuNPs. Therefore, gold nanoparticles synthesized using the polyphenol extract of *Artemisia selengensis* Turcz have potential clinical and medical applications.

4. Conclusion

In this study, AuNPs were successfully prepared from *Artemisia selengensis* Turcz polyphenol and demonstrated the potential to inhibit *P. gingivalis*. The antibacterial test showed that gold nanoparticles modified by *Artemisia selengensis* Turcz polyphenol could significantly inhibit the growth of *P. gingivalis*. This indicates that the antibacterial properties of gold nanoparticles are enhanced by the modification of *Artemisia selengensis* Turcz polyphenol. The results of this study provided potential candidates for the development of oral antibacterial agents and new strategies for the treatment of periodontal diseases. However, further studies are needed to gain insight into the mechanism of interaction between gold nanoparticles and *P. gingivalis* and to evaluate their safety

and biocompatibility in stomatology. It is hoped that the findings of this study will provide new directions and approaches for disease prevention and treatment in the field of oral health.

Acknowledgments

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