



Rosa damascena flower mediated phytofabrication of palladium nanoparticles, in-vitro and in-vivo applications

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ABSTRACT

The formation of palladium nanoparticles is described in this research work as being simple, affordable, and non-toxic. This study employed *Rosa damascena* flower extract to effectively reduce ions to palladium nanoparticles. Biogenic formation of palladium nanoparticles (PdNPs) is characterized using Ultraviolet spectrophotometers, FTIR, XRD spectrophotometers, and TEM. The SPR peak was recorded at 360 nm in UV-visible analysis of biologically formed palladium nanoparticles. The diameter of the synthesized palladium nanoparticles was around approx 50 nm with a spherical shape. These biologically formed palladium nanoparticles demonstrated notable antibacterial activities. After antibacterial testing of palladium nanoparticles further check for anti-inflammatory and analgesic activity on rat model. These finding indicate that the flower extract of *R. damascena* is an excellent bio-reductant for the formation of palladium nanoparticles and has promise for employment in several fields in biomedical application.

1. Introduction

Nanobiotechnology is a subfield of nanotechnology concerned with the development of nanosized materials inside biologically active complexes that are more abundant in nature [1,2]. The modification of materials having at least a single size between 1 to 100 nm is known as nanotechnology, as described by the national nanotechnology initiative in the United States [3]. The topic of nanotechnology has recently emerged as a result of the fusion of bio science and nanoscale innovations. This recently developed discipline is concentrated on production, control, and application of particles at the nanoscale for improved bioscience [4]. Some well-known materials have intriguing nanosized. A Scientists has demonstrated their expertise throughout time, developing dendrimer materials of nanocomposite as well as distinct nano-based nanoparticles [5,6]. The synthesis of nanoparticles can indeed employ biological processes that are considered environmentally friendly and durable and are among the most alluring features of modern nanotechnology innovation [7]. Using as few toxic chemicals as possible to protect the environment from contamination is the primary goal of biological synthesis [8,9]. Nanoparticles derived from bacteria, fungi, and plants offer lots of possibilities uses in biology. The finding of a suitable solvent for the atmosphere and a reducer are the fundamental conditions during the production of nanoparticles

[10,11,12]. The phytochemicals nearby in plant extract, such as flavonoid, alkaloid, phenolic, and terpenoid are principally capable of transforming ions into stable nanoparticles [13,14]. Metallic nanoparticles synthesis using green approach is becoming a prominent environmentally friendly invention in recent years. The work demonstrates an environmentally friendly natural generation of palladium nanoparticles from *Allium fistulosum*, *Basella alba*, and *Tabernaemontana divaricate* leaf extracts. For these synthesis used of palladium acetate precursor [15]. The principle aspires of this study was the formation of palladium nanoparticles (PdNPs) employing *Rosa damascena* aqueous flower extract serves bio-reducing agent. After formation, palladium nanoparticles were investigated and characterize using Ultraviolet-Visible spectroscopy, Fourier Transform Infrared spectroscopy, X-ray Diffraction, and Transmission Electron Microscopy. Subsequently the analysis of palladium nanoparticles, test for antibacterial activity, and further checked activity of PdNPs against anti-inflammation and analgesic on a rat model.

2. Materials & method

2.1. Chemical and reagents

The high analytical level chemical Palladium Chloride (PdCl₂)

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utilized in this investigation was all purchased by Sigma-Aldrich Company USA. *Rosa damascena* flower collected from the University campus (IFTM University, Moradabad).

2.2. Preparation of flower extract of *Rosa damascena* plant

Flowers of the *R. damascena* plant was cleaned with distilled water and dried for 10 days to remove moisture after dried, crushed the petals into fine powder. On heating mantle, 10 g flower powder were weighed and boiled in 100 ml distilled water for 20 min in 50 degrees C. After 20 min cool down extract at room temperature, after that Whatman filter paper used for the filtration of this extract. This extract was filtered and kept at room temperature until it was needed.

