Synthesis and Characterization of Eco-Friendly Synthesised Ocimum Tenuiflorum-Mediated Palladium Nanoparticles and its Antibacterial and Antibiofilm Activity

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**Abstract:** Plant-based green synthesis of metal oxide nanoparticles (NPs) is becoming more and more popular since it is easy to use, can be scaled up commercially, and can be combined with a variety of lower-risk, imperishable value-added components. Holy basil, or Ocimum tenuiflorum L., is an annual herb in the Lamiaceae family that has a powerful scent and soft hairs.Palladium nanoparticles (Pd-NPs), due to their distinct physicochemical characteristics, have demonstrated great potential in biomedical applications. studies have demonstrated the significant potential of Pd-NPs as gene/drug carriers, biosensors, prodrug activators, and photothermal, photoacoustic, and antimicrobial/antitumor applications. The aim of current work was to synthesize paladium nanoparticles using green technology and evaluate their effectiveness against bacteria and biofilms. The synthesis of Ag-NPs was visually deduced by a change in colour from light yellow to reddish-brown. UV-Visible Spectrometry, X-ray diffraction (XRD), and Transmission Electron Microscopic (TEM) analysis were then used to characterize synthesized nanoparticles. The antibacterial activity of the leaf extract and PdNP were tested against Streptocoocus mutants and Candida albicans. Statistical analysis was carried out to establish possible relations between the antibacterial and antibiofilm activities. Functional groups such as alcohol, aldehyde, nitrile, primary amines, carbonyl, and aromatic groups were confirmed by FTIR and XRD. Total phenol was higher in leaf extract, while total flavonoids were higher in the PdNps. The leaf extract of Ocimum tenuiflorum mediated the green synthesis of palladium nanoparticles and possess strong antioxidant and antibacterial potentials that can find application in various biomedical areas. XRD findings, the SEM image showed an irregular shape with an average particle size of 3.14 nm. When Pd-NPs made from Ocimum tenuiflorum extract were tested against both Gram-positive and Gram-negative bacteria, they demonstrated a sizable zone of inhibition against Candida albicans and Streptococcus variants. It was demonstrated that Pd-NPs had superior antifungal activity against Candida albicans.

**Keywords:** Antibiofilm, Antioxidant,Candida albicans, Ocimum tenuiflorum, Palladium, Streptococcus mutants

# Introduction

Plant-based green synthesis of metal oxide nanoparticles (NPs) is becoming more and more popular since it is easy to use, can be scaled up commercially, and can be combined with a variety of lower-risk, imperishable value-added components. One of the most exciting and rapidly expanding topics today is nanoscience and nanotechnology, which calls for careful attention to design environmentally friendly NP synthesis techniques that do not need the use of hazardous chemicals.[(Dwivedi & Gopal, 2010; *[No Title]*, n.d.-a; Rana, Frollini, et al., 2021; Rana, Potluri, et al., 2021; Wei et al., 2020)](https://paperpile.com/c/RbZvp9/liV7+6nd8+o3EP+htGp+0HNu) In this instance, using green chemistry minimizes the usage of or production of hazardous chemicals during the synthesis steps. As a result, there is a growing body of research on creating metal nanoparticles (NPs) via biological methods, particularly with leaf extracts. This is due to the ease of experimentation, environmental friendliness, cost-effectiveness, and reproducibility of biological approaches in contrast to techniques based on physics and chemicals.[(Aparna et al., 2021)](https://paperpile.com/c/RbZvp9/o3G9) As a result, interest in green nanotechnology is growing.[(Anastas & Warner, 2000; Shukla & Iravani, 2018; Vaidyanathan et al., 2010)](https://paperpile.com/c/RbZvp9/gyRg+bMS9+QQ2B) For the green production of nanoparticles, a wide range of bioactive phytochemicals found in micro and macro organisms such as algae, bacteria, fungi, and plants can be employed. However, compared to methods using microorganisms, plant leaf extracts have been utilized extensively in green nanotechnology because they are easily handled, widely accessible, and contain a variety of phytochemicals that function as stabilizing and reducing agents during the creation of nanoparticles. Undoubtedly, green technology has been used to synthesize a variety of unique NPs, including ZnO, CuO, Au, Ag, Fe, Pd, and so forth.[(Lakshmipathy et al., 2015; Machado et al., 2014)](https://paperpile.com/c/RbZvp9/8EEK+3B5b) . NPs have recently been investigated as nanomedicines for the early detection and treatment of numerous fatal illnesses, including cancer. Treatment for cancer cells such as osteosarcoma (a kind of bone cancer) , retinoblastoma (an ophthalmological cancer) [(Barani, Mukhtar, et al., 2021; Barani, Rahdar, et al., 2021)](https://paperpile.com/c/RbZvp9/SG7B+1aZP) [(Arshad et al., 2021)](https://paperpile.com/c/RbZvp9/jg2x) Furthermore, NPs are fulfilling their function as a more secure drug carrier in the effective drug delivery systems for medications such as deferasirox [(Rahdar et al., 2021)](https://paperpile.com/c/RbZvp9/SC60) and crosin [(Saravani et al., 2020)](https://paperpile.com/c/RbZvp9/QpbV) .

