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Biosynthesis, characterization, and antibacterial activity of gold nano particles

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A B S T R A C T

Background: Due to rising bacterial resistance and adverse effects of antibiotics, research over the past few decades has centered on alternative antimicrobials. The current study looked at how well-chosen human pathogens responded to gold nanoparticles' antibacterial properties.
METHODS: In this study, gold nanoparticles were created using panchagavya (PG), and the resulting nanoparticles (PG-AuNPs) were then examined using various spectroscopic methods. Additionally, the well-diffusion method was used to assess the antibacterial activity of PG-AuNPs against Escherichia coli, Bacillus subtilis, and Klebsiella pneumoniae.
Results: A color change and a UV-Vis spectrum with a maximum absorption peak at 527 nm were used to confirm the synthesis of AuNPs. From the UV results, the Tauc method calculated the bandgap energy to be 2.13 eV. FTIR analysis reveals that the presence of).

Introduction

Nanomaterials are becoming more widely used in medicine due to their drug delivery mechanism in cancer therapy, as well as their availability, material properties, and capacity to improve

drug selectivity against cancer cells [1]. Gold nanoparticles (AuNPs) have generated increased interest among diverse metallic nanopar- ticles because of their unique qualities, which include nano size, low toxicity, comparatively simple fabrication, and precise target-ing [2]. The antibacterial property of AuNPs has recently been a major research topic, making them a good candidate for antibiotic complementation. The antibacterial activity of AuNPs is mediated by the development of holes in the bacterial cell wall, resultingin cell death due to the loss of cell contents. Furthermore, AuNPs can inhibit multidrug-resistant pathogens by attaching to bacte-

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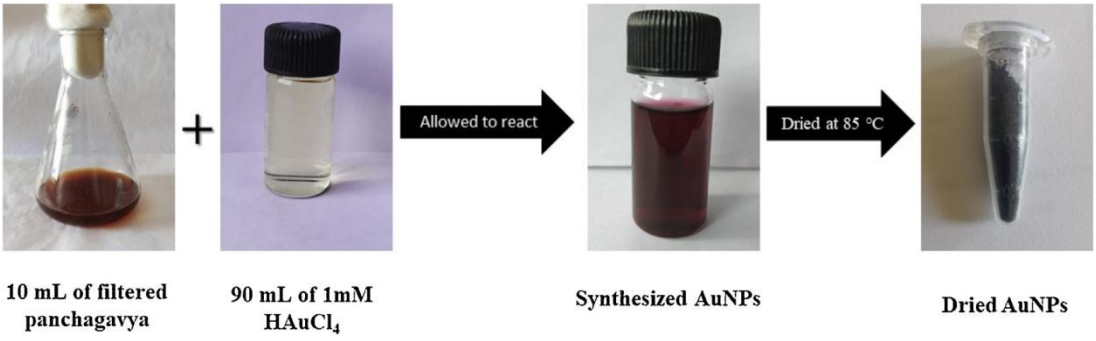

Fig. 1. Schematic diagram of panchagavya mediated gold nanoparticles.

rial DNA and blocking the uncoiling of DNA during transcription by binding to bacterial DNA [3]. For the synthesis of nanoparticles, a variety of technologies are used; among them, biological methods are less hazardous and environmentally benign.

The biological method was done by using bacteria, fungi, biomolecules, plants, and other natural raw materials for the syn- thesis of nanoparticles. Natural nanoparticles are employed as unique reducing and capping agents for the effective reduction of metallic ions into nanoparticles, whose chemistry is inextrica- bly tied to the characteristics of the raw materials, resulting in a synergy between both materials [4]. *panchagavya* (PG) is a mag- nificent combination of five cow-derived products: urine, dung, milk, curd, and ghee. Importantly, PG is a mixture of microorgan- isms, including *Azotobacter* sp. *Azospirillum* sp., *Pseudomonas* sp., probiotics, and a variety of other beneficial organisms. Moreover, carbohydrates, proteins, lipids, micronutrients, and antioxidants are present in the PG. Silver and copper nanoparticles synthe- sized from PG showed significant antibacterial and free radicals scavenging activities respectively [5,6]. However, no report on the synthesis of AuNPs using panchagavya has been published till date. Hence, the current study will be established as a future reference for the further investigations on PG nanoparticles. The goal of this study was to synthesize and characterize AuNPs utilizing PG as well as evaluate the antibacterial activity of synthesized nanoparticles (PG-AuNPs) against *Escherichia coli, Bacillus subtilis,* and *Klebsiella pneumoniae.*

Materials and methods

Preparation of panchagavya (PG)

The procedure given by Gandhi et al., [6] was used to prepare the PG. The prepared PG was filtered using Whatman No 1 filter paper and the filtrate was used to synthesize AuNPs the schematic diagram as shown in Fig. 1.

