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Fabrication of Gold Nanoparticles from *Prosopis juliflora* Leaves Extract by Green Method for Potential Antibacterial Application

K. S. V. Krishna Rao[1]*, P. Rama Subba Reddy[1,2], E. Chandra Sekhar[3]

[1]Department of Chemistry, Polymer Biomaterial Design and Synthesis Laboratory, Yogi Vemana University, Kadapa, Andhra Pradesh, India. [2]Centre of Polymer Systems, University Institute, Tomas Bata University in Zlin, Tř. T. Bati 5678, Zlin 760 01, Czech Republic. [3]Department of Humanities and Sciences, Kuppam Engineering College, Kuppam, Chittoor, Andhra Pradesh, India.

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ABSTRACT

Incorporation of green chemistry principles to nanotechnology is one of the key concerns in nanoscience research. There is growing need to develop environmentally benign metal nanoparticle fabrication progression that does not use toxic chemicals in the synthetic conventions to avoid antagonistic effects in medical applications. In this process using rapid, convenient and environment-friendly method for the fabrication of gold nanoparticles (Prosopis juliflora [PJ]-GNPs) by reducing aurum chloride with the aqueous extract of PJ leaves. To elevate the process parameters involved in the fabrication of PJ-GNPs by green method including extract concentration Au^{+3} ion concentration and time intervals. The antibacterial activity is carried out by disc diffusion method. The green synthesized PJ-GNPs are primarily confirmed by visual observations through color changing of the reaction mixture from light yellow to ruby red. The surface plasmon resonance of PJ-GNPs is obtained at 520 nm, and the effect of the phytochemicals present in PJ extract, including phenolic compounds, phytosterols, and quinones on formation of stable PJ-GNPs are investigated by ultraviolet-visible spectroscopy, Fourier transform infrared spectroscopy; the size of PJ-GNPs is confirmed by dynamic light scattering and transmission electron microscopy. The morphology and shape of the PJ-GNPs are determined by scanning electron microscopy and energy dispersive X-ray analysis. The antibacterial activity of PJ-GNPs is studied against pathogenic microbial strains.

Key words: *Biosynthesis, Prosopis juliflora extract, Gold nanoparticles, Antibacterial activity.*

1. INTRODUCTION

The synthesis of metal nanoparticles is a growing area for research due to its potentiality in the applications and development of advanced technologies. In general, nanoparticles are synthesized using chemical methods which are not eco-friendly. Nowadays, there is a growing need to develop environmentally benign nanoparticle synthesis process that does not use any toxic chemicals in the synthesis. At present, the noble metallic nanoparticles and nanostructured materials are increasing interest in recent research, because of their interesting properties exhibit completely new or improved properties as compared to the larger particles of the bulk material with specific characteristics - such as size, distribution, and morphology. The noble metallic nanoparticles - such as gold, silver, and platinum - are widely applied in products that directly come in contact with the human body such as shampoos, detergent, soaps, shoes, cosmetic products, and toothpaste, besides medical and pharmaceutical applications [1], and also make them useful for catalysis [2] sensor applications [3] biological labeling [4] optoelectronics recording media and optics [5] and the applications in biomedicine like antibacterial and antiviral, same that depends on their morphology and size [6-8]. The gold nanoparticles (GNPs) are the most extensively studied nanomaterials and have led to the development of innumerous techniques and methods for molecular diagnostics, imaging, drug delivery, and therapeutics [9].

However, in recent days, natural plant extracts are extensively used as reducing agents as well as capping agents to inhibit the agglomeration and stability [10-12] of the synthesized nanoparticles. Interestingly, the noble metallic nanomaterials are receiving supplementary

*Corresponding Author:
E-mail: drksvkrishna@yahoo.com

interest in recent research, due to their interesting applications exhibiting completely innovative and improved characteristic properties compared to the voluminous particles of the bulk materials with specific characteristics [13,14]. Detection of the formation of metal nanoparticles, the conventional chemical reduction system was used in the biosynthesis process [15].

Among the non-agriculture south Indian plants, *Prosopis juliflora* (PJ) is one of the most eco-friendly and ecologically important trees. It grows in dry areas without water along time in drought seasons. It provides shelter and food to many species of animals on its nectar, pollen, leaves, and fruits [16,17]. PJ leaves are rich in essential amino acids, tannins, flavonoids, and polyphenols but low in sulfur containing amino acids; Alkaloids and other chemical compounds are also present [18].

