

Green Synthesis of Palladium nanoparticles from Eucalyptus globulosa Leaves Extract

Characterization and Biological Activity studies

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Abstract: Effective biological methodologies have been broadly designed for the preparation of metal nanoparticles. Hence, the use of biogenic materials such as plants, bacteria biomass, algae, enzymes and fungi has been utilized to synthesize various metal nanoparticles as an efficient, economical and sustainable process. Aqueous extract of dried leaves of Eucalyptus globulosa is used as a biogenic reducing agent for ecologically sound synthesis of palladium nanoparticles. Dynamic Light scattering (DLS), UV-visible spectroscopy, Fourier-Transform infrared spectroscopy (FTIR), Energy Dispersive X-Ray analysis (EDAX) and Scanning electron microscope (SEM) analyses were used to characterize the formed Palladium nanoparticles. The synthesized palladium nanoparticles exhibited antimicrobial activities.

Keywords: PdNps, Bioreductant, Stabilizing Agents.

I. INTRODUCTION

Nanotechnology is the potentiality to construct material, systems and devices with atomic precision. Nanoscale science and technology are founded on the established concept that nanostructures should have qualities that differ from bulk materials in terms of electrical, optical, magnetic, chemical and mechanical capabilities. When comparing bulk material with nanoparticles in similar applications; it is proved that nanoparticles have superior performance properties².

Metal nanoparticles, particularly noble metal nanoparticles, appear to be of great interest, not only because of their vast surface areas, but also because of their unique function. Transition metal NPs' characteristics are becoming increasingly important. The seed mediated method¹, heatless synthesis², and weak reductant method³ can all be used to make nanoparticles with interstitial nanostructure. Nanomaterials are ideal for usage as possible catalysts because of their large surface area to volume ratio. Palladium is a highly efficient metal in catalysis. Palladium-based materials have been widely studied in a variety of catalytic applications, including the production of carbon-carbon bonds⁴, oxidations⁵, hydrogenation⁶, and electrochemical reactions in fuel cells⁷. Palladium's uses, on the other hand, are not limited to catalysis. PdNPs have a wide range of applications in the biological arena. Prodrug activators, antibacterial agents, photothermal agents, photoacoustic agents, gene/drug carriers, and anticancer agents have all been revealed for PdNPs.

II. LITERATURE REVIEW

Subramanyan Bharathiraja et al.⁸ used chitosan oligosaccharide to make structured PdNPs and devised a photothermal therapy to improve particle biocompatibility. They have the ability to eliminate the tumor. Yu Liu et al.⁹ created flower-like polypyrrole (PPy) coated PdNPs, and in-vitro photochemical heating of the nanoparticles in the presence of Hela cells resulted in cell death after 10 minutes when the cells were exposed to laser irradiation. Using Rosmarinus officinalis leaf extract at room temperature for 24 hours, Rabiee et al.¹⁰ created Pd nanoparticles with an average size of 15-90 nm and a semi-spherical and polyhedral shape, which showed antifungal activity against Candida parapsilosis, Candida albicans, Candida glabrata, and Candida krusei. Petla, R. K. et al.¹¹ used Glycine max (Soybean) leaf extract to create spherical PdNPs nanostructures around 15 nm that have antibacterial activity against M.luteus, E.coli, S.aureus and S.epidermidis. Thus, alkaloids, polyols, vitamin C, carbohydrates, glycosides, flavanoids, polyphenols, oxalic acids, amino acids, and other critical phytochemical bioreductants which are used to synthesize PdNPs that can be obtained from various plant parts such as stem, fruit, flowers,