Synthesis and characterization of AuNPs

For the genesis of AuNPs, 10 mL of PG extract was combined with 90 mL of $HAuCl_4$ (1 mM) and allowed to react at room temperature (RT); the colour of the reaction mixture varied over the incuba- tion period, indicating the synthesis of AgNPs. UV–visible (UV–vis)

spectroscopy in the range of 300 nm–800 nm demonstrated the reduction of silver ions into AuNPs spectrophotometrically. The PG-AuNPs solution was spun for 40 min at 25,000 rpm to attain pure PG-AuNPs in a dry state for further analysis. For the Fourier transform infrared spectrometer (FTIR) study, the dried PG-AuNPs were mixed with KBr and formed into pellets. With a voltage of 30 kV and a current of 20 mA, X-ray diffraction (XRD) analysis of PG-AuNPs was investigated with a scan speed of 2.0°/min and a scan width of 0.02° within the scan range of 20°–80° (Bruker D8). The size and charge of the PG-AgNPs were determined using a SZ- 100 dynamic light scattering analyzer. Micrographs generated from high-resolution transmission electron microscopy (HRTEM) com- bined with energy dispersive X-ray spectroscopy (EDS) were used to analyse the particle shape and elemental content of PG-AuNPs.

Antibacterial activity

The antibacterial activity of PG-AuNPs was investigated using the standard well diffusion method against *Bacillus subtilis* (MTCC 2394), *E. coli* (MTCC 448), and *K. pneumoniae* (MTCC 109) [7]. Freshly formed culture was spread on sterilized nutrient agar plates, and 8 mm wells in the agar plates were made with a sterile cork borer. The wells were loaded with different concentrations (10 µL, 20 µL, 30 µL, and 40 µL) of PG-AuNPs and 10 µL of ciprofloxacin was serving as a control. After that, the plates were maintained at 37 °C for a day, and the antibacterial activity of the PG-AuNPs was determined by measuring zones of inhibition (ZOI) around the well impregnated with PG-AuNPs.

Results and discussion

UV analysis

The shift in the colour of the solution from brown to ruby red verified the generation of PG-AuNPs [8]. UV–vis spectroscopic anal-ysis is a widely used approach for determining the formation of metal nanoparticles by examining the unique optical properties of the nanoparticles, which are dependent on their size and shape [9]. The synthesized AuNPs are indicated by a prominent single surface plasmon resonance (SPR) band at 527 nm in the UV–vis spectra as shown in Fig. 2. The SPR band was developed when free elec- trons in PG-AuNPs were excited while absorbing visible light [10]. Spherical nanoparticles produce a single SPR band in the absorp- tion spectra, according to *Mie's theory* [11]. Singh et al., [12] also reported a UV absorption peak at 527 nm for synthesized AuNPs nanoparticles *using Padina gymnospora.* The bandgap energy was determined from UV analysis using the *Tauc plot* method [13], and it was found to be 2.13 eV (Fig. 3). The low energy gap of PG-AuNPs ensures that visible light is adequately absorbed [14].

FTIR analysis

An infrared spectrum, which can be considered as a sample fingerprint by resulting absorption bands due to the bending or stretching of unique bonds. Different peaks were found in the FTIR spectra at 3192 cm^{-1}, 2937 cm^{-1}, 1576 cm^{-1}, 1091 cm^{-1}, 1013 cm^{-1}, and 857 cm^{-1} as shown in Fig. 4. The major stretching vibra- tions of the O H group were

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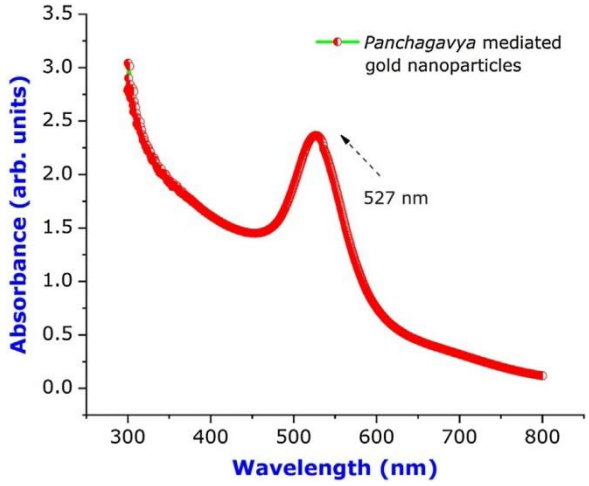
found at 3192 cm^{-1}. The band at