In this report, the rapid and single-pot bio-synthesis of PJ-GNPs by reducing corresponding aqueous salt solutions of both the metals using leaf extract. The PJ-GNPs can be prepared without taking part of annoying physical steps such as centrifugation, sonication, and annealing and without adding toxic chemicals (any reducing agents). The various operational parameters were evaluated for bio-synthesis route using PJ leaf extract, producing the PJ-GNPs. Till date, there is no report of synthesis of PJ-GNPs exploiting aqueous leaf extracts of PJ.

2. MATERIALS AND METHODS

Gold (III) chloride trihydrate ($HAuCl_4.3H_2O$) were purchased from Aldrich. PJ plant materials (leaves) are collected in the month of October 19, 2015, in Yogi Vemana University Andhra Pradesh; India. Throughout the experiment, double-distilled water was used and all the reagents were used without further purification.

2.1. Preparation of Extract

The PJ plant leaves are to be collected and dipped in distilled water to remove the surfaces adhered dust particles; the leaves are separated from water and let it be allowed for drying in dust free environment at room temperature for 48 h. These leaves are chopped finely into small pieces for further study. 20 g of leaves are to be boiled in 100 mL of sterile distilled water in 500 mL flask for 30 min at room temperature. The extract has to be filtered with Whatmann grade no.1 filter paper to attain clear solution and is used to synthesize PJ-GNPs. Collected extract is stored at 4°C for further experimental analysis.

2.2. Synthesis of GNPs

The aqueous leaf extract of PJ is used for the bio-reduction process to produce the GNPs with 0.001N aqueous solution of $HAuCl_4$. 0.5 mL of PJ leaf extract is added to 20 ml of aqueous solution of $HAuCl_4$ for the reduction of Au^{+3} ions and kept at room temperature for 1 h. To synthesize PJ-GNPs 1, 2, 3 and 4 mL of the aqueous PJ leaf extract (Figure 2a) and ultraviolet (UV)-vis absorption spectra of PJ extract (4 ml in each) and different concentrations of Au solutions (1, 2, 3, 4 ml) at 530-580 nm is carefully added to 10 mL of 0.001 N aqueous chloroauric acid solution in 250 mL Erlenmeyer flasks for the optimization of fine SPR band (Figures 1 and 2b). The absorbance variation and wavelength sift of PJ-GNPs is gradually increased with the increase of time is due to the rapid colloids of metal ions with the polyphenols present in the PJ leaf extract (Figure 2c). The flasks containing the mixture of leaf extract and metal ion solution were incubated on a rotary shaker at 200 rpm in the dark and at room temperature. A visual change

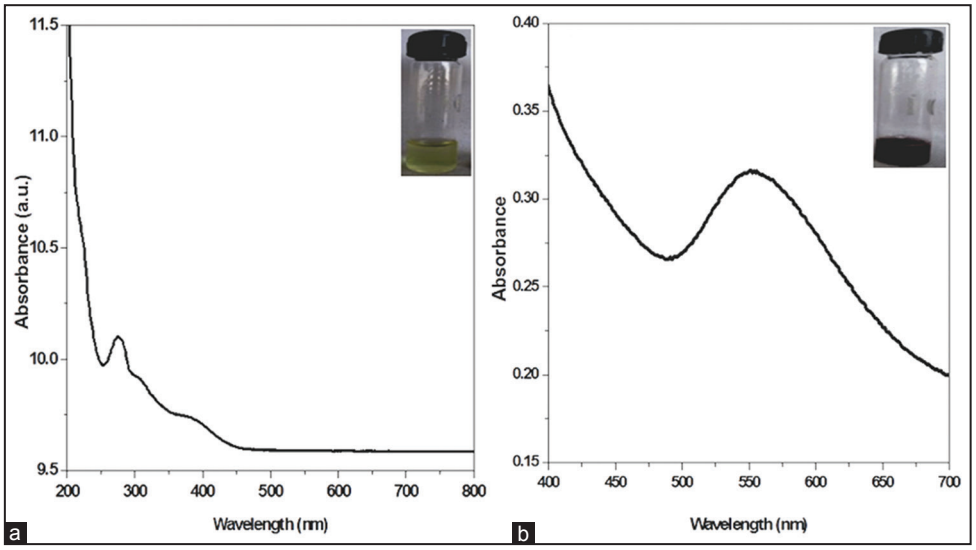

Figure 1: Ultraviolet-vis absorption spectra of bio-synthesized gold nanoparticles from *Prosopis juliflora* depicting peak at 550 nm. The inset shows extract before (a) and after (b) exposure to aurum chloride solution.

