

The Potential Antifungal Activity of the Developed Palladium Nanoparticles

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This research outlines that the manufacture of palladium nanoparticles (PdNPs) as being straight forward, inexpensive, and environmentally friendly. Rosa damascena flower extract was successfully used in this investigation to reduce palladium nanoparticles (PdNPs). UV-VIS, FTIR, XRD, TEM, EDX, DLS, and SEM were used to the characterized of the biologically formed of PdNPs. Upon UV-visible spectra of physico-chemically produced PdNPs, the SPR peak was measured at 360 nanometers. The TEM investigation indicated that the produced palladium nanoparticles had a spherical form and a diameter of around 50 nm. The biologically synthesized PdNPs demonstrated notable antimicrobial activity, including antifungal activity. PdNPs shows antifungal activity against some fungal species such as *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*. Based on findings, zone of inhibition of fungal strains was less than from fungal strains with Fluconazole. Bio-inspired production of PdNPs allows for adaptability to fungus strains. This makes PdNPs more suitable for biomedical applications.

Keywords: Antifungal Activity; FTIR; Green synthesis; Palladium nanoparticles; TEM; UV-Visible spectroscopy.

A new discipline of nanobiotechnology was recently formed due to the merging of nanometers-scale technologies with biological technologies. This newly developed area concentrates on the production, control, and application of nano-sized materials for emerging biotechnology¹. Globally, studies and research in nanotechnology have rapidly advanced quickly. Although the science of nanotechnology has the potential to expand, some people are still concerned on the possible risks and effects of nanoparticles on human health and the environment^{2, 3, 4}. A primary function of nanoparticles is to act as a link connecting larger particles and molecular or atomic structures⁵. The

multidisciplinary fields encompassing research and technology from physics, chemistry, and biology, usually known as nanotechnology, was initially introduced in 1974 by Prof. Norio Taniguchi.

In the last twenty years, the synthesis of nanoparticles has ushered in nanotechnology, nanoparticles synthesis, and the invention of materials with a variety of uses^{6, 7}. Among these, palladium, platinum, gold, and silver nanoparticles have been extensively studied to better understand their size and shape-dependent physicochemical properties and their interactions with various substances and molecules^{8, 9}. Much work has gone into creating innovative and promising

processes for making noble metal nanoparticles. Overall, top-down to bottom-up procedures are able of producing of nanoparticles. Top-down methods involve slicing original materials to create nanoparticles¹⁰. The bottom-up approach entails creating nanoparticles from smaller compounds like atoms and molecules, which then develop into nano-sized particles using different biological and chemical techniques¹¹.

The three most crucial requirements are choosing a safe material for stabilization, an appropriate non-toxic oxidizer, and an eco-friendly liquid (the most popular ones are ethanol, water, and their mixtures)¹². Many synthetic approaches have been utilized in order to produce nanoparticles; the most common method is biophysical, molecular, and biological. Biochemical processes usually contain the use of dangerous reagents. They are also quite expensive¹³. Among the most throughout comparison from the other methods like physical and chemical, plant-mediated processes offer a secure, non-toxic, and eco-friendly way to synthesis nanoparticles for a variety of functions such as physiological ones^{14,15}. The above synthesis process has been accomplished using algae, fungi, bacteria, and plants^{16,17}. However, plant extracts may be used to create nanoparticles with certain sizes, shapes, and contents. Furthermore, the range of physicochemicals present in their material might function as organic stabilizing and reducing agents for the nanoparticles production process^{18,19}. The biological formation of palladium nanoparticles has been investigated in types of plant species, such as *Annona squamosa*²⁰. In a well-diffusion assay, palladium nanoparticles were shown to have antimicrobial activity against anti-fungal strains²¹. A few species of the common, filamentous, mycotoxigenic fungus *Aspergillus*, including *A. niger* and *A. flavus*, are referred to as pathogens that like advantages of their surroundings²². *Candida* species cause chronic mycosis, which affects the mouth, throat, skin, ovaries, and the gut^{23,24,25}. The potential for harm from these species to humans also depends on how well-developed their immune systems when inhaled or injected with spores. They are responsible for 90-100% of infections, including invasive aspergillosis (IA)²⁶, which has a high fatality rate in critically ill and immunocompromised persons. As a result, they can spread locally or disseminate to other