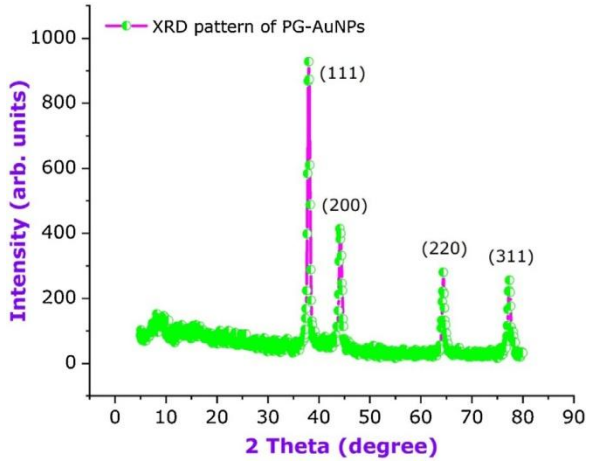


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Fig. 2. UV–vis absorbance spectrum of panchagavya mediated AuNPs.

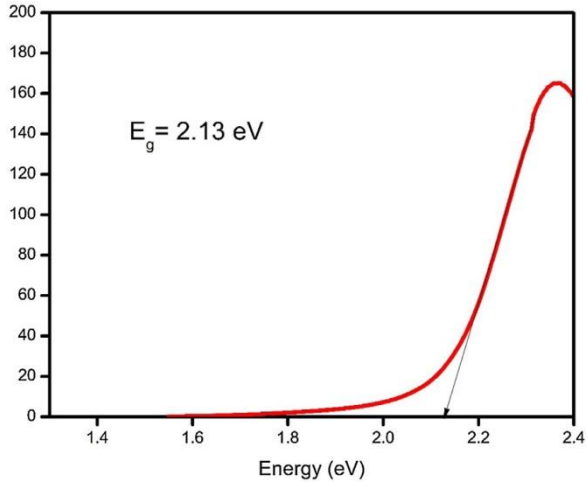

Fig. 3. Direct energy bandgap of panchagavya mediated AuNPs.

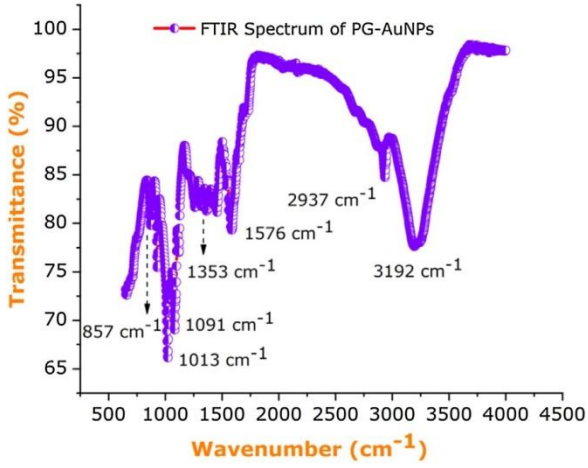

Fig. 4. FTIR spectrum of panchagavya mediated AuNPs.

2937 cm^{-1} corresponded to the C–H stretch of the protein's methy-lene groups [15] and the C C stretching of the benzene ring was observed at 1576 cm^{-1} [16]. The NH stretch vibration occurring in the amide bonds of the proteins was ascribed to the band at 1462 cm^{-1}. The NO symmetry stretching characteristic of the nitro compound is exemplified by the band at 1364 cm^{-1}. The small

Fig. 5. XRD pattern of panchagavya mediated AuNPs.

band at 1246 cm^{-1} representing CN stretching of amines [17].Secondary alcohols

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and/or the C-O- stretching ester group would be linked to a band at 1091 cm^{-1} and 924 cm^{-1} [18,19]. The CN stretching vibrations of aromatic and aliphatic amines are responsible for the band detected at 1013 cm^{-1} [20]. NH_2 primary amines were found in the band at 857 cm^{-1}. According to the FTIR results, amino acids and proteins have a greater ability to adhere to metal and are involved in the depletion and capping of AuNPs in the liquid media [21].