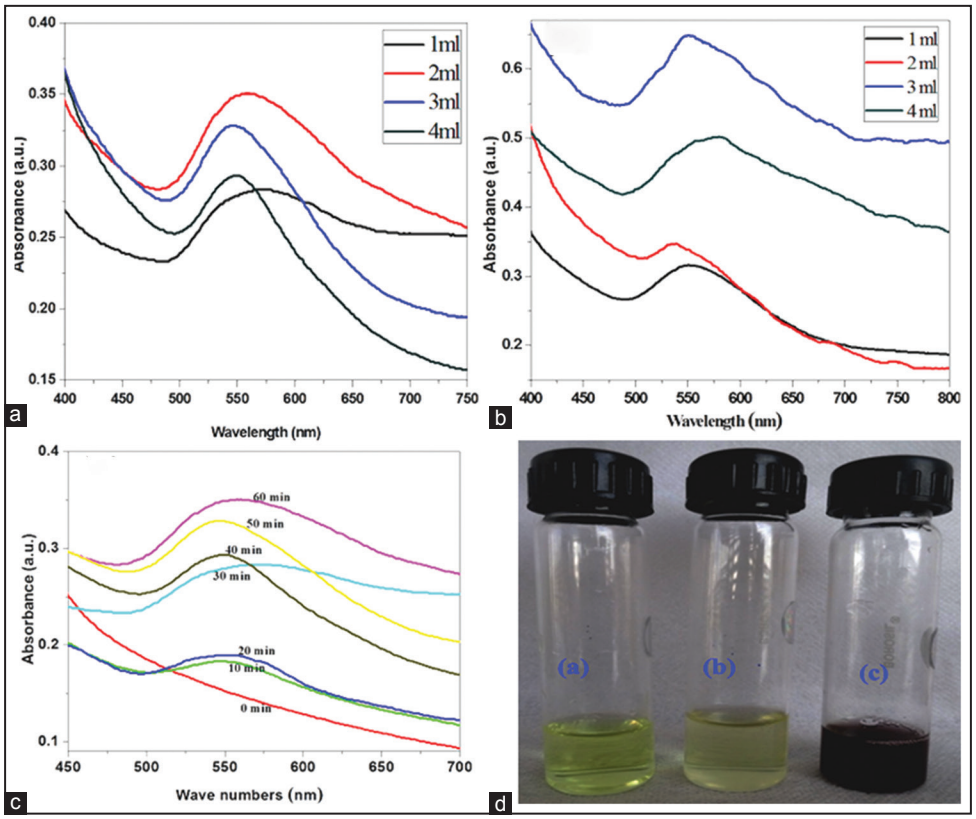

Figure 2: (a) Ultraviolet (UV)-vis absorption spectra of colloidal gold nanoparticles (GNPs) (4 ml of 0.001N) with different *Prosopis juliflora* (PJ) extract solution at 530-580 nm. (b) UV-vis absorption spectra of PJ extract (4 ml in each) and different concentrations of Au solutions (0.001N, 1, 2, 3, 4 ml) at 530-580 nm. (c) UV-vis absorption of time variation of PJ gold nanoparticles. (d) Digital micrograph showing the color change of (a) aurum ion solution, (b) PJ extract and (c) color change during the formation of PJ-GNPs.

in color of the colloidal solution occurred to primary confirmation of green synthesis of GNPs (Figure 2d).

