

# ***In Vitro Antimicrobial and Antioxidant activity of Biogenically Synthesized Palladium and Platinum Nanoparticles using *Botryococcus braunii****

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## **Abstract**

**Context:** The spreading of infectious diseases and the increase in the drug resistant among microbes has forced the researchers to synthesize biologically active nanoparticles. Improvement of eco-friendly procedure for the synthesis of nanoparticles is growing day by day in the field of nanobiotechnology. **Objectives:** In the present study we use the extract of green alga *Botryococcus braunii* for the synthesis of palladium and platinum nanoparticles and evaluation of their antimicrobial and antioxidant activity. **Methods and Material:** Green alga was collected from Udaisagar Lake Udaipur (Rajasthan, India) and isolated by serial dilution method and grown on Chu-13 nutrient medium. The characterization of alga synthesized palladium and platinum nanoparticles was carried out by using X-ray diffraction and scanning electron spectroscopy. The zone of inhibition was measured by agar well plate method and minimum inhibitory concentration was determined by agar dilution assay for antimicrobial activity. Antioxidant activity of nanoparticles was also studied by DPPH method. **Results:** Stable palladium and platinum nanoparticles were successfully produced by using green alga. XRD pattern revealed about crystalline nature and SEM micrographs have shown the morphology of biogenically synthesized metal nanoparticles. FT-IR measurements showed all functional groups having control over stabilization and reduction of the nanoparticles. Green synthesized nanoparticles exhibited antimicrobial activity against gram positive and gram negatives bacterial strains, antifungal activity against fungus and also demonstrates antioxidant activity. **Conclusion:** The biogenic synthesis of metal nanoparticles can be promising process for production of other transition metal nanoparticles and also new nanocatalyst will revolutionise the synthesis of organic heterocycles.

## Soyut

**Bağlam:** İlaca dirençli mikroplar arasında artış ve bulaşıcı hastalıkların yayılması biyolojik olarak aktif nano tanecikleri sentezlemek için araştırmacılar zorlamıştır. Nano tanecikleri sentezi için çevre dostu prosedür geliştirilmesi gün nanobiotechnology alanında büyüyor. **Amaç:** çalışmanın Yeşil alg *Botryococcus braunii* özü Paladyum ve platin nano tanecikleri sentezi ve kendi antimikrobiyal ve antioksidan etkinliği değerlendirilmesi için kullanıyoruz. **Yöntemleri ve malzeme:** Yeşil alg Udaisagar Gölü Udaipur (İstanbul, Türkiye) toplanan ve seri seyreltme yöntemiyle izole ve Chu-13 besin ortamda büydü. Alga sentezlenmiş Paladyum ve platin nano tanecikleri karakterizasyonu X-ışını kırim ve Tarama elektron spektroskopisi kullanılarak gerçekleştirilmiştir. İnhibisyon bölgisinin agar iyi plaka yöntemi tarafından ölçüldü ve minimum inhibitör konsantrasyonu agar seyreltme tahlili antimikrobiyal aktivitesi için tarafından belirlendi. Nano tanecikleri antioksidan etkinliği de DPPH yöntemi tarafından incelenmiştir. **Bulgular:** İstikrarlı Paladyum ve platin nano tanecikleri yeşil alg kullanarak başarıyla üretildi. XRD desen kristal doğası hakkında ortaya ve SEM Filmleri biogenically sentezlenmiş metal nano tanecikleri morfolojisi göstermiştir. FT-IR ölçümleri tüm Fonksiyonel grupların istikrar ve nano tanecikleri azalma denetiminizden gösterdi. Yeşil sentezlenmiş nano tanecikleri antimikrobiyal aktivite g karşı sergilenen. **Sonuç:** Metal nano tanecikleri biyojenik sentezi diğer Geçiş metalleri nano tanecikleri üretimi için umut verici bir süreç olabilir ve aynı zamanda yeni Nanokatalizör organik heterocycles sentezi devrim.

**Keywords:** Palladium, Platinum, Nanoparticles, Antimicrobial, Antioxidant, Biogenic.

## INTRODUCTION

Green synthesis of metal nanoparticles has three qualifying characteristics as an envirosafe solvent system, particle-stabilizing capping agent and eco friendly reducing agents have been selected.<sup>1</sup> Biological synthesis using algae is one of the green approaches for synthesis of d-block metal nanoparticles. Algae are eukaryotes, photoautotroph, aquatic and oxygenic organisms.<sup>2-3</sup> Algae have more

information in their genetic material to encode various reducing stabilizing agent that mediates the biogenic synthesis of metal nanoparticles. Algae acquire energy from sunlight through photosynthesis and convert inorganic carbon into organic material for their growth. Since algae are sustainable bio-resources, they can be used largely in the greener synthesis of metal nanoparticles.<sup>4</sup> Biogenic synthesis is the alternate route for synthesizing biocompatible metal nanoparticles to other synthesis processes as chemical and physical.<sup>5</sup> It is the newest possible way of linking nanotechnology and biotechnology in the developing field of nanobiotechnology.<sup>6</sup>

Transition metal nanoparticles were to be reckon as one of the important metallurgies in periodic elements because of biocompatibility greener approach, eco-friendly, adoptable nature and photo-synthesizing properties.<sup>7</sup> Many metal nanoparticles like Cu, Ag, Pt, Au, and Pd were used in different fields such as catalysts, labelling the biological substances, optoelectronics, photo thermal therapy and biological activities against microbes. In particular biogenic synthesis of palladium and platinum nanoparticles has fetched the attention of the researchers because it is cost effective, sustainable and eco friendly. Palladium and platinum nanoparticles are broadly used as heterogeneous and homogeneous catalyst,<sup>8</sup> drug carrier, as drug, used in many medical diagnoses without destructing the DNA structure,<sup>9</sup> in cancer treatment, used as nanocatalyst in environment remediation scavenging dye from textile industries, used in Suzuki coupling reactions,<sup>10</sup> also demonstrated antimicrobial activities,<sup>11</sup> and other discipline of biological sciences has been assessed.<sup>12</sup> There is a parallel increase in the use of methods for estimating the efficiency of such nanoparticles as antioxidants.<sup>13, 14</sup> One such method that is currently popular is based upon the use of the stable free radical diphenylpicrylhydrazyl (DPPH).<sup>14</sup>

Our aim in present contribution was to synthesize and characterize palladium and platinum nanoparticles from aqueous extract of green alga *Botryococcus braunii* and to evaluate their antimicrobial potential against bacterial and fungal species and antioxidant efficacy. Our study can be considered as the first report for synthesis of palladium and platinum nanoparticles using green alga *Botryococcus braunii*. Methods used in this work are elucidated and synthesized palladium and platinum nanoparticles were characterized by using different techniques including UV-visible

spectroscopy, Fourier transform infrared spectroscopy, Scanning electron microscopy and X-ray diffraction.