2.3. Procedure for green synthesis of PdNPs

An aqueous solution of 1 m Molar PdCl₂ was prepared and employed in the green synthesis of palladium nanoparticles. For the reduction of palladium ions into palladium nanoparticles, 10 ml of plant extract was mixed into 90 ml of 1mMolar aqueous solution of palladium chloride (PdCl₂ reagent). After mixing using of magnetic stirrer for the reaction mixture, after 15 min in magnetic stirrer color was changed from light yellow to dark brown. 24 h later, the mixture changed color from brown to dark black that shows the visualization analysis synthesis of palladium nanoparticles [16]. In accordance with the required palladium nanoparticles qualities, the synthesis can be conducted at an ambient temperature. The synthesis of palladium nanoparticles can affect how long the reaction last. To enhance the reaction rate of palladium nanoparticles, different quantities of the palladium chloride and the water-soluble flower extract of the *rosa damascena* plant can be used. After qualitative analysis further reaction mixture characterized as UV-Visible spectrophotometer, FTIR, XRD, and TEM were put to use to confirm the presence of palladium nanoparticles in the reaction mixture Fig.1.

3. Characterization

3.1. UV-Visible spectrometer

Characterization of biological synthesized palladium nanoparticles analyzed by Shimadzu double beam 1800 UV-Visible spectroscopy. The samples were scanned between 300 to 700 nm, with a one nm resolution. As a blank, deionized water was employed.

3.2. FTIR

Fourier Transform Infrared spectrum of the flower extract of the *R. damascena* plant and biologically synthesized palladium nanoparticles were examined used model FTIR Perkin Elmer, RX FTIR, USA. In this process, samples were operated into potassium bromide (KBr) matrix, combine gently and make pellets. RX-1 fast access FTIR to dependable Infra Red outputs of the samples. Resolution of FTIR spectroscope has 4000 cm⁻¹ to 650 cm⁻¹. FTIR spectrum recorded at room temperature. The purpose of the FTIR study was to identify bio-active components bound to the palladium nanoparticles.

3.3. XRD

X-ray diffraction broadly utilized to analyzed crystallographic form of the synthesized palladium nanoparticles used PANalytical XRD machine, and X'Pert PRO model. This distinguished technology has cut measuring durations. A diffractogram requires a sample on a glass disc with dimensions of 3.5 cm x 2.5 cm and an area of 0.2 cm, with an even surface on a single side.

3.4. TEM

The structure and dimension of the nanoparticles examined by the Transmission Electron Microscopy (TEM) model JEOL JEM-1400 used an acceleration voltage of 120 KV. The auto montage features (which come included) facilitate the simple to capture high-precision photos with a broad area of images. A extremely high power electron sources in KV passes across an extremely thin material, even the connections between the electron and the particles can potentially be exploited to study characteristics.

4. Results

4.1. Ultraviolet-visible spectrum of synthesized palladium nanoparticles

The Surface Plasmon Resonance, a particular features of palladium nanoparticles, is activated when the production of palladium nanoparticles starts, causing the solution to become yellow to dark black [17]. When palladium chloride mixed with *r. damascena* flower extract continuously stirrer at ambient temperature, the color altered from yellow to dark black after 24 h, revealing palladium nanoparticles. To characterize PdNPs, wavelength ranges from 300 to 700 nm were measured. Fig.2 depicts the UV-Vis Spectrophotometer of PdNPs. A

