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Exploring the Antidiabetic, Antioxidant and Antibacterial potential of Biogenic Platinum Nanoparticles

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Abstract:
In the present study, platinum nanoparticles (PtNPs) were synthesized via a green and eco-friendly approach using an aqueous extract of Azadirachta indica leaves, providing a cost-effective and sustainable alternative to conventional chemical methods. The physicochemical properties of the synthesized nanoparticles were systematically characterized using Field Emission Scanning Electron Microscopy (FESEM), Energy Dispersive X-ray (EDX) spectroscopy, UV–Visible spectroscopy, High-Resolution Transmission Electron Microscopy (HRTEM), Zeta potential analysis, and Fourier Transform Infrared (FTIR) spectroscopy. The biosynthesized PtNPs exhibited a dark brown coloration and were predominantly spherical, with particle sizes ranging from 20 to 50 nm, indicating uniform morphology and enhanced stability. A characteristic absorption peak at 230 nm in the UV–Visible spectrum confirmed the successful formation of PtNPs. Biological evaluation revealed that the synthesized nanoparticles exhibited significant antibacterial activity against both Gram-negative and Gram-positive bacterial strains, with comparatively higher efficacy against Gram-negative bacteria. In addition, the PtNPs demonstrated strong antioxidant potential, indicating their effectiveness as free radical scavengers. Furthermore, they displayed promising antidiabetic activity through concentration dependent inhibition of α-amylase enzyme, suggesting their potential to regulate postprandial glucose levels by delaying carbohydrate digestion.

Keywords: Platinum nanoparticles, Anti-oxidant assays, Anti-bacterial property, Gram-positive & Gram-negative bacteria, Phytochemical screening, Anti-diabetic assay.

1. Introduction –

The field of nanotechnology has revolutionized numerous scientific disciplines by enabling the manipulation of materials at atomic and molecular levels, typically within the 1–100 nm range. At this scale, nanoparticles exhibit distinctive physicochemical properties that differ significantly from those of their bulk counterparts. Their ultra-small size, tunable surface characteristics, enhanced solubility, and multifunctional nature collectively make them highly versatile materials for scientific innovation [1]. Consequently, nanoparticles have found widespread utility across a broad spectrum of fields, including electronics, optoelectronics, pharmaceuticals, biomedical sciences, therapeutics, environmental monitoring, cosmetics, chemical processing, food technology, optical devices, energy systems, and even space applications [2]. In recent years, nanoparticles have been synthesized through various approaches, including chemical methods and biologically mediated routes using plants, bacteria, and fungi. Among these, green synthesis i.e. plant based has emerged as a superior alternative because it avoids toxic reagents, provides natural capping agents, and is generally more cost-effective [3]. Numerous studies highlight that plant extracts are particularly advantageous for metal nanoparticle fabrication, as they contain abundant bioactive constituents, such as phenols, alkaloids, tannins, terpenoids, and other phytochemicals, that play key roles in reducing metal ions and stabilizing the resulting nanoparticles.

Noble metal nanoparticles, such as those of silver, gold, platinum, and palladium, have been widely explored for their versatile applications across material science, chemistry, and biomedical research [4]. Among them, platinum nanoparticles (PtNPs) have attracted exceptional interest owing to their distinctive structural and optical properties,

remarkable catalytic potential, large surface area, and strong resistance to corrosion, all of which make them highly suitable for medical and therapeutic uses [5]. Platinum-based compounds, including cis-diamminedichloroplatinum, are already established as effective chemotherapeutic agents [6]. Beyond biomedical applications, PtNPs are also utilized in energy-related technologies such as hydrogen storage systems and fuel cells [7], and play a significant role in the synthesis of various organic dyes [8]. Additionally, PtNPs have demonstrated promising antimicrobial properties against a broad spectrum of pathogen microorganisms through mechanisms involving disruption of membrane, oxidative stress induction, and interference with cellular metabolic processes.

The rapid emergence of antibiotic-resistant microorganisms has become a major global health challenge, undermining the effectiveness of many frontline antibiotics developed during the earlier "golden era" of antimicrobial discovery [9,10]. This rising resistance highlights the urgent need for alternative therapeutic strategies that circumvent conventional drug-target pathways [11]. Similarly, oxidative stress resulting from excessive production of reactive oxygen species (ROS) plays a critical role in pathogenesis of numerous chronic disorders such as cardiovascular diseases, diabetes, cancer, and various neurodegenerative conditions.

Diabetes mellitus represents one of the most prevalent metabolic disorders worldwide and is characterized by persistent hyperglycemia arising from defects in insulin secretion, action of insulin, or both [12]. Chronic hyperglycemia is associated with severe complications affecting multiple organ systems such as nervous, cardiovascular, renal, or visual systems. One of the widely accepted strategies for controlling this involves the inhibition of carbohydrate digesting enzymes such as α- amylase or α-glucosidase, thereby slowing glucose absorption and reducing blood glucose fluctuations [13]. Consequently, there is a growing interest in developing nanoparticle-based antidiabetic agents capable of overcoming these kind of problems.