## MATERIAL AND METHODS

### ***Chemicals and Test strains***

Green alga *Botryococcus braunii* was collected from Udaisagar Lake, Udaipur (Rajasthan, India). The reagents agar-agar, palladium acetate and hexachloroplatinic acid ( $H_2PtCl_6$ ) are of analytical grade and were purchased from Sigma Aldrich. Bacterial strains like *Pseudomonas aeruginosa* (MTCC 441), *Escherichia coli* (MTCC 442), *Klebsiella pneumoniae* (MTCC 109) and *Staphylococcus aureus* (MTCC 96) and a fungal strain *Fusarium oxysporum* (MTCC 2087) were purchased from Microbial Type Collection, Chandigarh (India).

### ***Isolation and Culturing of alga Botryococcus braunii***

Green alga was isolated by serial dilution method and grown on Chu-13 nutrient medium solidified by 1.5% agar-agar. Composition of Chu-13 medium:  $CaNO_3$  (0 .300g/l),  $MgSO_4 \cdot 7H_2O$  (0.025g/l),  $CaCl_2 \cdot 2H_2O$  (0.027g/l),  $K_2HPO_4$  (0.010g/l), Ferric citrate (0.0035g/l), Citric acid (0.0035g/l),  $Na_2CO_3$  (0.02g/l),  $Na_2SiO_3 \cdot 5H_2O$  (0.044g/l) and some micronutrients also added.<sup>15</sup> Algal colonies appearing after three weeks of incubation were isolated and inoculated into liquid medium. For Growth experiments algal species was grown for algal biomass in incubator at  $27 \pm 1^\circ C$ ,  $1.2 \pm 0.2$  Klux light intensity and 16:8 hrs light: dark cycle in nutrient medium. After standardization of optimal culture conditions in Chu-13 medium best results of growth of green alga were found.

### ***Preparation of algal extract***

Grown algal biomass was centrifuged; shade dried and 5g of algal biomass was taken in 250 ml Erlenmeyer flask along with 100 ml of distilled water. Then the mixture was autoclaved for 15 min and filtered through Whatman No.1 filter paper. The filtered extract was centrifuged and supernatant was used as reducing agent for preparing metal nanoparticles. Prepared algal extract was kept at  $4^\circ C$  in refrigerator for further experimental use<sup>16</sup>.

### ***Synthesis of Palladium and Platinum nanoparticles***

Both nanoparticles have been synthesized by using the following processes:

**Palladium nanoparticles:** 20 ml algal extract mixed with 80 ml, 1mM palladium acetate [Pd(OAc)<sub>2</sub>] aqueous solution in 250 ml Erlenmeyer flask, at pH 6-7 and put on magnetic stirrer at 60°C temperature for 3h. Simultaneously, a positive control of palladium acetate aqueous solution and algal extract and a negative control containing only palladium acetate aqueous solution were maintained under same conditions. The progress of process was regularly monitored by observing color change. In positive control the initial pale yellow solution turned to dark brown, indicating formation of palladium nanoparticles but in negative control no any change in color was found. After the formation of palladium nanoparticles, the solution was centrifuged for 30 min and the obtained nanoparticles were washed with deionised water to remove impurities. This process of centrifugation and washing was carried out thrice to get a better separation of nanoparticles. The obtained palladium nanoparticles were oven dried at 70°C<sup>17</sup>.

**Platinum nanoparticles:** 90 ml, 1mM aqueous solution of hexachloroplatinic acid (H<sub>2</sub>PtCl<sub>6</sub>) was added with 10 ml algal extract in 250 ml Erlenmeyer flask at pH 6-7. Mixture was put on magnetic stirrer at 95°C temperature for 4h. Simultaneously, a positive control of hexachloroplatinic acid aqueous solution and algal extract and a negative control containing only hexachloroplatinic acid aqueous solution were maintained under same conditions. In positive control the initial light yellow solution turned to brown and finally black color, consistent with the formation of platinum nanoparticles but in negative control no any change was found. Synthesized platinum nanoparticles were separated from mixture by centrifugation for 30 min and then washed with deionised water. This process of centrifugation and washing was repeated three times and finally obtained platinum nanoparticles were oven dried at 58°C for 4h<sup>11</sup>.

### **Characterization of metal nanoparticles**

The dried powders of metal nanoparticles were used for characterization as FTIR analyses were carried out on FTIR (Perkin-Elmer) in the range of 4000-450 cm<sup>-1</sup> using dried powder of metal nanoparticles. Samples for analysis was prepared at ambient conditions and mixed with KBr. X-ray diffraction measurements were carried out on Philips Xpert pro XRD system (DY 1650) for determining the size of synthesized metal nanoparticles. Scanning Electron Microscopic (SEM) images

obtained with the help of scanning electron microscope (Model-FEI Quanta 200 SEM) for analyzing the morphology of synthesized nanoparticles.