locations. Delaying the initiation of antifungal medication due to delayed diagnosis worsens the prognosis of IA^{27,28}. As a result, it is still difficult to definitively diagnose fungal infections, especially in immuno-compromised hosts. Furthermore, if any fungal infection symptoms exist, they are typically non-specific²⁹. The identification of the etiological agent may result from culture-based diagnostics, which has the potential to be a laborious procedure. Furthermore, the place of collection may have an impact on the sample's sensitivity, and contamination may go unnoticed³⁰. The abuse of currently available antifungal medications, such as fluconazole, azoles, echinocandins, and polyenes, has caused the appearance of clinical drug-resistant *Aspergillus* species in recent years^{31,32}. As an illustration,azole resistance is uncommon and results from a shift in the amount of ergosterol or from the creation of other forms of sterols that make up the membranes. Without impairing regular cell function, this process might be able to explain the resistance. Nevertheless, studies have shown that resistance toazole medications can have major clinical consequences; individual with aspergillosis that is resistant to therapy have a 25% increased risk of death³³. On the other hand, current research suggests that metallic nanoparticles be employed to treat *Aspergillus* sp.-caused fungal infections³⁴. Systemic candidaemia, a potentially fatal condition, can also result from *candida* infections³⁵. The yearly occurrences of *Candida* infections are estimated to be approx 4 million cases globally³⁶, with a death rate of roughly 40%³⁷. This leads to a substantial financial strain due to the high cost of medications and extended hospital stays³⁸. *Candida albicans* is the main pathogen regarding the pathogenic agents of *Candidiasis* due to its phenotypic flexibility and high infection frequency³⁹. Fungal virulence factors impact *C. albicans* colonization and infection. These variables comprise the capacity to develop at 37 degree C, structure transition, hemolytic activity, and release of hydrolytic enzymes, tissues adhesion, immune system evasion, filamentation ability, and biofilm formation^{40,41}. Recent years have seen a rise in research on nanotechnology, and the utilization of nanoparticles in clinical and diagnostics has been crucial to this field's expansion, essentially replacing traditional treatment modalities⁴². The size, shape, and metallic element of the particles all play a role. Biosynthetic

NPs offer several beneficial characteristics, including reduced toxicity and remarkable catalytic and physicochemical capabilities^{43, 44}. With significant efficacy against the growth of *A. niger*⁴⁵, *Aspergillus flavus*⁴⁶, and *Candida albicans*⁴⁷, PdNPs are particularly well-known for their antifungal activities. Since *Aspergillus* sp. and *Candida albicans* resistance to standard antifungal therapy essentially provides a principle obstacle to the prevention of diseases, the introduction of NPs as microbial drugs could significantly enhance the control of *Aspergillus* along with other species infections. Compared to chemical, and physical processes, biosynthesis procedure has several benefits, such as low environmental impact, more affordable, free from pollutants, and high sustainable. They have numerous applications in material science, medicine, and agriculture. Bio-inspired palladium nanoparticles from *Rosa damascena* plant extract increase antifungal impact and making nanoparticles suitable for site-specific administration. PdNPs also display improved biological recognition, enabling specific interaction with fungal cell components. Belonging to the family of *Rosaceae*, *Rosa damascena* uses in the various fields like pharmacological characteristics encompass antibacterial, antifungal, etc. This research investigates the green manufacturing of palladium nanoparticles used extract of *R. damascena* plant. Extract from flower was used in the manufacturing of palladium nanoparticles as a moderating agent. After green procedure of PdNPs *in-vitro* study was done, such as checking the activity of palladium nanoparticles against antifungal strains.

MATERIALS AND METHODS

Chemicals and plant

The high-level analytical chemicals substances utilized all of the materials used in this work were bought by Sigma-Aldrich Co. The deionized water used in every part of the studies. The flowers petals of the *R. damascena* were collected from the campus in the IFTM University in Moradabad 244102, U.P, and India.