Although several studies have reported the green synthesis of PtNPs using various plant extracts, comprehensive investigations exploring their multifunctional biological properties remail relatively less explored. In view of these considerations, the present work aimed to develop a rapid, eco-friendly, and inexpensive strategy to synthesize PtNPs using the aqueous leaf extract of Azadirachta indica as a natural reducing and stabilizing agent. The green-fabricated PtNPs were systematically characterized through UV–Visible spectroscopy, zeta sizer/potential analysis, X-ray diffraction (XRD), high – resolution transmission electron microscope (HR-TEM), field emission scanning electron microscope (FE-SEM), energy – dispersive X-ray analysis (EDX), and Fourier transform infrared spectroscopy (FTIR). Furthermore, the antioxidant potential was evaluated using the DPPH free radical scavenging assay, while their antidiabetic activity was assessed through α-amylase inhibition assay and antibacterial efficacy was investigated against selected pathogenic bacterial strains. The findings of this study are expected to contribute to the growing body of knowledge on green nanotechnology and provide insights into development of multifunctional platinum-based nanomaterials for future biomedical and pharmaceutical applications.

2. Experimental procedure

2.1 Chemicals and reagents

Chloroplatinic acid hexahydrate ($H_2PtCl_6.6H_2O$) & α-amylase from porcine pancreas were purchased from Sigma Aldrich. 2,2-Diphenyl-1-picrylhydrazyl, di-nitrosalicylic acid (DNS), isopropyl alcohol, sodium phosphate, ferric chloride, ascorbic acid, copper sulphate, potassium sodium tartarate, potassium iodide, and conc. H_2SO_4, ethyl alcohol, potassium hydroxide (KOH) pellets, chloroform, streptomycin, Muller-Hinton agar, Muller-Hinton broth, and conc. HCl of analytical grade was purchased from HiMedia.

2.2 Apparatus

High resolution transmission electron microscope (HR-TEM), Field emission scanning electron microscope (FE-SEM), Fourier transform infrared spectroscopy (FTIR, Bruker), UV-spectrophotometer (Shimadzu, UV 3600 PLUS), Zeta sizer/potential (Malvern Nano ZS), refrigerated centrifuge, laminar air-flow, etc.

2.3 Microorganisms

The bacterial strains included two Gram – positive bacteria, i.e. S. aureus (MTCC 96) and B. subtilis (MTCC 121) & two Gram – negative bacteria, i.e. E. coli (MTCC 443) and P. aeruginosa (MTCC 424). They were obtained from the Department of Microbiology and Biotechnology, M.D.U. (Rohtak), Haryana, India.

2.4 Collection of plant specimens and preparation of leaf extract

A. indica leaves were gathered from the nursery of M.D.U., Rohtak (Haryana). Leaves were cleansed with deionised water various times to eliminate any kind of impurities and debris. After that, the leaves were kept at room temperature overnight to air dry until they reached a sustained weight. Subsequently, dry leaves were crushed in a mortar and pestle to obtain a fine powder. 5 g from the obtained powder was mixed with 100 mL of DW, and then the solution was heated at 50 - 60° C and stirred at 500 rpm for approximately 30 min with the help of a magnetic stirrer. Eventually, the solution was filtered using Whatman filter paper, and the filtrate was kept in a glass beaker at 4.0 °C for further use [14].

2.5 Phytochemical screening of synthesized leaf extract

Various qualitative chemical tests were conducted to determine the chemical profile of the aqueous extract. The subsequent tests were performed on leaf extract to ascertain various phytoconstituents present in it [15].

Detection of Alkaloids

The dry powder sample (100 mg) was taken in a test tube, and 2 mL of ammonia solution i.e. NH_4OH was added to it. It was allowed to settle for a few minutes. Then, 5 mL of chloroform was put in to the test tube sample by shaking, and the mixture was then filtered to remove the powder sample. Chloroform was then vaporized using a water bath, and 1 mL of Mayer's reagent was added to it. A creamy-coloured precipitate was obtained, indicating the presence of alkaloids.

Detection of flavonoids

Two- three drops of NaOH solution were mixed with 0.5 mL of the test solution,i.e.aqueous leaf extract. A profound yellow colourappeared, which turned colourless after the addition of diluted H_2SO_4. This indicated the presence of flavonoids.

Detection of Anthraquinones

Add a few drops of isopropyl alcohol to the test solution. After that, 5 mL of 10% ammonia solution i.e. NH_4OH was added and shaken intensely for 30 sec. A red, violet, or pink-coloured solution indicated the existence of anthraquinones.

Detection of Tannins

To 1 mL of test solution, 3 mL of distilled water and 2-3 drops of 10% ferric chloride solution ($FeCl_3$) were added. A deep or dark blue-green colour indicated the presence of tannins.

Detection of Saponins

Froth test: The test sample in a clean test tube was mixed with 5 mL of DW, followed by shaking the mixture effectively. The formation of foam, which remains stable at room temperature for some time, showed the presence of saponins in the sample.