and leaves. PdNps prepared using various plant species were reported. PdNps have been synthesized utilizing *Curcuma longa*¹² (tuber extract) with a size of nearly 10-15nm, coffee and tea¹³ (powder extract) with a size of 5-100 nm, *Camellia sinensis*¹⁴ (leaves extract) with a size of 7nm, and *Cinnamomum camphora*¹⁵ (leaves extract) with a size of 3.2-6 nm. Biogenic reduction can also be done with bacteria, algae, diatoms, and human cells, in addition to plant elements. Such biogenic synthesis procedures are ecologically sound, sustainable, low cost, biocompatible, utilizing less energy and are alternative to toxic and expensive chemical and physical procedures. Hence these synthesis are also very much beneficial as they avoid noxious organic reagents and solvents as the plant element extract serves the property of both reducing and capping agent. However it is a remarkable challenge to fabricate and produce PdNps in a biogenic way as the process is one pot process. Hence biogenic nanotechnology deals with environment friendly methodologies and /economically feasible solvents and reagents.

The primary supporting aspect in modifying various sizes and shapes in nano particle synthesizing is the difference in concentration as well as confirmation of effective biomolecules of various plants¹⁶. Metal salt reductions using plant extract at ambient temperature is used to synthesize desired nano particles in a relatively comfortable environment. Normally, the metal solution and the plant extract are thoroughly combined. The size, morphology, and quality of the synthesized nanoparticles are influenced by a variety of parameters of the reaction solution such as metal salt concentration, plant extract volume and concentration, pH of reaction solution and other reaction variables, such as temperature, reaction time¹⁷. The preparation of NPs is greatly influenced by the pH approximation of the reaction solution¹⁸. Temperature plays an important role in influencing the characteristics of NPs during synthesis¹⁹. This study aims to alter the concentration of metal ion and amount of *Eucalyptus globulosa* leaves extract to fabricate the nanoparticles and determining its antimicrobial activity. Furthermore, in the clinical environment there is a common call for prevention of microbial infections like *S. aureus* and *E. coli*. This bacterium has rapidly evolved resistance to multiple drugs. *S. aureus* is a commensal organism that is commonly being a major hospital acquired pathogen. Although the majority of *E. coli* strains are safe, some of them can cause significant food poisoning in their hosts and can cause food contamination²⁰. *S. aureus* and *C. albicans* are the second and third leading causes of bloodstream infections. The opportunistic pathogen *Staphylococcus epidermidis* is regarded as important. *Staphylococcus epidermidis*, along with its more aggressive relative *Staphylococcus aureus*, is the leading cause of nosocomial infections. The current innovation deals with synthesis of PdNps using *Eucalyptus globulosa*. *Eucalyptus globulosa*: Common Names Tasmanian blue gum, bloodwood, gum tree, blue gum, ironbark, eucalyptus tree. *Eucalyptus* is a member of the Myrtaceae family which includes plants with antibacterial, antifungal, and anti-inflammatory effects. *Eucalyptus* is most vital among genera of Myrtaceae family; a wide genus of shrubs and evergreen trees including 700 species²¹. The essential oil of *Eucalyptus globulosa* is the plant's main product, and it has a wide range of therapeutic applications. Eucalyptol (1, 8-cineol)²² is present in the oil. It's also a good source of kinos, a plant gum made by a variety of plants and trees that's commonly used in medicine and tanning treatments. *Eucalyptus* oil has long been used to treat boils, wounds, and other ailments. *Eucalyptus* leaves include isoprenoids, a volatile phytochemical, as well as a variety of other compounds that have antibacterial properties²³. *Eucalyptus* extract is widely used in cosmetics and can be utilized as a food ingredient. Saponins, Flavanoids, Tannins, and Alkaloids are all found in *Eucalyptus* leaf extract. Antimicrobial action is seen in alkaloids and flavanoids²⁴.

III. METHODOLOGY

3.1 Reagents and Materials

PdCl₂, concentrated HCL, absolute alcohol of research Lab were purchased. Double distilled water was used to wash all the glassware properly and these glasswares were dried in oven. Throughout the experimentation, double distilled water was used.

Eucalyptus globulosa leaves collection and selection.