Preparation of Flower extract

Flower of *R. damascena* were plucked from campus of IFTM University and gently washed with distilled water. Petals of flower was

washed and then dried in air for 14-15 days in the air on wet paper. After removal of moisture from petals, crush and make a powder. A 10-gram powder was combined with 90 milliliters of distilled water and boiled for 20 minutes at 40°C on a heating mantle. The extract was cooled at room temperature after 20 minutes, and the Whatman filter paper was used to remove any leftover components. For later usage, the flower extract was maintained in a refrigerator at 40 degree C.

Biosynthesis of *R. damascena* capped PdNPs

According to our prior research, the formation of PdNPs was executed utilized the method describes in our investigation⁴⁸. The formation of PdNPs through metal ions (PdCl₂) 90 ml of a 1mM aqueous palladium chloride (PdCl₂) solution combines in 10 ml of *R. damascena* flower extract. A magnetic stirrer was used to mix this solution; after 15 minutes reaction, mixture color turn into brown. After 24 hours, the mixture of solution turns brown to black color, which shows the formation of palladium nanoparticles through flower extract⁴⁹.

Anti-fungal Study of PdNPs

The anti-fungal efficacy of PdNPs including *Aspergillus niger*, *Aspergillus flavus*, and *Candida albicans* was investigated. The antifungal study has been done through the Well diffusion method. According to the findings of the antifungal research conducted on PdNPs, the size of the nanoparticles had a significant impact in determining their antifungal activity, with smaller particles seeming to be more effective. The antifungal activity may also be affected by other parameters in addition to size, such as the fine-scale, the content of the nutrients, and the organisms that are being targeted.

RESULTS AND DISCUSSION

According to the findings of the research, floral extracts from *R. damascena* have the potential to produce PdNPs, which are metal nanoparticles that are safe for the environment, according to the research findings. *R. damascena* flowers have the potential to produce a wide range of secondary metabolites, which could potentially aid in the production of PdNPs. This approach was more effective than the conventional ones, which involve the use of hazardous chemicals and solvents. The

findings demonstrated exceptional antifungal effectiveness in opposition to antifungal strains, with the mechanism of action being connected to the emergence of disintegration.

UV-Visible analysis of PdNPs

A distinctive characteristic of PdNPs, Surface Plasmon Resonance peak at 360 nm has been studied in our previous research⁵⁰,