Phase analysis (XRD)

XRD is primarily used to identify phases and characterize the crystalline nature of PG-AuNPs. Fig. 5. illustrates the face-centered cubic (FCC) AuNPs because of XRD analysis, which is agreed with the FCC structure referenced in joint committee of powder diffrac- tion standard (JCPDS) card no. 04-0784 [22]. Furthermore, the XRD peaks at 2θ of 38.30°, 44.67°, 64.57°, and 78.76° might be assigned to the (111), (200), (220), and (311) crystallographic planes, indi- cating that the PG-AuNPs are cubic crystalline. These findings corroborate a previous report on the manufacture of gold nanopar-ticles by endophytic bacteria [23].

Dynamic light scattering studies (DLS) Particle size

The hydrodynamic size and zeta potential of nanoparticles are significant features for future uses in nanoparticle research. The hydrodynamic size was determined to be 53.29 nm with a polydis- persity index of 0.354, as illustrated in Fig. 6(a). The value of the polydispersity index is less than 0.7, indicating that the nanoparti- cles are of good quality. Similarly, Khandel et al., [24] found that the average size of *Alpinia calcarata* leaf extract mediated AgNPs was
45 2 nm, with a polydispersity index of 0.258, indicating that the nanoparticles were of acceptable quality.

Zeta potential analysis

A high absolute zeta potential value suggests that the nanopar- ticles have a large electric charge on their surface. It defines the particle's strong repelling forces, which prevent aggregation and stabilize Nanoparticles in the buffer solution. The zeta potential was discovered to be 9.8 mv in Fig. 6(b). Due to electrostatic repulsion, it was concluded that gold nanoparticles had warped with anionic substances and that the particles were relatively stable [25].

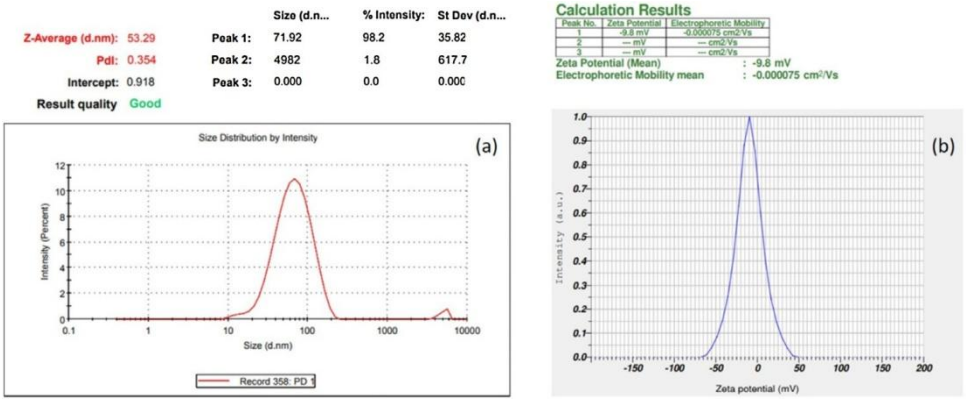

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Fig. 6. (a) DLS analysis of particle size and; (b) Zeta potential of PG-AuNPs.