### **Evaluation of Antimicrobial activity**

#### **Test microorganisms**

The antimicrobial activity of platinum and palladium nanoparticles were studied against two Gram negative bacterial strains *Pseudomonas aeruginosa* (MTCC 441) and *Escherichia coli* (MTCC 442), two Gram positive bacterial strains *Klebsiella pneumoniae* (MTCC 109 ) and *Staphylococcus aureus* (MTCC 96) and a fungal strain *Fusarium oxysporum* (MTCC 2087). Antibacterial and antifungal potential of synthesized nanoparticles were assessed in terms of zone of inhibition of microbial growth by agar well plate method and minimum inhibitory concentration was determined by agar dilution assay.

#### **Agar well plate method**

Bacterial cultures were maintained in Petri plates containing nutrient agar (NA) medium at 37°C. Nutrient agar media was prepared containing 10g beef extract, 2g yeast extract, 5g peptone, 5g NaCl and 15g agar in 1L of distilled water. The fungus *Fusarium oxysporum* was maintained on potato dextrose agar at 25°C. The nutrient agar and PDA (potato dextrose agar) was autoclaved at 121°C at 15psi for 15min and poured on to sterile petri plates to a uniform depth of approximately 4mm. Once the media get solidified the culture was spread on to the petri plates with the help of L-spreader. With the sterilized 5mm cork borer, wells were introduced into the agar and 20 µl of both platinum and palladium was added into the wells. Untreated algal extract and salt of platinum and palladium was used as negative control. The plates were incubated at 37°C and 25°C overnight as per requirements. The experiments were carried out in triplicates. The antimicrobial activity was evaluated by measuring the size of clear zone around each well<sup>18</sup>.

#### **Agar dilution method**

The MIC of these nanoparticles was determined by agar dilution technique where the stocks of 50 mg/ml of the synthesized nanoparticles were resuspended in 10%

DMSO to produce two-fold dilutions in the range of 25-30 mg/ml and so on. Each dilution of nanoparticles was put in to the melted agar. Pour the agar into petriplates and allow them to solidify. After this, bacteria prepared to a standard concentration are added as a spot to each plate, with  $10^4$  colony forming units (CFU) per spot. These dilution plates were then incubated at 37°C, with a control plate having no any antimicrobial agent. After incubation the growth of the microbial isolates on the agar plate were observed. The least concentration of nanoparticles that prevents microbial growth was considered to be as MIC value of those nanoparticles against that microorganism<sup>18, 19</sup>.

#### ***DPPH radical scavenging activity***

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method by Blois<sup>20</sup> in 1958. Briefly, an 0.1mM solution of DPPH in ethyl alcohol was prepared and 1mL of this solution was added to 3 ml of the solution of all extracts in methanol at different concentration (5, 10, 15, 20 & 25 $\mu$ g/ml).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 541 nm using a UV-Vis spectrophotometer. Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical was calculated by using the following formula.

$$\text{DPPH scavenging effect (\% inhibition)} = \{(A_0 - A_1)/A_0\} * 100$$

Where, A<sub>0</sub> is the absorbance of the control reaction, and A<sub>1</sub> is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

#### **RESULTS AND DISSCUSION**

In this study, we have synthesized palladium and platinum nanoparticles by the use of extract of green algae *Botryococcus braunii*. Algal extract appears to be a potential source of reducing and stabilizing agent without using any chemical as reducing agent. The complete process of formation of metal nanoparticles was initially confirmed by visual observation showed in Fig.1. Figure 1a demonstrates the change in color from pale yellow to dark brown and in Figure 1b light yellow color

changed into black of reaction mixture provides a convenient signature to indicate the formation of palladium and platinum nanoparticles respectively.<sup>15, 16</sup>