Fresh leaves of *Eucalyptus globulosa* were collected from Alibag, Nagaon region. *Eucalyptus globulosa* plant selection was done from the less polluted area where the trees and plants were densely populated on a small hilly area. The selected *Eucalyptus globulosa* plant was healthy and disease free. As the essential oil is the major product of the *Eucalyptus globulosa*, the bioactivity of the essential oil is dependent on the kind and type of ingredients. Species, season, climate, soil type, location, age of leaves, fertility regime, procedure used for drying the plant material, etc. are the prime factors for the

activity of this essential oil (Brooker and Kleinig²⁵, 2006). The *Eucalyptus globulous* leaves were plucked during rainy season or at the end of a rainy season.

3.2 Biogenic *Eucalyptus globulous* plant extract preparation

The *Eucalyptus globulous* leaves were washed with distilled water and further washed with absolute alcohol and shade dried for 5 days (Fig.1). The shade dried leaves were kept for 2 hours at 45°C. Later they were crushed to produce fine powder using a mixer grinder (Fig.2). The ground powder was stored in the airtight container for later use. In a conical flask, 10gm of ground powder was heated with 100 ml of distilled water. The extract was then filtered using Whatmann paper No. 1 and Millipore filter 0.22µm.

3.3 Synthesis of Palladium nano particles

The Palladium ion 2 mM solution was prepared using Palladium (II) chloride. 2 mM of Palladium chloride and 40 mL produced extract were combined with 400 mL double distilled water and stirred continuously at 1000 rpm at room temperature. The Palladium solution turns out from yellow (Fig. 3) to black (Fig. 4), indicating reduction to Palladium nanoparticle. The reaction mixture was monitored by UV visible spectroscopy. Palladium Nanoparticles were obtained and centrifuged at 10,000 rpm for 30 minutes later being rinsed with water.

3.4 Instrumentation

UV visible spectroscopic analysis was conducted on (Shimadzu UV-2450) with quartz cuvettes of 1cm path length. By examining the spectrum of reaction mixture, the reduction of Palladium into Palladium nano particles is estimated. The Zetasizer Nano ZS (Malvern Instruments Ltd. UK) was used to measure dynamic light scattering (DLS) using a photo diode detector with a quantum efficiency more than 50% at 633 nm and a Helium-neon laser at 633 nm. The Malvern dispersion technology software DTS 7.10 was used to unfold and estimate the data. The Perkin Elmer FTIR Frontier model was used for spectroscopic research. The surface morphological features of nanoparticles were examined using a scanning electron microscope (SEM) with an operating voltage of 5.00 kV, the FEI inspect F-50 FESEM. EDAX was used to determine Palladium's elemental presence.

3.5 UV-Visible absorption studies

The production of PdNps was studied using a UV-Vis Spectrophotometer (Shimadzu UV-2450) with quartz cuvette (1cm path length). Surface Plasmon resonance peak was recorded initially when 40 ml of *Eucalyptus globulous* extract was added to the Pd (II) solution (2mM) at ambient temperature. Later SPR peak was recorded for the reaction mixture containing measured amount of extract and 2mM Pd (II) solution after 12 hr which indicates the completion of reaction as the initial colour of the reaction mixture which was yellow and later on it turns to black coloration.

3.6 Dynamic size study for size and stability, Dynamic light scattering (DLS)

The Zetasizer Nano ZS was used to perform dynamic light scattering (DLS) studied (Malvern Instruments Ltd. UK). Formed PdNps were monitored at 298K through DLS so as to observe the acquired size. After the completion of the reaction which was indicated by the colour change (yellow to black) after almost 12 hours of duration the DLS measurements were recorded. The selected combination of *Eucalyptus globulous* extract and Pd (II) ion solution showed results in good terms.

3.7 Antimicrobial Study

Antimicrobial activity of the nano particles synthesized was tested with determination of a minimum inhibitory concentration using REMA (Resazurin Microtitre Assay). Both antibacterial and antifungal tests were carried out using the PdNPs. This study was carried out at National Facility for Biopharmaceuticals, G. N. Khalsa College, Matunga, Mumbai.

