

Phytochemical-Assisted Synthesis of Palladium Nanoparticles from Aqueous Blueberry Extract and their Antibacterial Activity

Research Article

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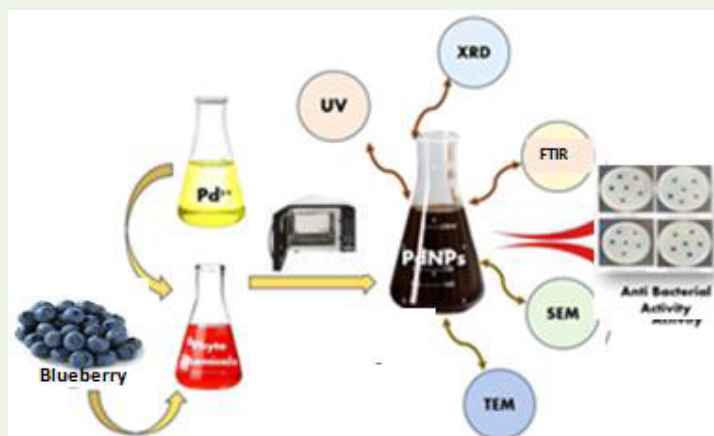
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Abstract

Green synthesis has emerged as a sustainable and cost-effective alternative to conventional chemical methods for the fabrication of metal nanoparticles. In current research, palladium nanoparticles (PdNPs) were synthesized using blueberry fruit extract through an eco-friendly green synthesis approach. The phytochemical constituents present in blueberry extract served as effective reducing and stabilizing agents, facilitating the formation of PdNPs without the need for hazardous chemicals. The synthesized nanoparticles were comprehensively characterized employing "scanning electron microscopy (SEM), ultraviolet-visible (UV-Vis) spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM). XRD results exhibited characteristic diffraction peaks at 39.98° (111), 46.49° (200), and 67.95° (220), confirming the crystalline nature and face-centered cubic (FCC) structure of PdNPs. Morphological investigations by SEM and TEM suggested formation of predominantly spherical nanoparticles with sizes ranging from 30 ± 5 nm. Furthermore, biogenically synthesized PdNPs displayed promising antibacterial activity against Gram-positive and Gram-negative bacterial strains. Biosynthesis "of palladium nanoparticles employing aqueous blueberry fruit extract and their potential application as" Antibacterial agents.



Introduction

The rapid development of nanoscience and nanotechnology has significantly transformed modern scientific research, making the field one of the most influential areas of the twenty-first century. The progress of nanotechnology largely depends on the ability to synthesize metal NPs with controlled size, morphology, shape and to integrate them effectively into complex systems for diverse applications. Among the various methods available for nanoparticle production, biological or green synthesis becomes “sustainable and environmentally friendly alternative to” conventional physical as well as chemical approaches [1-3]. Biogenic nanomaterials offer several advantages, encompassing lower energy consumption, reduced toxicity, and utilization of renewable biological resources. Various biological entities such as microorganisms, plants, and animal-derived materials “have been employed as reducing and stabilizing agents for nanoparticle synthesis [4-6]. In particular, plant-mediated synthesis has gained considerable attention because of its cost-effectiveness, eco-friendliness, simplicity nature. The abundance of phytochemicals present in plant extracts enables the efficient reduction” of metal ions from their oxidized states to their corresponding zero-valent metallic nanoparticles.