Holy basil, or Ocimum tenuiflorum L., is an annual herb in the Lamiaceae family that has a powerful scent and soft hairs. It naturally occurs from India and Sri Lanka eastward to Malaysia to southeast Asia. [(Pandiyan et al., 2022)](https://paperpile.com/c/RbZvp9/6OTU)Tulsi is referred to as Dashemani, Shwasaharni (antiasthmatic), and Kaphaghna (antikaphic medicines) in Ayurvedic medicine. Tulsi leaves and petals are used to cure purulent ear discharges, lumbago, asthma, bronchitis, and blood and heart disorders. O. tenuiflorum is rich in phenolic compounds and terpenes. Many research have reported on the main components of essential oils, such as methyl eugenol, methyl chavicol, β-caryophyllene, β-elemene, germacrene A, etc. Kaempferol, rosmarinic acid, caffeic acid, 3,4-dimethoxycinnamic acid, luteolin, diosmetin, apigenin, and genistein are among the other significant phytochemicals that have been found. O. tenuiflorum has antibacterial, hepatoprotective, analgesic, anti-inflammatory, anticancer, neuroprotective, antifertility, wound healing, and antidiabetic, hepatoprotective, and cardioprotective qualities.[(Merchant et al., 2022; *Phytochemical Constituents and Pharmacology of Ocimum Tenuiflorum L*, n.d.)](https://paperpile.com/c/RbZvp9/zIlO+s2bW)

Although palladium is found in column 10 of the periodic table, its outer electron shell structure (Pd = 4d10, 5s0) sets it apart from other elements in the group. Among the metals of the platinum group, it has the lowest melting point and is the least dense (Ru, Rh, Pd, Os, Ir, Pt). The remarkable qualities of palladium nanoparticles (Pd-NPs) include superb mechanical, optical, electrical, catalytic, and physical qualities, as well as a wide range of sizes and shapes. Preliminary studies have demonstrated the significant potential of Pd-NPs as gene/drug carriers, biosensors, prodrug activators, and photothermal, photoacoustic, and antimicrobial/antitumor applications. [(Hazarika et al., 2017)](https://paperpile.com/c/RbZvp9/d0co)[(Saldan et al., 2015)](https://paperpile.com/c/RbZvp9/Zz3y)[(Phan et al., 2019)](https://paperpile.com/c/RbZvp9/DR8D)

Palladium nanoparticles (Pd-NPs), due to their distinct physicochemical characteristics, have demonstrated great potential in biomedical applications. Pd-NPs have been produced using a number of conventional chemical and physical methods. [(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/RbZvp9/kzE0+FCy5) Conversely, these methods can include the use of dangerous substances and reaction circumstances that could be detrimental to the environment and public health. Consequently, rapid, efficient, and reasonably priced methods for the synthesis of Pd-NPs have been developed that are acceptable to the environment.[(Jain & Verma, 2022; Marya et al., 2022)](https://paperpile.com/c/RbZvp9/UHjJ+Cw9Q) Plants, yeast, fungi, bacteria, seaweeds, and plant extracts were used to make Pd-NPs.[(Anand et al., 2016)](https://paperpile.com/c/RbZvp9/78FO) [(Fahmy et al., 2020)](https://paperpile.com/c/RbZvp9/6f4u)[(*[No Title]*, n.d.-b)](https://paperpile.com/c/RbZvp9/ChUj)[(Gurunathan et al., 2015)](https://paperpile.com/c/RbZvp9/NE3O)

Developing more simple and ecologically friendly synthetic methods is essential for studying metal nanoparticles, particularly on a large scale. In an effort to reduce environmental risks, there has been a recent surge in interest in green chemical techniques. The synthesis of "greener" nanoparticles starts with the selection of a solvent machine that is safe for the environment, a profitable and problem-free renewable stabilizing agent, and a non-toxic reduction agent. Therefore, the goal of the current work was to synthesize paladium nanoparticles using green technology and evaluate their effectiveness against bacteria and biofilms.