IV. RESULTS AND DISCUSSIONS

Eucalyptus globulous which was used as a bioreductant also acts as stabilizing agent in the synthesis of palladium nanoparticles. 5 ml of 2 mM of PdCl₂, 4 ml of *Eucalyptus globulous* plant extract 40 ml of double distilled water. The total volume obtained was 49 ml. The prepared reaction mixture was kept for stirring for 12 hours at 1000 rpm at room

temperature.

4.1 Dynamic light scattering (DLS)

During the DLS studies the average size obtained was 76.4 nm (Fig. 5). This methodology was confirmed as the particles obtained showed size below 100 nm.

4.2 UV-Visible spectrum of palladium nanoparticles.

The reduction of Pd (II) ion was monitored through UV-Visible spectroscopy. Further the PdNPs thus obtained were separated by centrifuging at 10000 rpm for 30 min. Hence it was concluded that by mixing the diluted metal solution with eucalyptus leaves extract, nanoparticles of the desired size can be obtained. During the UV-visible monitoring the colour of the reaction solution changed from yellow to black. The obtained colour change is due to SPR (Surface Plasmon resonance) which is the result of the excitation of the surface electron on the conduction band²⁶. During the process, the Pd ion peak at 330 nm vanished and was shifted to 270 nm (Fig. 6) indicating the creation of PdNPs. Similar result were observed and peak shift was observed for *Prunus x yedoensis* leaf extract²⁷.

4.3 FTIR analysis of palladium nanoparticles.

In FTIR (Fig. 7), peak at 700 cm^{-1} indicates the reduction of Pd (II) to Pd (0). Strong and intense peak is seen at 1000 cm^{-1} denotes C-O stretching vibrations. Less intense and weak peak observed at 1350 cm^{-1} indicates -COO- carboxylic acid group. C=C stretching vibration is indicated by the weak peak at 2100 cm^{-1} . The 2980 cm^{-1} strong and sharp peak indicates -NH stretching. The broad nature in the range of $3400\text{ to }3000\text{ cm}^{-1}$ region can be attributed to presence of -OH group. The medium intense and sharp peak at 1650 cm^{-1} indicates aromatic ring. Due to these IR bands in the spectrum of Eucalyptus globulosa extract fabricated PdNPs concludes the presence of organic compounds of Eucalyptus extract, a bio-reductant as well as capping ligands on the surface of the PdNPs^{28, 29}.

4.4 Elemental and morphological analysis (SEM)

The morphology and size of the particles were analyzed using a scanning electron microscope. The obtained shape was distorted spherical along with particle size 60 nm to 100 nm. Elemental Dispersive X-Ray Analysis estimate the peak at 2.7 keV and 3.2 keV gives the indication of presence of palladium (Fig. 8). The peak at 0.1 keV and peak at 0.2 keV confirms the presence of carbon and oxygen. The carbon content was due to organic compounds of Eucalyptus leaves extract (Fig. 9). and the oxygen was either due to organic compounds from Eucalyptus leaves extract or may be from air²⁸. Gil et al. prepared PdNPs ranging 100nm using chaga mushroom. Nadagouda et al. prepared PdNPs which shows size of 5-100nm using coffee and tea extract¹³.

4.5 Antimicrobial analysis.

Antimicrobial activity of the nano particles synthesized was tested with determination of minimum inhibitory concentration using REMA (Resazurin Microtitre Assay)