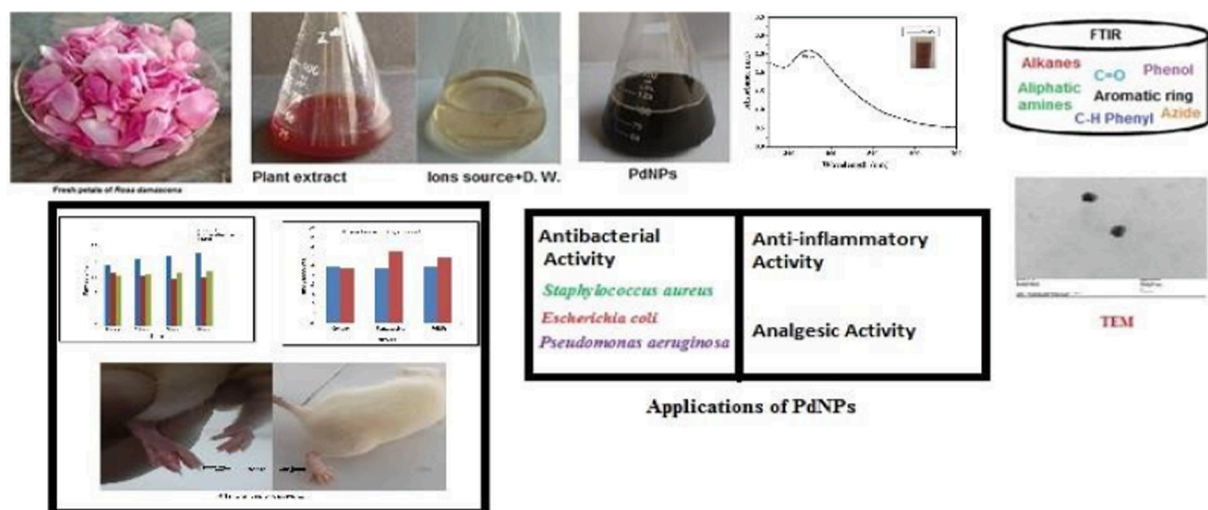


Fig. 1. Green synthesis of Palladium Nanoparticles.

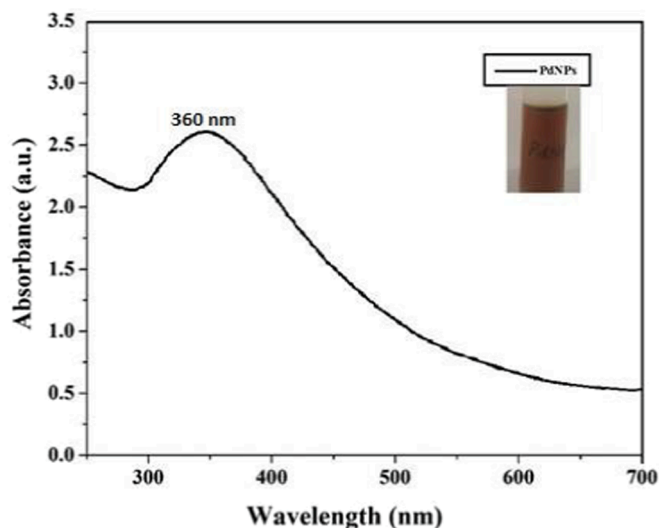


Fig.2. UV-Vis spectra of Palladium nanoparticles.

unique peak for palladium nanoparticles was detected at 360 nm, which correlates to the SPR of palladium nanoparticles production [18].

4.2. FTIR

Plant extract play a major role as reducing and stabilizing agent were measured by FTIR (fourier transform infrared) spectrophotometer. The FTIR images of plant extract of *Rosa damascena* as well as palladium nanoparticles was examined shown in Fig.3. The results of FTIR analysis showed various peaks in the distinctive regions shows the character of phytochemicals of plant extract for reducing palladium ions into

palladium nanoparticles. According to flower extract analysis, strong peaks were observed at 3333.9 cm^{-1} and 3287.6 cm^{-1} in the hydroxyl group specific to alcoholic and phenolic chemicals [19] The peak at 2932 cm^{-1} indicates the presence of C-H stretching, 2152 cm^{-1} for C=O stretching. 1958 cm^{-1} aromatic compound, 1708 cm^{-1} C=O stretching. The aromatic ring at 1606 cm^{-1} OH bonding at 1364 cm^{-1} and 1345 cm^{-1} , 1237 cm^{-1} C-N stretch, $1066\text{--}1045\text{ cm}^{-1}$ and 921 cm^{-1} C-O, C=C for $878\text{--}818\text{ cm}^{-1}$, 779 cm^{-1} and 776 cm^{-1} for C-H.

