Phytomolecules Loaded Palladium Nanomaterials by Leaf Extract of Eclipta Prostrata and its Antibiofilm Activity

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**Abstract:** The study aims to evaluate the anti biofilm activity of phytomolecules leaded palladium nanomaterials by leaf extract of *Eclipta prostrata.* Fresh leaves were fragmented, thoroughly cleaned three times in distilled water to get rid of any dust, and then added to water (1:10) at 60°C, stirring continuously for 30 minutes. The mixture was filtered, cooled and stored at 40°C for later use. Shaking the leaf extract with 90ml of Palladium (1.0mM) in DIW for 24 hours. Next, the nanoparticles undergo 2-hour centrifugation at 400 rpm and 60°C. Wash and separate the PdNPs after a 24-hour centrifugation. The development of PdNPs was indicated by the liquid's color changing from brown to a creamy, semi-solid hue. Antibiofilm activity was performed using a well diffusion assay by measuring the diameter and size of the inhibitory zone, the antibacterial efficacy. Antimicrobial activity shows at an OD of 1.23, the positive control lacking PdNPs showed growth. Significant colony growth suppression would reduce OD. When compared to the control group and other NP concentrations, the increased survival rate exposed to PdNPs at 150 μg/ml and 200 μg/mL may be explained by these anomalies. The increased heart beat were exposed to PdNPs at 150 μg/ml and 200 μg/mL, the reduced hatching rate were exposed to PdNPs at 150 μg/ml and 200 μg/mL, the increased malformation were exposed to PdNPs at 150 μg/ml and 200 μg/mL. Biosynthesised PdNPs are nontoxic and have antibacterial properties . PdNPs demonstrated a good zone of inhibition against both bacterial strains. The use of biological sources to synthesize nanoparticles adds a new dimension to all application areas. additional research is required to monitor the environment, understand the molecular mechanism and bioaccumulation of PdNPs, and offer a fresh perspective on all potential uses. The existence of certain bacterial species, like *E.faecalis,* inside the root canal system is the cause of endodontic failure. These bacteria can cause a prolonged intra- or extra-radicular infection because they are more resistant to disinfectants. Therefore, the focus of current research is on finding materials that have better disinfecting capabilities and less cytotoxicity.

**Keywords:** Green synthesis, palladium nanoparticles, *Danio rerio, Eclipta prostrata,*

# Introduction

Nanoparticles (NPs) are materials that differ from their bulk counterparts by having at least one dimension between 1 and 100 nm[(Nasim et al., 2022)](https://paperpile.com/c/f1mXL6/R6fB). They are regarded as the basic building blocks of nanotechnology[(Johnson et al., 2022)](https://paperpile.com/c/f1mXL6/TeFo). Particles of this size range have been used by many people and industries since the beginning of time[(Pandiyan et al., 2022; Saquib et al., 2020)](https://paperpile.com/c/f1mXL6/JZRS+QtlG). The NPs' large surface area and nanoscale size give them distinctive chemical and physical properties[(Kamath et al., 2022; Merchant et al., 2022)](https://paperpile.com/c/f1mXL6/DvRK+x90I). For many conversion reactions, including Heck, Suzuki, and Stille coupling reactions, PdNPs have been studied as the best reagent[(Melcer, 2014)](https://paperpile.com/c/f1mXL6/z5Ga). Because PdNPs ability to function as a catalyst depends on both their size and morphology[(Anna Thomas et al., 2022)](https://paperpile.com/c/f1mXL6/mPtf).

Palladium nanoparticles are silvery-white precious metals with a high density[(Aparna et al., 2021; Inamuddin & Asiri, 2020)](https://paperpile.com/c/f1mXL6/AAhw+ENos). It has been reported that a variety of plant species, including Anogeissus latifolia, Cinnamomum zeylanicum, Cinnamomum camphora, Curcuma longa, Doipyros kaki, Gardenia jasminoides, Glycine max, Musa paradisica, Ocimum sanctum, Pinus resinosa, and Pulicaria glutinosa, can be used to produce palladium and platinum nanoparticles through biosynthesis[(Chokkattu et al., 2022; Nudelman et al., 2023)](https://paperpile.com/c/f1mXL6/xB33+VxYd)[(Jayaseelan et al., 2022)](https://paperpile.com/c/f1mXL6/hp2B).