# Materials and methods

## Preparation of Leaf Extract

Fresh leaves were collected and thoroughly washed un-der the tap water to remove any dust or lose material. After washing, the leaves were again rinsed with distilled water to get rid of any remaining contaminants. After the leaves were dried to eliminate any remaining moisture, precisely 25 grams of dry Aloe barbadensis miller or 10 grams of Ocimum tenuiflorum leaves were finely diced and well-pounded with a mortar and pestle.Moreover, 100 milliliters of distilled water were added to well-minced leaves and cooked for approximately 15 minutes in each case. The hot extract solution was boiled, then allowed to cool before being filtered through Whatman No. 1 filter paper. In order to use the plant leaf extract for the biogenesis of Pd NPs, it was refrigerated after extraction.

## Synthesis of palladium nanoparticles

Distilled water was used to prepare the stock aqueous solutions of the precursor palladium nitrate (PdNO3), which had concentrations of 5, 10, and 50 mmol·kg−1. Next, a flask with a flat bottom was filled with the prepared known concentration solution and submerged in the oil bath.The solution's temperature was kept constant at 70±1∤C. Then, with continuous magnetic stirring, 20 ml of the previously produced Ocimum tenuiflorum plant leaf extract was added dropwise at a rate of around 5 ml/min. The Ocimum tenuiflorum leaf extract was added, and after about 15 minutes, the pH of the reaction mixture was maintained at 11.5 by adding freshly made 0.2mol·kg−1KOH solution. The creation of NPs is first confirmed by the solution's color changing from colorless to reddish brown and the appearance of dark black precipitates. For four hours, the reaction mixture was allowed to reflux at 70±1∘C while being constantly stirred. Black precipitates were refluxed and then allowed to settle at room temperature. The resulting nanosamples were then cleaned with ethanol after being rinsed three to four times with distilled water. Ultimately, the material was dried in a hot air oven at 55–60°C.

## Characterisation of palladium nanoparticles

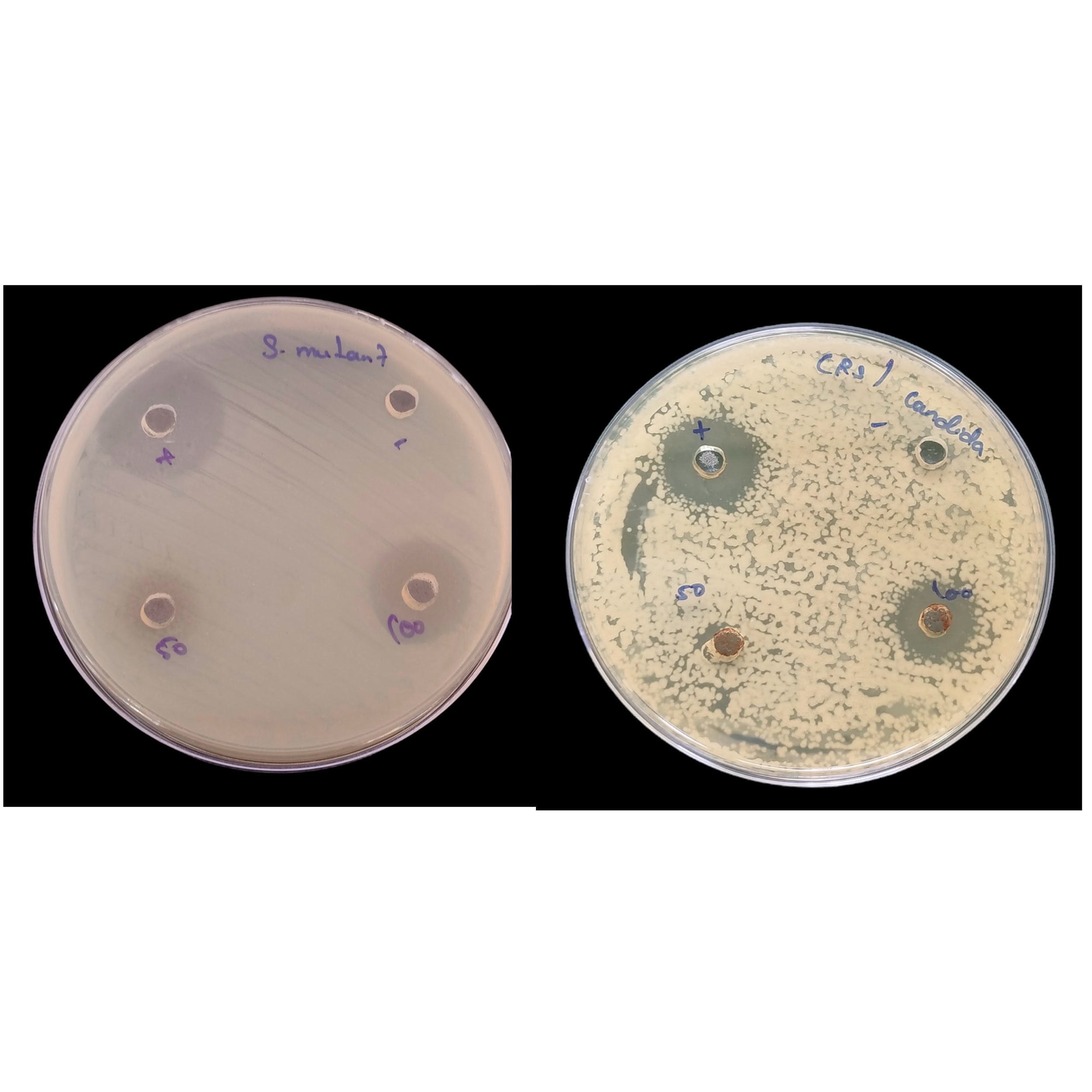
The materials that were synthesized have been verified through spectral analysis using UV-Visible, XRD, SEM, and EDS techniques. The spectrum studies have been conducted using the Cary 100 Bio UV-Visible instrument, a double-beam spectrophotometer that can collect data in the 190–900 nm range. The samples' phase identification and crystallinity have been examined using the Panalytical Empyrean XRS-45 kV XRD equipment.Additionally, the JEOL JEM2100F-200 kV transmission electron microscope and the Carl-Zeiss Ultra 55field emission-scanning electron microscope were used to analyze the morphology and shape/size of the synthesized NPs. On the other hand, the synthetic nanoparticles' elemental detection examination was done using an electron detector coupled to a scanning electron microscope.