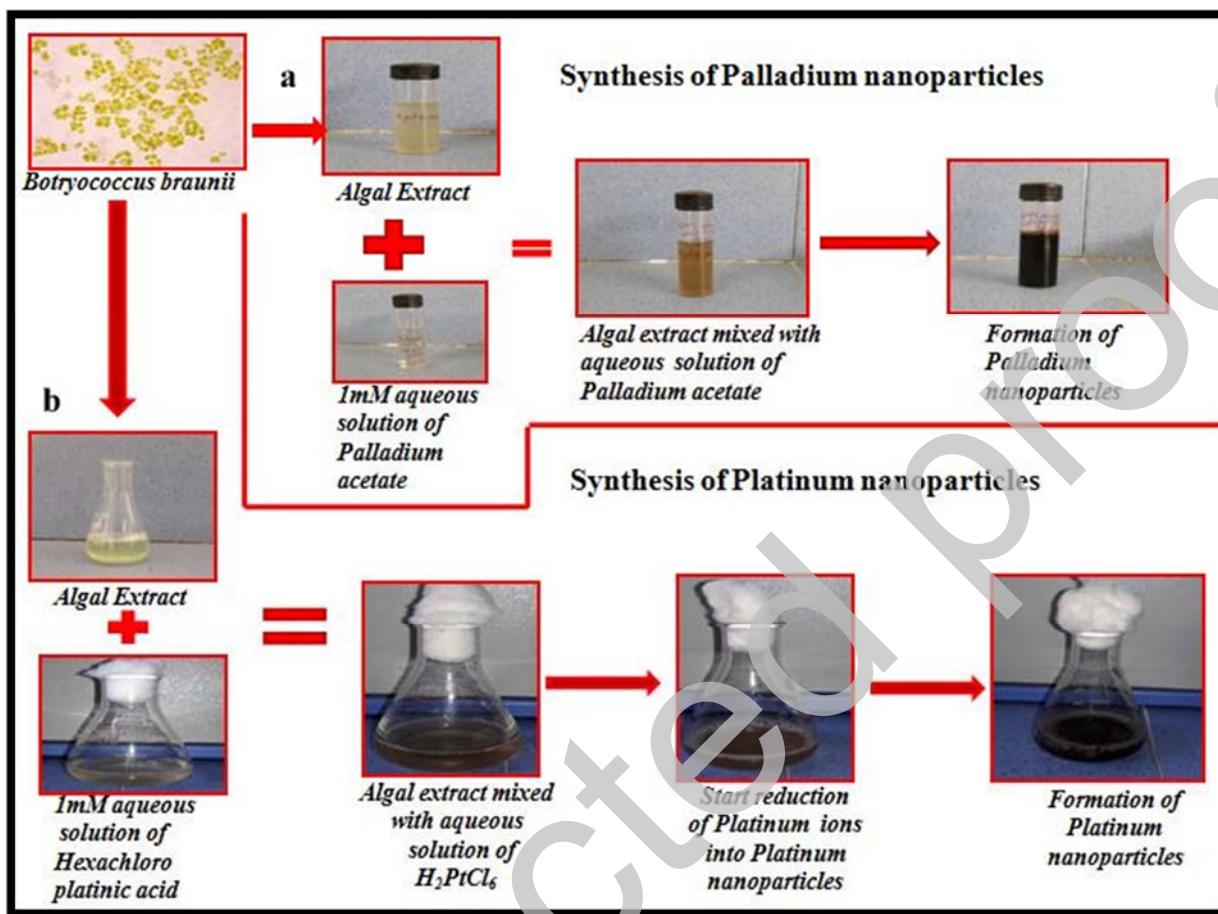


Fig. 1 Biogenic synthesis of metal nanoparticles (a) Palladium nanoparticles (b) Platinum nanoparticles

### Fourier transforms infrared (FTIR)

Fourier transform infrared (FTIR) spectrum of experimental samples revealed two types of vibrations as stretching and bending in the wave length range of 4000-450cm<sup>-1</sup>. FTIR spectrum measurements were demonstrated to identify the major functional groups present in green algae *B. braunii* to examine their possible involvement in the synthesis of palladium and platinum nanoparticles. Different peaks positioned at 3435.88, 2923.49, 2852.33, 1637.82, 1559.61, 1414.42, 1384.79, 1069.01, 1056.17, 837.53, 781.32, 714.25, 695.06, 657, 618.16 and 532.74 cm<sup>-1</sup> in FTIR spectrum of algal extract of green algae *B. Braunii*. The peaks at 3435.88 due to N-H and O-H stretching vibrations.<sup>21, 22</sup> 2923.49 and 2852.33 cm<sup>-1</sup> bands arose due to asymmetrical C-H stretching vibrations of-CH<sub>2</sub> and -CH<sub>3</sub>.<sup>22</sup> 1637.82 cm<sup>-1</sup> peak is characteristic of N-H bending vibrations in amide of protein as

capping agent.<sup>23, 24</sup> The peak at 1559.61 cm<sup>-1</sup> showed the presence of carboxyl group and weak band at 1414.42 and 618.16 cm<sup>-1</sup> due to COO<sup>-</sup> in amino acid residue of protein.<sup>25</sup> The peak observed around 1384.79 cm<sup>-1</sup> can be assigned to C-N stretching vibrations of amine. C-H bending vibrations by carbohydrates (glucose residue by C-OH bond) showed the peak at 1037.17cm<sup>-1</sup>.<sup>26</sup> 873.53, 781.32, 695.06 and 532.74 cm<sup>-1</sup> bands were demonstrated due to O-C=O bending vibrations of CO<sub>3</sub><sup>2-</sup>, C-H rocking of lipids, N-H wagging of amine and alkyl halide respectively. The results of present study have shown that hydroxyl groups have strong ability to interact with nanoparticles. The main peaks existing in the spectrum of alga are also present in the spectrum of synthesized palladium and platinum nanoparticles with lower intensities and slight shift. Therefore, it may be evidenced that proteins, polysaccharides, amides and long chain fatty acids are responsible biomolecules for bioreduction and act as capping and stabilizing agents.<sup>27, 28</sup>

### ***Scanning electron microscopy (SEM)***

The shape and size of both biogenically synthesized nanoparticles were elucidated with the help of Scanning electron microscopy (SEM) Fig.2a,b. Scanning electron microscopy showed the presence of cubical, spherical and truncated triangular shaped palladium and platinum nanoparticles were synthesized.<sup>29, 30</sup> The size distribution histogram shows that the average size of synthesized nanoparticles was 4.89 nm and 86.96 nm for palladium and platinum nanoparticles respectively. From the SEM images the number of nanoparticles (total 50 particles for each sample) counted by ImageJ software. The following equation was used for calculating statistical properties of nanoparticles named as number average diameter (D<sub>n</sub>), weight-average diameter (D<sub>w</sub>), and polydispersity index (PDI).

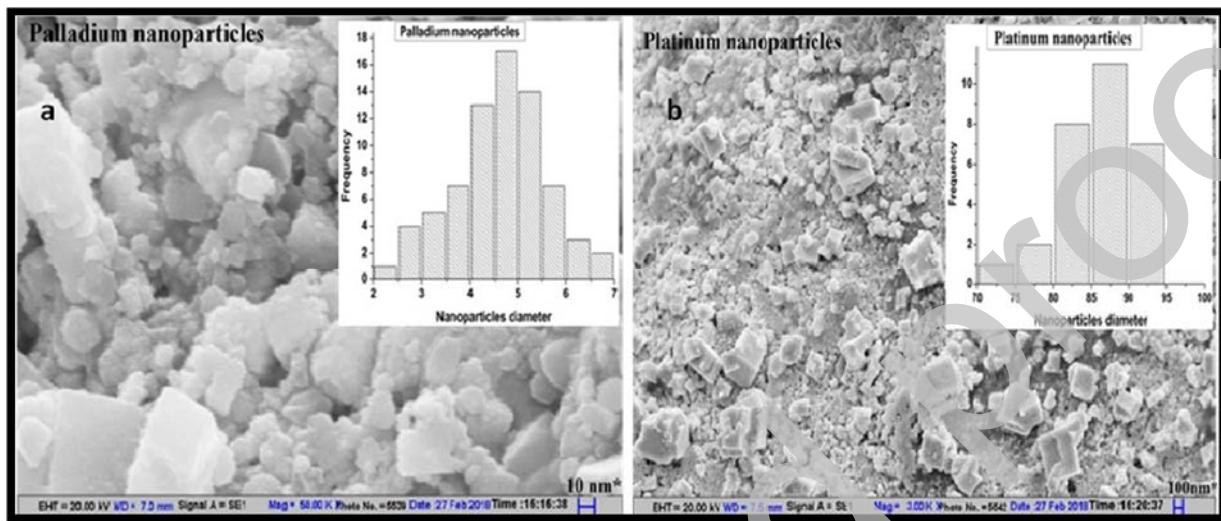
$$D_n = \frac{\sum d_i}{n}$$

$$D_w = \frac{\sum (d_i)^4}{\sum (d_i)^3}$$

$$PDI = \frac{D_w}{D_n}$$

Here d<sub>i</sub> is the diameter of microspheres and n represents the number of nanoparticles.