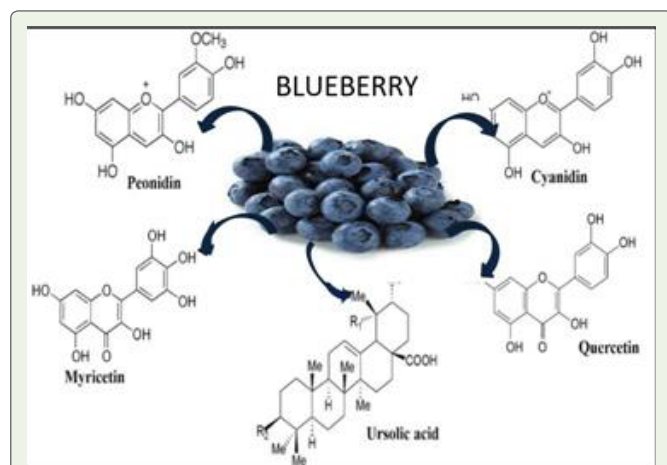


Figure 1: Cyanidin “and peonidin are the major anthocyanin glycosides found in fruit. Quercetin glycosides are the major flavanols in blueberry fruit; myricetin glycosides are present in lesser quantities. The triterpenoid ursolic acid is also present in” blueberry fruits.

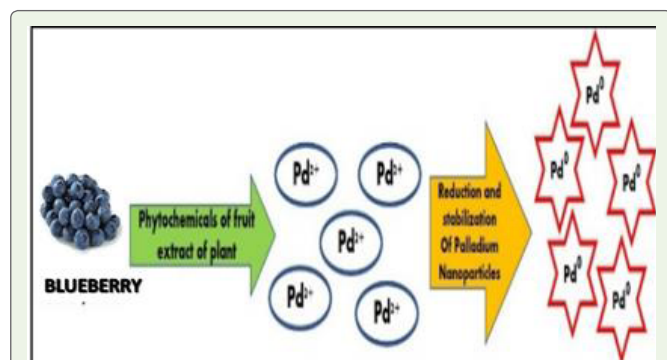


Figure 2: Graphical “representation of the green synthesis of PdNPs using phytochemicals present in the” ABE.

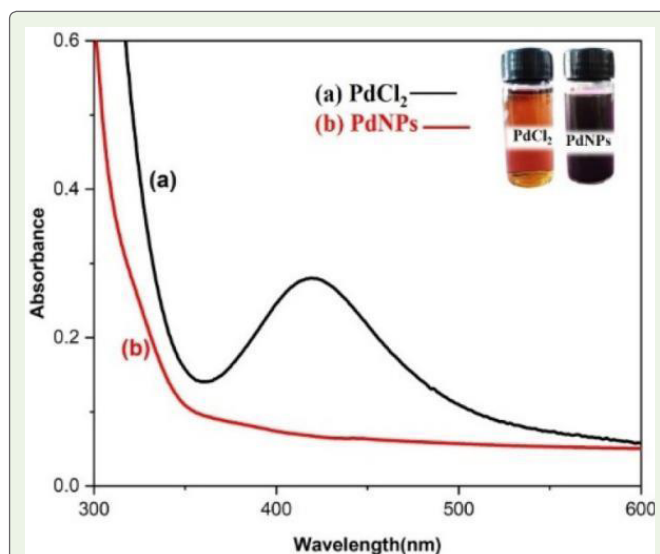


Figure 3: UV-vis absorption spectra of biogenic ABE PdNPs.

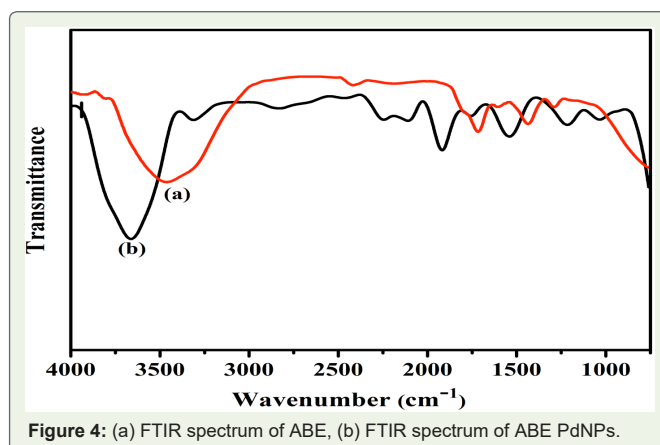


Figure 4: (a) FTIR spectrum of ABE, (b) FTIR spectrum of ABE PdNPs.