4.3. XRD

X-ray pattern of biologically synthesized palladium nanoparticles from *Rosa damascena* flower extract showed in Fig. 4. Palladium nanoparticles were examined used of XRD pattern to analyze/ confirm the nanoparticles's crystallographic morphology. The spectrophotometer of *R. damascena* flower extract-mediated palladium nanoparticles confirms the presence of 37.201 , 50.484 , 60.229 , 72.456 at 2 theta. Since there are no crystallographic impurities in the nanocrystalline palladium nanoparticles, the XRD spectrum indicates high purity. XRD pattern agrees well with the Transmission Electron Microscopy result such as ($\approx 50\text{ nm}$).

4.4. TEM

Transmission Electron Microscopy (TEM) was used to examine the structure and dimension of palladium nanoparticles synthesized from *R. damascena* flower extract. Palladium nanoparticles formed from *R. damascena* flower extract were approximately 50 nm in diameter and spherical in shape. Fig 5 shows the dimensions and shape of palladium nanoparticles formed by biological method using transmission electron microscopy.

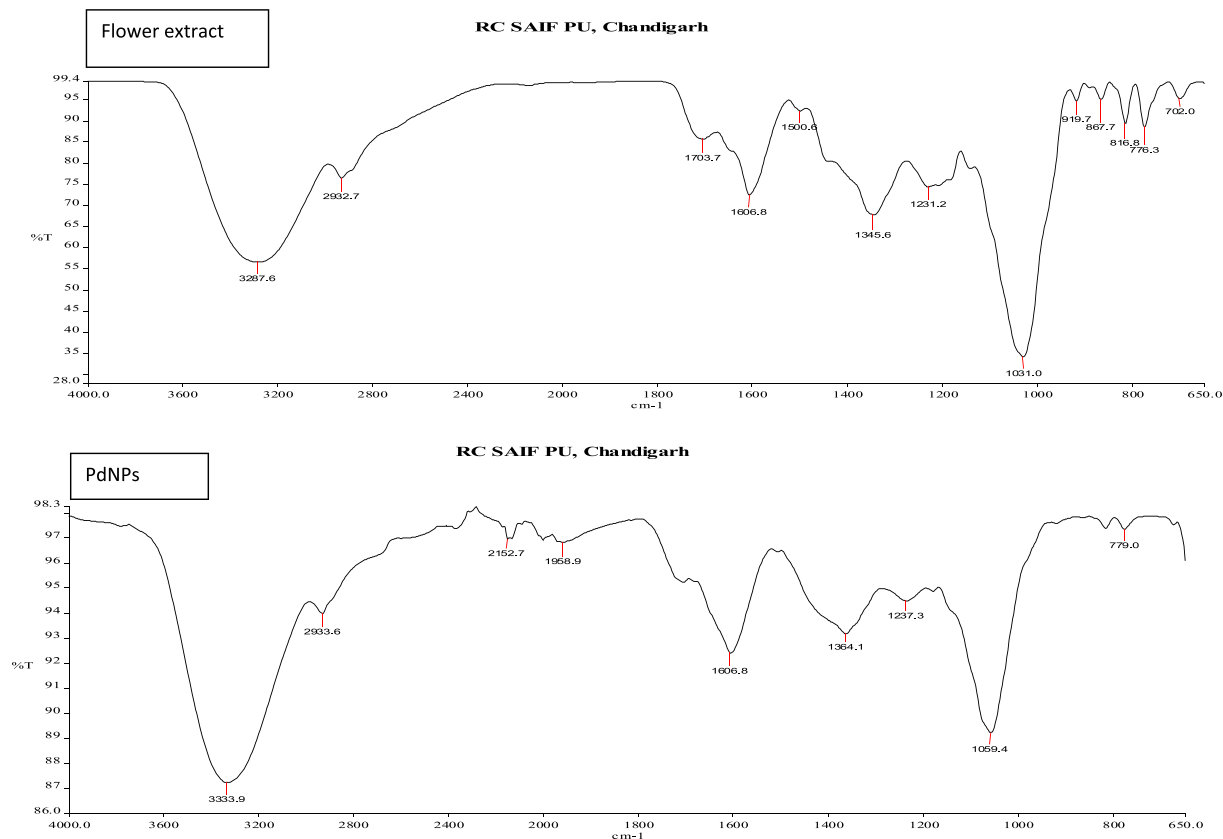


Fig. 3. FTIR.

