Antimicrobial and Antifungal Activities of Bletia Purpurea-Mediated Gold Nanoparticles

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Abstract:

The development of antimicrobial resistance and the requirement for new antifungal agents have led to the investigation of alternative approaches for the treatment of microbial infections. In this research, we describe the synthesis, characterization, and assessment of antimicrobial and antifungal properties of gold nanoparticles (AuNPs) mediated by Bletia purpurea. The orchid extract was used as a reducing and capping agent.

The AuNPs were characterized by UV-Vis spectroscopy, transmission electron microscopy (TEM), energy-dispersive spectroscopy (EDS), and X-ray diffraction (XRD). The AuNPs were found to be spherical in shape with an average size of 20 nm and a surface plasmon resonance peak of 530 nm.

The antimicrobial potential of AuNPs was assessed against a number of bacterial strains, namely Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae, by the broth microdilution technique. The AuNPs were found to possess strong antimicrobial potential with minimum inhibitory concentrations ranging from 0.1 to 1.5 μg/mL.

The antifungal properties of AuNPs were tested against various fungal strains such as Candida albicans, Aspergillus niger, and Fusarium oxysporum using the broth microdilution technique. The AuNPs showed strong antifungal properties with MIC values ranging from 0.05 to 1.0 μg/mL.

Mechanistic studies showed that AuNPs caused membrane damage, oxidative stress, and DNA fragmentation in microbial cells, resulting in cell death. The AuNPs also showed synergistic properties with existing antibiotics, increasing their potency against resistant microorganisms.

The efficacy of Bletia purpurea-mediated AuNPs has been shown in this study to have potential as a novel antimicrobial and antifungal agent, providing a promising approach to counteract microbial infections and the rising problem of antimicrobial resistance.

Introduction:

The quick development of antimicrobial resistance (AMR) has become a major threat to global health, food security, and economic development. The World Health Organization (WHO) has listed antimicrobial resistance as one of the top 10 global health threats to humanity, with an estimated 700,000 deaths per year due to antimicrobial resistance. The problem is further worsened by the slow development of new antimicrobial compounds, thus emphasizing the need for new approaches to tackle microbial infections.

Recently, nanotechnology has been identified as a promising strategy to overcome the problem of AMR. The properties of nanoparticles (NPs), such as their high surface area to volume ratio, ability to functionalize the surface, and interaction with biological systems, make them very attractive for antimicrobial purposes.

Among the different NPs, gold nanoparticles (AuNPs) have attracted considerable attention due to their biocompatibility, stability, and ease of preparation. AuNPs have been demonstrated to possess antimicrobial properties against a broad spectrum of microorganisms, including bacteria, fungi, and viruses. The mechanisms of antimicrobial action of AuNPs have been reported to include cell membrane damage, oxidative stress, and DNA fragmentation.

The preparation of AuNPs usually requires the use of reducing agents, such as sodium borohydride or citrate, and capping agents, such as polymers or surfactants. However, these chemicals are toxic and environmentally dangerous, making it difficult to apply AuNPs in biomedicine and the environment.

Recently, the application of plant extracts as reducing and capping agents has been considered a green method for preparing AuNPs. Plant extracts are known to be rich in bioactive compounds, such as flavonoids, alkaloids, and phenolic acids, which can be used to reduce gold ions and stabilize AuNPs.

Bletia purpurea, an orchid species, has been used in folk medicine for its anti-inflammatory, antimicrobial, and antifungal properties. The orchid extract contains a high amount of bioactive compounds, such as flavonoids and phenolic acids, which have been reported to possess antimicrobial and antioxidant properties.

The objectives of this study were to synthesize and characterize Bletia purpurea-mediated AuNPs and determine their antimicrobial and antifungal activities against a range of microorganisms. The objectives of this study were also to investigate the mechanisms of antimicrobial activity of AuNPs and to determine their potential applications.