PDI value 0.198 for platinum nanoparticles and 0.862 for palladium nanoparticles was calculated and these values showed uniform size of synthesized nanoparticles. The PDI values used as an indicator for the size distribution of synthesized nanoparticles.<sup>31</sup>

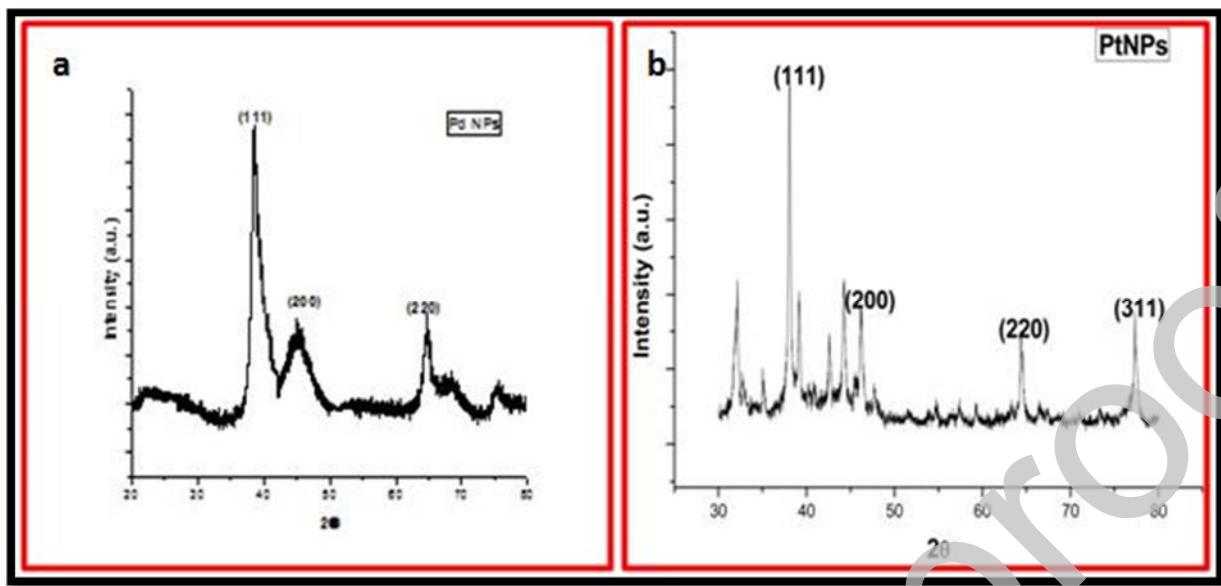


**Fig. 2** Scanning electron microscopy (SEM) images of green synthesized (a) Palladium nanoparticles (b) Platinum nanoparticles

### X-ray diffraction

The synthesized metal nanoparticles were further evidenced by XRD measurements. The XRD analysis of green synthesized palladium nanoparticles in Fig.3a showed major diffraction peaks at  $2\theta$  of  $40.1^\circ$ ,  $46.6^\circ$ ,  $68.0^\circ$ , which corresponds to (111), (200) and (220) planes of face-centred cubic structure of palladium nanoparticles (JCPDS no. 05-0681). The crystallite size of palladium nanoparticles was calculated from (111) plane of fcc palladium using Scherrer's equation. The crystallite size of synthesized palladium nanoparticles is calculated to be around 5 nm.<sup>32, 33</sup>

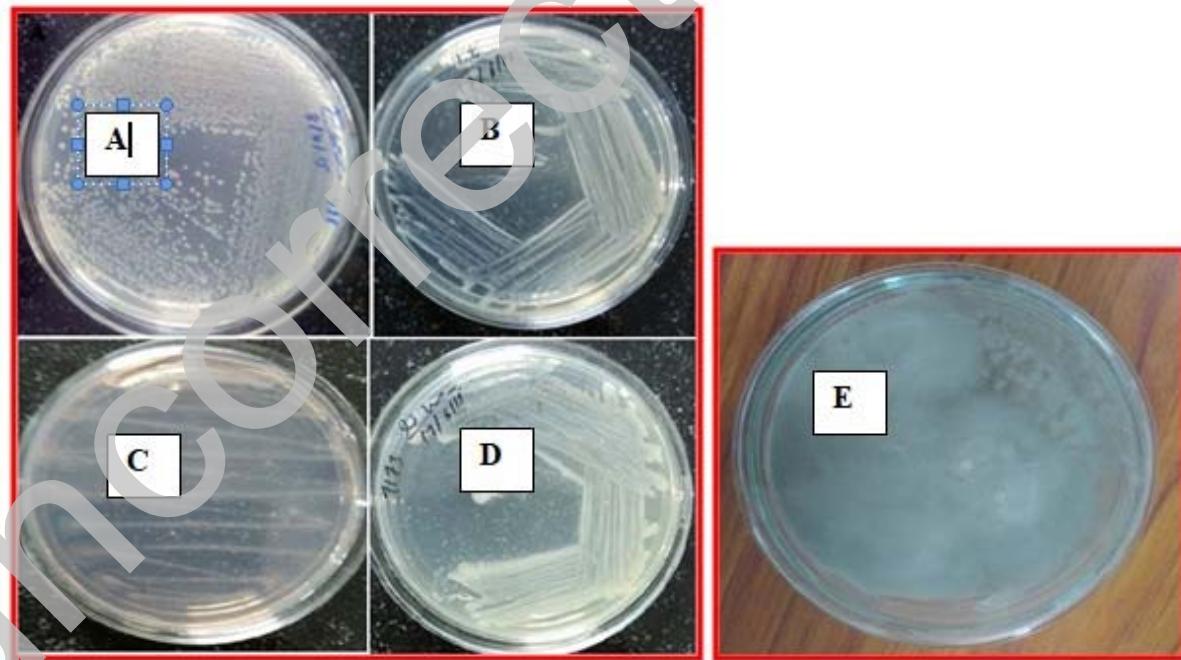
Furthermore in Fig.3b the diffraction lines at about  $2\theta$  on  $38.10$ ,  $46.60$ ,  $64.70$ ,  $77.40$  which matched to (111), (200), (220), (311) planes of the face-centred cubic (fcc) crystal lattice of platinum (JCPDS No. 88-2343). The crystallite size of platinum nanoparticles was calculated from (111) plane of fcc using Scherrerr's equation. The crystallite size of synthesized platinum nanoparticles was found to be 87 nm.<sup>34, 35</sup>