## Anti-bacterial activity of silver nanoparticles

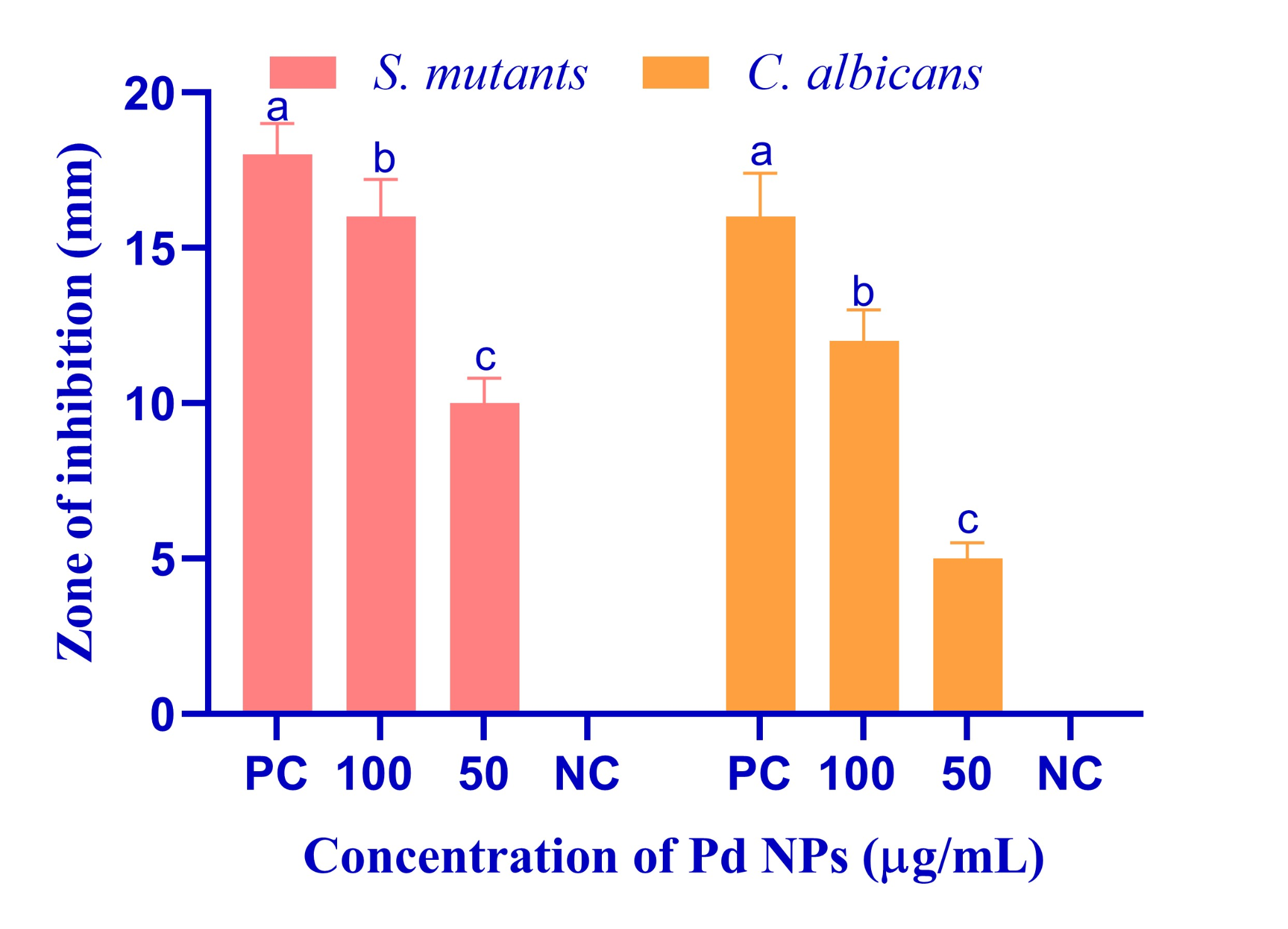
Using the agar well diffusion method, the antibacterial activity of these paladium oxide nanoparticles has been evaluated against the pathogens Streptococcus mutants and Candida albicans . Throughout the experiments, the protocols of the Clinical and Laboratory Standards Institute (CLSI) and the National Committee for Clinical Laboratory Standards (NCCLS) were adhered to in order to determine the MBC (minimum bacteria concentration) and the zone of inhibition, respectively.