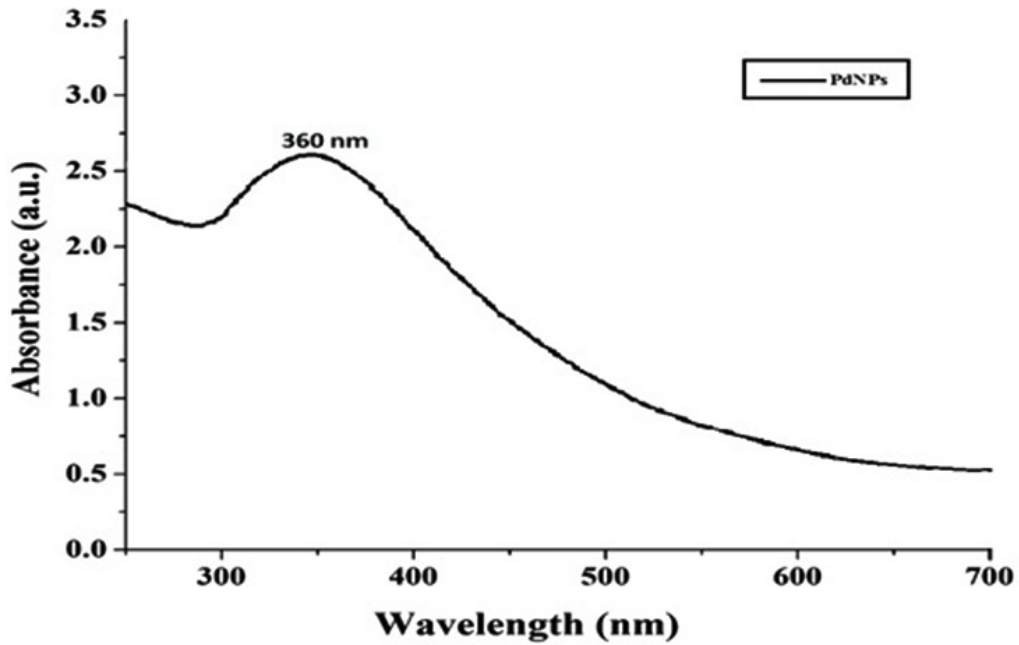


Fig.1. UV-Vis analysis

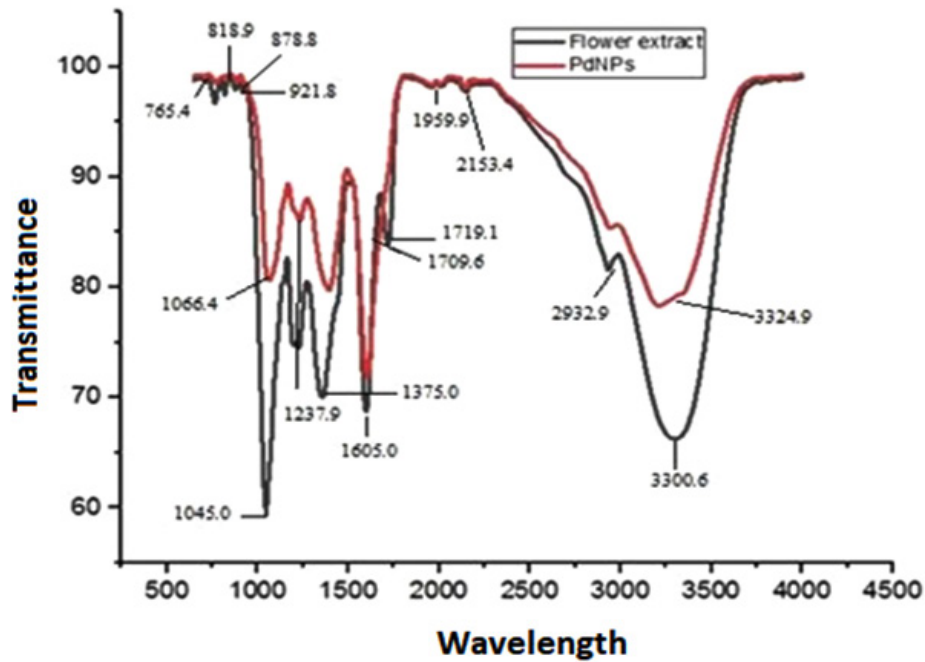


Fig. 2. FTIR analysis

shown in the UV-Visible graph, was excited as the biosynthesis of PdNPs in Fig. 1. Turning the solution color change yellow to black after 24 hours that visualized formation of PdNPs. This variation in color denotes the initial formation of PdNPs.

FTIR Characterization of PdNPs

Fourier Transform Infrared spectrophotometer for the *R. damascena* extract performed significantly as stabilization and reducing agent. The plant extract and the PdNPs were analyzed using the FTIR spectra shown in Fig.2. The FTIR analysis revealed a variety of peaks in the characteristic areas that indicate the

phytochemicals in plant extract which have ability to reduce palladium ions into nanoparticles. Strong peaks in the hydroxyl group typical to alcoholic compounds were found at 3333 cm^{-1} , according to the floral extract analysis⁵¹. The C-H bond is located at 2932 cm^{-1} , while the C=O bond at the peak of 2152 cm^{-1} . C=O bond at 1958 cm^{-1} , and 1708 cm^{-1} . Aromatic ring of OH at 1606 cm^{-1} , and 1364 cm^{-1} at C-N, 779 cm^{-1} at C-H region.

