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Green synthesis of Platinum nanoparticles from *Moringa oleifera* Lam. and its antimicrobial efficacy.

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Abstract

Integration of nanoscience in medicine leads to the development of biomedical products that helps the society in a faster and safer manner.In the present studies platinum nanoparticles were synthesized by green synthesis route using aqueous extracts of *Moringa oleifera* and characterization was done through UV, SEM, TEM, FT-IR and XRD.The synthesized nanoparticle were spherical in shape with an average size of 20 nm. Various bioactive compounds present in aqueous extract of this plant were responsible for bio reduction of nanoparticles. In antimicrobial activity, It was observed that against E. *col*i maximum zone was at 80µg/ml (IZ-18mm). Against *B. subtilis* no activity was observed at initial concentration, however nanoparticles showed zone at 60 and 80µg/ml respectively (IZ-10 and 12 mm). Further against *P. aeruginosa* nanoparticles showed activity at all dilutions. *S. aureus* was found to be resistant as no activity was observed except at 60µg/ml. Further when we tested these nanoparticles against different fungal strains it was observed that against *F. oxysporium* sample showed activity at all concentration, however maximum zone was observed at 60µg/ml (IZ-14mm). Against *P. funicolusum* it was observed that activity was low as compared to other strains. It was observed that *C. albicans* was found to be resistant at all concentrations except 80µg/ml where some activity was observed (IZ-12mm. Further it was observed that against *T. reesei* nanoparticles had best activity at all concentrations. Maximum activity was observed at 80µg/ml (IZ-18mm) .Result showed the biosynthesis of platinum nanoparticles using aqueous extract of *Moringa oleifera* is a clean, inexpensive and safe method that is free from toxic substance and consequently does not have any side effects.

Keywords: Green synthesis; SEM; TEM; Moringa oleifera ; Antimicrobial activity.

INTRODUCTION

Nanotechnology isdescribed as portentsat nanometre scale which is generally stated in range of 1-100 nm . It manipulates various substances at the atomic, molecular along with macromolecular levelto innovate substances atnanometre scale, andsystems that have unique features and functions. The eco- friendly approach by green route of synthesis of nanoparticles is easy, efficient, in comparison to chemically synthesised or through use of microbes. Plant extracts could be an alternative to conventional chemical routes for the synthesis of metallic nanomaterials in a clean, nontoxic and ecologically sound manner (Rai *et al.,* 2008). The key advantages of this route of synthesis of nanoparticles from plant extracts is that they possess significant phytochemicals which can contribute in the reduction of platinum ions and be synthesizing faster than microbes. These phytochemicals include ketones, aldehydes, amides, terpenoves, flavones, which are directly related to the reduction of ion and the formation of platinum nanoparticles.

Platinum nanoparticles (PtNPs) have attracted scientific communities for their significant therapeutic applications (Dreaden et al., 2012; Allen & Cullis, 2004). They possess exclusive optical features that can be optimized by modifying their sizes and shapes. They possess a localized surface Plasmon resonance, lead to a idiosyncratic absorption band in the UV/Vis area, which is not displayed by the heavy groups (Perelshtein *et al.,* 2008).

Moringa oleifera Lam is categorized in Moringaceae family having 14 species among which *M. oleifera* is most commonly found. In India conventionally this plant is known by the name ***'Sahanjana***". Its pods and leaves have rich source of biomolecules like it contain 2.5 and 6.7 g protein/100 g, respectively. The plant is also recommended in folk remedies for conjunctivitis, high blood pressure, abdominal, boils, cold, discomfort, tumors, relapsing fever, hysteria and skin diseases, etc. It also bears certain bioactivity, viz., anti-inflammatory, anti-asthmatic, antioxidant and hepatoprotective, antitumor etc. *Moringa oleifera* is consumed in asian diet since many decades as raw food source.

Recently existence andbanquet of antimicrobial resistance is a serious issue in both developing and developed nations and which can leads to global crisis (Xia, 2008). A stratagem for the repression of resistance needs to be innovated, executed and evaluated which should be focused on improving rational use of antimicrobials and reducing prospects for spread of resistant organisms (Sumathi and Thomas., 2017). It has been reported that, the metallic nanoparticles are meticulously being explored and broadly explored as potential antimicrobials. The antimicrobial potency of the nanoparticles is known to be a function of the surface area in communication with the microorganisms. Therefore, the search for new antimicrobial drugs from nanoparticles derived from natural sources has increased as ansubstitute to commercial drugs.

Considering the importance of nanoparticle metal synthesis especially platinum using different plants, the aim of this study was green synthesis of platinum nanoparticles using aqueous extract of *M. oleifera* and investigation of its antimicrobial activity.

