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Topic: **Synthesis and Characterization of Cyanobacterial Gold Nanoparticles**

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Findings

Green nanotechnology overcomes the limitations of physical and chemical approaches as it allows the synthesis of nanoparticles in an aqueous medium in ecofriendly manner. Gold nanoparticles (AuNPs) became a promising subject in the research area, due to their unique chemical, physical and biological characteristics. Biogenic AuNPs are playing prominent roles and have wide applications in the different fields of science, industries, and nanomedicines. Because of their biocompatible nature, AuNPs are frequently used in the medical area (Alavi et. al., 2022). AuNPs are used as potent antifungal, antiviral, and antibacterial agents (Umamaheswari & Abirami 2023; Dehghani et. al., 2023; Ifijen et. al., 2023). It is frequently used in cancer diagnosis and treatment, imaging, therapy, and drug delivery at the cellular and molecular levels (Kang et. al., 2010; Aldahhan et. al., 2022; Abdelghany et. al., 2023); Murphy et al., 2008; Yang et al., 2019; Karagoz et. al., 2014; Beik et al., 2019). AuNPs can interact with cancer cells and have the ability to load and release drug at a specific spot via a variety of administration ways. By conjugating medications (in smaller amounts) with AuNPs (with equal or greater efficacy), side effects of traditional drugs have been reduced, and this has improved patient quality of life (Kneipp et. al., 2010).

In our findings, considering the biomedical and pharmaceutical use of biogenic AuNPs, in present study aqueous cell-free extracts of all 30 cyanobacterial strains were screened for the

synthesis of AuNPs based on minimum time for synthesis and smallest size.

The color change of reaction mixture was first indication of AuNPs synthesis which was further confirmed by UV spectra (350-700 nm) at different peaks lies within 520-570 nm. All cyanobacterial strains were capable of synthesizing AuNPs within 5 hours. Mostly spherical AuNPs were obtained during screening which was confirmed and analyzed via SEM. *Phormidium sp.* NCCU-104 synthesized smallest sized AuNPs (08 nm).

Their synthesis was optimized by altering reaction conditions parameters (temperature, pH, reaction time, concentration of extract, and metal precursor). The best conditions for AuNPs synthesis were found to be 1mM $HAuCl_4$, 45 ml of extract volume, pH 6.5, 60 °C, and 35 min. Which resulted in a reduction of AuNPs size from 08-23 nm to 04-07 nm. Optimized AuNPs were characterized by physiochemical and biological method. Spherical-sized nanoparticles were observed and the average size was 04 nm confirmed by TEM and AFM.

AuNPs showed a 0.570 PDI value and -29 mV ZETA potential was which ensured AuNPs were and not aggregated under aqueous conditions. AuNPs exhibited antibacterial property and Minimum Inhibitory Concentration obtained using 96-well plate microtiter method were 31.25 ± 1.56 µg/ml, 62.5 ± 1.52, 125 ± 2.15, 62.5 ± 1.62 against *E. coli*, *K. pneumonia*, *S. aureus* and *B. cereus* respectively. AuNPs also showed antifungal activity and Minimum Inhibitory Concentration obtained using 96-well plate microtiter method were 125 ± 1.45 and 62.5 ± 1.59 against *Candida albicans* and *Candida glabrata* respectively.

Antioxidant activity was observed with dose dependent manner. During ABTS and DPPH assays obtained IC_{50} value of AuNPs were, 11.18 µg/ml and 15.072 µg/ml respectively.

AuNPs possess anti-inflamatory activity, during proteinase inhibition maximum inhibition 74.06% ± 0.42 noted with 150 µg/ml AuNPs and 94.23% ± 0.29 with 60 µg/ml with Aspirin (standard). Antidiabetic potential of AuNPs to inhibition of both α-amylase and α-glucosidase. Maximum inhibition was 84.76% and 81.95% for α-amylase and α-glycosidase with 150 µg/ml

AuNPs. AuNPs can also act as anticancer drugs as elucidated by MTT and DAPI assay using an A549 cancer cell line. AuNPs strongly inhibited the proliferation of cancerous cells. The IC50 < 16.50 μg/ml AuNPs was noted after 48 h of exposure. AuNPs also induced apoptosis at IC_{50} value as visualized by fluorescent microscopy.

AuNPs were proved their biocompatible nature during hemolysis and antithrombotic activity that test on human RBCs. Maximum hemolysis was 2.05%, which is much less than the permissible limit >5%. Highest antithrombotic activity was 88.13% with 150 μg/ml of AuNPs.

The findings of present study suggested that all cyanobacterial strains were capable of synthesizing AuNPs in an eco-friendly way which is the need of the hour. Biosynthesized AuNPs derived from *Phormidium sp.* extract showed antioxidant, antibacterial, antifungal, anti-inflammatory, antidiabetic, and anticancer activity. The biocompatible nature of biosynthesized AuNPs further rendered them safe for biomedical purposes i.e. AuNPs appeared as a good candidate for developing a new generation of novel biomedicine. Thus, this study established that *Phormidium sp.* is a novel source for the biosynthesis of AuNPs and has a great scope in future for various medical applications.