4.6 Minimum inhibitory concentration

Inoculum preparation: - In a Luria Bertani broth, a loopful of culture was implanted and grown for 24 hours at 37°C . Fungal culture was grown in Sabouraud's broth incubating at room temperature. To get $1.5 \times 10^8\text{ CFU/ml}$, the culture OD was scaled to the 0.5 McFarland standard. A final density of $1 \times 10^6\text{ CFU/ml}$ was achieved by homogenising the cell suspension. **Sample preparation-** 30 μg of Pd nanoparticles in 1.0 ml double distilled water. A 100 μl sample was used for actual studies. Using the resazurin microplate test, the samples' minimum inhibitory concentration (MIC) against the test organism was evaluated. The testing was conducted out on 96-well transparent microplates with a flat bottom. Every column's first row was mixed with 100 μl of Mueller Hinton broth with conventional antibiotics, while the second row was filled with 100 μl of media. 100 μl 2X media as well as 100 μl samples were placed in the third row (each column with different sample). Then, starting with the fourth row, every well was poured with 1X Media (Mueller Hinton broth). From the third till the twelfth row, equivalent two-fold serial dilutions were prepared. Finally, all of the wells were completely stirred. The resultant concentrations of the 3rd to 12th rows ranged from 50% dilutions to 0.1 percent dilutions, and microbial cells were introduced to every well as a final cell count of $1 \times 10^6\text{ CFU/ml}$. The cultivated microplates were capped with lids and

incubated for 24 hours at 37 degrees Celsius. After adding 5L of 5mg/mL Resazurin to those wells and incubating at 37 °C for 30 minutes, the MIC of the samples was evaluated. The change in colour of Resazurin in the microplate wells was used to assess microbial growth (blue when there is no growth and pinkish-red formazan when there is growth). The MIC was established as the minimum sample concentration that did not change colour (was clear) and completely inhibited microbial growth. The inhibition percentages for *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Candida albicans* are shown in Table – I.

V. CONCLUSION

This research work is an effective and quick synthesis of stable nanostructured palladium particles was demonstrated using *Eucalyptus globulus*. The *Eucalyptus globulus* leaves extract serve as a reducing as well as capping agent. By changing the concentration of Palladium salt and amount of extract stable PdNPs were effectively generated using a easy, cheaper, ecologically sound and ideal technique involving low energy consumption. This methodology avoid the use of hazardous chemical making the process green. As a result, this research leads to the invention of a simple biological process for the preparation of palladium nanoparticles.

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Fig. 1 – Eucalyptus Leaves: Shade dried



