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PHYTOFABRICATION OF NANO-CRYSTALLINE PLATINUM PARTICLES BY LEAVES OF *CERBERA MANGHAS* AND ITS ANTIBACTERIAL EFFICACY.

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ABSTRACT

Biological synthesis of Platinum nanoparticles by leaves of *Cerbera manghas* extract through a green route was reported in this study. The properties of prepared nanoparticles were characterized by Fourier Transform Infrared Spectroscopy (FT-IR), Transmission Electron Microscopy (TEM), X-ray diffraction (XRD). The formation of Platinum nanoparticles was confirmed by the presence of an absorption peak at 263 nm using UV–visible spectrophotometer. TEM image revealed that most of the particles were in spherical shape with size ranging from 9.60 nm to 11.70 nm. The nanoparticles were crystalline in nature and it was confirmed by XRD pattern. From the FTIR measurements it was noticed that reduction has been carried out by carboxyl and alcoholic groups present in the leaves of *C. manghas*. Furthermore, biologically synthesized Platinum nanoparticles were found to be effective against selected bacterial pathogens.

KEYWORDS: ***Cerbera manghas*, Leaf extract, Platinum Nanoparticles, TEM, Antibacterial activity.**

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INTRODUCTION

In recent years, the application of bionanotechnology is gaining tremendous impetus. Research in bionanotechnology has shown to provide reliable, eco-friendly processes for synthesis of noble nanomaterials. Various physical and chemical methods are available for crystallization of these metals from ionic form. But these techniques require extreme conditions like high temperature, pressure and large quantities of harmful Chemicals, all which reflects on the environment in post-production[1]. Biosynthetic methods employing either biological microorganisms or plant extracts have emerged as simple and viable alternate to chemical synthetic procedure and physical methods[2]. Indeed, over the past several years, plants, algae, fungi, bacteria and viruses have been used for low-cost, energy efficient and non-toxic production of metallic nanoparticles[3]. The plant species that have been explored, include *Helianthus annuus*[4], *Tagetes erecta*[5], *Cassia fistula*[6], *Pomegranate*[7], *Momordica charantia*[8], *Lonicera japonica*[9], *Dalbergia sissoo*[10], *Toona ciliata*[11], *Caesalpinia pulcherrima*[12], *Senna siamea*[13], *Piper betle*[14], *Ananas comosus*[15], *Amaranthus spinosus*[16] and *Achyranthes aspera*[17]. Platinum is one of the rarest and most expensive metals. It has high corrosion resistance and numerous catalytic applications including automotive catalytic converters and petrochemical cracking catalysts. Platinum nanoparticles are usually used in the form of colloid or suspension in a fluid. They are the subject of extensive research due to their antioxidant properties. The key application areas of platinum nanoparticles include Electrocatalysts and catalytic converters, Magnetic nanopowders, Polymer membranes, Cancer therapy[18], Coatings, plastics, nanofibers and textiles. So far there is no report on the development of Platinum nanoparticles by utilizing *Cerbera manghas* plant. *Cerbera manghas* an evergreen coastal tree belongs to family Apocynaceae commonly known as Sea Mango. It grows preferentially in coastal salt swamps and in marshy areas. *C. manghas is naturally and abundantly* distributed from the Seychelles Islands in the Indian Ocean eastward to French Polynesia. It attributes lowland and coastal habitats, and is often associated with mangrove forests like Sundarbans. In Bangladesh, it is distributed mainly in the Sundarbans and southern region. The folk medicinal uses of leaves and seeds are Constipation and Surgical ointment as topical anesthetic [19]. The fruits are used for manufacturing bioinsecticides and deodorants. The leaves of *Cerbera manghas* showed cytotoxic activity against two breast cancer cell lines (T47D and MCF7) and two ovarian cancer cell lines (SKOV3 and CAOV3) and a normal (Vero) cell line[20]. Our aim in the present contribution was to synthesize and characterize platinum nanoparticles from aqueous extracts of *C. manghas* and to assess its antimicrobial potential. Our study can be considered as the first report for the synthesis of platinum nanoparticles using *Cerbera manghas.*

MATERIALS AND METHODS

2.1. Materials

Hydrogen hexachloroplatinate hexahydrate (H_2PtCl_6. $6H_2O$) were obtained from Hi-Media chemicals (Bangalore, India). All other reagents were of the highest commercially available grades. The glassware's have been washed with Lavelone and distilled water and dried in oven before use.