# Results and Disscusion

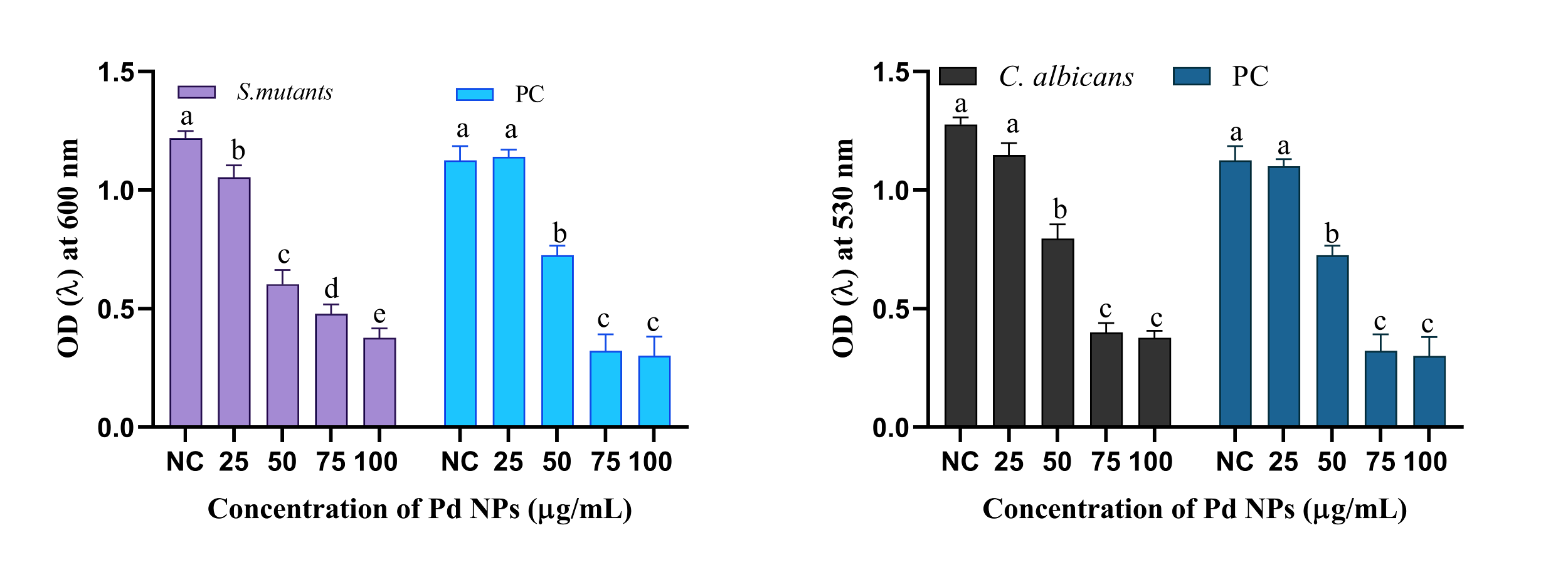
Since the color change was brought about by the activation of surface plasmon vibrations in the palladium nanoparticles, the shift in the solutions' color from light yellow to yellowish-black to deep black implies the formation of palladium nanoparticles. UV-Vis spectra demonstrate the monitoring of Pd-NP production as PdCl2 and propolis extract concentrations increased. These results are in line with earlier studies on the synthesis of palladium nanoparticles.[(Fahmy et al., 2020)](https://paperpile.com/c/RbZvp9/6f4u) [(Basavegowda et al., 2015)](https://paperpile.com/c/RbZvp9/korR)