Detection of Steroids

2 mL of acetic anhydride was mixedwith 0.5 mL of the test solution,i.e. aqueous leaf extract. After that few drops of conc. H_2SO_4 was added to it. The colour change from violet to blue or green demonstrated the presence of steroids in the sample.

Detection of terpenoids

Approximately 2-3 mL of the test sample was mixed with 3 mL of chloroform in a clean test tube. The sample was then transferred to a petri plate for the evaporation of chloroform. After that few drops of conc. H_2SO_4 was added to the solution, and it was warmed up for about 2 minutes. A grey-coloured solution indicated the presence of terpenoids in the extract.

2.6 Synthesis of Platinum nanoparticles (PtNPs)

PtNPs were prepared by following [16] with slight modifications. First of all, 1 mM $H_2PtCl_6.6H_2O$ solution was prepared in 10 mL of DW in a 50 mL Erlenmeyer flask. After that, 2 – 3 mL of the prepared plant extract was mixed to the above solution. The mixture solution was heated to 90°C with stirring for approximately 1 h, until a colour change from light yellow to dark brown was observed, showing the synthesis of PtNPs (Figure 1). Then the cleansing step was accomplished by centrifuging the synthesized nanoparticles, and then accumulating the precipitated pellets and cleansing them 3-4 times consecutively using deionized water, to remove any impurities. Finally, the purified PtNPs were stored at 4 °C for extended use.

Figure 1. Green synthesis of PtNPs using Azadirachta indica leaf extract

2.7 Characterization of synthesized PtNPs

To detect morphology, size, stability and elemental composition, various characterization techniques were performed. The biosynthetic reduction process of platinum (Pt^{4+}) ions into PtNPs was observed by a double-beam UV-Visible Spectrophotometer (Shimadzu, UV 3600 PLUS) in the scanning range of 200 – 800 nm at Aryabhata Central Instrumental Laboratory (ACIL), M.D. University, Rohtak. Size and shape were studied using HRTEM measurements with a TECNAI (200 kV) at SAIF, AIIMS, New Delhi. An FTIR spectrum over the range of 4000 - 600 cm^{-1} was obtained to evaluate the plausible functional groups present in the sample at the UIET department (M.D. University, Rohtak). FESEM-EDX analysis (ZEISS Sigma 360) and Zeta sizer/potential (Malvern Nano ZS) were conducted to study the elemental composition and stability, respectively, at ACIL, M.D. University, Rohtak (Haryana).

2.8 Anti-oxidant properties of synthesized PtNPs (DPPH assay)

The free radical scavenging potential of plant extract and PtNPs was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay [17]. Briefly, a 0.1 mM of DPPH solution was synthesized by dissolving 4 mg of DPPH solution in 100 mL of ethanol. Test samples of different concentrations (20 to 100 µg/mL) were then mixed with 3 mL of the 0.1 mM DPPH solution. Then, incubation was performed at room temperature for approximately 30 minutes in dark conditions. For positive control, ascorbic acid was used in the experiment. Following the incubation, the absorbance of the reaction mixture was measured at a specific wavelength, i.e., 517 nm. 3 mL of DPPH solution was taken as a control. The percentage (%) radical scavenging activity was assessed using the given formula,

$$\text{RSA}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.9. Anti-diabetic properties of synthesized PtNPs

2.9.1. α – amylase inhibition assay

First of all, the α-amylase solution was prepared by dissolving 0.5 g of enzyme in 0.02 M phosphate buffer (pH 6.9). The varying concentrations of PtNPs of 12.5, 25, 50, 100, and 200 µg/mL were mixed to 1 mL of above prepared enzyme solutions and incubation was done at 37ºC for 15-20 min. After this, 0.5% starch solution (1 mL) prepared in phosphate buffer was added to the above mixture and incubation was done for another 10 min. Lastly, DNS solution was added to stop the reaction and mixture was boiled for 5-10 min in water bath at 80-85ºC. Acarbose was used as a positive control and for blank, instead of sample phosphate buffer solution was taken. The reaction mixture was mixed well and the absorbance was taken at 540 nm and percentage inhibition of α-amylase activity was calculated by using the following equation [18] :

$$\%\ \text{Inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

2.10 Anti-bacterial properties of synthesized PtNPs

The anti-bacterial properties of PtNPs were evaluated using the agar well diffusion technique [19]. Inoculation of various selected bacterial strains was performed on developed culture plates using the streak plate technique. Wells were created on the agar surface by using a cork borer of 8 mm diameter. After that, nanoparticles of varying concentrations were streamed into the wells employing a sterile syringe. Incubation was performed at 37°C ± 2°C for 24-48 hours to assess bacterial activity. The plates were evaluated for the clearance of zone all over the wells.

The inhibition zone was calculated by measuring its diameter around the wells (in mm) in conjunction with the well diameter.

2.10 Statistical analysis

All experimental tests were carried out in triplicate, i.e., n = 3, while the gathered data were represented as the mean value.