XRD Characterization of PdNPs

Fig.3. displays the X-Ray image of originally produced PdNPs derived from *R. damascena* floral extract. The crystalline shape

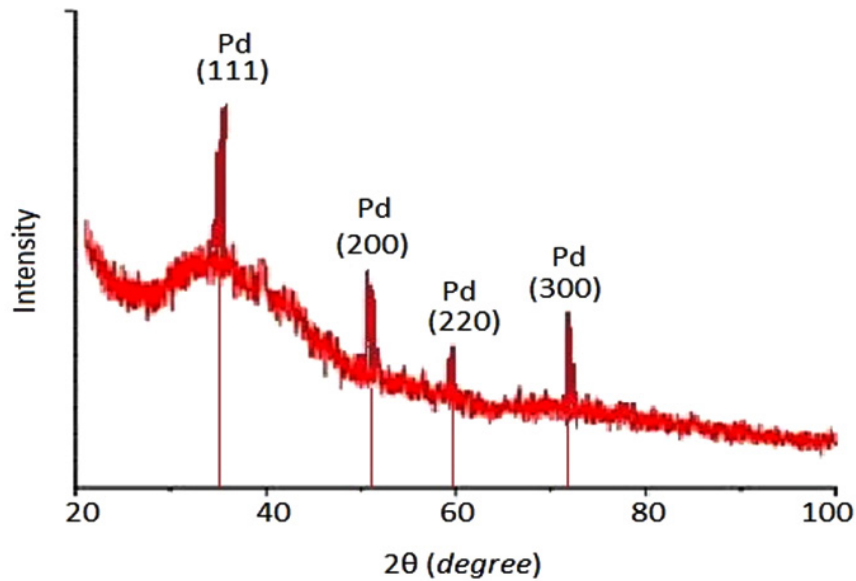


Fig. 3. XRD analysis

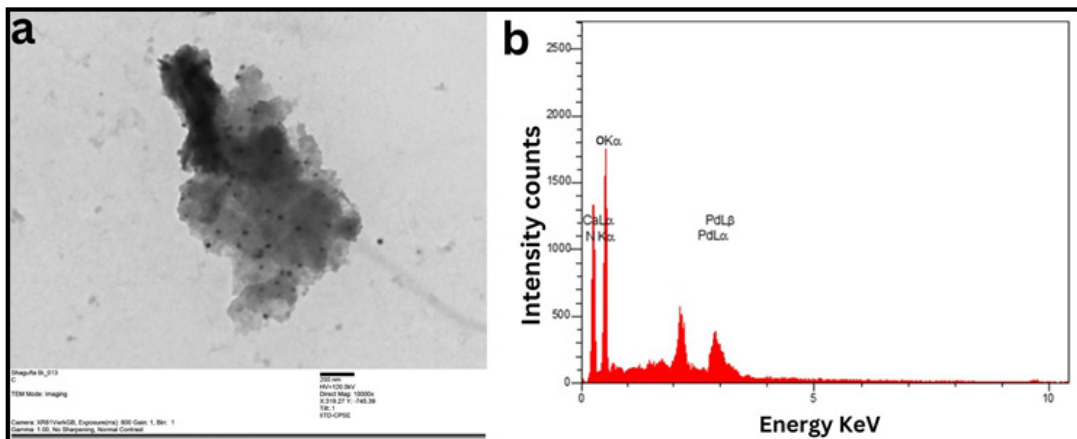


Fig. 4. TEM (a), and EDX (b) analysis

of PdNPs was analyzed and confirms using XRD pattern spectroscopy. X-ray diffraction, which was performed employed through “X’Pert PRO” spectroscopy, a technique that was often used for determining the crystalline organization of nanoparticles. A spectrophotometer used to measure PdNPs synthesized by plant extract indicates the existence of different regions at 2θ . XRD pattern shows great purity because the nanocrystalline PdNPs were free from crystallographic imperfections.

TEM and EDX of PdNPs

Transmission Electron Microscopy was used for the synthesized PdNPs for analyze particles size. Biologically synthesized PdNPs were found at 50 nm with a spherical shape shown in Fig.4 (a). Energy Dispersive X-Ray Spectrophotometer used for the examined of elements in the solution of synthesized PdNPs that confirm the palladium is presently shown in Fig.4 (b).