**Fig.3** XRD patterns of biogenically synthesized (a) Palladium nanoparticles and (b) Platinum nanoparticles

#### **Antimicrobial activity of synthesized palladium and platinum nanoparticles**

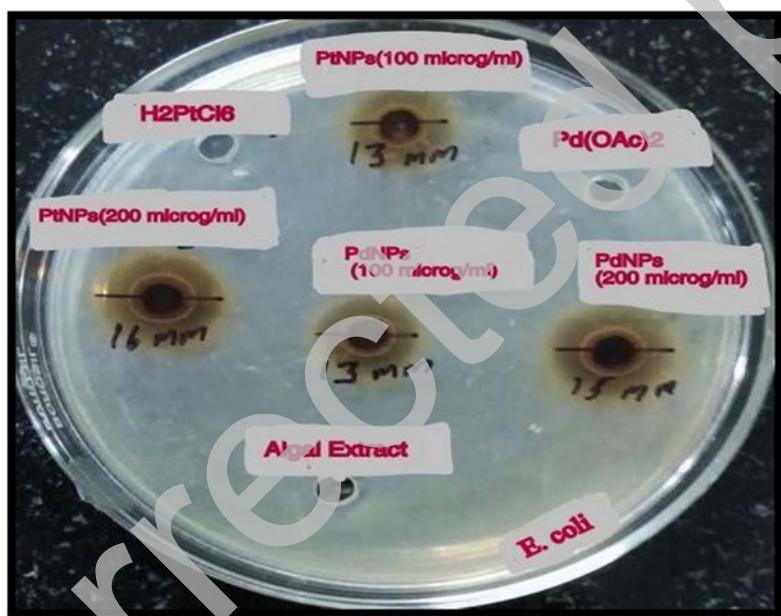
Revived bacterial strains were maintained on nutrient agar medium as shown in Fig.4 and the fungal strains maintained on potato dextrose agar is also shown.



**Fig. 4** Revival culture of [A] *K. pneumonia*, [B] *S. aureus*, [C] *P. aeruginosa*, [D] *E. coli* [E] *F. oxysporum*

## Assay of biological activity

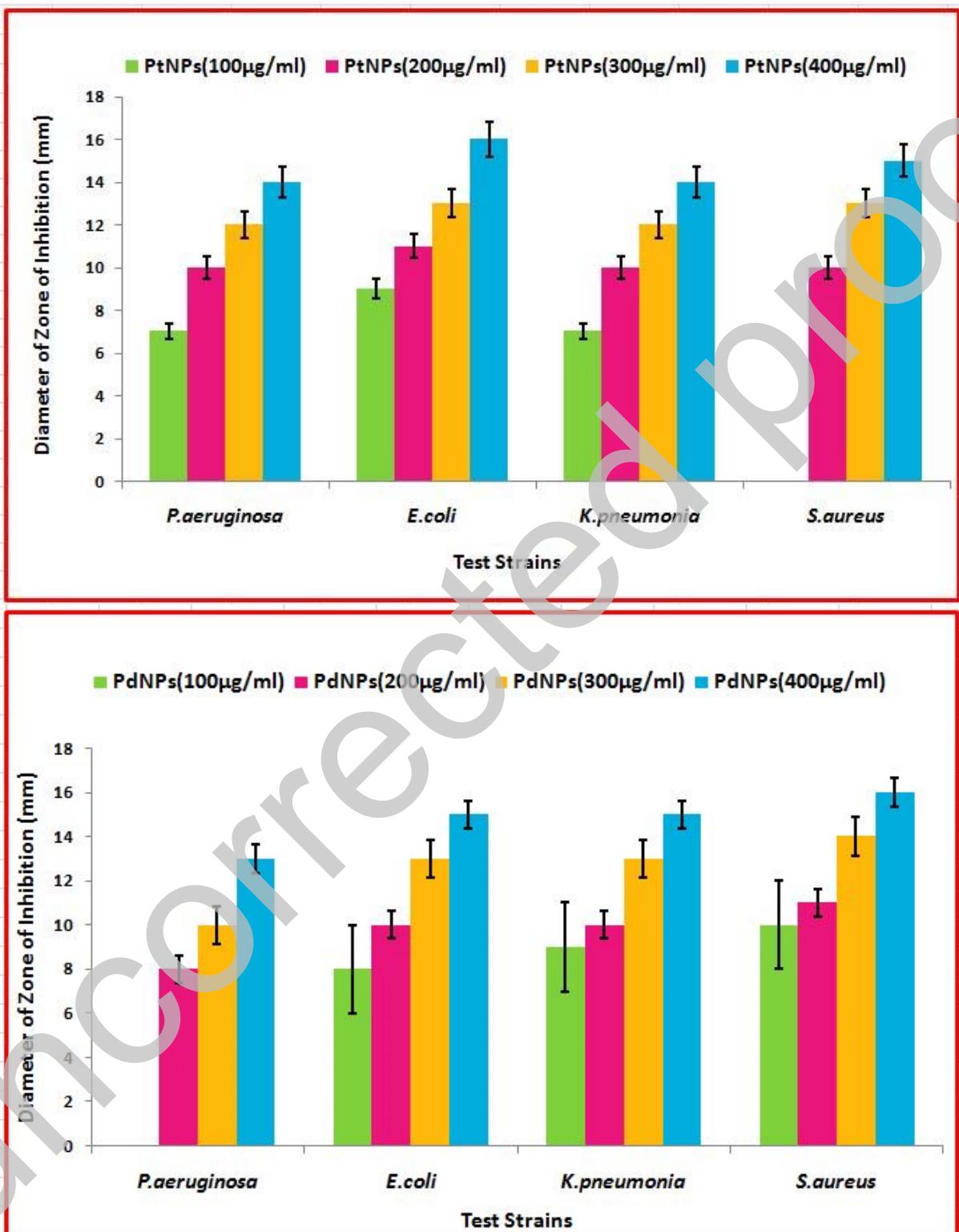
Biological activity of algal extract and synthesized nanoparticles was done against both bacteria (Gram-positive and Gram-negative) and fungus using agar well diffusion method.<sup>36, 37</sup> Figure 5 shows the different zone of inhibition formed by synthesized platinum and palladium nanoparticles, antibiotics, algal extract, salts of platinum and palladium against test strains. The well filled with algal extract did not show any zone of inhibition but the nanoparticles synthesized from that algal culture shows both antibacterial and antifungal activity with zone of inhibition ranging from 7-16mm Table 1 and Fig 6.<sup>38</sup> PtNPs and PdNPs at 400 $\mu$ g/ml concentration showed maximum zone of inhibition against test strains.