3. CHARACTERIZATION STUDIES

The bio-reduction of pure Au^{+3} ions to $Au^{\bullet}$ is monitored by measuring the UV-vis spectrum by sampling of aliquots (0.3 ml) of GNPs solution diluting the sample in 3 ml distilled water. UV-vis spectral analysis was performed using UV-vis spectrophotometer (UV-3092, LAB INDIA), at the range of 200-800 nm. Fourier transforms infrared (FTIR) (PerkinElmer Spectrum Two, UK) analysis is used to find the chemical interactions of phytochemicals in the leaf extract with the metal solution. Dynamic light scattering study (DLS): Mean diameter and size circulation of the NPs were determined by DLS method using a Brookhaven BI-9000 AT instrument (Brookhaven Instruments Corporation, USA). PJ-GNPs were prepared by placing a drop of the colloidal solution on carbon-coated copper grids, allowing the films on the transmission electron microscopy (TEM) (HR-TEM, JEOL JEM-2010, Accelerating voltage of 200 kV) grids to stand for 2 min, removing the excess solution with blotting paper, and letting the grid dry before measurement. X-ray diffraction (XRD) analysis of the bio-synthesized PJ-GNPs cast onto glass slides were recorded using a Rigaku diffractometer (Cu radiation, λ=0.1546 nm) running at 40 kV and 40 mA and were recorded with 2θ angle from in the angle of 10-70°C. Scanning electron microscopy (SEM) (Icon analytical, FEI Quanta 200) experiments were performed to characterize size and shape of bio-reduced PJ-GNPs.

The antimicrobial activity of PJ-GNPs is evaluated against *Escherichia coli* and *Bacillus subtilis* by disc diffusion method. Discs were prepared using Whatmann No.1 filter paper and placed on Mueller-Hinton agar plates. The sample of bio-synthesized PJ-GNPs is placed on the disc with the help of micropipette. The plates were incubated at 37°C overnight and measured the zone of inhibition.

4. RESULTS AND DISCUSSION

4.1. UV-vis Analysis of PJ-GNPs

By using PJ extract, we fabricated GNPs at room temperature, and the formations of GNPs were observed by visual color change and conformed by UV-visible spectroscopy. There is increased productivity of GNPs as shown by sharp and intense SPR bands at regular intervals of time and optimal conditions. The color change observed from light

yellow to ruby red within 15 min after the addition of PJ leaf extract to aqueous $HAuCl_4$, which is the characteristic for the formation of PJ-GNPs due to the excitation of surface plasmon vibrations of Au^+ ions. It is well known that the optical properties of the metal nanoparticles are strongly dependent on their size and shape. The SPR vibrations of produced a peak at around 520 nm indicated the reduction of $HAuCl_4$ into GNPs (Figure 1b), whereas no intense peak is observed in Figure 1a for the pure PJ leaf extract. According to the Mie theory, the small GNPs exhibit only one SPR absorption band, whereas anisotropic particles show two or three SPR bands [19].

4.2. FTIR Analysis of GNPs

FTIR measurements are carried out to identify the possible phytochemicals in the leaf extract and responsible for capping and efficient stabilization of the PJ-GNPs. The position and intensity of the emission band are found to depend on composition of nanoparticles indicating the possibility to these nanoparticles utility in the therapeutic applications. Figure 3a shows the absorbance peaks at 3324, 2112, 1636 and 512 cm^{-1} is evidence to the presence of functional groups present in the PJ leaf extract. Figure 3b revealed that the splitting of above strong bands at 3413, 2924, 2854, 1536, 1456 and 1400 cm^{-1} along with other two bands is due to the formation of GNPs using the polyphenols PJ leaf extract. The band at 1400 cm^{-1} belongs to weaker aliphatic nitro compounds. The both bands at 2854 and 2924 cm^{-1} can be assigned to carbonyl groups and secondary amines, respectively. The intense broadband at 3413 cm^{-1} is the characteristics of the hydroxyl functional group in alcohol and phenol compounds. The absorbance at 527 cm^{-1} is a very weak band indicates that PJ-GNPs synthesized using PJ leaf extract are surrounded by some proteins and secondary metabolites such as alkaloids having functional groups of hydroxyl, amines, alcohols, phenol, and carboxylic acids.

4.3. Morphology and Size Studies

Particle size ascertainment of the produced GNPs is shown under heading like size distribution by intensity. The size of PJ-GNPs dispersed is drifted widely from around 20-25 nm; the average particle size is predictable in the range of 22 nm and the peaks width was found 2.5 (Figure 4). The particles are not highly monodispersed but seem slight agglomerated as this could be due to the fact that the presence of some important bio-organic compounds in the plant extract (Figure 5a and b) and seems to act as a ligand which effectively stabilizes the formed GNPs. Selected area of electron diffraction pattern showing the characteristic crystal planes of elemental gold and their particle size distribution histogram (insert of Figure 5a). In the XRD pattern, three prominent peaks were observed at 2θ=24°, 31° and 41° (Figure 5c), which corresponds to (111), (200) and (222) Bragg's reflections of the face-centered cubic structure of TC-silver nanoparticles, respectively. Figure 5d explored the size and morphology revealed the fact of synthesized PJ-GNPs is almost spherical.