DLS Characterization of PdNPs

Dynamic light scattering instrument name (DynaPro-TC-04) analysis of the particles size

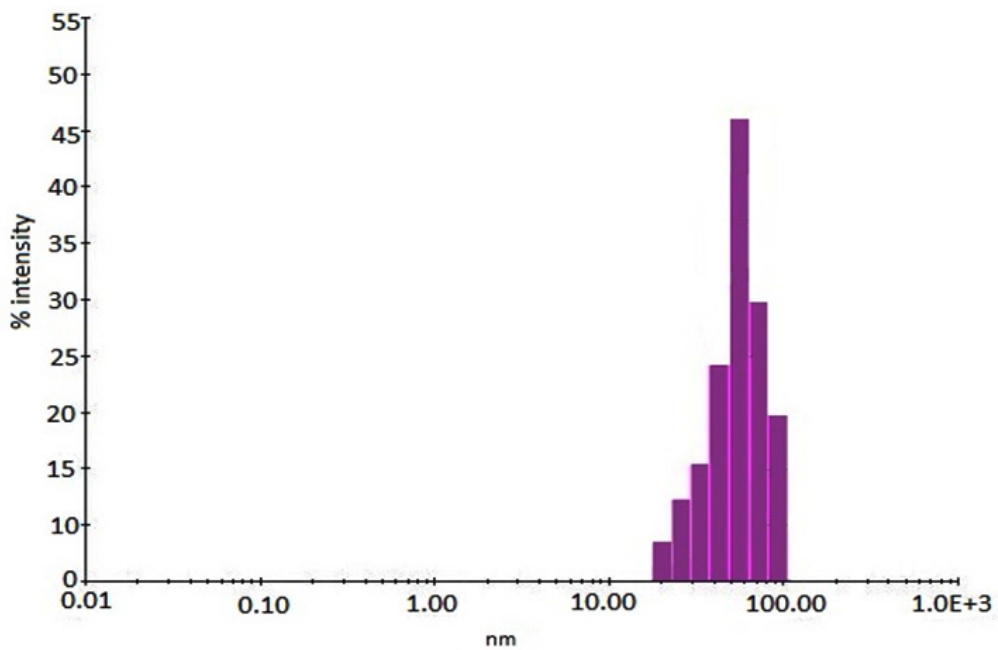


Fig. 5. DLS analysis



Fig. 6. SEM analysis

distribution of the nanoparticles is shown in Fig.5. PdNPs ranged from 20 nm to 100 nm. The DLS pattern shows the major particles as 50, 60 and 70 nm. This estimated through the intensity gain during the dynamic light scattering evaluation.

SEM Characterization of PdNPs

The size of the biologically produced PdNPs via *R. damascena* flower extract was approximately 42-47 nanometers through the used of the ZEISS EVO 18 equipped with an acceleration voltage ranging from 0.2 to 30 kV. Scanning electron microscopy tests were also used to establish the diameter and structure of PdNPs. The structure of the PdNPs was barley spherical, and mostly PdNPs aggregates non-spherical illustrate in Fig. 6.

Antimicrobial Efficacy of PdNPs

The antifungal action of the biosynthesized PdNPs from the *R. damascena* plant was evaluated with the well-diffusion process. The process was employed for perform the antifungal action in opposition to the fungal strain chosen: *Aspergillus niger*, *Aspergillus flavus*, and *Candida albicans* shown in Fig 7 (a, b). There were 4 wells in which each well has a distinct zone of inhibition against fungal strains illustrated in Table.1 shows the zone of inhibition in diameter that was calculated. 25ml of SDA agar media was added to every petri plate which was sterile and was left to set. For positive control of fungal strains, 1000 ppm Fluconazole was used.