Blueberry (*Vaccinium Corymbosum*), belonging to *Ericaceae* family, rich source of diverse phytochemicals and bioactive compounds. The fruit contains substantial amounts of polyphenols, flavonoids, anthocyanins, proanthocyanidins, and Vitamin C, Vit K1, Vit E, and other minerals that display a variety of biological activity [3]. blueberries have been “reported to possess antibacterial, anti-inflammatory, antiviral, antioxidant, antimutagenic, anticarcinogenic, antitumor, and antiangiogenic properties [7]. Due to these therapeutic benefits, they have long been” employed in conventional medicine for treating and preventing microbial infections [8].

Concentration and composition of phenolic compounds in blueberries vary with cultivar, geographical location, climatic conditions, fruit maturity, harvesting time, and storage conditions. blueberries are recognized for their health benefits [9-10], including the prevention of dental caries, periodontal diseases, urinary tract infections, inflammation, digestive disorders, and hypercholesterolemia.

In current research, PdNPs were synthesized employing aqueous blueberry extract (ABE) and evaluated for their biological

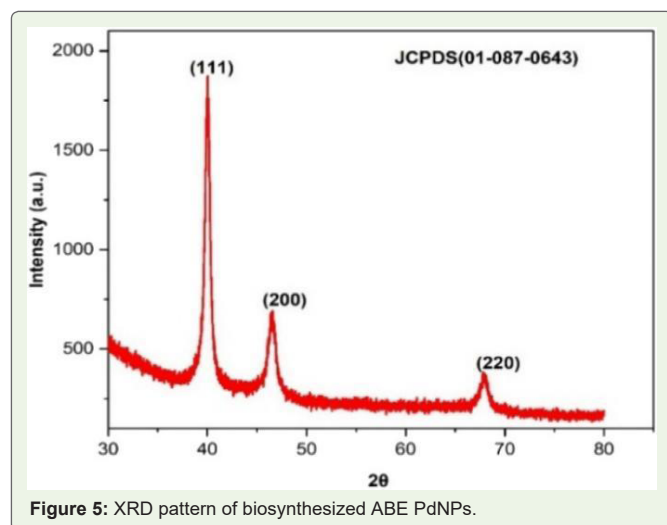


Figure 5: XRD pattern of biosynthesized ABE PdNPs.

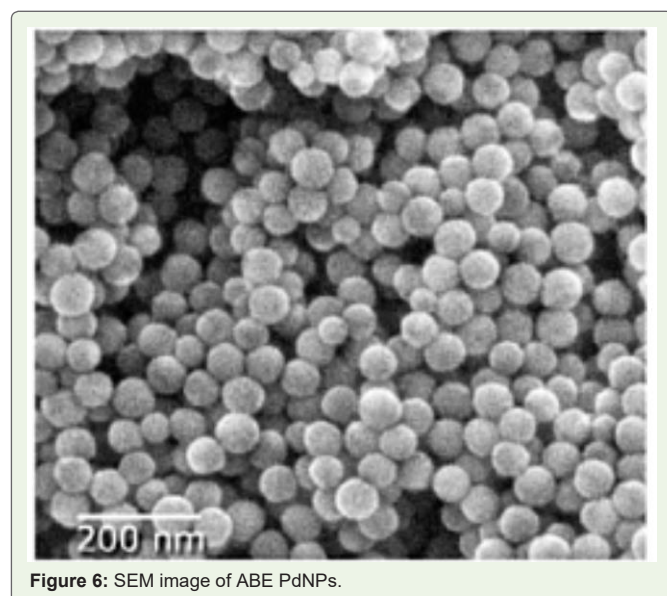


Figure 6: SEM image of ABE PdNPs.

