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Original article

Bryonia laciniosa Linn mediated green synthesized Au NPs for catalytic and antimicrobial applications

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ABSTRACT

Gold nanoparticles (Au NPs) were synthesized via a green and sustainable approach using the seed extract of *Bryonia laciniosa* (shivlingi seeds). The prepared AuNPs were characterized by UV–visible spectroscopy, transmission electron microscopy (TEM), Fourier transforms infrared spectroscopy (FTIR), and particle size analysis using DLS. UV–visible and FTIR results elucidated that the prepared Au NPs showed absorption band at 533 nm and signals for biomolecules that were responsible for the stabilization of Au NPs such as phenolic components. The Au NPs were spherical with a diameter of 20–40 nm as revealed by TEM measurements. The synthesized Au NPs demonstrated excellent catalytic potential by reducing 4-nitropenol within 15 min. Furthermore, Au NPs demonstrated substantial antibacterial potency against *Bacillus subtilis* and *Escherichia coli* bacteria, with clearance zones of 5 and 16 mm, respectively.

1. Introduction

Nanoparticles (NPs) have several important applications in biology, material science, physics, and chemistry which lies in their unique physicochemical characteristics. Numerous materials have been explored in recent decades with varying capabilities, however Au NPs garnered the most interest due to their wide range of applications, which include catalysis of nitro pollutants, heavy metal ion sensing, drug administration, and antibacterial activity (Abd El-Ghany et al., 2021; Liu et al., 2021; Saravanan et al., 2021; Soni et al., 2021; Sperling et al., 2008; Usman et al., 2019). Traditional methods have been utilized for several years, although studies have shown that green approaches are more efficient for the development of NPs because they have almost negligible toxicity to the environment, are less expensive, and are easier to characterize. In the last decade, these methods have gained increased attention, particularly for silver (Ag) and gold (Au) NPs, that are quite more stable than other metal NPs. Green methods for synthesizing NPs may readily be scaled up, and thus have a number of advantages, including eco-friendliness, affordability, safety, and fast synthesis, as well as organic capping and improved stabilization (Almaary et al., 2020; Ghotekar et al., 2020; Gnanasangeetha and Suresh, 2020; Kyzioł et al., 2021; Mani et al., 2021a, 2021b; Sasireka and Lalitha, 2021; Singh et al., 2018a). Microorganisms and plants are used as green resources in the biosynthesis of NPs, and they are the good alternatives to other chemical/physical procedures that use expensive/noxious chemicals and energy-intensive processes. Various bio-molecules found in plant extracts are relentlessly required in redox reactions. These function as bio-reductant and provide a sustainable platform for the fabrication of metallic NPs in the solution. Green reducing agents contain an extensive range

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of natural components, viz phytochemicals, polysaccharides, and microbe-released molecules (Mondal and Sharma, 2016; Narayanan and Sakthivel, 2010; Singh et al., 2020). The plant extract works as a reducing and protective agent in the solution and also eliminates particle agglomeration. The most frequent type of Au complexes can be found in one of six oxidation states ranging from −1 to +5, with an increased electronegativity.

Several studies have shown that plant extracts reduce metal salts and cap metal NPs such as *Ocimum sanctum*, green tea leave, *Cinnamomum zeylanicum*, *Diopyrus kaki*, *Centella tulsi* leaves, ananas leaves, *etc* (Rani et al., 2020; Singh et al., 2019, 2018b). There are a several methods to prepare NPs, and green synthesized Au NPs are generally effective and biocompatible. The popular phytocompounds such as flavonoids, phenol, tannin, terpenoids, steroids, proteins, saponins, oils, and lipids are also found in *B. laciniosa* seed extract.