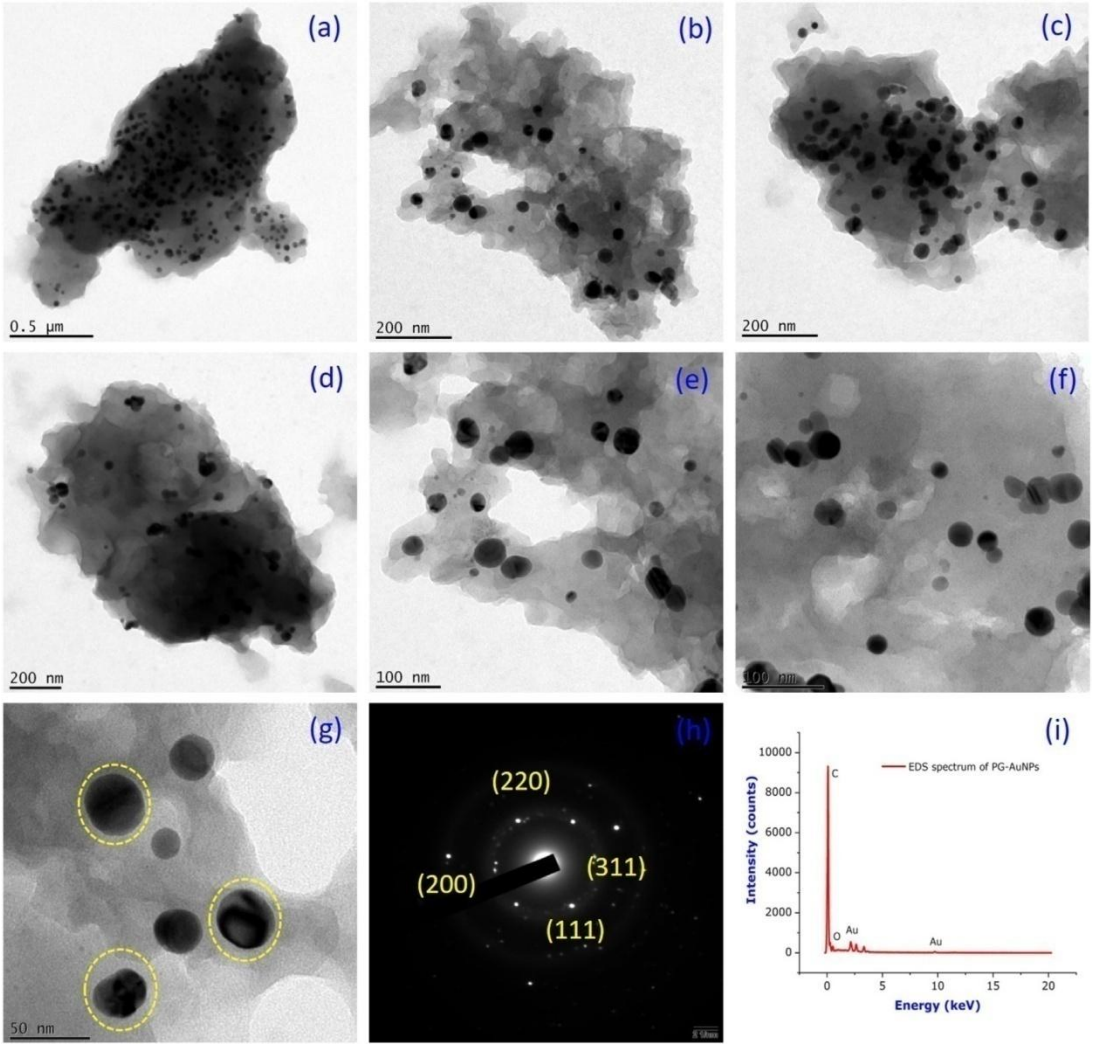

Fig. 7. HRTEM analysis of PG-AuNPs. (a-g) Spherical shaped PG-AuNPs; (h) SAED pattern of PG-AuNPs; (i) EDS spectrum of PG-AuNPs.

Table 1
ZOI of tested bacterial strains at different concentration of PG. The results were expressed as mean ± standard deviation.

Strain	ZOI (mm)				
	Control (10 µL)	10 µL	20 µL	30 µL	40 µL
B. subtilis	12.33 ± 0.17	9.17 ± 0.17	9.33 ± 0.33	10.16 ± 0.17	11.42 ± 0.58
E. coli	14.33 ± 0.53	9.33 ± 0.17	10.16 ± 0.17	11.17 ± 0.33	12.67 ± 0.24

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K. pneumoniae 26 ± 0.57 13.16 ± 0.17 13.33 ± 0.17 15.17 ± 0.3317.12 ± 0.14

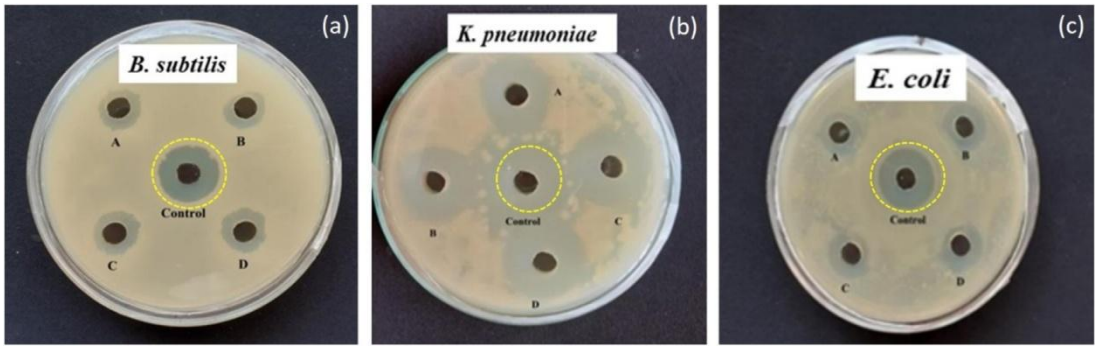

Fig. 8. Antibacterial activity of PG-AuNPs against gram-positive and gram-negative organisms at 10 µL (A), 20 µL (B), 30 µL (C) and 40 µL (D). *Bacillus subtilis* (MTCC 2394),
E. coli (MTCC 448), and *K. pneumonia*.