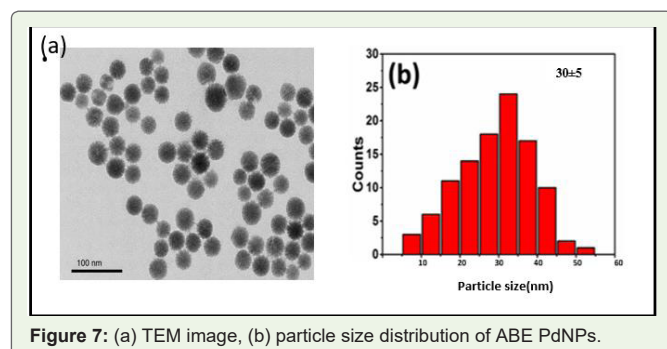


Figure 7: (a) TEM image, (b) particle size distribution of ABE PdNPs.

applications. PdNPs possess excellent oxidation resistance, stability, biocompatibility, and catalytic activity, along with superior optical, electronic, and plasmonic properties. Their “face-centered cubic (fcc)” crystal structure enables formation of diverse morphologies

[11-12], while their exceptional hydrogen absorption capacity makes them valuable for sensing, storage, and catalytic applications.

Conventional “physical and chemical synthesis methods frequently necessitate expensive equipment, high energy input, and toxic reducing agents, generating” hazardous by-products that limit biomedical applications. In contrast, green synthesis offers simple, environmentally friendly, cost-effective alternative. Biological resources like “plant extracts act as natural reducing and stabilizing agents, enabling controlled NP synthesis. ABE serves as an efficient reducing and capping agent for the one-step synthesis of eco-friendly PdNPs. This sustainable approach offers a promising route for forming functional PdNPs with potential applications in catalysis, environmental” remediation, and biomedicine [13-15].

Furthermore, a comprehensive survey of the existing literature revealed that blueberry (*Vaccinium Corymbosum*) fruit extract has previously been discovered for green synthesis of PdNPs. Therefore, we reveal eco-friendly synthesis and stabilization of PdNPs employing ABE as “both reducing and capping agent. The synthesized PdNPs were thoroughly characterized using a range of spectroscopic and microscopic techniques, including Transmission Electron Microscopy (TEM), Fourier Transform Infrared (FTIR) spectroscopy UV-Visible spectroscopy. In addition, the biological efficacy of the prepared nanoparticles was evaluated by investigating their antibacterial properties, signifying their potential for biomedical” and therapeutic applications.