Fig. 2 – Eucalyptus Leaves: Finely ground powder

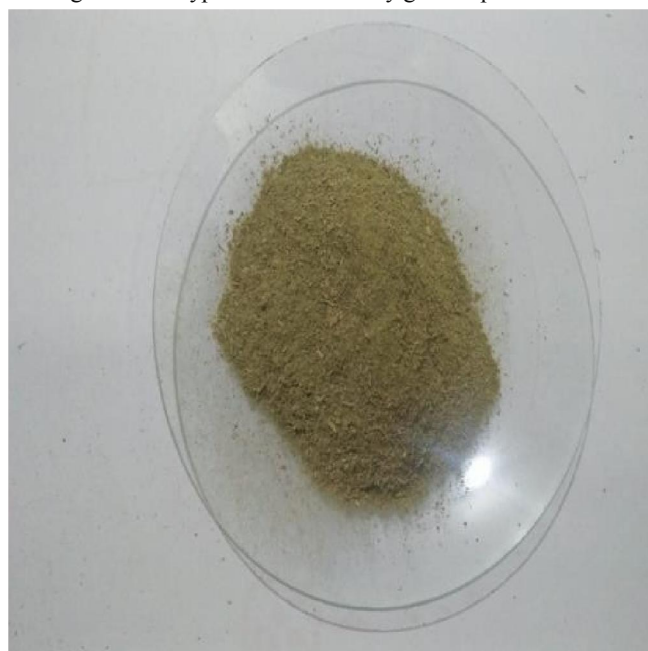


Fig. 3 – Initial colour



Fig. 4 – Final colour

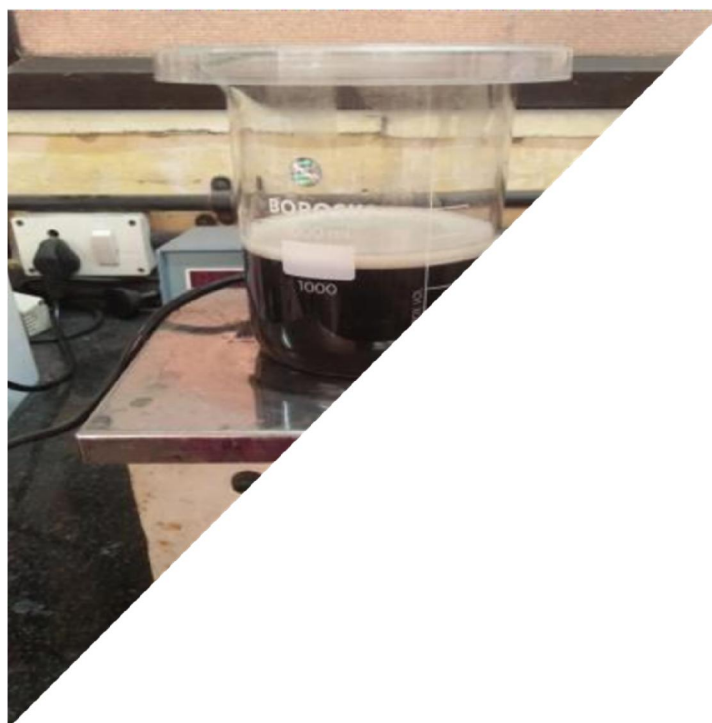


Fig. 5 – Dynamic light scattering

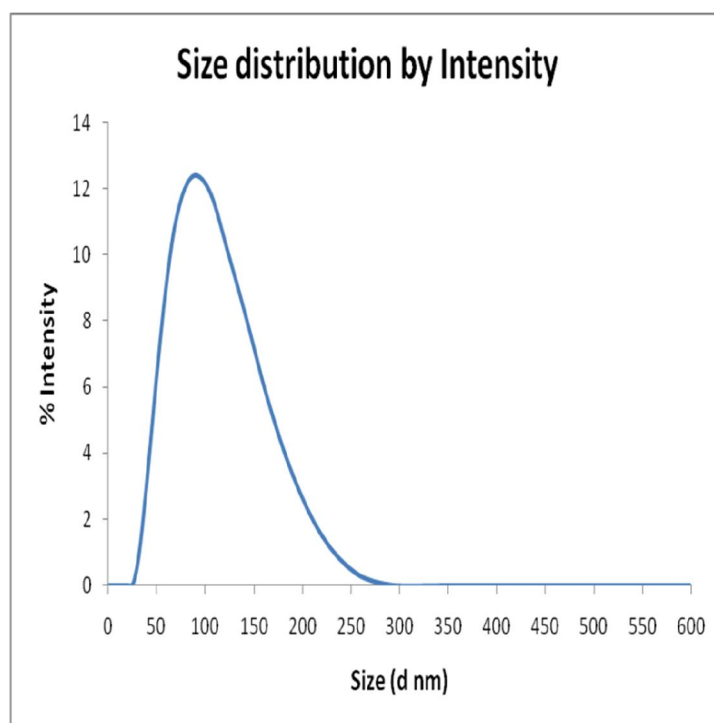


Fig. 6 – UV-Visible Spectroscopy