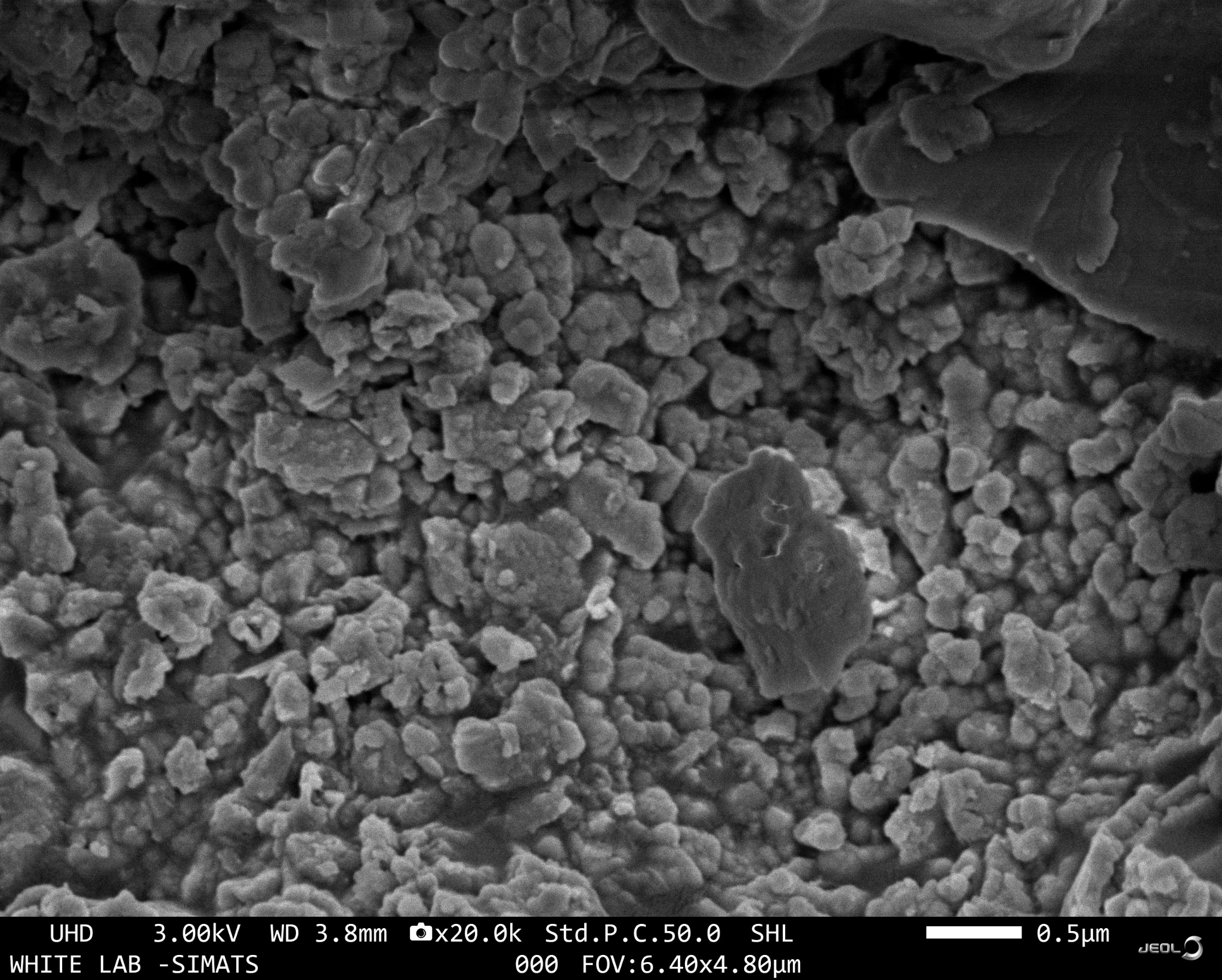
**Fig.1.** Antibacterial activity of synthesized Pd NPs against dental caries pathogens.



**Fig. 2.** Zone of Inhibition on Pd-NPs against S. mutants and C. albicans. Mean values within the column followed by the same letter in superscript are not significantly different at P< 0.05 level.