3. Results and discussion

3.1 Analysis of phytochemical constituents of leaf extract

The results of phytochemical screening showed that the A. indica leaf extract comprised alkaloids, flavonoids, tannins, saponins, and anthraquinones (Table 1). The phytochemical screening test helped in isolating and characterizing the chemical components present in leaves extract. These secondary metabolites present possess the reducing ability, which further assisted in the reduction process of Pt^{4+} ions into PtNPs.

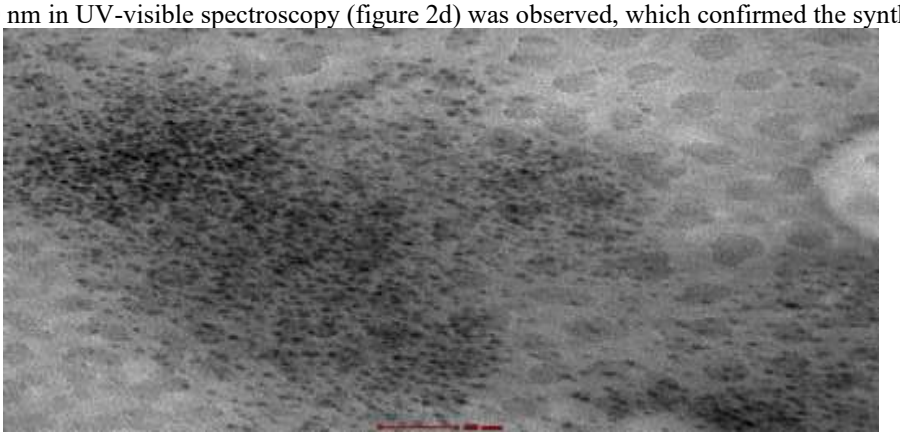
Table 1. Phytochemical screening of leaf extract of A. indica

S. No.	Tests	Results
1.	Terpenoids	-
2.	Alkaloids	+
3.	Flavonoids	+
4.	Anthraquinones	+
5.	Tannins	+
6.	Saponins	+
7.	Steroids	-

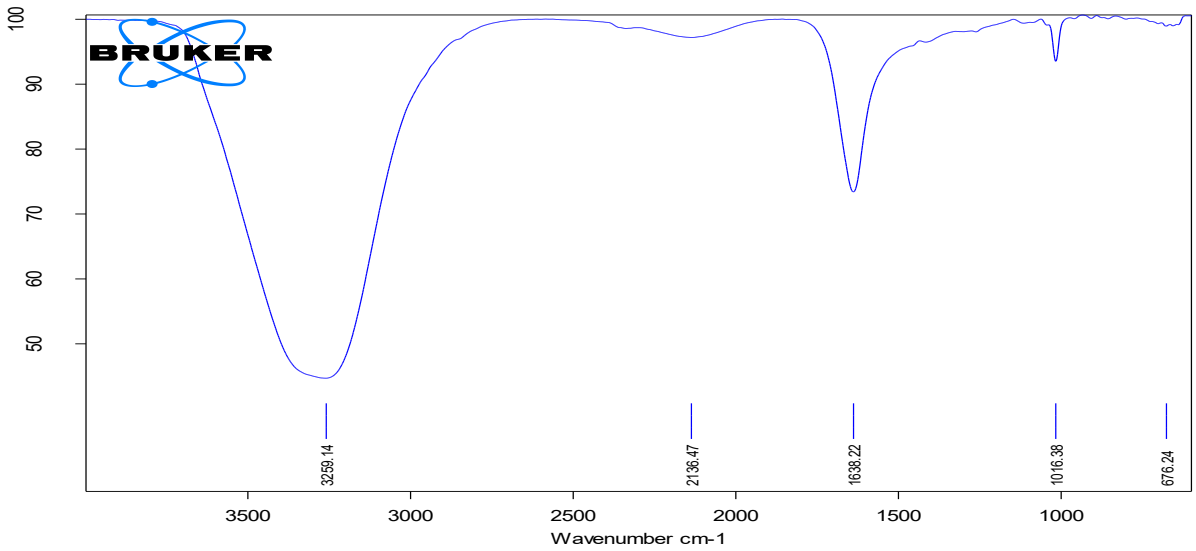
+ = Presence; - = Absence

3.2 Characterization of green synthesized PtNPs

HR-TEM is commonly employed for collecting vital information about nanoparticles such as their shape, size and morphology. PtNPs' average particle size came out as 50 – 100 nm, as shown in Figure 2a, which was in good agreement with previously reported works [20]. The spherical shape of nanoparticles was seen predominantly. In FTIR spectra (Figure 2b), the peak at 3259.14 cm^{-1} was due to a strong hydroxyl group present in phenolic and flavonoid compounds. The other peaks, present at 1638.22 cm-1 and 1016.38 cm-1, were subsequently linked to conjugated C=C and C-N amine stretching vibrations. The presence of all characteristic peaks strongly recommended stabilization and effective capping of nanoparticles. The present results were in accordance with other preceding published papers that were related with the green synthesis of PtNPs [21]. The surface charge and stability of PtNPs was measured with the help of zeta sizer/potential instrument and the value came out as -9.42 mV (Figure 2c) confirming the stable nature of nanoparticles. The negative value showed that the nanoparticles were capped with different biomolecules of negative charge as a result of that repulsion or dispersion occurred among them and also their stability was augmented [22]. A broad peak at ~230 nm in UV-visible spectroscopy (figure 2d) was observed, which confirmed the synthesis of PtNPs.