Moreover, green Au NPs can also be used as remediation agents for increasing environmental pollution. Huge quantities of toxic chemicals are routinely discharged into the soil, water, and atmosphere by industries and mills posing a threat to the environment (Paul et al., 2022; Zhou et al., 2021). Pesticides, dyes, and pharmaceuticals all are known to contain 4-nitrophenol (4-NP) (Ayad et al., 2020; Serrà et al., 2020). It is a common pollutant discharged to soils and water. The 4-NP has a low breakdown rate, and it can be degraded using a variety of ways, namely photocatalytic degradation, microbial degradation, microbial catabolism, and electrochemical processes (Chen et al., 2019; Fan et al., 2018; Kalantari et al., 2019; Li et al., 2019; Samuel et al., 2018; Seo et al., 2017; Strachan et al., 2020). For the degradation of 4-NP, the common method is a chemical reduction but it requires harsh conditions. 4-aminophenol (4-AP), produced as a result of 4-NP reduction, can cause genetic problems when inhaled, however it is less hazardous than 4-NP. The best an efficient way for the reduction of 4-NP is the optimized use of metal NPs.

Furthermore, antimicrobial resistance (AMR) is also a global issue (Morrison and Zembower, 2020; Varela et al., 2021). Generally, there are two different bacterial types Gram +ve and Gram −ve and *B. subtilis* and *E.coli* from these two types, respectively serve as the test organisms for the determination of antimicrobial activity. *B. cereus* is anaerobic Gram-positive bacteria with a thicker cell wall and is commonly found in soil and vegetation. It quickly revives at the room temperature. The main illnesses caused by this bacterium are diarrhea, nausea, and intestinal infections. *E. coli*, a rod-shaped Gram-negative bacterium with a thinner cell wall, is another bacteria used in antibacterial assays. The six different strains of *E. coli* cause diarrhea, which are most commonly known for *E. coli* mediated food contamination (Badineni et al., 2019; Gandhi et al., 2021; Parasuraman et al., 2019; Renuka et al., 2020). Au NPs harm microbial cells by producing oxidative stress, mutations, protein degradation, and cellular membrane disruption. Moreover, literature based on ayurveda reports that *B. laciniosa* extracts have a extensive assortment of antibacterial activity against a group of microorganisms that cause the most prevalent bacterial illnesses. *B. laciniosa* can be used as a bitter tonic, hepatoprotective, anti-pyretic, laxative, and to rectify metabolic imbalances. This plant contains bryonin, saponin, punicic acid, goniothalamine, and glucomannan (Patel et al., 2012; Supe, 2018).

In lieu of the above discussion, Au NPs have been synthesized via a novel, green and cost-effective approach using *B. laciniosa* (Shivlingi seeds). Furthermore, they were checked for use as an efficient catalyst and antimicrobial agent against 4-nitrophenol reduction and two bacterial strains, respectively. This is the first investigation of green synthesis of Au NPs by utilizing *B. laciniosa* seeds as a sustainable technique.

2. Experimental portion

2.1. Materials and methodology

The seeds of *B. laciniosa* (shivlingi seeds) were collected from the local market of Punjab, India. Two bacterial strains were received from Sri Guru Granth Sahib World University, Punjab, India. $AgNO_3$, 4-NP NaOH, and $NaBH_4$ were obtained from Merck, Germany. All glassware were washed with aqua regia and then rinsed with deionized (DI) water before use.

2.2. B. laciniosa leaf extract preparation

First, 10 g of *B. laciniosa* were washed with deionized water and heated for 1 h at 90 °C in 100 mL of DI water, yielding 50 mL of seed extract. The extract was filtered through Whatman No.1 filter paper, which is used to remove large particulate matter and insoluble substances from the extract. The filtered extract was then kept at room temperature for a while before being employed in synthesis.

2.3. Preparation of Au-NPs

A 100 mL deionized water was heated for 1 h at 100 °C. Subsequently, 100 μL of 10^{-3} M chloroauric acid ($HAuCl_4$) was pipetted to the resulting solution. After 15 min of stirring, 1 mL shivlingi seeds extract was added dropwise to the stirred solution. The colour of the solution changed from brown to wine-red very instantly, indicating the presence and growth of Au NPs (Fig. 1).