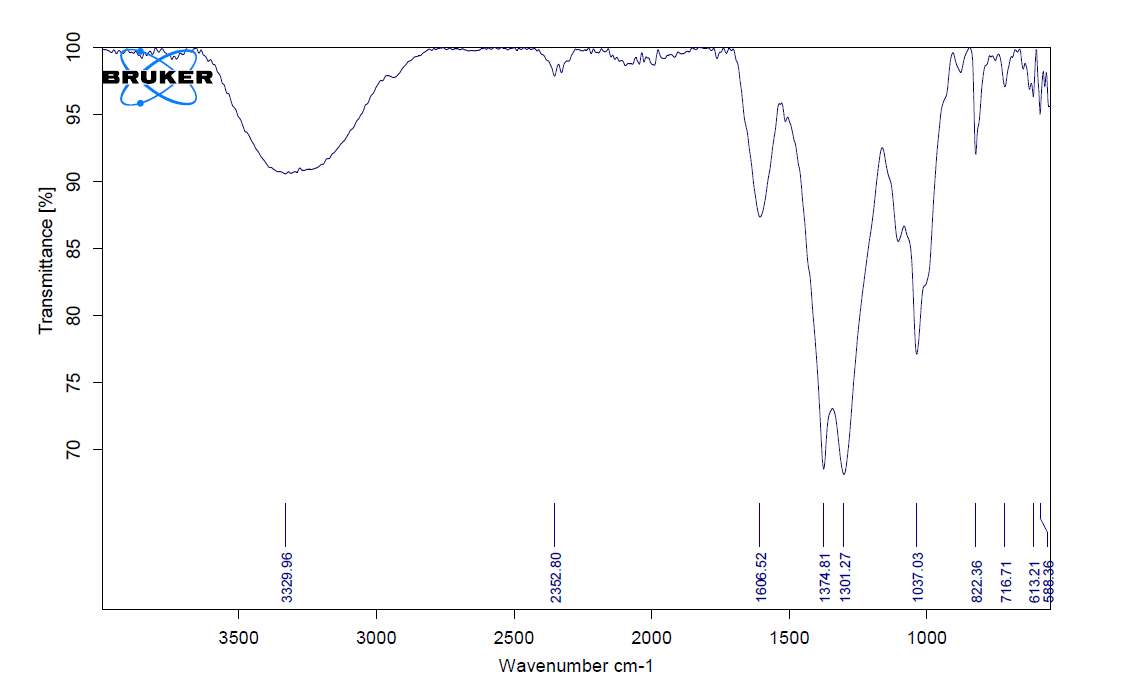


**Fig.3.** In vitro biofilm production. *S. mutants* and *C. albicans* were grown in 96-well microtiter plates and their biofilm was quantified through staining with crystal violet after 48h of cultivation. Mean values within the column followed by the same letter in superscript are not significantly different at p< 0.05 level.



**Fig. 4.** SEM image of biosynthesized palladium nanoparticles

The presence of flavonoids, responsible for the inherent hue of propolis powder and liquid extract, is signified by FTIR peaks within the 2300–1900 cm−1 range (Figure 8). It is observed that in Pd-NPs, this band gets wider and stronger (Chehelgerdi et al., 2023). Biomolecules such as flavonoids, phenolic compounds, and terpenoids included in propolis extract decrease palladium ions to Pd-NPs. It has been documented how flavonoids work mechanistically to produce metal NPs by acting as a capping and reducing [(Hussain et al., 2019)](https://paperpile.com/c/RbZvp9/Isjr). The largest functional peak, at 3430 cm−1 , displays the presence of phenolic hydroxyl groups in the structure, which practically confirms the existence of friedelin, lupeol, and β-sitosterol. The strong peak in the area 1640 cm−1 relates to the aromatic stretching of C–H, which is linked to the phenolic ring structure. The presence of such groups in the aromatic ring structure is indicated by the unsaturated C=C structure, as demonstrated by the peak in the 2065 cm−1 area .