(a)



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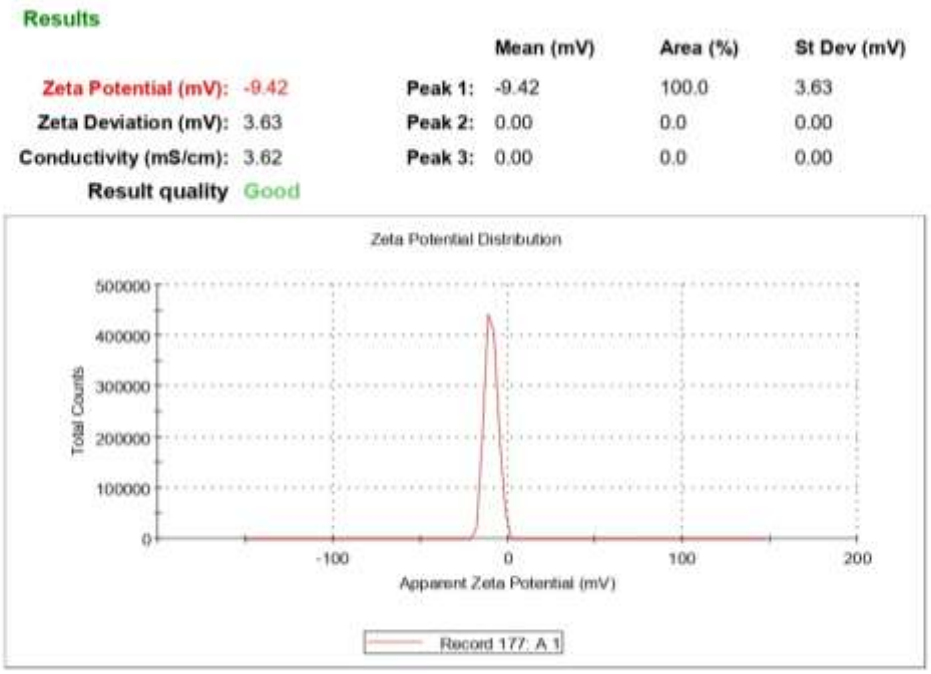

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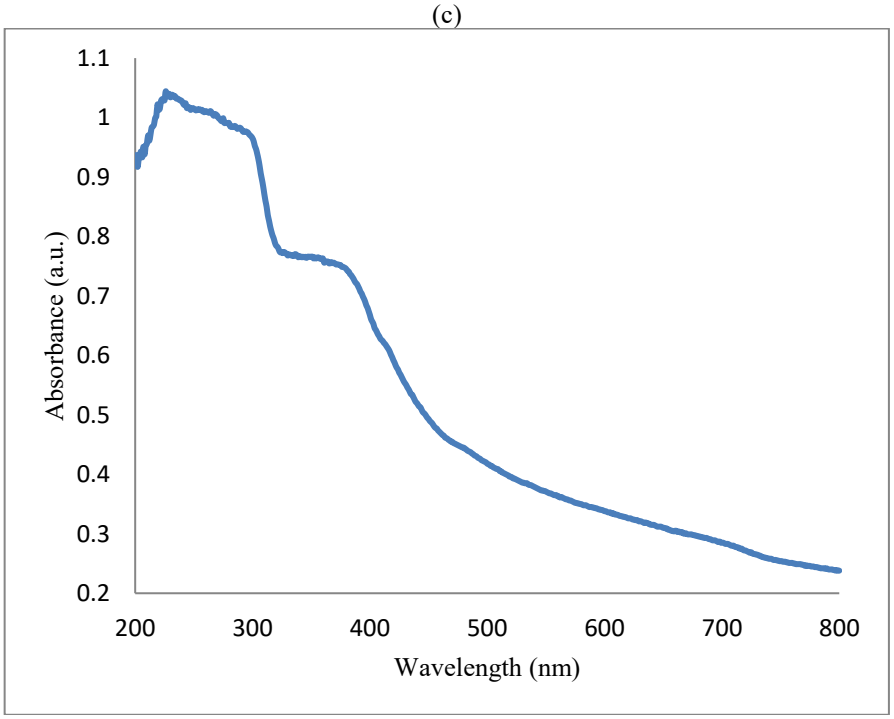


(d)