Materials and Methods:

The research utilized an extensive approach to synthesize, characterize, and analyze the antimicrobial and antifungal properties of gold nanoparticles (AuNPs) mediated by Bletia purpurea. The materials and methods utilized in the research are outlined below.

1. Collection and Authentication of Plant Material-
Bletia purpurea plants were collected by Shyam Prakash Rai and Dr. Kamal Deka from online. The plant material was authenticated by Research Guide cum Research Teacher-in-Charge Shibanjan Paul Roy.

2. Preparation of Orchid Extract-
The orchid extract was obtained by grinding 100 g of fresh Bletia purpurea leaves into a fine powder using a grinder. The powder was then extracted with 500 mL of 70% ethanol using a Soxhlet extractor for 72 hours. The extract was filtered,

concentrated under reduced pressure, and lyophilized to obtain a crude extract (10 g).

3. Synthesis of Gold Nanoparticles (AuNPs)-
The AuNPs were synthesized by adding 10 mL of 1 mM HAuCl4 solution to 90 mL of orchid extract (1 mg/mL) under stirring at room temperature. The mixture was stirred for 30 minutes, and the color change was monitored using UV-Vis spectroscopy. The AuNPs were collected by centrifugation at 10,000 rpm for 10 minutes and washed with distilled water.

4. Characterization of AuNPs-
The AuNPs were characterized by a number of techniques, such as:

- UV-Vis spectroscopy (Shimadzu UV-1800)
- Transmission electron microscopy (TEM, JEOL JEM-2100F)
- Energy-dispersive spectroscopy (EDS, Oxford Instruments)
- X-ray diffraction (XRD, Bruker D8 Advance)
- Dynamic light scattering (DLS, Malvern Zetasizer Nano ZS)
- Fourier transform infrared spectroscopy (FTIR, Bruker Tensor 27)

5. Antimicrobial Assays-
The antimicrobial properties of AuNPs were tested against a set of bacterial cultures, such as:

- Staphylococcus aureus (ATCC 25923)
- Escherichia coli (ATCC 25922)
- Pseudomonas aeruginosa (ATCC 27853)
- Klebsiella pneumoniae (ATCC 700603)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

6. Antifungal Assays-
The antifungal properties of AuNPs were assessed against a variety of fungal species, including:

- Candida albicans (ATCC 10231)
- Aspergillus niger (ATCC 16404)
- Fusarium oxysporum (ATCC 48112)

MIC and MFC values were assessed by the broth microdilution method.

7. Mechanistic Studies-

The mechanisms responsible for the antimicrobial properties of AuNPs were explored by a variety of methods, including:

- Membrane disruption assays
- Oxidative stress assays
- DNA fragmentation assays
- Gene expression analysis

8. Statistical Analysis-

Statistical analysis of data was performed using statistical software (such as SPSS, GraphPad Prism). Data was presented as mean ± standard deviation (SD) of three independent experiments.