**Fig. 5.** FTIR spectrum of Paladium nanoparticles

The mixture's optimal absorbance value occurred at a pH of 6. When propolis extract is used as a reducing agent, Pd nanoparticles are seen to be uniformly distributed and generally persistent in solution. Because of the negative surface charges, the particles get larger, which causes an increase in absorbance. The functional groups act as a capping agent to stop the particles from aggregating. It is simple to link the variance in nanoparticle stability to the pH effect. The electrolyte's pH has a big impact on the particle formation process. This suggests that the size of the nanoparticles is decreasing, which could be because the full charge of the clusters enhanced the repulsive electrostatic interactions, reducing the amount of particle aggregation. [(Rehbock et al., 2013)](https://paperpile.com/c/RbZvp9/BzIu) [(Sathishkumar et al., 2009)](https://paperpile.com/c/RbZvp9/3VLg) [(Aziz & Jassim, n.d.)](https://paperpile.com/c/RbZvp9/HLt0) . 75 °C was the ideal temperature for Pd-NP production (Saadh et al., 2024). This result showed that, in comparison to room temperature, increasing the mixture's temperature will speed up the formation of palladium nanoparticles. It is hypothesized that as temperature increased, the absolute negative charge increased, suggesting that higher temperatures generated more nanoparticles.[(Chokkattu et al., 2023; Solanki et al., 2023; Sreevarun et al., 2023; Wadhwani et al., 2022)](https://paperpile.com/c/RbZvp9/9jzQ+SBfJ+LqLo+8w2L). The Pd(II) ions in the PdCl2 solution were changed into zero-valence Pd atoms by the reducing agents in the propolis extract. In an attempt to lower the overall surface energy, reduced Pd atoms tried to cluster together, and small particles tried to merge into larger particles, which ultimately led to the formation of larger Pd particles. The organic long chains stopped the Pd particles from growing larger by preventing their agglomeration into nanoscale particles.[(Laghari et al., 2023; Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/RbZvp9/MOUg+rVf2)

As a result, Pd-nanoparticles' crystalline and amorphous structures are obtained. The XRD results of Pd/extract [33–35] indicate that palladium NPs have a face-centered cubic (fcc) structure. (1 1 1), (2 0 0), and (2 2 0) were the fcc lattice planes. [(Liu & Zhang, 2009)](https://paperpile.com/c/RbZvp9/HEls) [(Sheny et al., 2012)](https://paperpile.com/c/RbZvp9/GjLX) [(Lu et al., 2003)](https://paperpile.com/c/RbZvp9/zyZt)

The primary factor influencing the antibacterial and anticancer properties of palladium nanoparticles is currently unidentified, however it can be linked to the Pd+ ions' mechanical action against bacteria, viruses, and cancer, where the accumulation of Pd-NPs in the aqueous . The saturation of the enzymes and proteins within the cell is greatly aided by the solution [(*[No Title]*, n.d.-b)](https://paperpile.com/c/RbZvp9/ChUj). Three antibacterial and anticancer mechanisms are believed to be involved in palladium nanoparticles. The first method involves the adhesion of tiny palladium particles to the cell wall, which impedes development and expansion of bacteria and cells, which disrupts the cell wall and incapable of defending the interior of the cell [(Rai et al., 2012)](https://paperpile.com/c/RbZvp9/FkQi). The second method involves Pd-NPs penetrating the bacterial cell and changing how nucleic acids normally form, which results in cell death or, at the very least, damage to DNA [(Murugesan et al., 2020)](https://paperpile.com/c/RbZvp9/5PoB). The third method involves the irreversible disruption of the bacterial cell wall caused by Pd+ ions interacting with proteins in the cell wall that contain sulfur. This proposed mechanism was also determined to be the main antibacterial mechanism when antimicrobial activity was tested . [(Keat et al., 2015)](https://paperpile.com/c/RbZvp9/5V8o) [(*[No Title]*, n.d.-c)](https://paperpile.com/c/RbZvp9/rGFh)

# Conclusion

Palladium nanoparticles were successfully synthesized using aqueous *Ocimum tenuiflorum* extract and aqueous Pd solution. This biosynthesis was controlled by using optimum conditions (concentrations, ratio of volumes, temperature, pH, and reaction time). UV-visible spectrum analysis was used to confirm and monitor the development of Pd-NPs. Under ideal conditions, the reduction response time was very rapid and completed within 30 min to form Pd-NPs. FTIR analysis looked into the flavonoid group that led to a reduction of Pd+2 metal ions. XRD analysis showed a mean particle size of 4.62 nm, confirming the crystalline phase and face-centered cubic structure of Pd-NPs. The chemical composition and morphology of the biosynthesized Pd-NPs were investigated using energy-dispersive X-ray fluorescence spectrometry (EDX) and scanning electron microscopy (SEM), which unveiled the nanostructure and the influence of *Ocimum tenuiflorum* extract. In agreement with the XRD findings, the SEM image showed an irregular shape with an average particle size of 3.14 nm. When Pd-NPs made from Ocimum tenuiflorum extract were tested against both Gram-positive and Gram-negative bacteria, they demonstrated a sizable zone of inhibition against *Candida albicans* and *Streptococcus variants*. It was demonstrated that Pd-NPs had superior antifungal activity against *Candida albicans*. According to the preceding studies' findings, biologically manufactured Pd-NPs could pave the way for significant medical advancements.

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