Many chronic diseases and infections are treated with plants in traditional medicine in a variety of therapeutic ways. Because traditional medicine and healthcare are more widely accepted in society and are more reasonably priced, many people still rely on them[(Morel et al., 2024; Ramamurthy et al., 2022)](https://paperpile.com/c/f1mXL6/77kW+ujrF). The discovery and development of contemporary medications has benefited greatly from the bioactive chemicals derived from plants. Therefore, it is important to investigate the medicinal worth of diverse plants in light of their pharmacological relevance and prospective use in various products[(Chokkattu et al., 2022)](https://paperpile.com/c/f1mXL6/VxYd). The medium-sized, branching, annual herb *E. prostrata* is native to tropical and subtropical regions of the world and bears white flowers[(Gou et al., 2023; Marya et al., 2022)](https://paperpile.com/c/f1mXL6/CcMz+Yc5q). It spreads easily throughout China, India, and other parts of the world and thrives primarily in damp areas such as the boundaries of rice fields, rivers, lakes, and swamps. It is extensively found in South America, Africa, and Asia's tropical and subtropical climates[(Chinnasamy et al., 2023; Jayaseelan et al., 2022)](https://paperpile.com/c/f1mXL6/hp2B+2OAn). It has historically been used to treat a variety of skin issues, including dermatitis, wounds, and preventing hair loss[(Feng et al., 2019)](https://paperpile.com/c/f1mXL6/KQpk). In Brazil, China, and India, the leaves are used to cure snake bite injuries. Infants suffering from catarrh can be cured with a mixture of leaf juice and honey[(Jain & Verma, 2022; Jayaseelan et al., 2022; Wadhwani et al., 2022)](https://paperpile.com/c/f1mXL6/hp2B+t8g6+Nllj). To encourage hair development, the juice of E. prostrata is applied topically or consumed orally[(Lee et al., 2018)](https://paperpile.com/c/f1mXL6/vr3V).

Reducing the use of hazardous chemicals is the primary goal of green synthesis in order to shield the environment from pollution[(Kamaraj et al., 2023; Sreevarun et al., 2023)](https://paperpile.com/c/f1mXL6/lpv6+dGLo). As a result, the production of nanomaterials via biogenic processes is growing in popularity. Therefore our aim of the study is to evaluate the anti biofilm activity of phytomolecules leaded palladium nanomaterials by leaf extract of *Eclipta prostrata[(Bhattacharyya & Law, 2022)](https://paperpile.com/c/f1mXL6/f83f).*

# Materials and Methods

## Collection of plant extract

Following their collection, the leaves of *Eclipta prostrata* were thoroughly rinsed under running water, left to dry at room temperature, and then chopped into little pieces in order to remove any residual dust.

## Preparation of leaf extract

To make an aqueous extract of *Eclipta prostrata*, 10 g of chopped leaves and 100 ml of double-distilled water were mixed in a 500 ml Erlenmeyer flask. The mixture was filtered through Whatman no. 1 filter paper after being left to stand at 60 °C for 20 minutes. The Palladium nanoparticles were created using the filtered bark extract. Extra leaf solution was kept at -20 °C until it was needed again.

## Preparation of nanoparticles

Fresh leaves were fragmented, thoroughly cleaned three times in distilled water to get rid of any dust, and then added to water (1:10) at 60°C, stirring continuously for 30 minutes. The mixture was filtered, cooled and stored at 40°C for later use. Shaking the leaf extract with 90ml of Palladium (1.0mM) in DIW for 24 hours. Next, the nanoparticles undergo 2-hour centrifugation at 400 rpm and 60°C. Wash and separate the PdNPs after a 24-hour centrifugation. The development of PdNPs was indicated by the liquid's color changing from brown to a creamy, semi-solid hue. Plant extracts and other phytochemicals present in biomaterials can operate as reducing agents, converting metal precursors into metal nanoparticles (NPs). Because phytochemical-containing materials contain ant-oxidants and non-toxic compounds, they can function as both stabilizing and reducing agents.