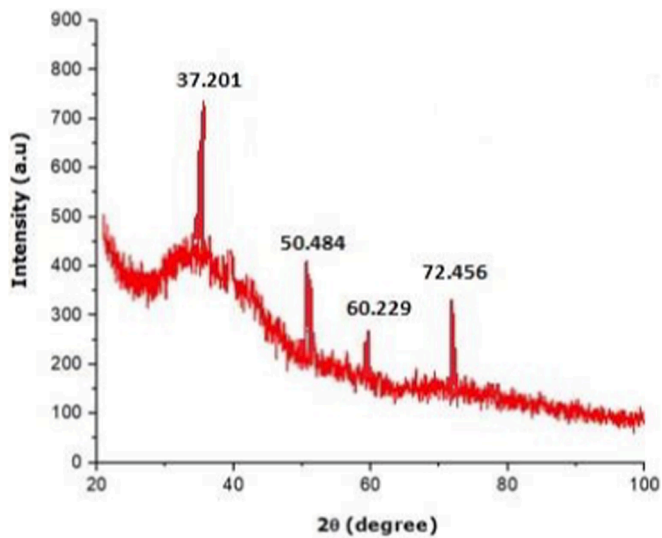


Fig. 4. XRD.

5. Application of palladium nanoparticles

5.1. In-vitro application

5.1.1. Anti-bacterial

The antibacterial activity of the biosynthesis of palladium nanoparticles from *R.damascena* flower extracts an examined using a well-diffusion procedure. The well-diffusion procedure was used for performing the anti-bacterial activity against the 3 microorganisms that were chosen, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* shown in Fig.6. After preparing and autoclaving the agar media, 25 ml of each Petri plate was poured and allowed to harden. Culture media 100 micro liters were distributed on a sterile Petri plate and left to set for 10 min before loading 20 micro liter of solution into different wells. One thousand ppm antibiotics (Levofloxacin) served as a +ve (positive) control for bacteria. After loaded Petri plates were placed under laminar air flow for 20 min before being incubated at 37 degree C for 24 h. Every well had its unique zone of inhibition shown in Table 1. The diameter in millimeters that were measured [20].zone of inhibition measured after incubation for bacterial strains such as *P.aeruginosa*, *S. aureus*, and *E.coli*. All of the bacterial strains examined are susceptible to palladium nanoparticles, and there is no zone of inhibition for negative control. The inhibition zone of palladium nanoparticles was observed against *P.aeruginosa* 17 ± 0.44 mm, *S.aereus* 16 ± 0.57 mm, *E.coli* 30 ± 1.25 mm which is comparatively higher than the zone of inhibition of