Materials and Methods

Biological reduction of Platinum nanoparticles by Green synthesis route

Initially Platinum hexachloride ($PtCl_6$) was purchased from Sigma Aldrich and they were reduced biologically using the fresh leaves of ***Moringa oleifera***. 0.3gm leaves were weighed and were grinded properly in the mortar and pestle for 15 to 20 minutes in distilled water. Now these grinded laves were taken in the vials (3-4) and it was centrifuged at 4ºC, 1000rpm for 5 minutes. Further supernatant was collected in fresh vial. 0.01gm, Platinum sulphide was taken in 100ml autoclaved distilled water in a washed and clean conical flask, which was then kept on magnetic stirrer. Supernatant collected in the vial was added to the flask containing the Platinum nanoparticle. The mixture was kept overnight and the alteration in the color of the solution was observed. The change in the color of the solution is the indicator of reduction of synthesized Platinum sulphide.

Antimicrobial activity

Antimicrobial activity of the synthesised nanoparticles was investigated by agar well diffusion method (Malarkodi et al., 2013) and activity index was calculated as :

$$\text{ACTIVITY INDEX} = \frac{\text{Zone of inhibition of sample}}{\text{Zone of inhibition of standard}}$$

Statistical analysis

The statistical error of mean was calculated by the following formula:-

$$S.E. = \frac{\sigma}{\sqrt{n}}$$

Where
σ = standard deviation
n = number of observations
The test of significance (t-test) was calculated by the following formula

$$t = \frac{m_1 - m_2}{\sqrt{(SEM_1)^2 + (SEM_2)^2}}$$

Where,
m_1 = mean of one set of values.
m_2 = mean of second set of values.
SEM_1 = standard error of the first set of values.
SEM_2 = standard error of the second set of values.

The probability 'p' for obtaining 't' value of at least as great as the calculated one for a given number for the degree of freedom was found in the Fisher's table.
The p - values were signified according to the following conventions.
$P<0.05$ = difference was almost significant.
$P<0.01$ = difference was significant.
$P<0.001$= difference was highly significant.

3 RESULTS and DISCUSSION

Biologically synthesized platinum nanoparticles

In the present research for the green synthesis of Platinum nanoparticles leaves extract of *Moringa oleifera* was used as it is enriched with lot of phytochemicals using recommended protocols. When we added the extracts from leaves to solution of Platinum oxide it was observed that the color of the reaction concoction was gradually changed from light yellow to dark brown, thus showing the synthesis of nanoparticles (**Fig.1**).

UV

UV-visible spectroscopy is known globally and one of the most commonly recommended techniques for structural elucidation of nanoparticles. The absorption spectrum revealed that a surface plasmon absorption band at **400** nm, representing the incidence of an array of spherical shaped nanoparticles **(Fig. 2)**

SEM and TEM

In the present research morphology and the topography of synthesized nanoparticles was determined by SEM and TEM . SEM appearance showed that the platinum nanoparticles were mostly spherical in shape but showed some aggregation and clustering. TEM image which revealed that synthesized nanoparticles were spherical in shape and well distributed, with an average size of 20 nm. The present results from TEM image were in accordance with the SEM image.**(Fig. 3 and 4)**

FT-IR

FTIR spectroscopy was employed to determine the possible biomolecules and functional groups involved in reduction, capping and efficient stabilization of newly synthesized nanoparticles (**Fig. 5**). From this technique we observed about the engaged bioactive compounds and functional moieties which are responsible for reduction, coating and prominent sustainability of novel designed nanoparticles**.** The sharp and pointed absorption spectra at 3357, 2500, 2225, and 1000 cm^{-1} were detected. The major peaks at 3357 corresponds to O-H stretches showing presence of alcoholic groups. Peaks at 2500 and 2225 reveals presence of strong C-H stretching showing alkanes as functional groups while peaks at 1000 showing presence of CO-O-CO stretching thus showing presence of anyhydride group.

XRD

X-ray diffraction (XRD) studies were carried out to confirm the synthesis of Platinum nanoparticles and characterize crystallinity and the phase pattern of the nanoparticles. It was observed that 2Θ (in degrees) were in the range of 25to 69.5°C (**Fig. 6**). These were compared with the JCPDS, Cu file no. 04-0836. The said 2θ values of peaks were in accordance with the standard of JCPDS.The XRD study confirms that the resultant particles were nanoparticles. Furthermore, it also confirms that the synthesized nanoparticles was free of impurities as no other characteristics XRD peaks were observed. The mean grain crystalline size of green synthesized Platinum was calculated using the Debye–Scherrer formula

$$\frac{D = K\lambda}{\beta \cos\Theta}$$

where D is the average crystalline diameter size (Å), K is a constant (0.9), λ is the wavelength of the X-ray used (k = 1.54 Å), 'β' is the angular line width at the half maximum of diffraction (radians) and 'Θ' is the Bragg's angle (degrees)

Antimicrobial activity

In the present research it was observed that platinum nanoparticles synthesized from green synthesis method showed prominent antimicrobial activity at different dose level ranging from 20µg/ml to 80µg/ml on clinically important microbes. It was observed that against E. *col*i maximum zone was at 80µg/ml (IZ-18mm). Against *B. subtilis* no activity was observed at initial concentration, however nanoparticles showed zone at 60 and 80µg/ml respectively (IZ-10 and 12 mm). Further against *P. aeruginosa* nanoparticles showed activity at all dilutions. *S. aureus* was found to be resistant as no activity was observed except at 60µg/ml (IZ- 16mm) (**Table 1 and Fig. 7**).