4.4. Antimicrobial Activity

The bio-synthesized PJ-GNPs show the antimicrobial activity against the pathogenic microbial strains (*B. subtilis* [ATCC-6633] and *E. coli* [ATCC-25922]) and gentamycin as positive control by using disc diffusion method. All the glassware, media and reagents used were sterilized in an autoclave at 121°C, 103 kPa of pressure for 20 min [16]. The PJ-GNPs shows zone of inhibition against ATCC-6633 and ATCC-25922 studied bacteria. Growth curves against the both strains in the presence of PJ-GNPs showed inhibition of growth suggesting antibacterial property.

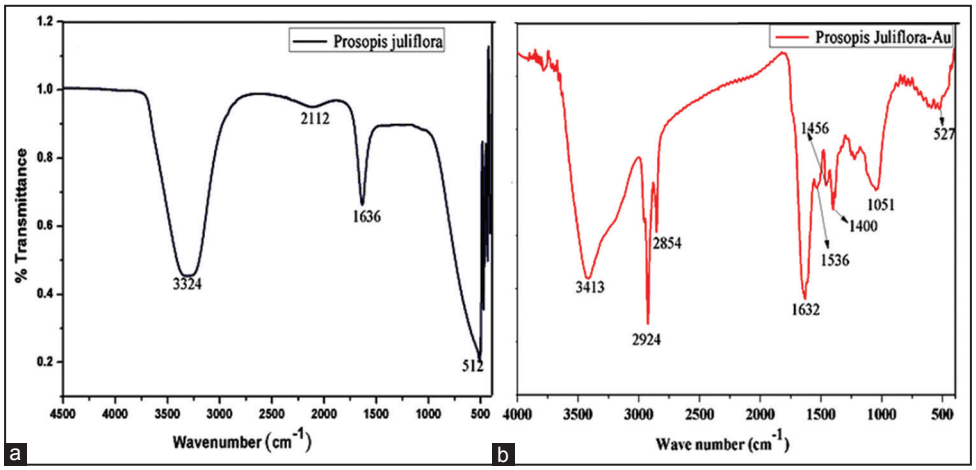

Figure 3: (a and b) Fourier transform infrared spectra of *Prosopis juliflora* (PJ) leaf extract and synthesized PJ-gold nanoparticles.

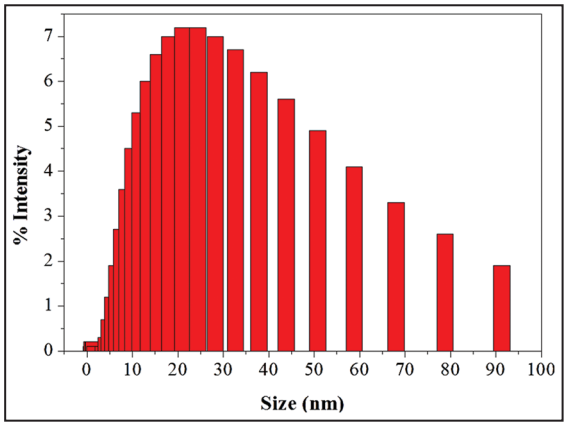

Figure 4: Size measurement of synthesized *Prosopis juliflora*-gold nanoparticles by using dynamic light scattering.

Pure PJ leaf extract and $HAuCl_4$ have minor inhibition (Figure 6) when compared to gold chloride and PJ-GNPs. This clearly indicates that the antimicrobial activity is increased when GNPs associated with tested bacteria [20,21].