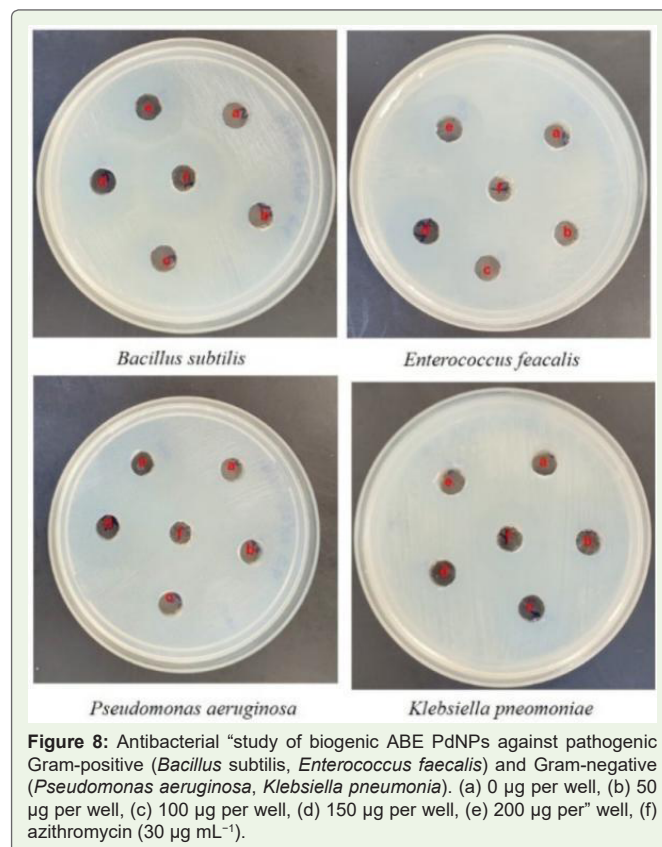


Figure 8: Antibacterial “study of biogenic ABE PdNPs against pathogenic Gram-positive (*Bacillus subtilis*, *Enterococcus faecalis*) and Gram-negative (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*). (a) 0 µg per well, (b) 50 µg per well, (c) 100 µg per well, (d) 150 µg per well, (e) 200 µg per well, (f) azithromycin (30 µg mL⁻¹).

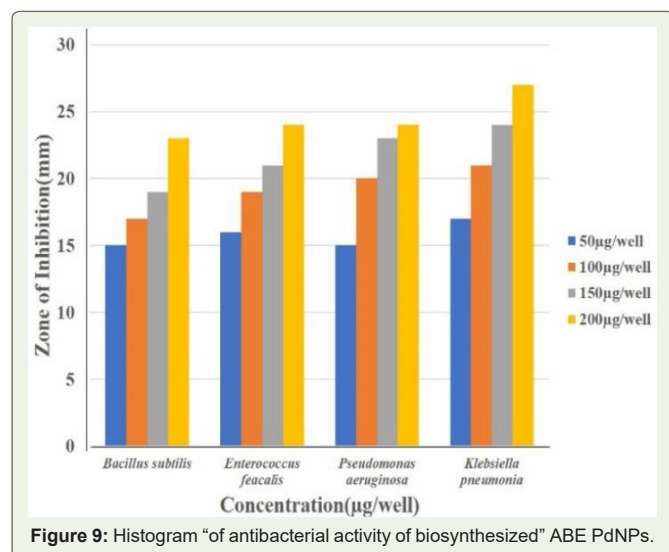


Figure 9: Histogram "of antibacterial activity of biosynthesized" ABE PdNPs.

Materials and Methods

Materials

Ripen fruits of blueberry were purchased from a local market in Hyderabad, India our university garden. We bought palladium chloride (PdCl_2) from Mumbai, India's S.D. Fine Chemicals. Analytical-grade reagents were all utilized without additional purification. During experiment, double-distilled (DD) water was utilized.

Preparation of Aqueous blueberry Fruit Extract

To remove dust, surface contaminants, and fungal spores, the fruits were thoroughly cleaned multiple times with distilled water. 10 g of fresh blueberries were crushed and homogenized in 100ml of DD water to make the aqueous extract. Resulting mixture then centrifuged to separate the solid residues and obtain a clear extract. The supernatant was collected and kept at 5 °C for subsequent usage in biosynthesis of PdNPs.

Green Synthesis of Palladium Nanoparticles (PdNPs)

They were synthesized employing aqueous blueberry extract as a bioreducing and stabilizing agent. Briefly, adding dropwise 1mL of aqueous blueberry extract to 10mL of a 1mM aqueous palladium (II) chloride (PdCl_2) solution under continuous stirring. Reaction mixture was treated with microwave irradiation for 2min. Reduction occurs slowly by the appearance of an intense black color, highlighting reduction of Pd^{2+} ions as well as formation of PdNPs. The PdNPs synthesis has been initially confirmed by visual observation and subsequently verified using UV-Visible spectroscopic analysis in wavelength ranging 300 to 500nm. Synthesized nanoparticles were then collected by centrifugation at 6000 rpm, washed to remove residual impurities, and dried for further characterization and applications.

Antibacterial Activity

The agar "well diffusion method" biosynthesized PdNPs' antibacterial activity [16–17]. Four bacterial strains were chosen as test organisms: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*,

(Gram-negative bacteria), and *Enterococcus faecalis*, *Bacillus subtilis* (Gram-positive bacteria).