2.4. Antimicrobial activity

For the determination of antimicrobial activity of synthesized Au NPs well diffusion technique was used. In brief, Luria Bertani broth (LB) and agar (type 2) solutions were placed on petri plates and incubated overnight at 37 °C for microbial culture growth. The bacteria (*E. coli* and *B. subtilis*) on the LB plates were then spread. After that wells were punched on the plates and the as prepared Au NPs were incorporated at various volumes (20, 80, 100, 120, 140 μL) and then placed over nigh at 37 °C for 24 h.

2.5. Catalytic potency

The catalytic activity of 4-nitrophenol was determined by UV–Visible absorption spectra. Firstly, 200 μL $NaBH_4$ of 10^{-1} M reaction

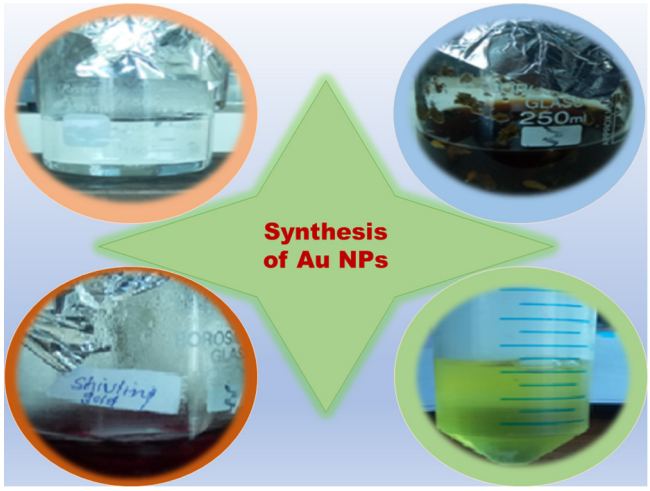

Fig. 1. Stepwise process for the synthesis of AuNPs.

with 30 μL 4 NP 10^{-2} M and 2.7 mL DI water spectrophotometrically monitored at various time intervals. During the reaction, the color of the solution progressively changed from bright yellow to colorless, confirming the release of 4-AP. Varying the volume of catalyst (5, 10, 15 μL) were added and note the time interval during the reduction process.

2.6. Characterization of Au-NPs

The AuNPs were analyzed by using UV-3600 UV–Vis- spectrophotometer (Shimadzu, Japan). The Au-NPs were analyzed and characterized by Transmission electron microscope (TEM; model JEM-200CX, Tokyo, Japan) operated at an acceleration voltage of 160 kV. FTIR instrument was used to detect functional groups of Au-NPs reduction and DLS.

3. Results

The Au-NPs were obtained by mixing *B. laciniosa* extract with 1 mM aqueous HAuCl4, which turns pink wine crimson in hue. This aqueous $HAuCl_4$ is employed as a gold precursor for the synthesis of Au NPs, while *B. laciniosa* seed extract is employed as stabilizing and reducing agent. The production of Au NPs was indicated by the color change.

3.1. UV–Visible spectral analysis

The UV–visible spectra give information about the formation and presence of Au NPs. The results indicate that there is a sharp peak of *B. laciniosa* (shivlingi seeds). A sharp peak was observed at 533 nm due to the addition of tetrachloroaurate ($HAuCl_4$) (Fig. 2). It is evident that the creation of Au-NPs takes only a few minutes and was accompanied by a color change.

3.2. FTIR analysis of AuNPs

The FTIR spectrum of Au-NPs showed the prominent absorbance bands at 3855, 3745, 3618, 2934, 1695, 1524, and 657 cm^{-1} (Fig. 3). The intense broad absorbance peak at 3745 and 3618 cm^{-1} corresponds to O–H stretching of phenols and carboxylic acids (Oh et al., 2018; Yuan et al., 2017). Further, the bands at 2934, 1695 cm^{-1} inferred the C–H and C=O stretching of alkyl and possible amide carbonyl moiety (Rajeshkumar, 2016). The peak at 1524 cm^{-1} corresponds to possible C–C stretching vibration of aromatic or unsaturated compounds (Sharifi-Rad et al., 2020).