## Antimicrobial activity

Using a well diffusion assay, the antibacterial efficacy of the produced ZnO NPs was evaluated against a variety of bacterial species. *S. mutants* and *E. faecalis* were the bacteria employed in the experiment. The bacteria were cultured at 37 °C for 24 hours in nutritional broth. After that, samples were mixed with saline solution to achieve 0.5 McFarland standard turbidity (108 CFU/ml). Mueller-Hinton agar plates were equally distributed with aliquots of each culture. After drilling 6 mm wells in the agar plates, different NP concentrations were added, and the mixture was incubated for 16 hours at 37 degrees Celsius. By measuring the diameter and size of the inhibitory zone, the antibacterial efficacy was assessed.

## Fish and fish maintenance

A breeding population of 6–20 month old wild-type (ABWT) zebrafish (Danio rerio) was employed to produce eggs. The fish were raised in a pumped-back system (Aquaschwarz, Germany) with a 1:1 sex ratio, a regular water exchange rate of 10% at 27oC (pH 7.4), conductivity of 550 µS, and a light-dark cycle of 14 h:10 h. The stock density of the fish was 5 adult fish/L. Fish were given Artemia nauplii once daily (Ocean Nutrition, Belgium) and finely ground dry feed (CIBA Fish Food, India) twice a day.

## The collection of eggs and experiment development

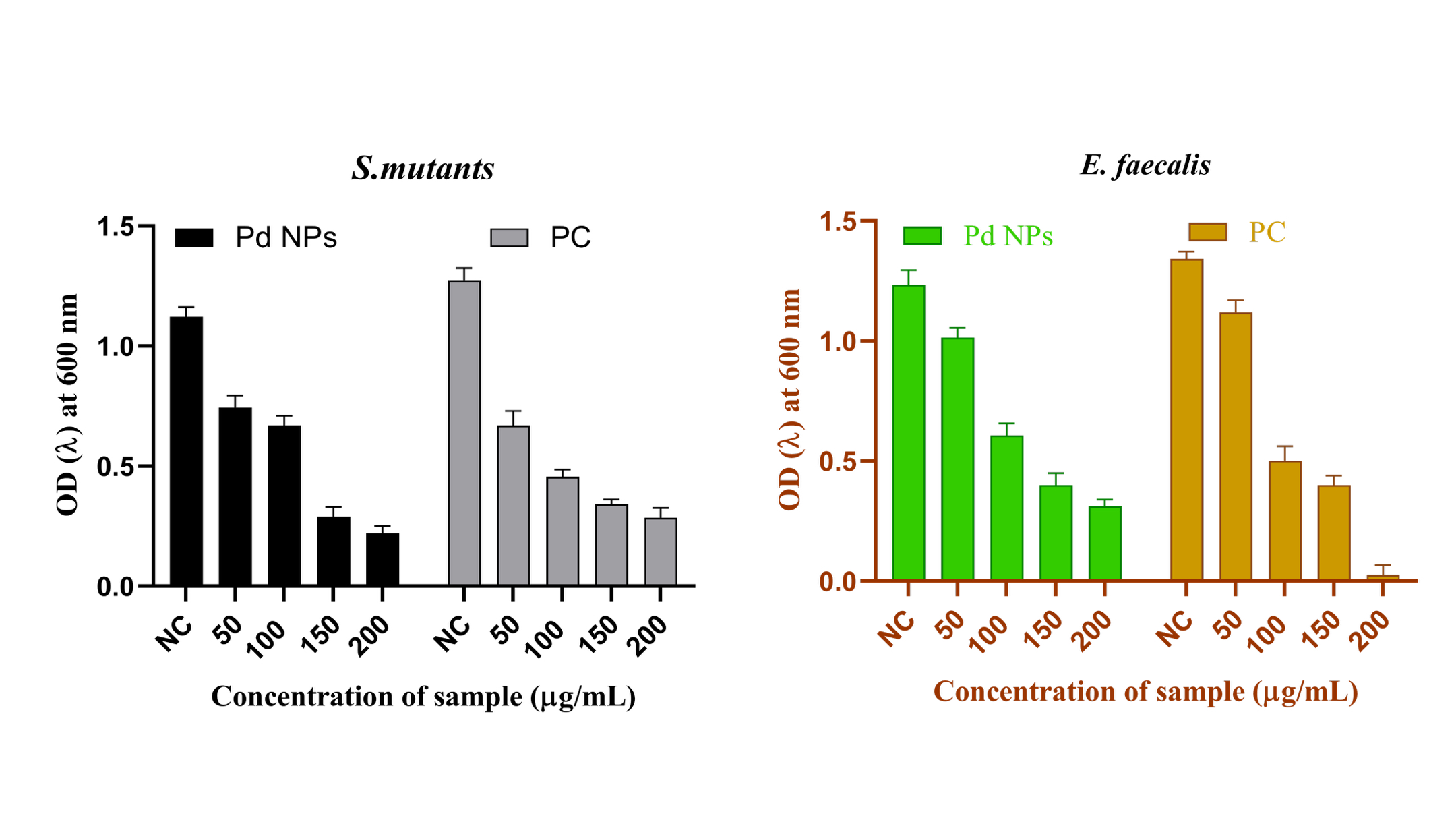