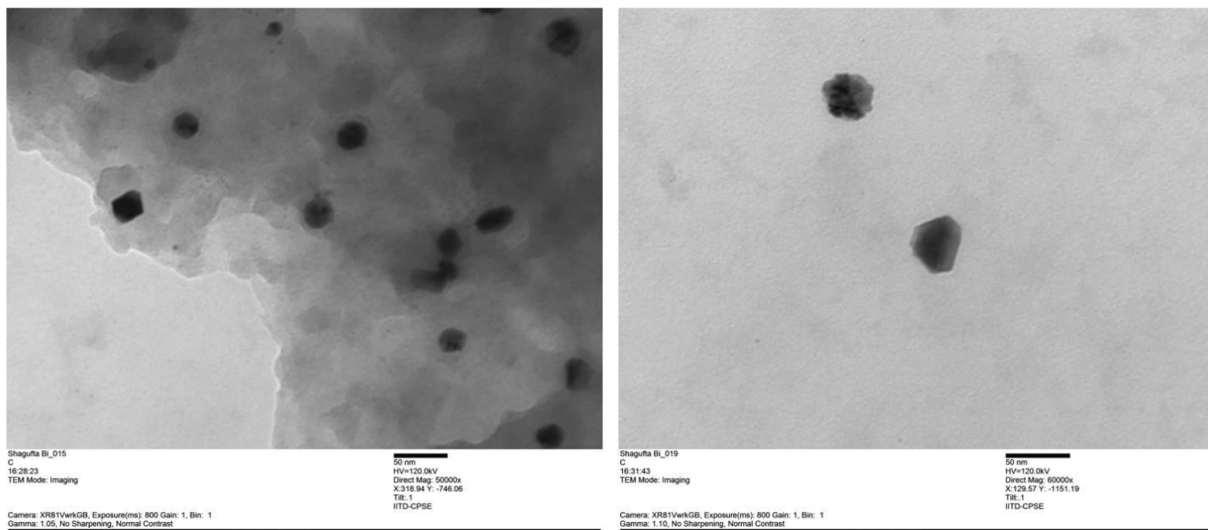


Fig. 5. TEM.

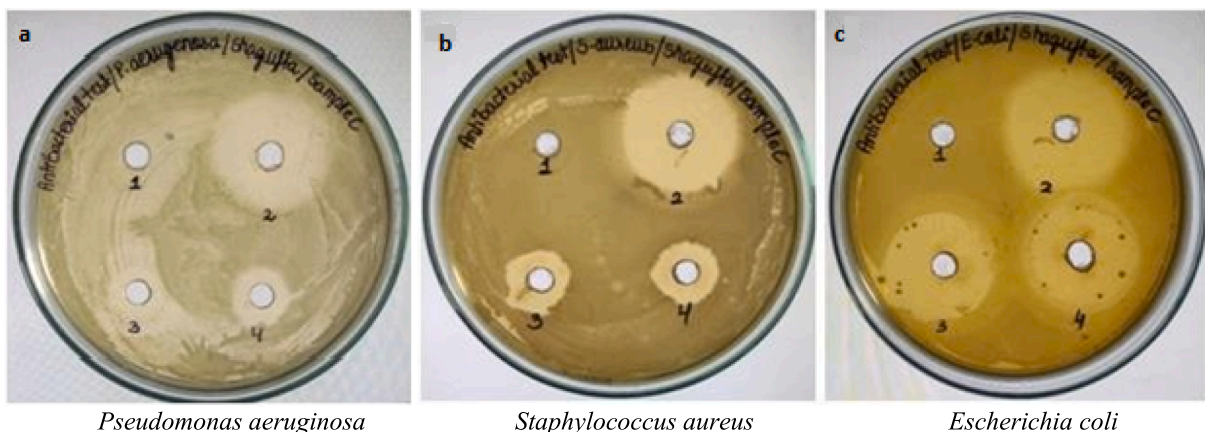


Fig. 6. Antibacterial Activity.

Table 1
Inhibition zone.

Bacterial Strains	Positive control (Levofloxacin 1000 ppm)	<i>R.damascena</i> mediated PdNPs 20 µl	PdNPs + Levofloxacin
<i>Pseudomonas aeruginosa</i>	16 ± 0.50	17 ± 0.44	30 ± 0.70
<i>Staphylococcus aureus</i>	18 ± 0.55	16 ± 0.57	29 ± 1.0
<i>Escherichia coli</i>	29 ± 0.70	30 ± 1.25	32 ± 1.30

antibiotics (Levofloxacin) such as *P.aeruginosa* 16 ± 0.50 mm, *S.aureus*, 18 ± 0.55 mm, and *E.coli* 29 ± 0.70 mm. Palladium nanoparticles along with Levofloxacin inhibition zone which is higher than other zones are *P.aeruginosa* 30 ± 0.70 mm, *S. aureus* 29 ± 1.0 mm, *E.coli* 32 ± 1.3 mm respectively.