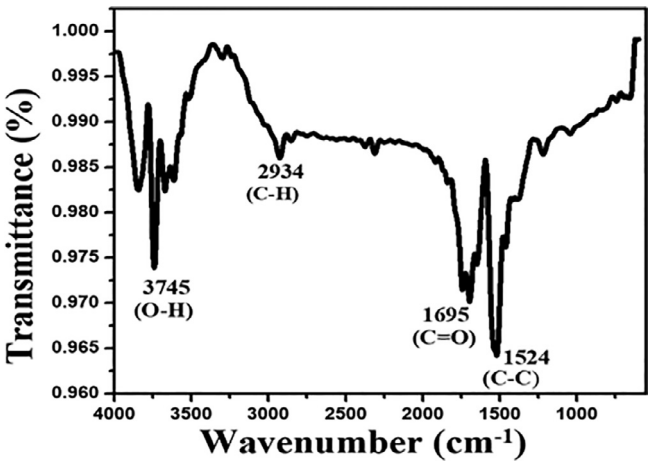

Fig. 3. FTIR spectra of *B. Laciniosa* mediated AuNPs.

3.3. Transmission electron microscopy

Transmission electron microscopy (TEM) is an analytical technique that detects the structure and content of NPs by bombarding them with electrons. The electron beam having energy around 100–300 keV was used. TEM micrograph shows the synthesized Au NPs and it revealed that Au NPs are well dispersed and spherical and some of them were found to be spherical and isotropic in structure. TEM analyses also revealed the size of Au NPs that was in the range of 20–40 nm and the crystalline nature of Au NPs was confirmed through selected area electron diffraction (SAED) pattern. The SAED results also indicate the crystalline conforming to (111), (200), (210) and (311) lattice planes of the *fcc* structure of Au NPs (Deokar and Ingale, 2016; Ndeh et al., 2017) (Fig. 4).

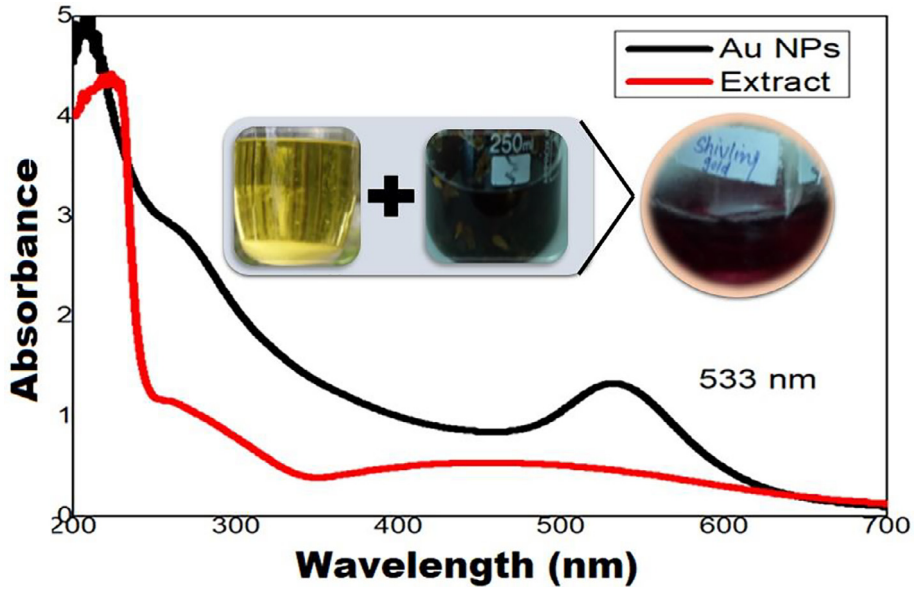

Fig. 2. UV–visible spectra of synthesized AuNPs and *B. Laciniosa* seed extract.