To encourage reproductive behavior, fish were put in aquariums the night before the experiment at a temperature of 27°C. The tanks were filled with empty pipette boxes coated in 4 mm x 4 mm mesh, which measured 10 x 6 x 4 cm in length, breadth, and height, respectively. The following morning, around two hours after fish spawning began, eggs were taken out of the boxes and cleaned in sterile water from the fish facility (Chehelgerdi et al., 2023). Fertile and unfertilized eggs could be distinguished from one another under a dissecting microscope (magnification x 6.34–6.40) (Leica S6E, Germany). For the fish embryo acute toxicity (FET) test, fertilized eggs between stages of 4 and 128 cells were used. The FET test was carried out at a temperature of 27°C in compliance with OECD test guideline 236 (OECD, 2013). Using 2.5 mL of test solution per well plate, fertilized eggs were distributed (Ref. 83.3922500, Sarstedt, Germany). Six concentrations of biosynthesised PdNPs (0.25, 0.5, 50, 250, 500µg/mL, and 1 mg/L) were generated (raising medium) from a standard solution mixed in sterile, facility-system-filtered water (Saadh et al., 2024). There were twenty eggs on each plate, suspended in the separate test fluid. A separate plate with 24 eggs in the growth medium was utilized as a negative control. Under a dissection microscope (Leica S6E, Germany), embryos were examined 24, 48, 72, 96, and 120 hours after treatment. According to Malafaia et al. (2019), four features of embryo demise were noted: 1) proportion of embryo survival; 2) number of heartbeats; 3) length of body; and 4) percentage of hatchability.