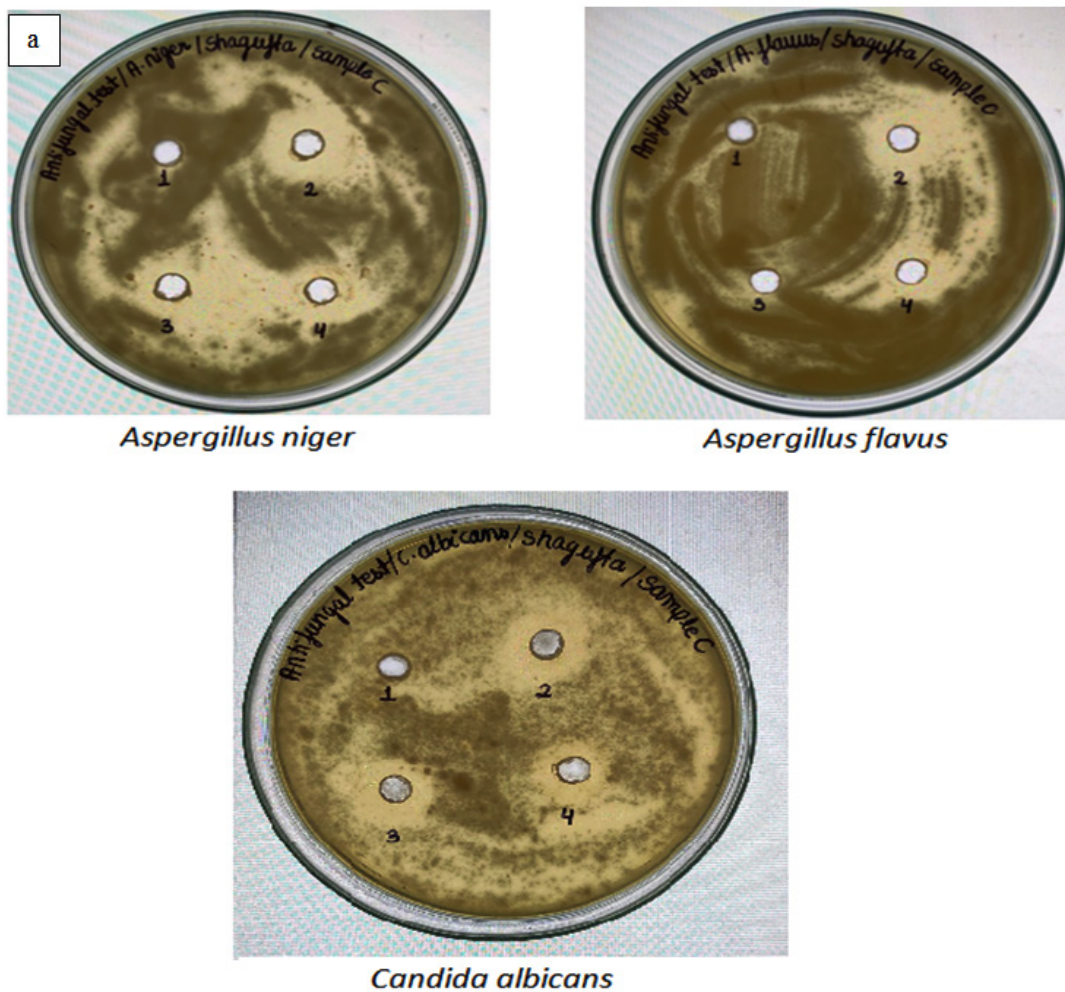


Fig. 7a. Antifungal images of the synthesized PdNPs

Table 1. Inhibition zone in mm

S.No.	Fungal Strain	Inhibition Zone (mm)		
		Positive control	PdNPs	PdNPs + Fluconazole
1	<i>Aspergillus niger</i>	15± 0.12	16 ±0.24	18±0.30
2	<i>Aspergillus flavus</i>	15 ±0.43	8 ± 0.42	15±0.55
3	<i>Candida albicans</i>	14 ±1.0	16 ±0.22	19±0.48