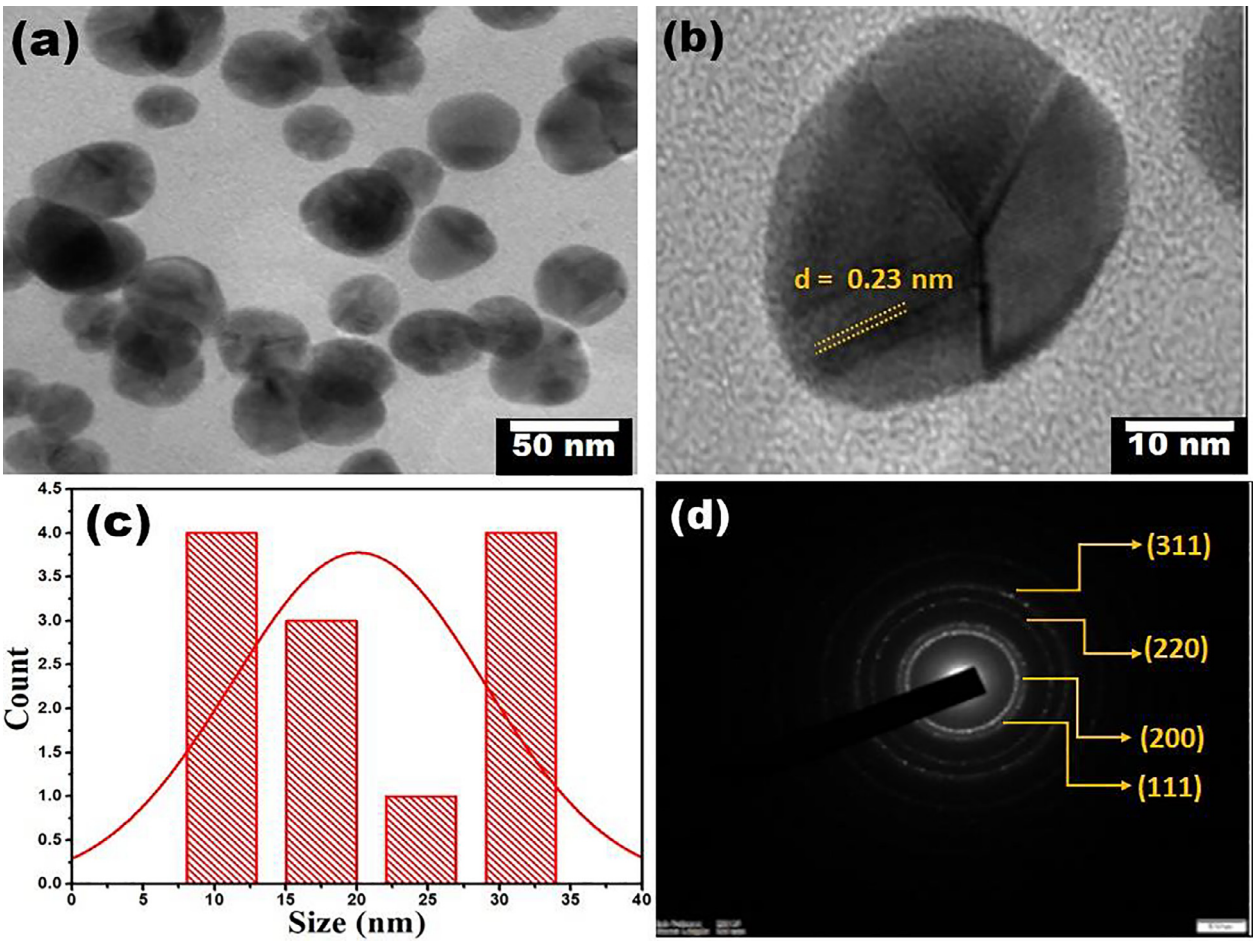

Fig. 4. Microscopic analysis of Au NPs: (a-b) HR-TEM micrographs with lattice d-spacing, (c) histogram with size distribution curve, and (d) SAED pattern with corresponding crystal planes.