## Statistical analysis

One-way analysis of variance (one-way ANOVA) and multiple comparison versus control were used in the statistical analysis, which was carried out using Graph Pad Prism for Windows version 12.0 (Systat Software, San Jose, CA, USA). The statistical significance threshold was established at p < 0.05.

# Results

## Antibiofilm activity of Nanoparticles incorporated plant extract

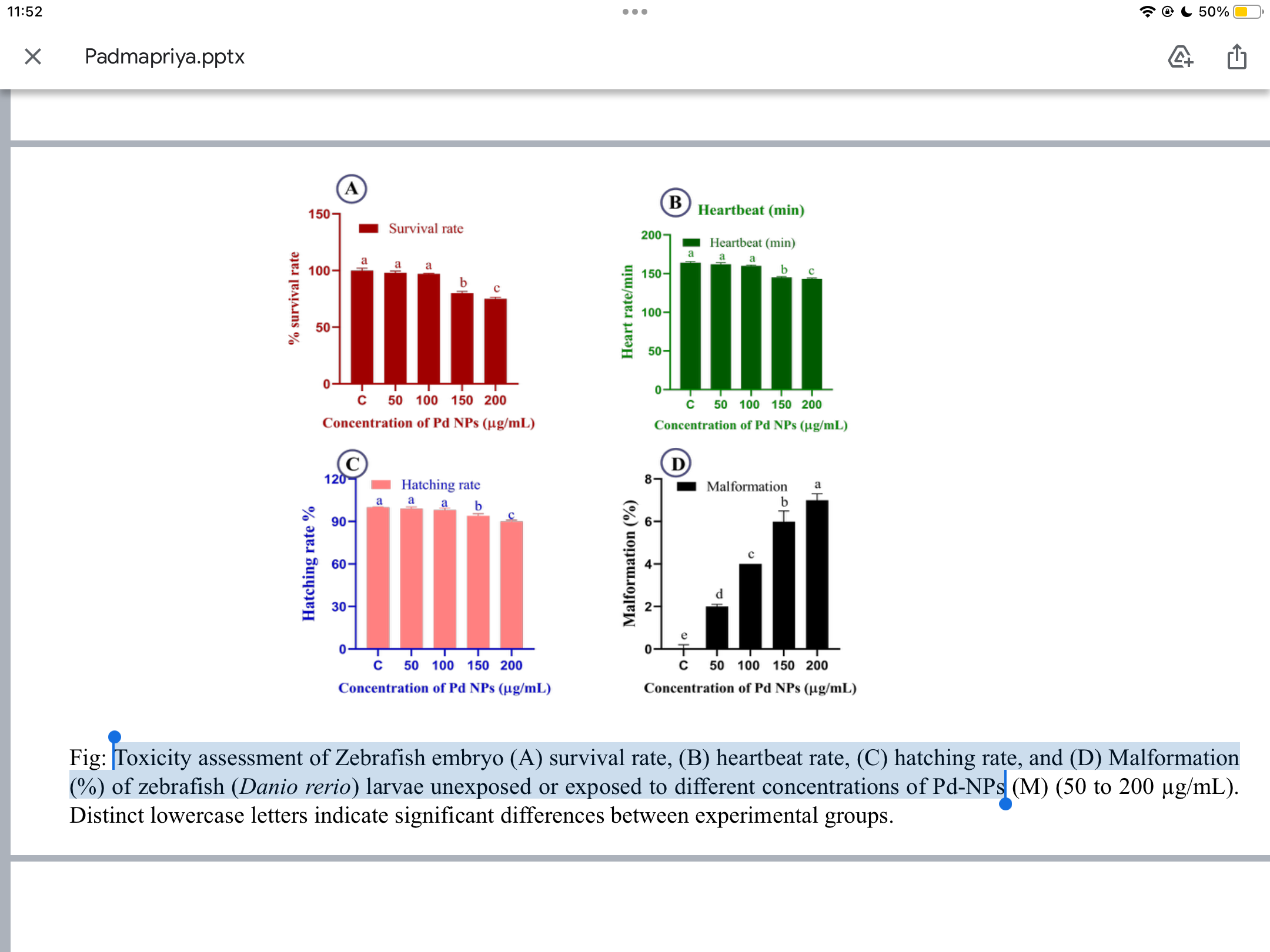


**Fig.1.** Antibiofilm activity of Pd-NPs against *S. mutants* and *E. faecalis*. Crystal violet was quantified by measuring absorbance at 600 nm