2.2. Plant Material and Preparation of Extract

C. manghas leaves were collected from Devamote, on the tributaries of Sharavethi river, Honnavar, Uttara Kannada District, Karnataka (Lat. 14°15'43" N Long. 74°28'31" E) and dried for 2 days at room temperature under shade. Leaf extract was prepared by taking 2.0 g of thoroughly washed and finely ground tuber powder in a 100mL Erlenmeyer flask with 100mL of sterile distilled water and the mixture was left in a shaking incubator operating at 200 rpm, 25°C for 24 h. The extract obtained was filtered through Whatman filter paper No. 1. The

filtrate was used for PtNPs synthesis. Antibacterial activities of Chloroformic extract were tested against gram positive and gram negative bacterial pathogens[21]. The inoculums suspensions were swabbed uniformly in different plates. Cavities were made in each plate using a well-cutter and it was filled with crude extract (100 mL) and then incubated at 37°C, zone of clearance is considered as an indication of antibacterial activity.

2.3. Synthesis of Platinum Nanoparticles using Extract of C. manghas

Reduction of Pt^{6+}ions was initiated by addition of 5mL of *C. manghas* to 95mL of 10^{-3}M aqueous H_2PtCl_6. $3H_2O$ solution in a 250mL Erlenmeyer flask respectively. The mixture solution was left on constant magnetic stirring at room temperature (25°C) and observed for change in color.

2.4. Preliminary Characterization of Platinum nanoparticles

2.4.1. Visual Observation

The color change in reaction mixture (metal ion solution + plant extract) was recorded through visual observation[22].

2.4.2. UV-Vis Spectroscopy

The bioreduction of Pt (VI) in aqueous solution was monitored by Scanning of the colored solution in the Ultraviolet-Visible spectroscopy (200 - 600 nm) UV-vis spectroscopy. UV-Vis analysis was performed on a Perkin Elmer Lambda 25 spectrophotometer operated at a resolution of 1 nm[23]. Synthesized platinum nanoparticles were extracted easily by centrifugation with 12,000 rpm for 15 min at 4°C and the pellets were lyophilized for further analysis.

2.5. Characterization of Platinum nanoparticles

Crystallographic information about the samples was obtained from X-ray diffractometer (PANalytical, Philips PW 1830) in the range of 20°- 70° with 2°/min scanning rate, operating at 40 kV and a current of 30 mA with Cu Kα radiation (λ = 1.5404 Å) was used. The colloidal suspension containing metal nanoparticles was dried on a small glass slab. Transmission Electron Microscopy (TEM) was carried out to study the size distribution, shape and morphology of the PtNPs[24]. FTIR is used to identify the biomolecules responsible for reduction of platinum ions to nanoparticles[25] and the spectra were recorded in the range of 500 - 4000 cm^{-1} at a resolution of 4 cm^{-1}.

2.6. Evaluation of antibacterial activity of platinum nanoparticles

Antimicrobial activity of the synthesized Platinum nanoparticle solution (100 mL) was checked against the pathogens used for initial screening and Hexachloroplatinic acid was used as negative control and streptomycin of 0.25 mg/mL concentration were used as a positive control antimicrobial agent. The formation of a clear zone around the cavity is an indication of antibacterial activity[26]. Zone of clearance was expressed in mille meters.

RESULTS AND DISCUSSION

3.1. Visual Observation

The nanoparticles were primarily characterized by Visual observation and UV-vis spectral analysis. After addition of *C. manghas* extract to the H_2PtCl_6 aqueous solution, the yellow color of the reaction mixture kept at room temperature (25°C) under constant stirring gradually turned into black color after 2 h (Figure 1). It is well known that PtNPs exhibit a black color in aqueous solution due to the excitation in UV-visible spectrum depending upon the particle size[27]. The appearance of a black color in solution containing the extract suggested the formation of platinum nanoparticles[28].

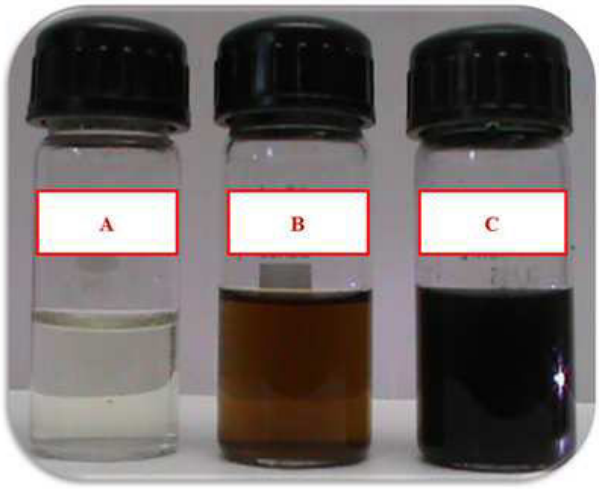

Figure 1
1mM concentration of H_2PtCl_6 (A) Aqueous extract of C. manghas (B) Platinum nanoparticles (C)