3.4. Particle size distribution analysis of AuNPs by DLS

The average size of Au NPs recorded through DLS was around 80 nm (Fig. 5). The polydispersity index (PDI) was found near 0.628 nm which indicates the mono dispersive nature of synthesized AuNPs. DLS measures the hydrodynamic diameter of NPs that are connected to the surface and migrate alongside NPs in solution, whereas TEM assesses size distribution based on numbers without capping agent.

3.5. Catalytic reduction

The catalytic effectiveness of formed Au NPs was tested by reducing the 4-nitrophenol into 4-aminophenol. The UV–Visible spectrum shows an absorption edge at 400 nm because of the creation of nitrophenolate ion (t = 0). The catalytic potential was determined by using different amounts such as 5 µL, 10 µL and 20 µL (Fig. 6a-c). The kinetic study depicts that the reduction process follows a pseudo-first-order reaction and the rate constant

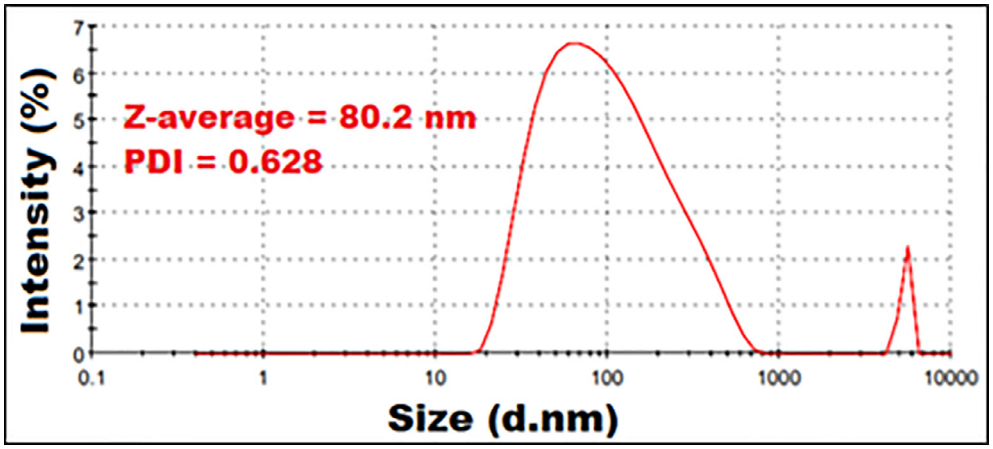

Fig. 5. Zetasize analysis of *B. laciniosa* mediated biogenic Au NPs.