**Fig. 5** Antibacterial assay: Zone of inhibition against *E.coli*

**Table 1** Diameter of zone of inhibition observed against in different test strains

Microbial Strain	Diameter of zone of inhibition (in mm)								
	Palladium nanoparticles concentration( $\mu$ g/ml)				Platinum nanoparticles concentration( $\mu$ g/ml)				
	100	200	300	400	100	200	300	400	
<i>Pseudomonas aeruginosa</i>	-	8±1.56	10±1.4	13±1.23	7±1.86	10±0.5	12±1.2	14±1.16	
<i>Escherichia coli</i>	8±0.6	10±1.5	13±1.8	15±1.66	9±1.26	11±1.4	13±1.2	16±1.96	
<i>Klebsiella pneumonia</i>	9±1.5	11±1.5	13±0.55	16±0.76	7±0.53	10±0.1	12±0.5	14±0.33	
<i>Staphylococcus Aureus</i>	10±0.1	11±1.5	14±1.56	16±0.86	-	10±0.7	13±0.3	15±0.2	



**Fig. 6** Comparative representation of zone of inhibition diameter formed against test strains

### Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration <sup>39</sup> required to inhibit the growth of microbes is less in case of platinum as compared with palladium Fig.7. These synthesized nanoparticles show least activity towards tested fungus, *Fusarium oxysporum*. The positive control drug used against both Gram positive and Gram negative bacteria was chloramphenicol and ampicillin. Nystatin and griseofulvin was used as positive control for fungus *Fusarium oxysporum*. The antibiotic ampicillin does not show any activity against *P. aeruginosa* as compare to PtNps and PdNps which show significant activity. Antimicrobial activity of nanoparticles was considered to be good if its MIC was less than 100 µg/ml, moderate if MIC was from 100 to 500 µg/ml and poor over 500 µg/ml in Table 2 <sup>40, 41, 4</sup>.