3.2. UV-vis Spectroscopy

The optical properties of PtNPs were calculated by UV-Vis absorption spectroscopy, an important and most commonly used technique, to ascertain the formation stability of metal nanoparticles. Due to surface plasmon resonance (SPR), a strong absorption of electromagnetic waves is exhibited by metal nanoparticles in the visible range. The SPR phenomenon arises when nanoparticles are irradiated with visible light, because of the collective oscillations of the conduction electrons[27].The strong surface plasmon resonance centered at ca. 263 nm, the characteristic wavelength ranges for PtNPs (Figure 2). Spectrum in this range attribute to the surface plasmon resonance (SPR) of PtNPs[29]. No presence of secondary peak indicated that the nanoparticles were spherical in shape[30]. The SPR band reveals the spherical shape of platinum nanoparticles which was further confirmed by TEM.

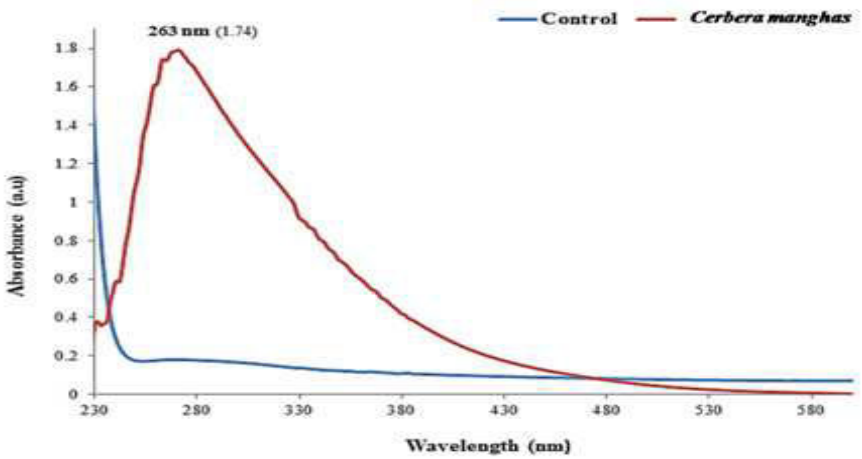

Figure 2
Absorbance spectrum of Platinum nanoparticles

3.3. XRD diffraction

The XRD pattern showed four intense peaks in the whole spectrum of 2 theta values of 38.16° corresponds to 111 planes for Platinum nanoparticles (Figure 3). This feature indicates that the nanocrystals are (111)-oriented. The data obtained was matched with the database of Joint Committee on Powder Diffraction Standards (JCPDS) file No. 04-0783. For all the patterns, the peaks in the range 38°-41° correspond to the (111), reflections confirming the successful synthesis of Pt nanoparticles as shown in electron diffraction patterns indicating that they are crystalline in nature which was similarly reported earlier[31].

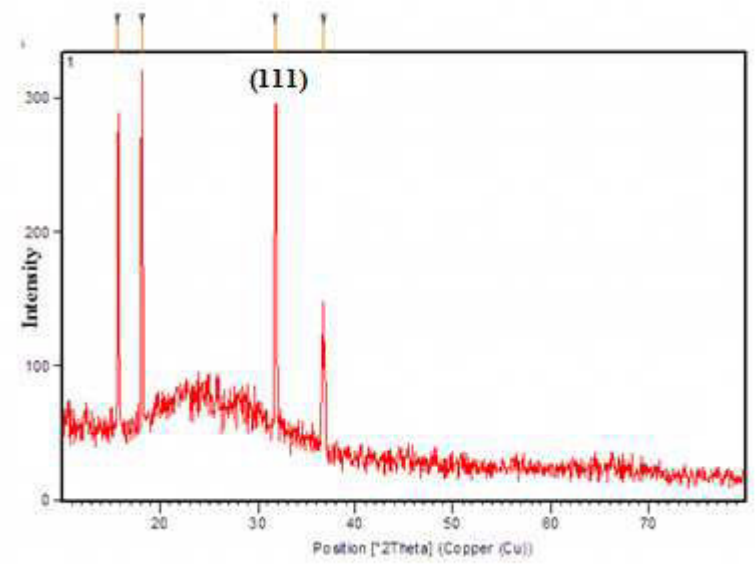

Figure 3
XRD spectrum of Platinum nanoparticles.