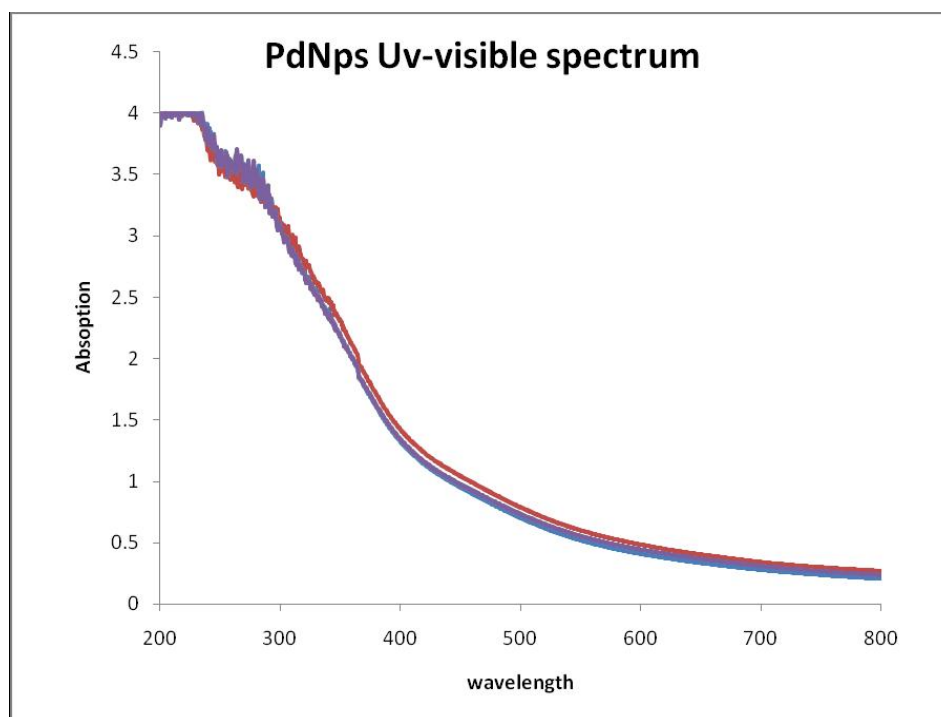


Fig 7 – Fourior Transform Infrared Spectroscopy

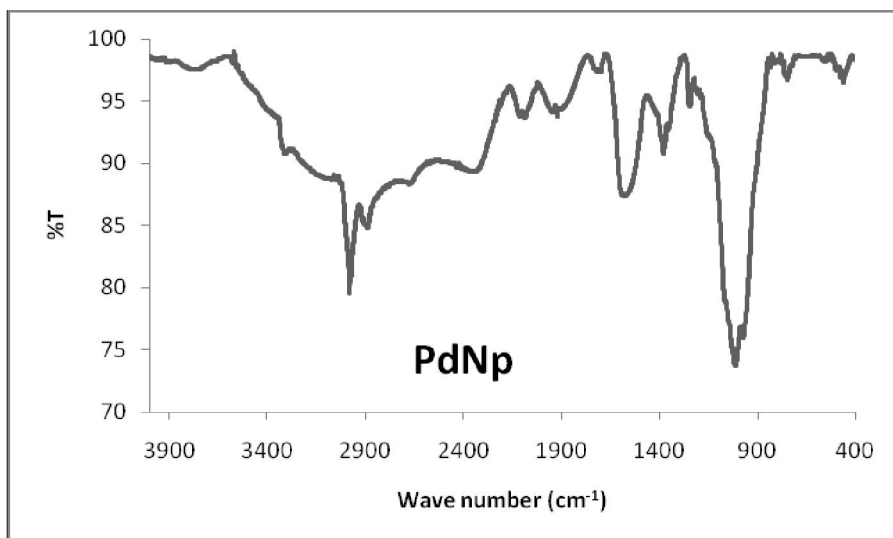


Fig. 8 – Scanning Electron Microscopy

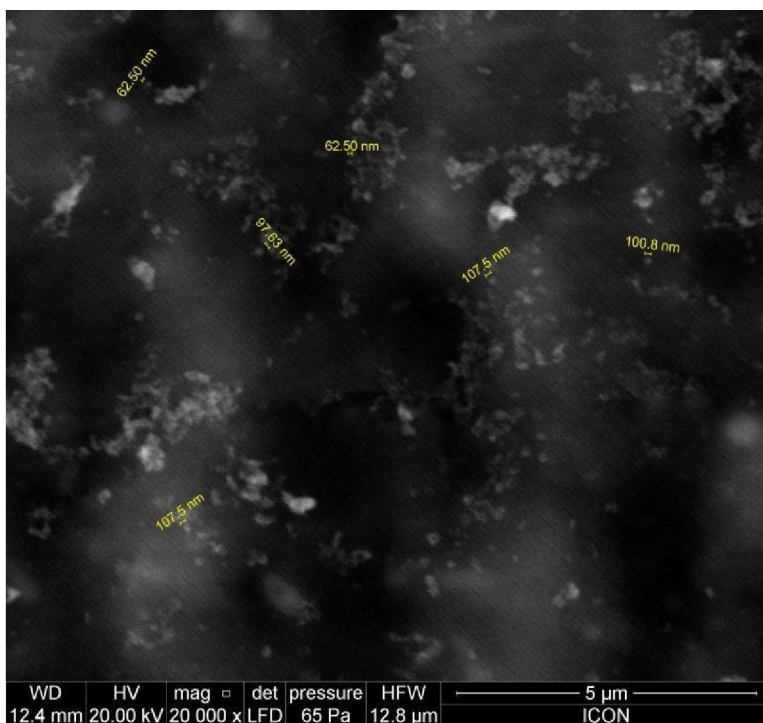


Fig. 9 – Energy Dispersive X-Ray Analysis

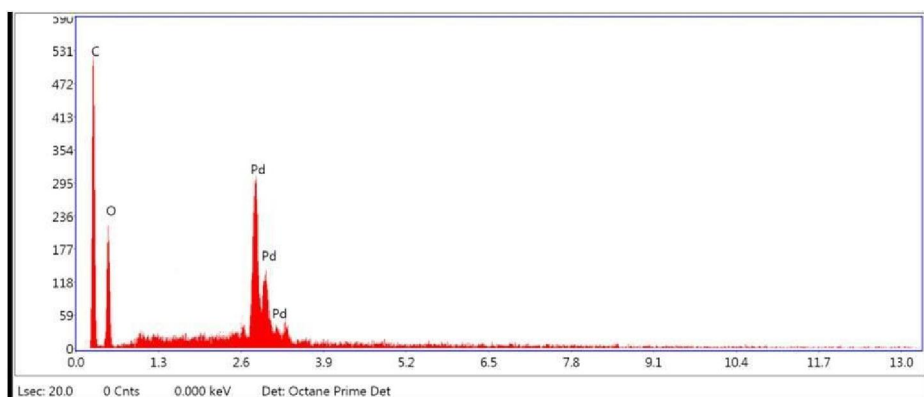