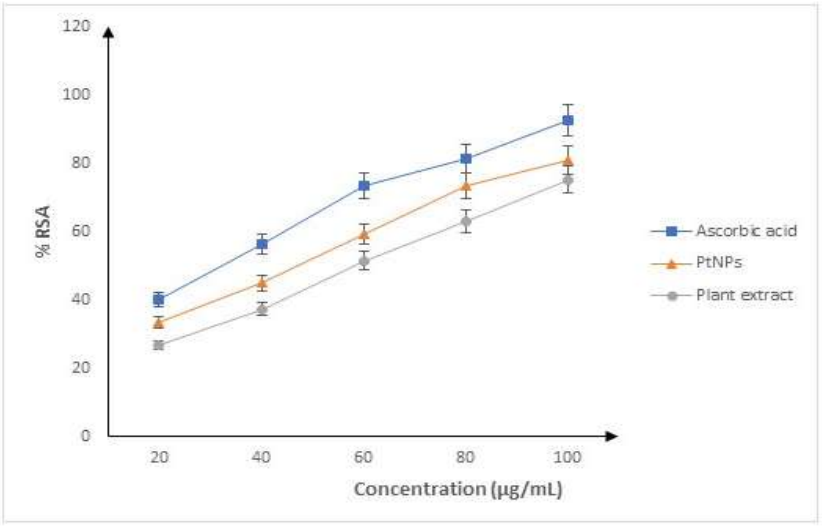
Figure 2. Characterization of green synthesized PtNPs by (a) TEM image showing 100 nm size (b) FTIR spectra; (c) Zeta potential (-9.42 mV); (d) UV-visible spectra i.e. peak ~230 nm;

3.3 Anti-oxidant study (DPPH Radical Scavenging Assay)

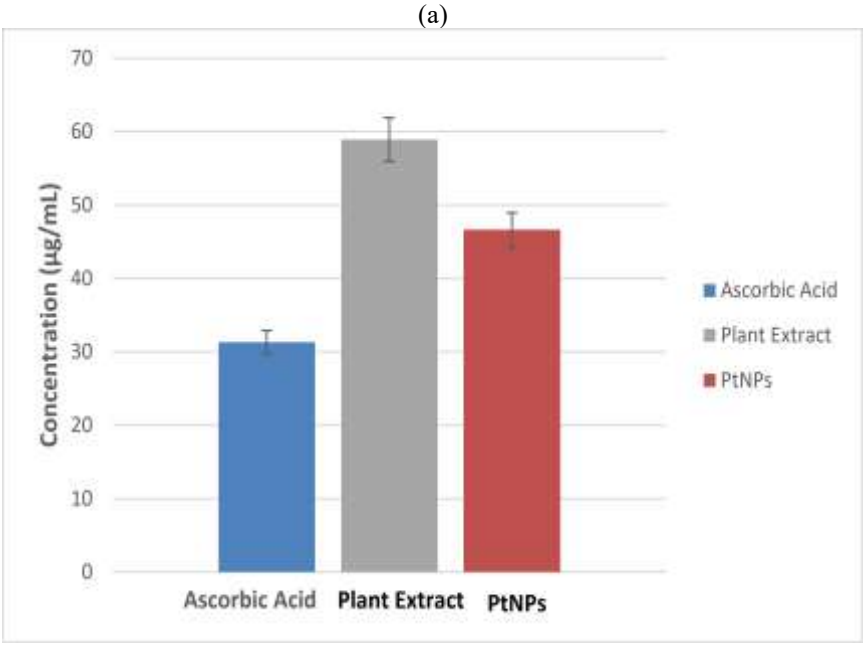
DPPH had been contemplated as one of the common and highly significant free radicals having the ability to damage the cells of human beings [23]. It was an uncharged, stable free radical, characterized by a deep purple colour and exhibiting strong absorbance at 517 nm. The mechanism of action was that aqueous leaf extract or PtNPs released hydrogen atoms or electrons for interaction with DPPH to neutralize it. The verdicts of the experiment demonstrated that the anti-oxidant potential of synthesized PtNPs was more than that of aqueous leaf extract, but less than that of standard, i.e. ascorbic acid (Figure 3a).

The standard reference used in the experiment was ascorbic acid, which displayed antioxidant potential with an IC_{50} value of 31.3 µg/mL. The aqueous leaf extract and synthesized PtNPs exhibited antioxidant efficacy with an IC_{50} value

of 58.92 µg/mL and 46.64 µg/mL, respectively. The concentration of an antioxidant – containing compound required to scavenge 50% of the initial free radicals, i.e. DPPH, is known as the IC_{50} value of that compound. The lower the IC50 value, the better the scavenging activity/antioxidant capacity of the compound. PtNPs showed less IC_{50} value than the leaves extract, hence greater antioxidant potential than the aqueous leaf extract (Figure 3b).



(a)



(b)

Figure 3. (a) Antioxidant potential of green synthesized PtNPs, plant extract and standard, (b) Comparative IC_{50} value of green synthesized PtNPs, plant extract and standard

3.4 Anti-diabetic activity of PtNPs (α-amylase inhibition assay)

The antidiabetic potential of synthesized PtNPs was evaluated using α-amylase inhibition assay and compared with standard antidiabetic drug, acarbose (Figure 4). The results demonstrated a concentration – dependent increase in inhibitory activity for both acarbose and PtNPs over the concentration range of 12.5 – 200 µg/mL. At the highest tested concentration of 200 µg/mL, PtNPs achieved approximately 74% inhibition, whereas acarbose showed nearly 90% inhibition. The observed effect may be attributed to interaction of nanoparticles with α-amylase enzyme, leading to reduced catalytic acitivity and delayed hydrolysis of starch into glucose. Additionally, the phytochemicals adsorbed on nanoparticle surface may attribute synergistically to enzyme inhibition. The high surface area and enhanced surface reactivity of PtNPs provide numerous active sites for enzyme interaction, thereby improving their biological efficacy.