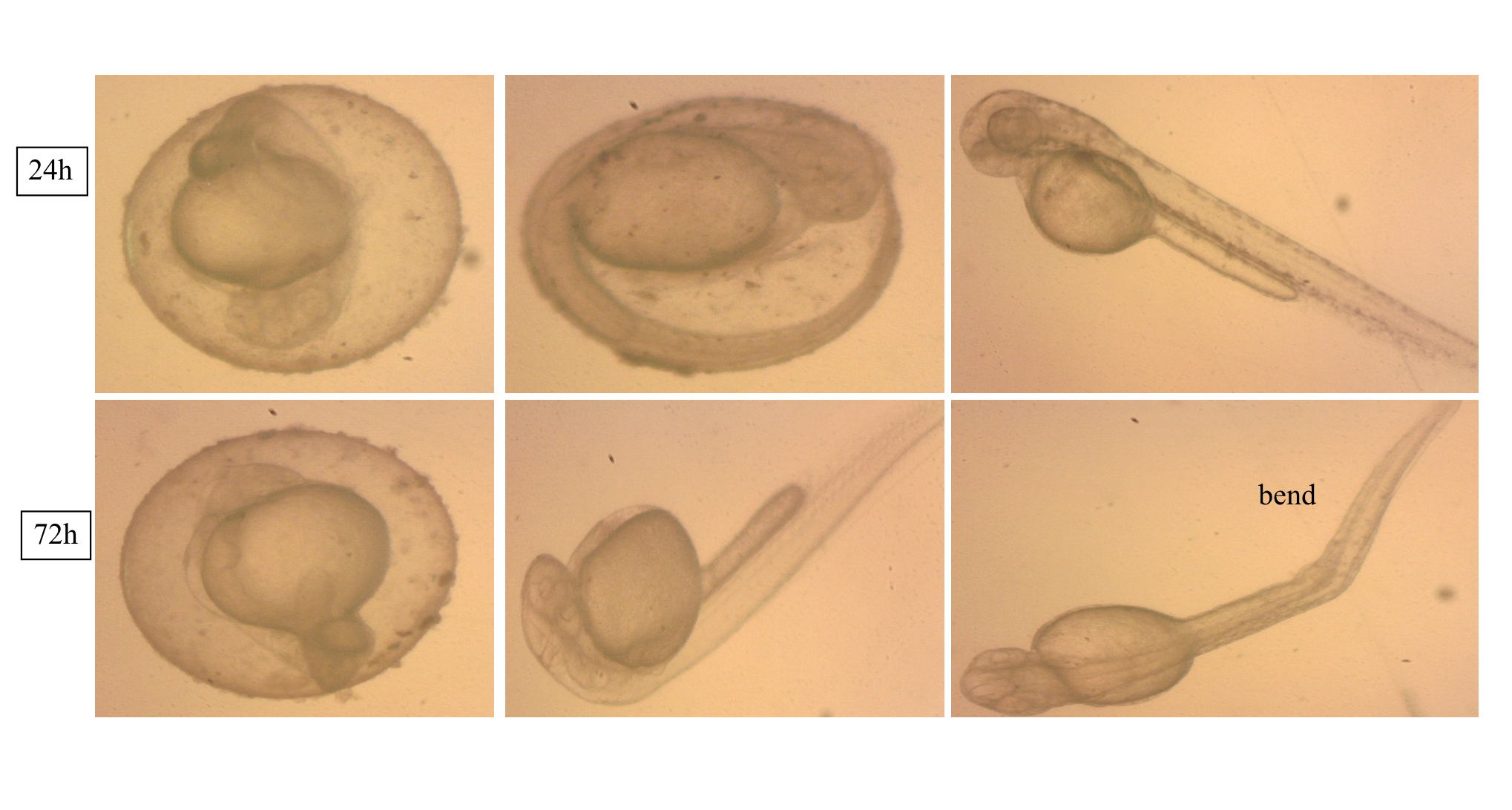
By assessing biofilm formation with crystal violet in the presence of different PdNPs concentrations, the antibiofilm activity of PdNPs was assessed. PdNPs at concentrations of 150 µg/ml and 200 µg/ml effectively inhibited the production of biofilms. PdNPs demonstrated notable antibiofilm action against *S.mutans* and *E.faecalis* in a dose-dependent manner. At an OD of 1.23, the positive control lacking PdNPs showed growth. Significant colony growth suppression would reduce OD.

## Toxicity assessment of Zebrafish embryo

When biosynthesized PdNPs were tested for toxicity in vivo on zebrafish embryos, only concentrations of 150 and 200 mg/L showed a discernible effect from the nanomaterials. We saw a considerable decrease in the rate at which eggs hatched in these groups, as well as adverse effects on the animals' growth (measured by body length) and heartbeat variations. Additionally, between 8 and 120 hours after conception, zebrafish embryos subjected to PdNPs greater than 150 μg/mI showed obvious defects like a bent spine, egg aggregation, tail deformation, and delayed hatching. When compared to the control group and other NP concentrations, the increased survival rate exposed to Pd-NPs at 150 μg/ml and 200 μg/ml may be explained by these anomalies. The increased heart beat were exposed to PdNPs at 150 μg/ml and 200 μg/ml, the reduced hatching rate were exposed to PdNPs at 150 μg/ml and 200 μg/ml, the increased malformation were exposed to PdNPs at 150 μg/ml and 200 μg/ml.