5. CONCLUSION

This paper reports the simple, one-step and effective green synthesis of gold nanoparticles using naturally occurring Prosopis juliflora leaf extract, without using any chemical reducing agents. This green method uses water as a benign solvent and plant extract as a reducing agent. These biologically synthesized PJ-GNPs play a key role in protecting our environment as green. Synthesized PJ-GNPs are characterized using UV-vis, FTIR, DLS, XRD, TEM, and SEM. UV-vis spectroscopy reveals the surface plasmon property and

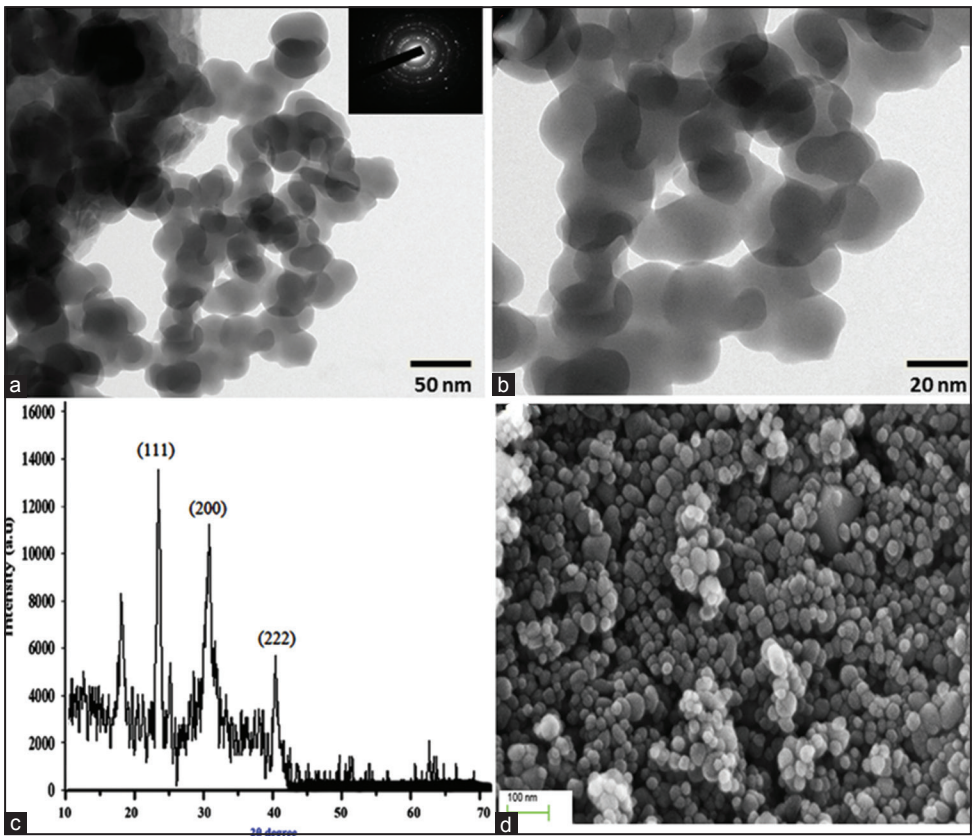

Figure 5: (a and b) Transmission electron microscope of *Prosopis juliflora*-gold nanoparticles (GNPs) and inset images indicate the selected area electron diffraction pattern of PJ-GNPs; (c) X-ray diffraction spectrum of synthesized PJ-GNPs confirming the crystalline nature (d) scanning electron microscopy image confirming the spherical shape.

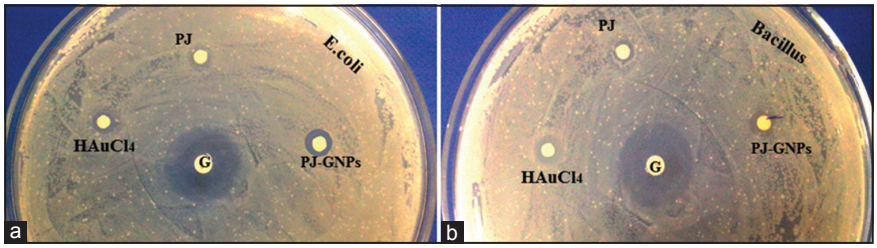

Figure 6: (a and b) Antimicrobial activity of pure leaf extract (*Prosopis juliflora* [PJ]), aurum ion solution ($HAuCl_4$), PJ-gold nanoparticles and gentamycin (G) an antibiotic against representative pathogenic bacterial strains.

formation of GNPs, while DLS reveal the nano nature of the prepared samples. It is proven that the PJ-GNPs synthesized from PJ extract seem to be promising and effective antimicrobial agent against the *E. coli* and *B. subtilis*.

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