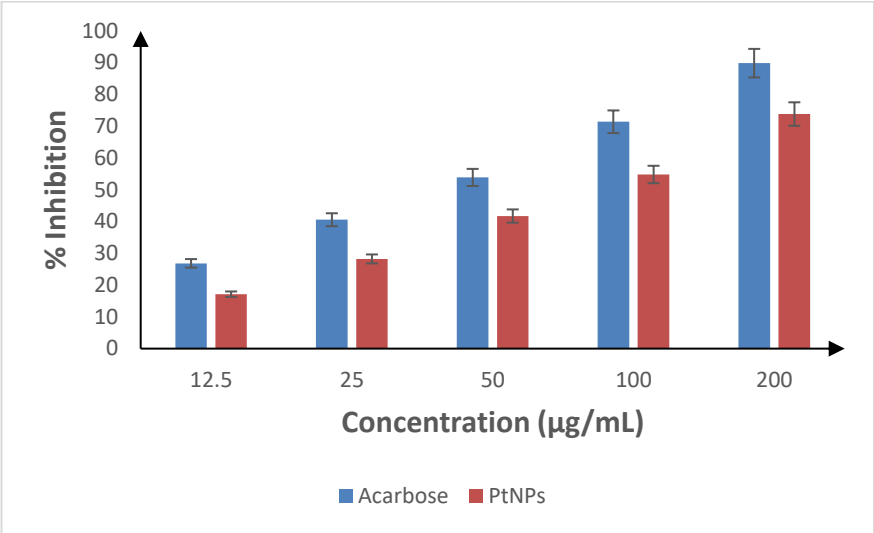


Figure 4. Antidiabetic efficacy of Acarbose (Positive control) and PtNPs against α-amylase

3.5 Antibacterial activity of green-synthesized PtNPs

Different concentrations of synthesized PtNPs were prepared (25, 50, 75 and 100 µg/mL) for evaluation of antibacterial potential of nanoparticles. The nanoparticles showed antibacterial activity against both Gram - positive and Gram - negative strains, as determined by the inhibition zone (Table 2). The strongest antibacterial activity was discerned against E. coli (20 mm), followed by P. aeruginosa (15 mm), S. aureus (14.5 mm), and B. subtilis (12 mm), but it was less effective than the standard drug (positive control), as shown in Figure 5. The noticeable antibacterial activity against Gram – negative bacteria may be since these have a minimal coating of peptidoglycan in their cell wall, which can be smoothly violated by the nanoparticles.

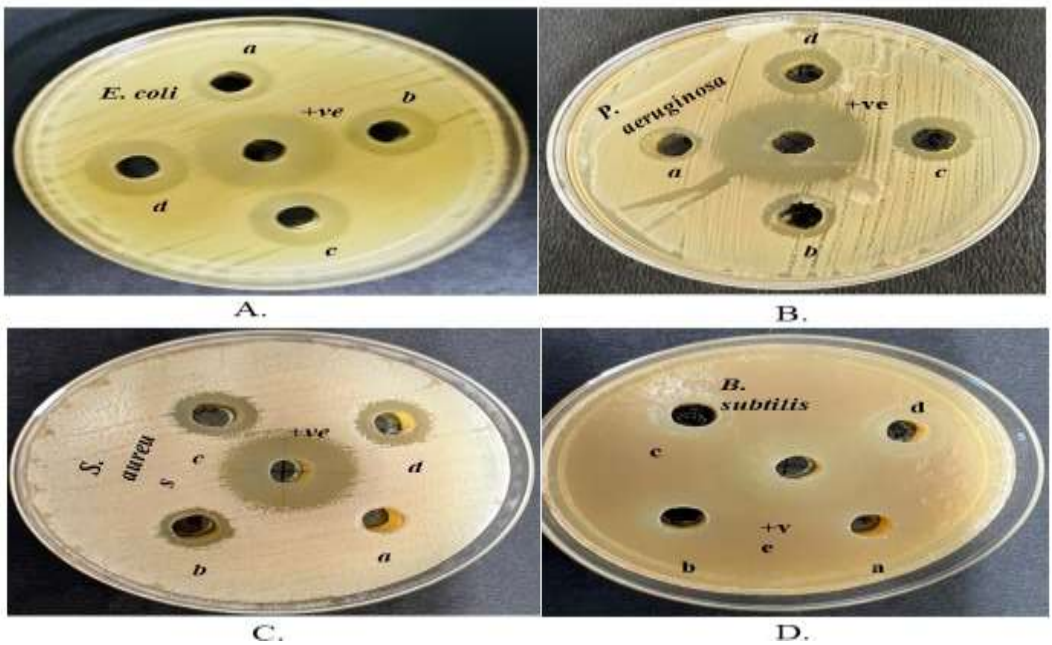


Figure 5. Antibacterial activity of green synthesized PtNPs at various concentrations (a - 25µg/mL, b - 50µg/mL, c - 75µg/mL and d – 100 µg/mL) and standard (+ve) i.e. antimycin (100µg/mL) against A. E. coli B. P. aeruginosa C. S. aureus D. B. subtilis