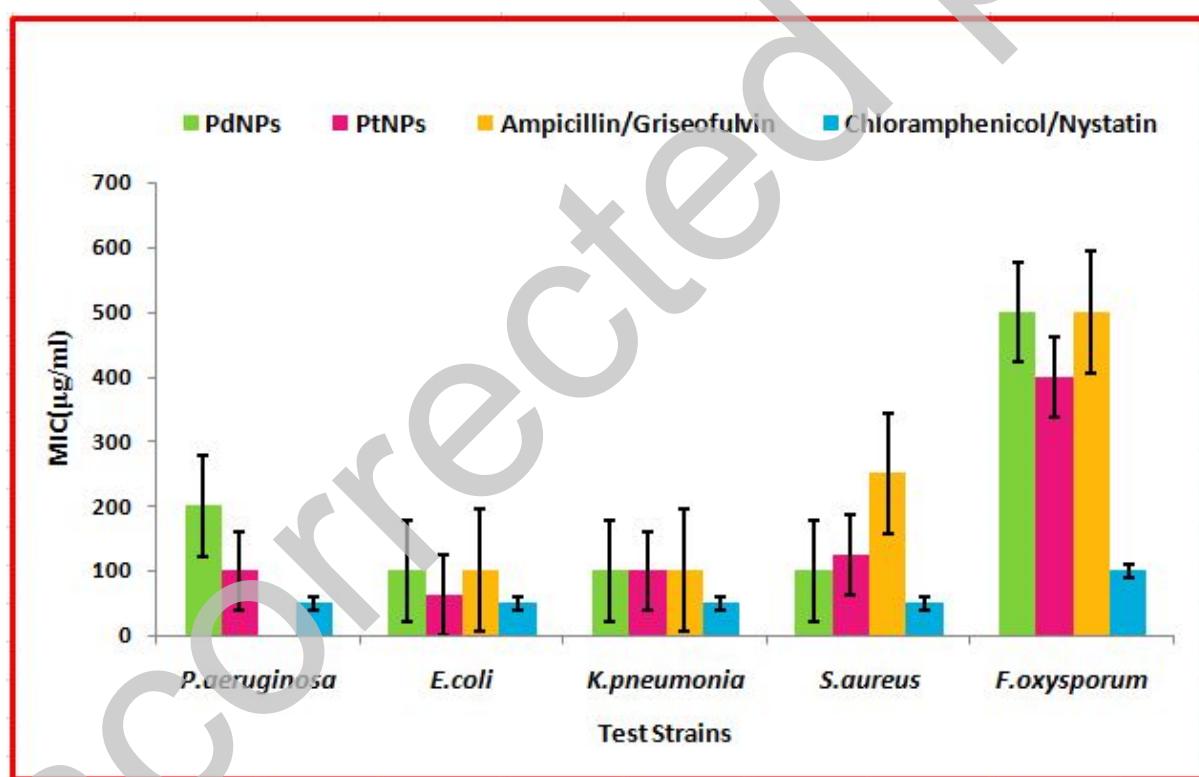


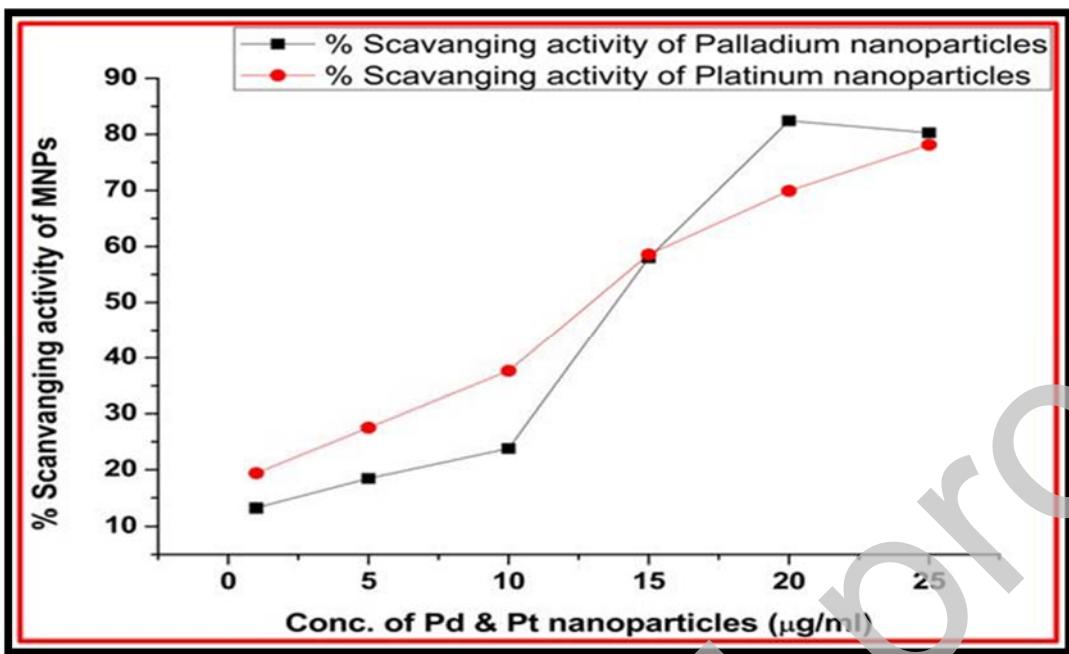
Fig. 7 Representation of MIC value against test strains

**Table 2** Minimum inhibitory concentration observed against different test strains

Nanoparticles (500µg/ml)	Minimum Inhibitory Concentration (µg/ml)				
	Gram negative Bacterial strains		Gram positive Bacterial strains		Fungal strain
	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Fusarium oxysporum</i>
Palladium	200	100	100	100	500
Platinum	100	62.5	100	125	400
Ampicillin	-	100	100	250	-
Chloramphenicol	50	50	50	50	-
Nystatin	-	-	-	-	100
Griseofulvin	-	-	-	-	500

### **Antioxidant activity**

The antioxidant potential of the green synthesized palladium and platinum nanoparticles were evaluated by quantifying the DPPH free radical scavenging activity Fig.8 and Table.3. In the presence of nanoparticles, the color of the DPPH solution gradually changed from purple to pale yellow with time. The percentage scavenging of DPPH increased linearly with an increase in nanoparticles concentration from 1 to 20 µg/ml and reached 82.43% within 30 min at 20 µg/ml in case of palladium and to 78.14% at 25 µg/ml in case of platinum. However, the positive control ascorbic acid showed 94.0% of scavenging activity at a concentration of 50 µg/ml. The negative control wells loaded with algal extract did not show any color change from purple.<sup>41, 42</sup>



**Fig. 8** Graph representing % Scavenging activity of nanoparticles

**Table 3** Comparison of DPPH scavenging activity of Palladium and platinum nanoparticles

Concentration of Palladium / Platinum nanoparticles ( $\mu\text{g}/\text{ml}$ )	% Scavenging activity of Palladium nanoparticles	% Scavenging activity of Platinum nanoparticles
1	13.22	19.37
5	18.44	27.51
10	23.78	37.66
15	57.96	58.57
20	82.43	69.93
25	82.27	78.14

## CONCLUSION

From the present work, a successful, rapid and combustion method is demonstrated for the synthesis of stabilized nano-scale palladium and platinum particles for the first time with the use of algal extract of green alga *Botryococcus braunii* as a reducing stabilizing capping agent. Biogenically synthesized nanoparticles were characterised by different techniques including Fourier transform infrared spectroscopy, Scanning electron microscopy, X-ray diffraction. FTIR spectrum confirms the interaction of algal biomolecules and formation of palladium and platinum nanoparticles. From SEM images and XRD patterns, the prepared nanoparticles exhibited cubical,

spherical and truncated triangular shape with 4.89 nm and 86.96 nm of palladium and platinum nanoparticles respectively. Green synthesized nanoparticles exhibited antimicrobial activity against gram positive and gram negatives bacterial strains, antifungal activity against fungus and also demonstrates antioxidant activity. This conversion of metal ions into metal nanoparticles will one day replace the other methods of synthesis of nanoparticles and could possibly used for large-scale synthesis of technologically important applications.

## **ACKNOWLEDGEMENT**

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## **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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