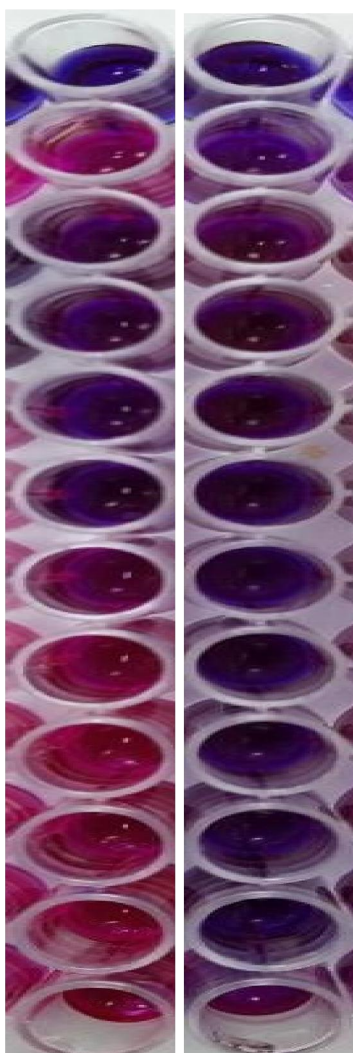


Fig. 10 – Minimum Inhibitory Concentration



S.aureus E.coli S.epidermidis C.albicans

Table – I: Inhibition Percentage

Name of Organism	Sample (in %)
Escherichia coli	0.20
Staphylococcus aureus	12.50
Staphylococcus epidermidis	0.78
Candida albicans	12.50