Table 2. Antibacterial activity of bio-synthesized Platinum nanoparticles (PtNPs)

S. No.	Name of the bacterial strain	Concentration of PtNPs added and Zone of inhibition (ZOI) (mm)			
		25 µL	50 µL	75 µL	100 µL
1.	E. coli	8	12	18	20
2.	P. aeruginosa	7	12	13.5	15
3.	S. aureus	-	11.5	14	14.5

4.	B. subtilis	-	6.5	10	12

3.5 Possible Antibacterial Mechanism

One possible antibacterial approach that can be employed by PtNPs is to alter the structure of the cell membrane of bacteria and prevent normal budding owing to loss of membrane integrity, as previously mentioned in studies [24]. The mechanism, especially, involves nanoparticles inserting into the cell wall of bacteria, followed by the release of reactive oxygen species (ROS), which further causes DNA down – regulation, oxidative stress, and ultimately leads to apoptosis or programmed cell death of bacterial cells (Figure 6) [25]. The persuasive antibacterial activity of PtNPs may be accreditted to their spherical shape, small size, and uniform dispersion. Nanoaprticles having small particle size and spherical in shape typically exhibit a higher surface area-to-volume ratio, thereby enhancing their efficacy compared to larger, irregularly shaped counterparts [26].

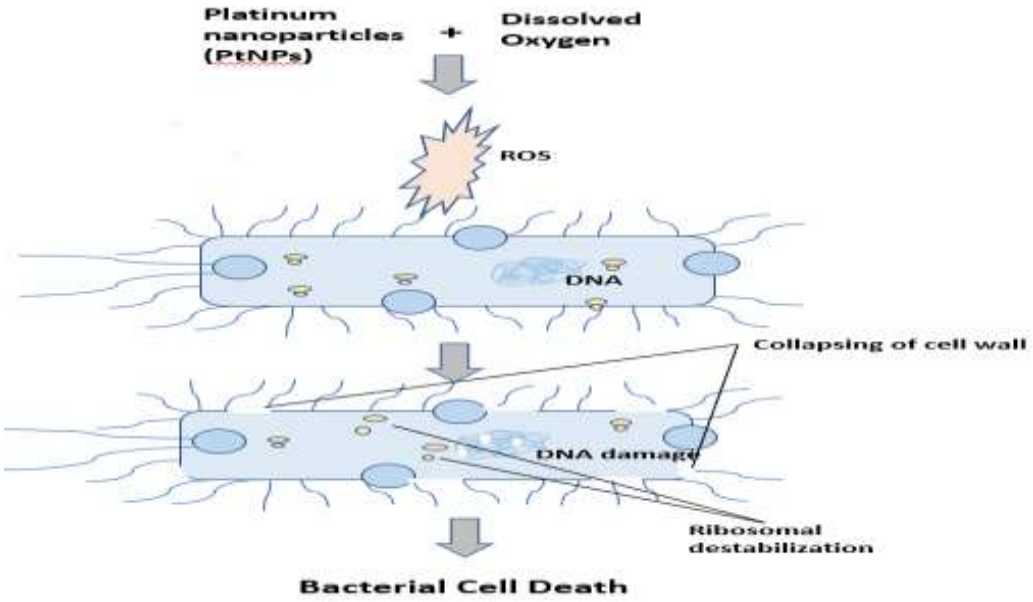


Figure 6. Possible mechanism of antibacterial activity of PtNPs against selected bacterial strains

4. Conclusion

The present work successfully demonstrated a rapid, cost-effective and eco-friendly approach for the synthesis of PtNPs employing the aqueous extract of A. indica leaves. The synthesized nanoparticles were successfully characterized through varying techniques such as HRTEM, FESEM, UV spectroscopy, FTIR and zeta potential, affirming their shape, size and morphology. The PtNPs were screened for their antibacterial activity against both gram - positive and gram - negative strains. The greater activity of PtNPs against bacterial strains can be assigned to their small size, large surface area and uniformly distribution. The nanoparticles also showed remarkable anti-oxidant activity, specifying their capacity to neutralize free radicals and eventually minimize the oxidative stress. In addition, the synthesized PtNPs showed promising antidiabetic activity by showing concentration – dependent inhibition of the α-amylase enzyme. Overall, the findings of present study highlighted that green synthesized PtNPs are multifunctional nanomaterials with significant antioxidant, antibacterial and antidiabetic properties, showing their potential for various biomedical and pharmaceutical applications. Future research should focus on elucidating the molecular mechanisms underlying these activities through advanced biochemical and proteomic studies.

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