The "antimicrobial activity of AgNPs has been assessed utilizing agar well diffusion approach. Preparing nutrient agar plates and allowed to solidify under laminar airflow conditions. After solidification, bacterial" cultures uniformly swabbed onto agar surface. Wells created in agar utilizing sterile 1 mL micropipette tips and filled with nanoparticle "solution at a concentration of $100\mu\text{g mL}^{-1}$. Following 37°C, zones" of inhibition have been assessed.

Results and Discussion

UV-Visible Spectral Analysis

The formation of palladium nanoparticles was initially confirmed through UV-visible spectroscopy by comparing the absorption characteristics of the precursor palladium chloride solution and the synthesized nanoparticles. Unlike AuNPs & AgNPs, exhibiting characteristic localized surface plasmon resonance (LSPR) bands responsible for their vivid colors, palladium nanoparticles generally do not display a distinct surface plasmon resonance peak. Instead, they are characterized "by a broad and continuous absorption profile across the visible region ([18-19].

(Figure 3) presents the UV-visible spectra of the PdCl_2 solution and the PdNPs obtained after microwave irradiation using blueberry fruit extract. The PdCl_2 solution exhibited a prominent absorption band around 425 nm, attributed to ligand-to-metal charge transfer (LMCT) transitions and d-d electronic transitions related to Pd (II) ions. Following nanoparticle formation, this characteristic absorption peak disappeared, demonstrating complete reduction of Pd (II) ions" to metallic palladium. In contrast, the synthesized PdNPs displayed broad absorption band extending from the near-ultraviolet to visible region, which is characteristic of PdNPs and confirms their successful formation.

FTIR

The successful formation of palladium nanoparticles (PdNPs) stabilized blueberry fruit extract was further confirmed by FTIR analysis via identifying various "functional groups in NPs reduction and stabilization. Figure 3 represents FTIR spectra of" the fruit extract and the synthesized PdNPs. O-H as a broad absorption band at 3285 cm^{-1} , whereas aliphatic C-H stretching vibrations in the fruit extract were identified as a weak peak at 2851 cm^{-1} . The bands detected at 1602 & 1404 cm^{-1} were "attributed to the symmetric and asymmetric stretching vibrations of COO^- and C=O groups, correspondingly. Carbonyl" and carboxylate functional groups' C-O stretching vibrations were linked to a strong, abrupt peak at 1013 cm^{-1} [42].

Comparison of FTIR spectra of fruit extract and PdNPs revealed slight shifts in peak positions and variations in peak intensities, indicating the involvement of these biomolecules in nanoparticle formation. Additionally, a new peak appeared at 1720 cm^{-1} in the PdNP spectrum, corresponding to stretching vibration of C=O group. Such spectral changes suggest interactions between functional groups of phytochemicals and palladium ions, leading to "reduction of Pd(II) to Pd(0) and" subsequent stabilization of synthesized nanoparticles.

XRD Analysis

XRD analysis took place for investigating crystalline structure of

biogenically synthesized PdNPs. As illustrated “in (Figure 5) XRD pattern exhibited distinct diffraction peaks” at 2θ values 39.98° , 46.49° , & 67.95° , which were “indexed to (111), (200), and (220) lattice planes, respectively. The” presence of these reflections confirms the successful formation of crystalline metallic palladium with FCC structure. Additionally, observed “diffraction pattern closely matches standard” JCPDS data for palladium, verifying phase purity as well as crystallographic identity of the synthesized NPs.[20]

Among the observed reflections, (111) plane “exhibited the highest intensity, suggesting that crystal growth preferentially occurred along this” direction. Applying “equation, full width at half maximum (FWHM) of (111) diffraction peak was computed for” determining “average crystallite size of the produced PdNPs. The” average crystallite size determined was roughly 6.7nm.