High resolution transmission electron microscopy (HRTEM)

HRTEM analysis was used to evaluate the morphology of gold nanoparticles [26]. The image of PG-AuNPs with a spherical shape was given in Fig. 7(a–i), EDS analysis of PG-AuNPs showed strong signals corresponding to gold along with weak signals corre- sponding to oxygen in Fig. 7(i). The weak signals are raised from macromolecules in the PG extract, such as proteins/enzymes and salts [27]. The results are consistent with previous reports of gold nanoparticles discovered using various synthesis methods [28,29]. Selected area electron diffraction (SAED) of nanoparticles revealed the crystalline nature of the PG-AuNPs yet again. Bright circular pattern rings were discovered in this study. The PG-AuNPs in cubic crystal form was also indicated by circular patterns of spots match-ing to reflections from the (111), (200), (220), and (311) of lattice planes as shown in Fig. 7(h). Our findings were in line with those of Doan et al., [15] and Qian et al., [29].

Antibacterial activity

The antibacterial activity of PG-AuNPs against selected bacterial strains were displayed in Fig. 8(a–c). The acquired result showed that the PG-AuNPs were harmful to all the tested bacterial strains at various doses. Among the bacterial strain examined, PG-AuNPs exhibited the strongest inhibitory activity against *K. pneumoniae* followed by *E. coli*, and *B. subtilis* with ZOI of 17.12 ± 0.14, 12.67 0.24, and 11.42 0.58 mm, respectively at the highest concentra-tion (40 µL) Table 1. When metal ions in solution come into contact with a bacterial cell, they become generally dispersed in the envi-ronment around the bacterial cell, with no specific localisation. NPs that engage with the bacterial cell wall, on the other hand, form a focused source of ions that constantly release ions, increasing cell toxicity [30]. Our findings showed that the PG-AuNPs have a bet-ter antibacterial effect against gram-negative bacteria. This could be because gram-positive bacteria has strong cell wall, whereas gram-negative bacteria has thin cell wall so the PG-AuNPs eas-ily

penetrate to the cell membrane of the gram-negative bacteriaand causes damages to the cell [31,32]. The antibacterial prop-erty of gold NPs was achieved in two processes. They inhibited themetabolic process by changing the membrane potential and low-ering adenosine triphosphate (ATP) synthase activity. Second, they rejected the ribosome's subunit for *t*RNA binding, effectively dis-mantling its biological process. They were also found to be lessharmful to mammalian cells [33]. Gold nanoparticles in various dimensions and shapes are the most widely investigated nano-materials for antibacterial and anti-biofilm applications, accordingto a recent literature review [34]. Electronic effects are producedby gold NPs with a small size and increased surface area, whichare advantageous for increasing the surface reactivity of NPs. Fur-
thermore, the high surface area percentage immediately interacted with the bacterium to a large amount, resulting in improved bac- teria interaction. The antibacterial activity of the NPs with large surface area was considerably increased by these two critical fea- tures [35]. Previous research has shown that gold nanoparticles play an important role in drug delivery and can be used to combat a wide range of pathogenic bacteria due to their effective antibac- terial properties [36–38]. The findings suggest that PG-AuNPs may be a useful tool for antibacterial drug for now and in the future.

Conclusion

In conclusion, we discovered that AuNPs were successfully synthesized utilizing PG as a reducing agent, and they were char- acterized using various techniques. The formation of AuNPs was primarily detected when the colour solution changed, and UV absorption spectra revealed a maximum absorbance peak at 527 nm. The FTIR data revealed PG-AuNPs with a variety of functional groups includes amino acids and proteins. XRD, SAED, TEM, and DLS displayed crystalline nature, spherical morphology, elemen- tal composition, and the particle size was discovered to be 53.29 nm. The synthesized nanoparticles showed a higher and moder- ate antibacterial activity against gram negative and gram-positive bacteria respectively.

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Competing interests

None declared.

Ethical approval

Not required.

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