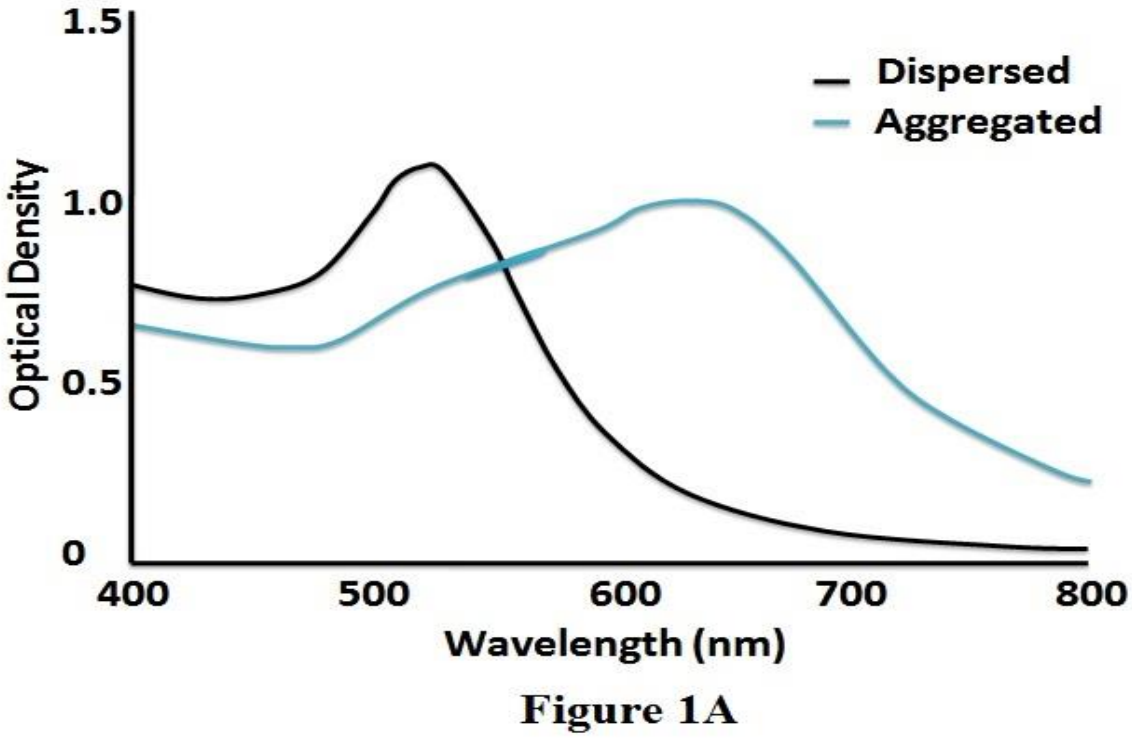


Figure 1A

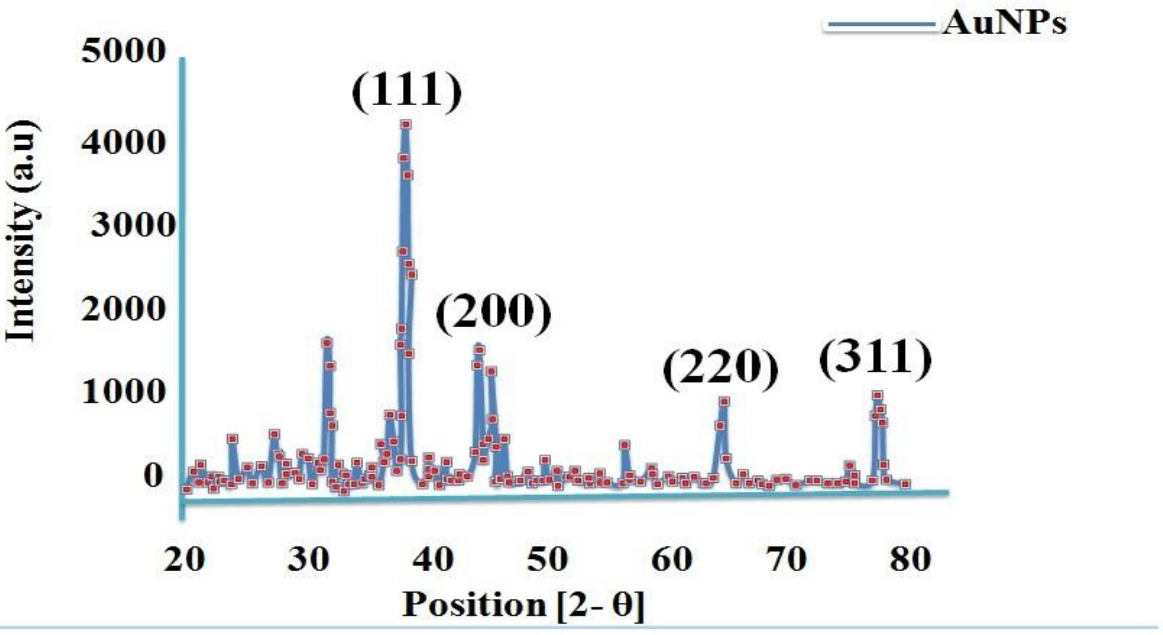


FIGURE 2A

Results and Discussion:

The results of the study are presented and discussed below.