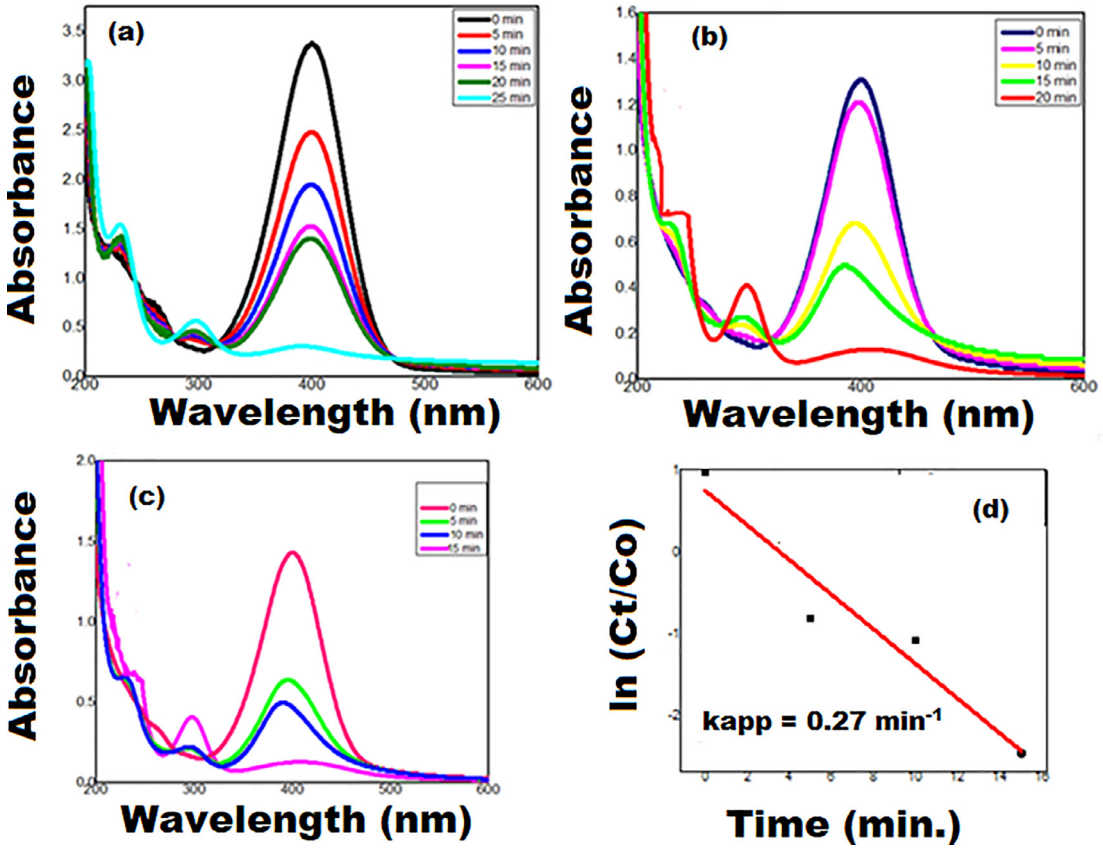

Fig. 6. (a–c) Catalytic efficacy of 4-NP in various concentrations (5, 10, and 15 μL) and (d) kinetic studies.

value is 0.273 with a regression coefficient (R^2) of 0.902 (Fig. 6d). The fact that R^2 is higher indicates that the catalytic reaction is a pseudo-first-order kinetic process (Alhaji et al., 2019; Kayalvizhi et al., 2022; Subbareddy et al., 2020). Plant-mediated NPs have been suggested to have a remarkable catalytic potential. The explanation for this is because green synthetic NPs have a greater specific surface area as well as more surface active sites (because of presence of phytochemicals on the surface of NPs), which improves 4-nitrophenol adsorption (Joseph and Vijayanandan, 2021; Kaur et al., 2019). Furthermore, because Au NPs have a huge quantity of reactive sites and a large surface area, they operate as electron intermediates, accelerating the electron relay mechanism between donor and acceptor components (Fig. 8a).

3.6. Antimicrobial potential of biogenic Au NPs

Au NPs were tested for antibacterial efficacy against two bacteria: *B. subtilis* and *E. coli*. Gram-positive microbes possess stronger cell wall that comprises peptidoglycan layer which are generally less prone to AuNPs, whereas Gram-negative bacteria have a weaker cell wall. The AuNPs enhances toxicity owing to the small size of NPs that helps to damage bacterial cell walls. The bacterial culture was disseminated on petri plates in various amounts in microliters, and then wells were formed on each plate and loaded with 20, 40, 60, and 80 μL suspensions of Au NPs. AuNPs were added in each well that shows higher antimicrobial activity towards both of these bacterial strains. In the end, it was observed that the zone of inhibition (ZOI) increased with the concentration of the NPs solution (Fig. 7, Table 1). The maximum zone of inhibition was around 16 mm and the minimum zone of inhibition was 2 mm. The AuNPs enhances toxicity owing to the small size of NPs that helps to damage bacterial cell walls. According to recent antimicrobial studies, AuNPs assaulted cell walls via mitochondrial dysfunction, gene mutation, protein degradation, and cell membrane collapsing (Fig. 8b).