**Fig.2.** Toxicity assessment of Zebrafish embryo (A) survival rate, (B) heartbeat rate, (C) hatching rate, and (D) Malformation (%) of zebrafish (*Danio rerio*) larvae unexposed or exposed to different concentrations of PdNPs



**Fig. 3.** Morphology of zebrafish embryo treated with PdNPs at different time intervals

# Discussion

Actinomycetes and Enterococcus faecalis are the most well-known bacterial species that cause chronic root canal infections [(Kamaraj et al., 2023)](https://paperpile.com/c/f1mXL6/lpv6). Of these, Enterococcus faecalis is the most researched bacterium in the fight against bacteria in the canals since it has been identified as the primary suspect in recurrent cases of apical periodontitis [(Cherian et al., 2022; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/f1mXL6/Mxtj+DTRs). The previously described factors make nanoparticles more effective at fighting bacteria, many are being tested to see how well they work against Enterococcus faecalis, a strain of bacteria that is known to withstand severe temperatures and nutrient-free environments[(Jayaseelan et al., 2022; Poornima et al., 2021)](https://paperpile.com/c/f1mXL6/hp2B+jJ2I). In this investigation, we examined the antibacterial activity of phytomolecules loaded PdNPs against the bacteria responsible for biofilm formation, S. mutans and E. faecalis. Microbial colonies known as biofilms have the ability to stick to abiotic surfaces and then grow there[(Ganapathy 2021)](https://paperpile.com/c/f1mXL6/jJ2I+ZEHk). There are numerous findings on the antibiofilm properties of different nanoparticles against human bacterial infections, such as S.mutans and E. Faecalis highlighting the efficacy of the treatments[(Kamaraj et al., 2023)](https://paperpile.com/c/f1mXL6/lpv6). Previous studies conducted antibiofilm activity against various pathogens like Pseudomonas, E.coli and S.epidermidis with silver nanoparticles [(Rai & Kon, 2015)](https://paperpile.com/c/f1mXL6/ShFZ). Certainly, comparing various articles on the green synthesis of PdNPs against E. faecalis reveals a diversity of approaches. Some studies focus on plant extracts, emphasizing the role of specific phytochemicals in reducing and stabilizing nanoparticles[(Cherian et al., 2022)](https://paperpile.com/c/f1mXL6/Mxtj). Others explore the use of microorganisms, highlighting the microbial-mediated synthesis process. The choice of precursor materials, reaction conditions, and characterization methods also varies across studies, influencing the size and morphology of the nanoparticles produced.[(Kamath et al., 2022; Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/f1mXL6/DvRK+oaBz) Understanding these nuances can contribute to a more comprehensive view of the field[(Grumezescu, 2016)](https://paperpile.com/c/f1mXL6/1UVL).

The combination of PdNPs and *Eclipta prostrata* is an area of interest in nanobiotechnology[(Kamaraj et al., 2022)](https://paperpile.com/c/f1mXL6/Rawq). Eclipta prostrata extracts can act as a reducing and stabilizing agent in the green synthesis of palladium nanoparticles. Research suggests that *Eclipta prostrata* extracts, when integrated with nanoparticles like silver or zinc oxide, exhibit increased antibiofilm efficacy[(Chokkattu et al., 2023; Thamilchelvan et al., 2023)](https://paperpile.com/c/f1mXL6/bJkM+FB84). These formulations have demonstrated inhibitory effects on biofilm formation by various bacteriaThe combination leverages both the inherent antimicrobial properties of *Eclipta prostrata* and the unique features of nanoparticles to enhance the overall antibiofilm activity[(*Website*, n.d.)](https://paperpile.com/c/f1mXL6/M4SL). These nanoparticles, in turn, may exhibit unique properties and applications[(Rajakumar & Abdul Rahuman, 2011; Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/f1mXL6/ojHW+dfym). The potential antimicrobial activity of palladium nanoparticles, coupled with the bioactive compounds present in *Eclipta prostrata*, could be explored for various purposes, including medical and environmental applications[(Priya et al., 2023; Solanki et al., 2023)](https://paperpile.com/c/f1mXL6/e2hq+pr9O). Similar results revealed the antiplasmodial activity of environmentally friendly manufactured PdNPs against *Plasmodium berghei* in Swiss Albino Rats using *Eclipta prostrata* extract[(Ragavendran et al., 2023)](https://paperpile.com/c/f1mXL6/bhi6). The research has demonstrated that the proliferation of *P. berghei* parasites was dramatically suppressed by produced Pd NPs. In fact, 78.13 percent of the produced Pd NPs demonstrated strong antimalarial efficacy against *P.berghei* parasites[(Laghari et al., 2023; Rokkarukala et al., 2023)](https://paperpile.com/c/f1mXL6/mBxm+f5qf). In this study we have evaluated the efficacy of eclipta prostrata against various pathogens like *S.mutans* and *E.faecalis*, our study shows an OD of 1.23, the positive control lacking PdNPs showed growth. Significant colony growth suppression would reduce OD[(Rajakumar & Abdul Rahuman, 2011)](https://paperpile.com/c/f1mXL6/ojHW)[(Ramasundaram et al., 2023)](https://paperpile.com/c/f1mXL6/BKTD).