3.4. Transmission Electron Microscopy measurements

Transmission electron microscopy (TEM) has been used to identify the size, shape, and morphologies of nanoparticles.TEM images of the platinum nanoparticles were observed in 100 nm scale (Figure 4 A&B). It was observed that the nanoparticles formed were spherical and predominantly monodisperse with diameter ranging from 9.60 to 11.76 nm and is not in physical contact with each other. The average particle size was 10.68 nm. Similar observation was noticed in the reduction of gold particle by the ethanolic leaf extract of *Piper betle* ranges from 10 to 35 nm[14].

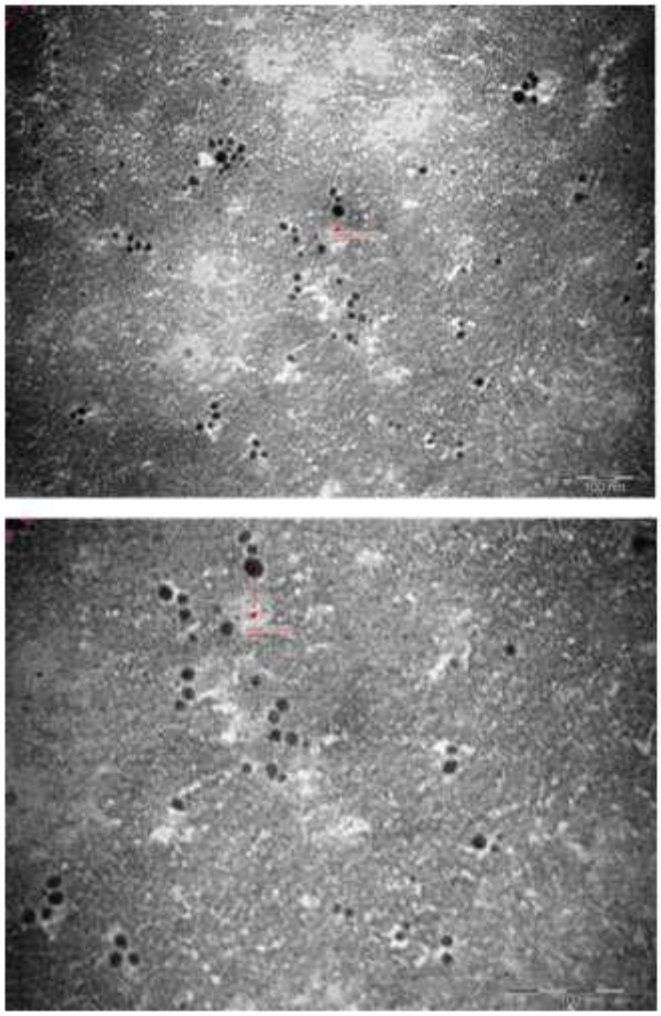

Figure 4
TEM Micrograph of Platinum nanoparticles.

3.5. FTIR Measurements

FTIR spectrum of *C. manghas* shows absorption bands at 1381, 1325, 1247 cm^{-1}. The absorption band at 1381 cm^{-1} assigned to C=O Carboxylic acid. The intense band at 1381 cm^{-1} is characteristic of C=O stretching vibration of carboxylic acid group[32,33]. The IR band at 1325 cm^{-1} corresponds to O-H stretch due to the presence of surface adsorbed primary and secondary alcohols (Carbohydrates etc.,) of *C. manghas*. The peak at 1247 cm^{-1} is due to C-O aromatic ethers (Figure 5A). The total disappearance of these bands after bioreduction suggested that carboxylic group is mainly responsible and alcohols might be partly responsible for the reduction of platinum ions [32]. The synthesized Platinum nanoparticles manifest absorption peaks at about 1398, 1118 cm^{-1}. Furthermore, the new peak near 1396 cm^{-1} was assigned to O-H stretch due to carboxylic acid. Metal nanoparticles can bind to proteins through free amino group or carboxylate groups in the protein[34]. The IR band at 1396 cm^{-1} in Pt nanoparticles (Figure 5B) are characteristic of the C=O stretching modes[35, 36, 37, 38] of the carboxylic acid group. Hence it is possible that proteins/ enzymes play a role in the reduction of metal ions by the oxidation of aldehydes to carboxylic acid.