5.2. In-vivo application

In vivo, experiments were conducted on Wistar albino rats weighing 150–200 g. For comfort the rats were housed in polypropylene cages at 23 degree, with nighttime and daytime cycle, as well as 50 to 60 % relative moisture rats were used before the experiments given adapt climate for 14 days and 12 h before the study food was suspended. The Institutional Animal Ethical Committee (AIEC) of IFTM University in Moradabad, Uttar Pradesh, India permitted this protocol with approval number: (IAEC/2022/2/06) vide resolution number is 2022/837ac/Ph.D./05.

5.2.1. Anti-inflammatory activity by carrageenan-induced paw edema procedure

Experimental Animal n=4 in each group (3 groups).

The effect of anti-inflammation according to time showed in Table 2. The carrageenan-induced through injection into the hind paws to produce edema in all groups of animals. Edema produced in control of animals, local edema in the following groups in 30 min increased. After 30 min it is reached at highest level after carrageenan-induced. The significance of the drug indomethacin shows the highest according to respect to the time shown in Fig.7. Palladium nanoparticles exhibit a less significant effect on rat correspondence to time.

5.2.2. Analgesic activity of palladium nanoparticles

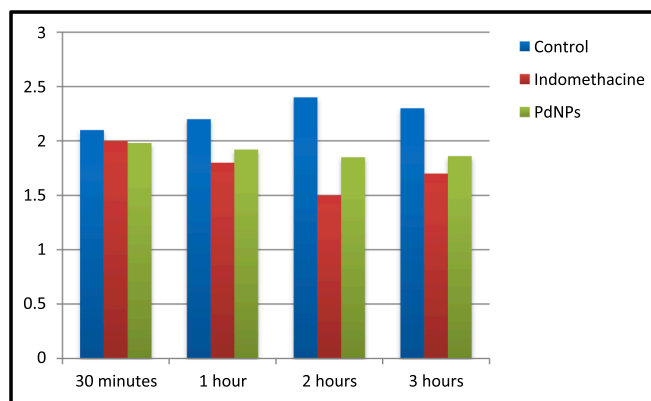
Analgesic activity of palladium nanoparticles according to time described in Table 3. Significance of standard drug and palladium nanoparticles before treatment is less than after treatment significance level was high with the respect to the time shown in Fig.8. Pentazocine dug administered orally to rat and respect to time inhibition percent 42.50% and palladium nanoparticles 08.00%.

Table 2
Effect of Palladium Nanoparticles on rat paw edema induced by Carrageenan.

Treatment	Dose (mg/ kg)	30 min	Edema 1 h	2 h	3 h
Control (1% CMC)	–	2.20 ± 0.06	2.25 ± 0.05	2.40 ± 0.05	2.30 ± 0.07
Indomethacin	10 mg/ kg	2.00 ± 0.06	1.18 ± 0.05	1.15 ± 0.07*	1.17 ± 0.02**
PdNPs	0.3	1.98 ± 0.03***	1.92 ± 0.01**	1.85 ± 0.01***	1.86 ± 0.01***

Values written as mean ± SEM in each group n=3. *P<0.05, **P<0.01, ***P<0.001 compare with the control group.

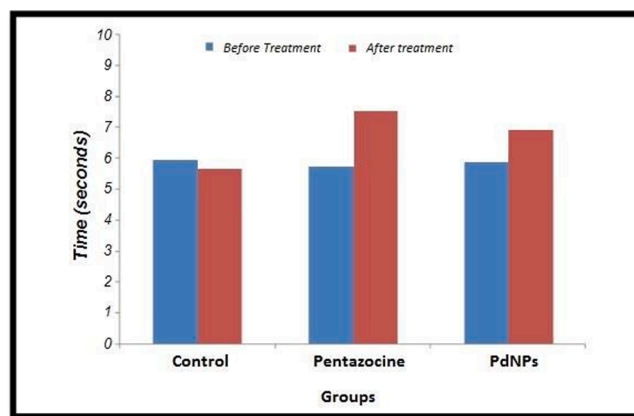
Abbreviation: PdNPs, Palladium nanoparticles.