Toxicity assessment of Zebrafish embryos was done under 4 categories: they include survival rate, heartbeat rate, hatching rate and Malformation (%) of zebrafish (*Danio rerio*) larvae unexposed or exposed to different concentrations of Pd-NPs[(“Synergetic Effects of Polyethylene Microplastic and Abamectin Pesticides on the Eyes of Zebrafish Larvae and Adults through Activation of Apoptosis Signaling Pathways,” 2023)](https://paperpile.com/c/f1mXL6/C4WZ). When compared to the control group and other NP concentrations, the increased survival rate exposed to Pd-NPs at 150 μg/ml and 200 μg/mL may be explained by these anomalies. The increased heart beat were exposed to Pd-NPs at 150 μg/ml and 200 μg/ml, the reduced hatching rate were exposed to Pd-NPs at 150 μg/ml and 200 μg/ml, the increased malformation were exposed to Pd-NPs at 150 μg/ml and 200 μg/ml[(Sudhakaran et al., 2023; “Synergetic Effects of Polyethylene Microplastic and Abamectin Pesticides on the Eyes of Zebrafish Larvae and Adults through Activation of Apoptosis Signaling Pathways,” 2023)](https://paperpile.com/c/f1mXL6/C4WZ+AszS). Similar findings were made by earlier studies employing silver nanoparticles, which showed that Ag-NPs exposed to zebrafish embryos showed shorter bodies than groups that were not treated[(Adel et al., 2023; Chinnasamy et al., 2023)](https://paperpile.com/c/f1mXL6/2OAn+s1N5). When 500 mg/mL of the biogenically produced AgNPs were given to Danio rerio, no negative effects were seen. The present discoveries corroborated the results of earlier research showing that higher concentrations of PdNPs exposure would be harmful to fish eggs and pose health risks[(*Anti-Inflammatory Potential of a Mouthwash Formulated Using Clove and Ginger Mediated by Zinc Oxide Nanoparticles: An In Vitro Study*, n.d., “Evolvulus Alsinoides-Wrapped Palladium Nanoparticles: A Potential Photocatalyst Rhodamine Blue, Inactivation of Human Pathogens and Non-Toxicity Assessments on Daphnia Magna and Danio Rerio,” 2023)](https://paperpile.com/c/f1mXL6/3f0j+SUe9). The process flow for creating biogenic PdNPs, characterizing them, and verifying their capping using several methods in conjunction with an aqueous extract of *E. prostrata* is shown in.

# Conclusion

Biosynthesised Pd NPs are nontoxic and have antibacterial properties . Pd nanoparticles demonstrated a good zone of inhibition against both bacterial strains. The use of biological sources to synthesize nanoparticles adds a new dimension to all application areas. The process of synthesizing Pd NPs using green chemistry has numerous benefits, including the capacity to easily scale up the process, economic feasibility, and a safe method of producing nanoparticles.The information in our study adds to the emerging field of nanomaterials as a potential alternative therapy in the future. Consequently, the outcomes are very positive, showing a significant rise in the activity of the unaffected elements. Nonetheless, additional research is required to monitor the environment, understand the molecular mechanism and bioaccumulation of PdNPs, and offer a fresh perspective on all potential uses.

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