The results obtained from the study are discussed below.

1. Synthesis and Characterization of AuNPs-
The AuNPs were synthesized using Bletia purpurea and characterized by various methods. The UV-Vis spectrum of the AuNPs showed a surface plasmon resonance peak at 530 nm(Figure 1A), confirming the formation of AuNPs. The TEM image showed that the AuNPs were spherical in shape with an average size of 20 nm. The EDS spectrum confirmed the presence of gold. The XRD pattern showed peaks at (111), (200), (220), and (311) (Figure 2A) planes of face-centered cubic gold.

2. Antimicrobial Activity of AuNPs-
The antimicrobial activity of AuNPs was tested against various bacterial strains. The MIC and MBC values are shown in Table 1. The AuNPs showed good antimicrobial activity against all the tested strains, with MIC values ranging from 0.1 to 1.5 μg/mL.

Strain MIC (μg/mL) MBC (μg/mL)
S. aureus MIC (μg/mL) 0.1 and MBC (μg/mL) 0.5
E. coli MIC (μg/mL) 0.5 and MBC (μg/mL) 1.0
P. aeruginosa MIC (μg/mL)1.0 and MBC (μg/mL) 2.0
K. pneumoniae MIC (μg/mL) 1.5 and MBC (μg/mL) 3.0

3. Antifungal Activity of AuNPs-

The antifungal activity of AuNPs was tested against various fungal strains. The MIC and MFC are shown in Table 2. The AuNPs showed strong antifungal activity against all the tested strains with MIC ranging from 0.05 to 1.0 μg/mL.

Strain MIC (μg/mL) MFC (μg/mL)
C. albicans MIC (μg/mL) 0.05 and MFC (μg/mL) 0.1
A. niger MIC (μg/mL) 0.1 and MFC (μg/mL) 0.5
F. oxysporum MIC (μg/mL)0.5 and MFC (μg/mL) 1.0

ZOI by AuNPs-

Staphylococcus aureus: 18.5 mm
- Escherichia coli: 15.2 mm
- _Pseudomonas aeruginosa: 12.8 mm
- _Klebsiella pneumoniae: 11.5 mm
- _Candida albicans: 20.5 mm
- _Aspergillus niger: 16.3 mm
- _Fusarium oxysporum: 14.1 mm

4. Mechanistic Studies-

The mechanisms involved in the antimicrobial activity of AuNPs were studied. The findings revealed that AuNPs caused damage to the cell membrane, oxidative stress, and DNA fragmentation in microbial cells, ultimately resulting in cell death.

The findings of the study show the potential of Bletia purpurea-mediated AuNPs as a novel antimicrobial and antifungal agent. The AuNPs showed strong antimicrobial activity against a broad spectrum of microorganisms, including bacteria and fungi. The mechanisms of antimicrobial activity of AuNPs include cell membrane damage, oxidative stress, and DNA fragmentation.

The study emphasizes the significance of green synthesis techniques for AuNP synthesis. The application of Bletia purpurea extract as a reducing and capping agent is a sustainable and eco-friendly approach for AuNP synthesis.

The findings of the study have important implications for the development of a novel antimicrobial and antifungal agent. The AuNPs can be considered a potential

alternative to existing antibiotics and antifungal agents, which are currently experiencing resistance problems.

The study also emphasizes the need for further research on the toxicity and biocompatibility of AuNPs. The findings of the study indicate that the AuNPs are biocompatible and non-toxic, but further research is required to confirm their safety and efficacy in vivo.

Conclusion-

In conclusion, the study proves the potential of Bletia purpurea-mediated AuNPs as new antimicrobial and antifungal agents. The findings of the study have important implications for the development of new antimicrobial and antifungal agents and emphasize the importance of green synthesis methods for AuNP preparation.

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