SEM and TEM Analysis

Employing morphology of synthesized PdNPs was investigated. The “NPs had a mostly spherical morphology and a rather uniform distribution, as” seen in (Figure 6a).

Further structural and size characterization was carried out using TEM. The TEM images suggested the formation of well-dispersed nanoparticles with homogeneous morphology under optimized synthesis conditions [21]. Particle size analysis obtained from the histogram plot (Figure 7 b) indicated an average particle size is 30 ± 5 nm. TEM demonstrated that the nanoparticles were composed of aggregated nanocrystalline domains arranged into extended and well-defined network-like structures. These observations confirm the successful formation of small, crystalline, and uniformly distributed palladium nanoparticles.

Antibacterial Activity

The agar has been employed to assess the biogenic PdNPs' antibacterial activity. Test organisms included “two Gram-positive bacterial strains (*Enterococcus faecalis* and *Bacillus subtilis*) and two Gram-negative strains (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*). The antibacterial activity of synthesized nanoparticles was verified by development of a distinct inhibition zone surrounding PdNP-treated wells following a 24-h incubation period (Figure 13).

Significant antibacterial action against both Gram-positive as well as Gram-negative bacteria was also demonstrated by” aqueous cranberry fruit extract utilized for PdNP production [22–25]. Primary mechanism of metal NPs antibacterial action is their ingestion and contact with bacterial cell membranes, which disrupts membrane transport. Additionally, nanoparticles may interact with cellular enzymes and DNA, resulting in respiratory inhibition and cell death.

Findings of present study revealed “that the biogenically synthesized palladium nanoparticles exhibited greater antibacterial activity against Gram-negative bacterial strains than against Gram-positive strains, as” “summarized in (Table 1). These observations align with previously reported research on antimicrobial properties of palladium nanoparticles. As illustrated in (Figures 8) (Figure 9), the PdNPs synthesized using aqueous blueberry fruit extract showed pronounced bactericidal effects, particularly against Gram-negative bacteria.

Enhanced susceptibility of “Gram-negative bacteria may be attributed” to their relatively thin peptidoglycan layer, facilitating easier penetration” of nanoparticles into the bacterial cell. Once internalized, the PdNPs can interfere with essential cellular and metabolic functions, ultimately leading to bacterial growth inhibition and cell death. Conversely, thicker peptidoglycan wall present in Gram-positive bacteria might act as a protective barrier, reducing nanoparticle penetration and antibacterial effectiveness.

Furthermore, the phytochemical compounds from the blueberry fruit extract that remain adsorbed on the nanoparticle surface as capping agents may contribute synergistically to the observed antimicrobial activity, thereby enhancing the overall bactericidal performance of the synthesized PdNPs against Gram-negative microorganisms.

Conclusion

By blueberry fruit extract with a PdCl_2 solution, green techniques were successfully employed to generate palladium nanoparticles. This has been validated by UV-visible spectrum analysis, eliminating SPR peak. PdNPs' face-centered cubic structure and crystalline character were validated by XRD analysis. The biosynthesized PdNPs' spherical shape, particle size 30 ± 5 nm demonstrated by TEM images. PdNPs antibacterial activity generated from aqueous blueberry fruit extract was assessed “against Gram-positive (*Enterococcus faecalis*, *Bacillus subtilis*), Gram-negative (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) bacteria. Results showed a significant zone of inhibition.

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Table 1: The antimicrobial properties of ABE PdNPs.

Name of the organism	Zone of inhibition (mm)					ZOI (mm) standard (azithromycin)
	0 μg per well	50 μg per well	100 μg per well	150 μg per well	200 μg per well	30 μg per well
<i>Bacillus subtilis</i>	—	15	18	19	21	25
<i>Enterococcus faecalis</i>	—	14	16	19	20	26
<i>Pseudomonas aeruginosa</i>	—	14	19	23	25	24
<i>Klebsiella pneumoniae</i>	—	17	20	22	26	26

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