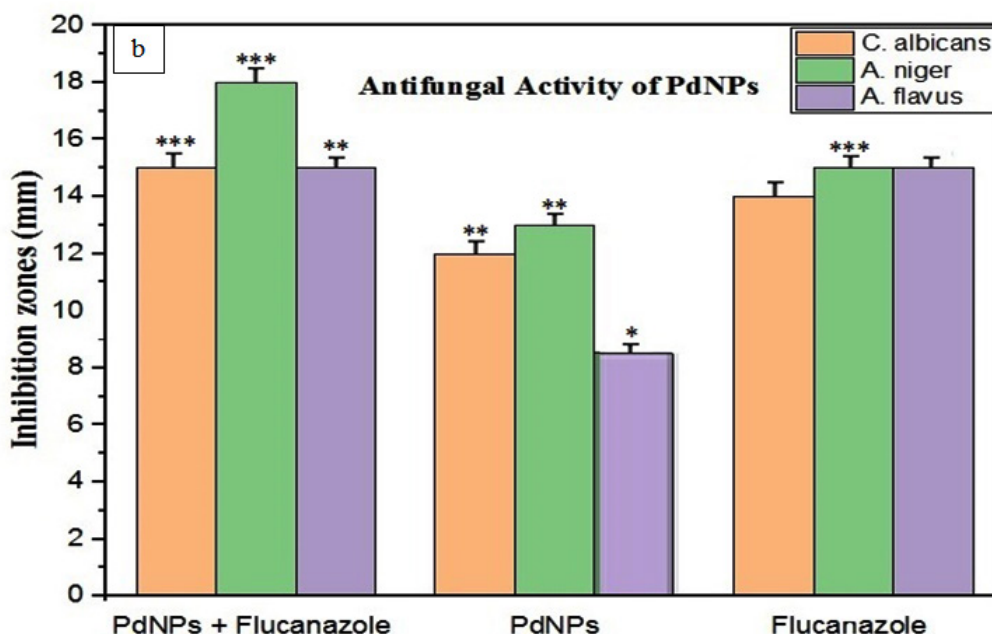


Fig. 7b. Graphically representation of antifungal activity of the synthesized PdNPs

For the showing fungal strain’s inhibition zone, plates were incubated in incubator for 1 day. The inhibit area of Fluconazole were measured 15 mm for *Aspergillus niger*, 15mm for *Aspergillus flavus*, and *Candida albicans* 14mm, and the inhibition zones of PdNPs against *Aspergillus niger* 13mm, *Aspergillus flavus* 8mm, and 12mm for *Candida albicans*. The PdNPs’s inhibitory zones with fluconazole were measured in opposition to as 18 mm for *Aspergillus niger*, 15 mm for *Aspergillus flavus*, and 15 mm for *Candida albicans* respectively.

CONCLUSION

The use of the *R. damascena* plant extract stabilizes and reduction of Pd ions to PdNPs production. The study investigated the

production of PdNPs using a range of techniques, including FTIR, UV-Visible spectrophotometer, XRD, TEM-EDX, DLS, and SEM. The Pd content was validated by EDX analysis, which revealed that the green-produced PdNPs were spherical and about 50 nanometers in size. The extract’s phytochemicals create nanoparticles with potent antifungal activity against a wide range of fungus. More research is needed to identify the individual phytochemicals employed to decrease palladium chloride and produce PdNPs, which have biological antifungal properties and hence merit additional treatment studies. The green manufacture of PdNPs was an alternative to conventional chemical procedures that is both safe for the environment and sustainable. By making use of plant extracts, enzymes, and microbes, this method reduces the amount of waste produced, reduces the amount of

harmful chemicals that are used, and reduces the expenses. PdNPs that have been synthesized using environmentally friendly methods possess potent antifungal properties, which make them appropriate for usage in antifungal drugs. The fact that they are so small and have such a large surface area allows them to come into contact with the membranes of fungal cells. This causes the integrity of the cells to be compromised, which in turn makes it more difficult for the fungal cells to reproduce. Due to the fact that these nanoparticles are biocompatible, it is quite unlikely that they will have a significant influence on either people or the environment.

Future prospective

The use of nanostructures for targeted drug delivery, detection, diagnostics, and bioimaging has recently shown encouraging results. In this instance, nanoparticles are likely to affect medicine positively. Different factors affected the synthesis of PdNPs from plant of *R. damascena* extract. Palladium nanoparticles have been used against antifungal activities. Green syntheses of PdNPs can be used in nano-pharmaceuticals soon join the nano-medical as quickly as possible. In order to improve efficiency and scalability, future research could concentrate on optimizing the synthesis process, which could result in more environmentally friendly ways to produce nanoparticles. Evaluating the benefits and drawbacks of biologically generated vs conventionally synthesized PdNPs could yield important information.

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

The manuscript incorporates all datasets examined throughout in this research study.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that

requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

Author Contributions

Shagufta Bi: Methodology, Data curation, Writing—draft manuscript, Writing—original draft, Writing—review and editing; Rashi Srivastava: Review & Supervision; Tanzeel Ahmad: Supervision.

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