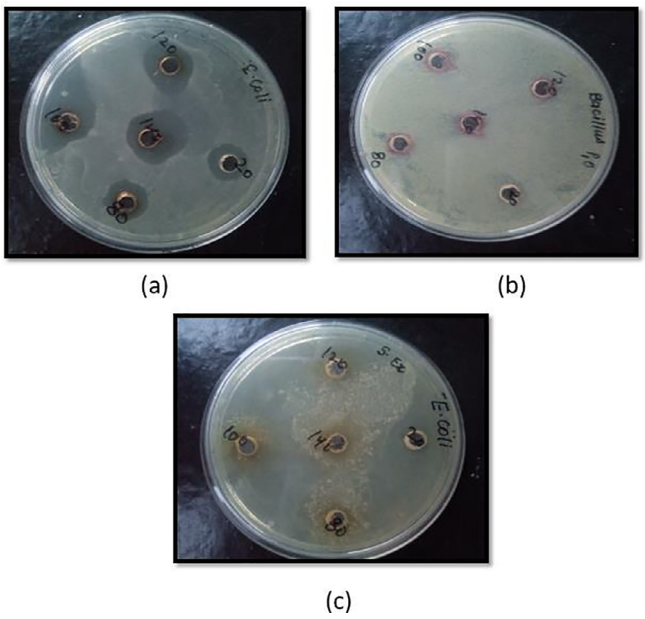

Fig. 7. Antimicrobial activity of AuNPs against (a) *E. coli* (b) *B. subtilis* bacteria (c) control of *B. laciniosa* extract.

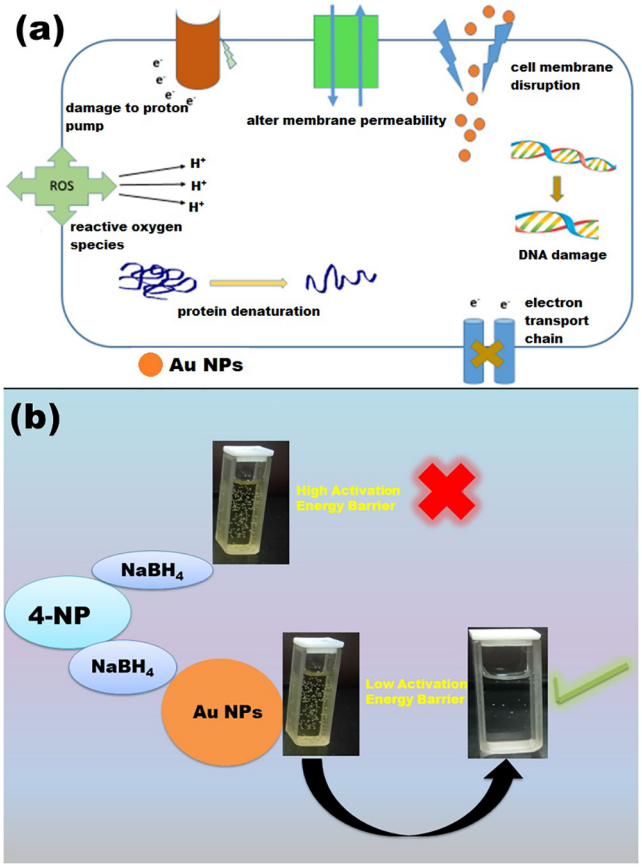

Fig. 8. Schematic representation of mechanism of catalytic reduction and antimicrobial study.

Table 1
Zone of clearance with different amounts of Au NPs.

S. No.	Au NPs (μl)	*E. coli* (mm)	*B. subtilis* (mm)
1.	140	16	5
2.	120	11	5
3.	100	10	4
4.	80	9	2
5.	20	5	2

4. Conclusions

Conclusively, we have successfully synthesized the Au NPs via the seed extract of *B. laciniosa* seeds. The formation of biogenic Au NPs was confirmed in a variety of optical and microscopic techniques. The obtained Au NPs are an efficient catalyst and antimicrobial inhibitor against the degradation of 4-NP and Gram-positive and Gram-negative bacterial strains. Thus, this study provides new insights into green/cost-effective Au NPs based effective remediation platform.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this manuscript

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