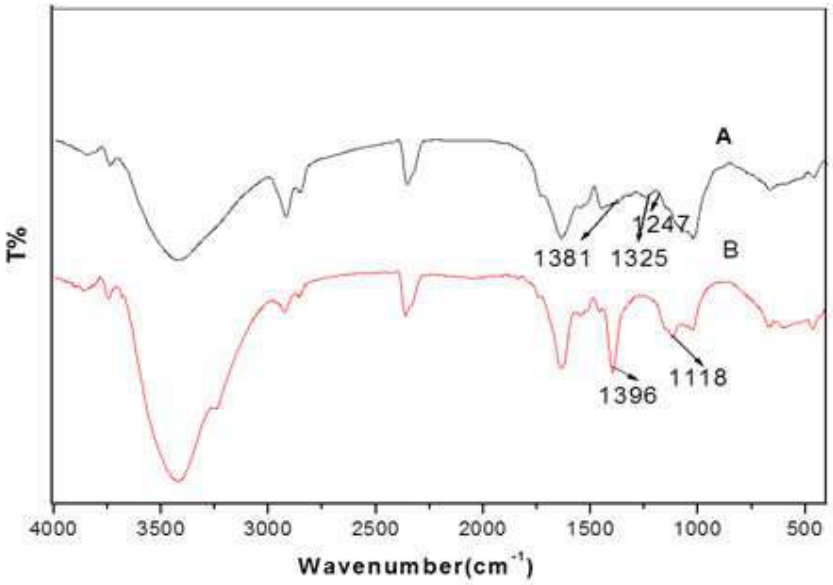

Figure 5
Spectrum of uninteracted C. manghas (A) Platinum nanoparticles (B).

3.6. Antimicrobial activity of Platinum nanoparticles

The Pt nanoparticles synthesized showed inhibition zone against almost all the test organisms (Table. 1). Maximum zone of inhibition was found against *V. cholerae* (20 mm) which was higher than that of the positive control streptomycin. The next maximum zone of inhibition was recorded in *S. aureus* (19 mm) followed by *S. pyogens* (12.8 mm) and *S. typhi* (11.7 mm) respectively and minimum zone of inhibition was recorded against *E. coli* (11 mm) (Figure 6). Furthermore, the nanoparticles synthesized by green route are found to be highly effective against gram negative bacteria. Antimicrobial properties of PtNPs against Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria[39].

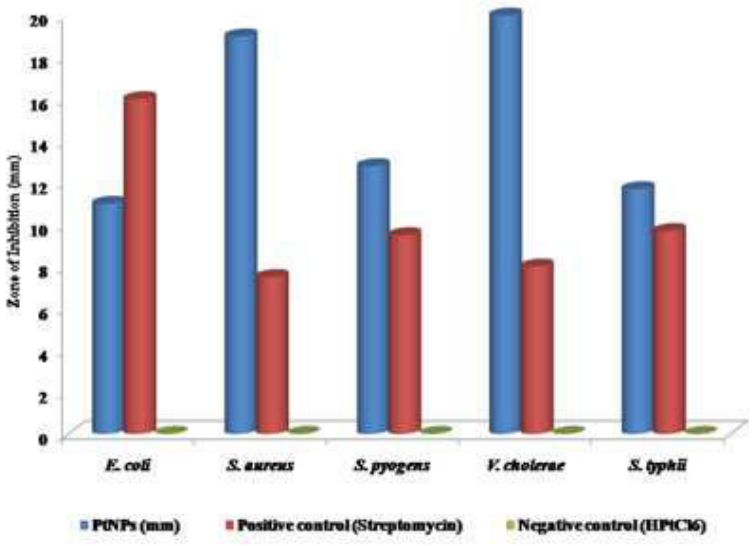

Figure 6
Antibacterial activities of Platinum nanoparticles against some selected human bacterial pathogens.

S. No	Bacterial Pathogens	Crude Extract (mm)	PtNPs (mm)	Positive control (Streptomycin)	Negative control ($HPtCl_6$)
1	*E. coli*	6	11	16	0
2	*S. aureus*	9	19	7.5	0
3	*S. pyogens*	7	12.8	9.5	0
4	*V. cholerae*	11	20	8	0
5	*S. typhii*	7	11.7	9.7	0

Table 1
Antibacterial activities of Platinum nanoparticles

4. CONCLUSION

In conclusion, we developed a simple, room-temperature, and efficient biological method for synthesis of platinum nanoparticles using marine plant *C. manghas* which could be used as an efficient biomaterial for the rapid and consistent synthesis of Platinum nanoparticles and also as an effective antibacterial agent.

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