**Fig. 7.** Anti-inflammatory Activity.**Table 3**
Effects for analgesic activity on Rats through Radiant Heat Tail Flick Model.

Treatment	Dose (mg/kg)	Before treatment (Mean ±SEM)	After treatment (Mean±SEM)	Percentage Inhibition %
Control 1% (CMC)	–	6.05 ± 0.165	5.5 ± 0.110	0.00%
Pentazocine	30 mg/ kg	5.26 ± 0.139	7.648 ± 0.282***	42.50%
PdNPs	0.025	5.95 ± 0.156	6.35 ± 0.237*	08.00%

Notes: control group compared with treated groups. Calculate by One Way ANOVA. *P<0.05, **P<0.01, ***P<0.001.

Abbreviation: PdNPs, Palladium Nanoparticles; SEM, standard error of the mean.

**Fig. 8.** Analgesic Activity.

6. Discussion

The analysis of the research shows that employing *Rosa damascena* flower extracts can produce environmentally friendly metal nanoparticles, including palladium nanoparticles (PdNPs). When compare to other approaches like physical and chemical, the biological synthesis method for producing nanoparticles is advanced because it is relatively reproducible, environmentally friendly, and widely accessible and gives frequently results in stronger substances. Numerous analyses have documented the impact of these techniques on the structure, dimension and uses of nanoparticles in biogenic system. Additionally, green synthesized palladium nanoparticles from *R.damascena* flower extract

shows results against anti-inflammatory and analgesic activity on rat model. These activities were less significant to standard drugs.

7. Advantages and limitations

Rosa damascena-mediated palladium nanoparticles have demonstrated antibacterial, antifungal, anti-inflammatory, analgesic activities. These properties are crucial for medical applications such as drug delivery, antibacterial, biosensors, anti-inflammatory and analgesic activities etc. Furthermore, produced palladium nanoparticles were discovered to have increased anticancer activity in comparison with other conventional anticancer medicines. These results might result in the creation of new anticancer and antibacterial medications that are safer, less harmful to the environment, and more efficient compared to other conventional equivalents.

Scientists looked at a range of readily available plants in the area and found they provide good building blocks for making greener nanoparticles. These studies imply that plants may be fully used, although it is challenging to synthesize significant amounts of nanoparticles globally. Utilizing basic elements in actual manufacturing can prove difficult due to time limitations. Time limitations could high temperatures, lengthy synthesizing times, and a partial knowledge of the formation route provide difficulties for biological formation. Plant extracts are not appropriate for enormous scale manufacture because the dimension and form of the nanoparticles they create are highly variable. A key problem in production is controlling the dimensions of nanoparticles. Make it difficult to use raw material in actual manufacturing.

8. Conclusion and future prospective

Flower extract of the *Rosa damascena* plant behave in the role of stabilization and capped agents in the formation of palladium nanoparticles. Flower extract accommodates various phytochemicals compound which assists as well in stabilizing and capped of nanoparticles. The biological production of palladium nanoparticles was analyzed through an Ultraviolet–Visible spectrophotometer, Fourier Transform Infrared spectrophotometer, X-ray Diffraction, and Transmission Electron Microscopy. Palladium chloride is employed in this study to inhibit the biological production of palladium nanoparticles via flower extract. The palladium nanoparticles were spherical in shape and size approx 50 nm. The biologically generate palladium nanoparticles have antibacterial, anti-inflammatory, and analgesic activities. Following current encouraging results, nanoparticles give significant prospects for tailored medication administration, detection, diagnostics, and bioimaging. In this scenario, nanoparticles are rapidly on track to having a positive influence on medicine. Palladium nanoparticles using considerable medicinal benefits may be employed more effectively to establish a developing and beneficial bond with a wide range of macromolecules and targeted drugs for cancer, inflammation, and other disorders. Future research should focus on exploring the full potential of *rosa damascena* flower-mediated palladium nanoparticles in various biomedical applications.

CRedit authorship contribution statement

Shagufta Bi: Visualization, Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Rashi Srivastava:** Supervision.

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.

Data availability

Data will be made available on request.

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