Further when we tested these nanoparticles against different fungal strains it was observed that against *F. oxysporium* sample showed activity at all concentration, however maximum zone was observed at 60µg/ml (IZ-14mm). Against *P. funicolusum* it was observed that activity was low as compared to other strains. It was observed that *C. albicans* was found to be resistant at all concentrations except 80µg/ml where some activity was observed (IZ- 12mm. Further it was observed that against *T. reesei* nanoparticles had best activity at all concentrations. Maximum activity was observed at 80µg/ml (IZ- 18mm) (**Table 2 and Fig. 8**)

The application of nanotechnology is garnering an increasing amount of attention within the food sector. This can be attributed to an improved knowledge of the possible benefits that may be derived through the utilization of this technology. This is happening as a direct result of a growing understanding of the possible benefits that may be acquired through the utilization of this technology (Vadlapudi & Kaladhar, 2014). The awareness of these potential benefits has been expanding in recent years. The knowledgeable person believes that within the next few years, nanotechnology will play a role that is increasingly crucial in the manufacture of one-of-a-kind food products. This opinion was expressed by the individual who was informed. This forecast was made by the person who was aware of everything.

This study provides a condensed explanation of how NMs can be utilized in plant research. It focuses in particular on the absorption, mobilization, and biological ramifications of the interactions between NMs and plants that are considered to be the most essential. There may be new ways to improve plant defense, growth, and development, as well as crop output, through the use of polymeric soft NMs (NMs). All of this is done in the interest of enhancing plant defenses and/or promoting plant growth and development, with the ultimate goal of raising harvest yields (Mendieta & Nestor, 2012). In order to bring the qualities, measurements, and varieties of nanomaterials that are currently in use in the real world into conformity with the standards that have been outlined in the paragraphs that came before this one, it is necessary to improve the quality of the nanomaterials themselves as well as the measures and types that are used to create them. In this fast emerging area of research, it clears the path for brand new prospects that are unprecedented in the scale of their potential impact (Malarkodi & Manoharan, 2013).

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Table. 1 Antibacterial activity of Platinum Nanoparticle prepared by green synthesis route from *M. oleifera*

Concentration (in µg/ml)	***E.coli***	***Bacillus subtilis***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
20	IZ- 14 AI- 0.58	IZ- Nil AI-	IZ-12 AI-0.46	IZ- Nil AI-
40	IZ- 16 AI-0.66	IZ- Nil AI-	IZ-14 AI-0.53	IZ-Nil AI-
60	IZ-16 AI-0.66	IZ-10 AI-0.35	IZ-14 AI-0.53	IZ-Nil AI-
80	IZ-18 AI- 0.75	IZ-12 AI-0.42	IZ-16 AI-0.61	IZ-16 AI-0.72
Standard (Ciprofloxacin)	IZ -24	IZ -28	IZ -26	IZ -22

Table. 2 Antifungal activity of of Platinum Nanoparticle prepared by green synthesis route from *M. oleifera*

Concentration (in µg/ml)	***Fusarium oxysporum***	***Penicillium funiculosum***	***Candida albicans***	***Trichoderma reesei***
20	IZ-8 AI-0.4	IZ-6 AI-0.25	IZ- Nil AI-	IZ- 14 AI-0.5
40	IZ-10 AI-0.5	IZ-8 AI-0.33	IZ- Nil AI-	IZ-16 AI-0.57
60	IZ-10 AI-0.5	IZ-8 AI-0.33	IZ-Nil AI-	IZ-16 AI-0.57
80	IZ-14 AI-0.7	IZ-10 AI-0.41	IZ- 12 AI-0.38	IZ-18 AI-0.64
Standard (Ketokenazole)	IZ -20	Z -24	IZ-26	IZ -28

Ketokenazole (as Standard at 1mg/ml), IZ- Inhibition Zone (in mm) , AI- Activity index

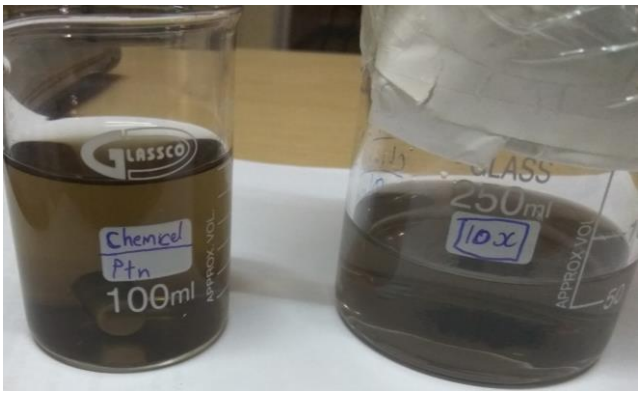

Fig. 1 showing in change of color which indicates formation of nanoparticles

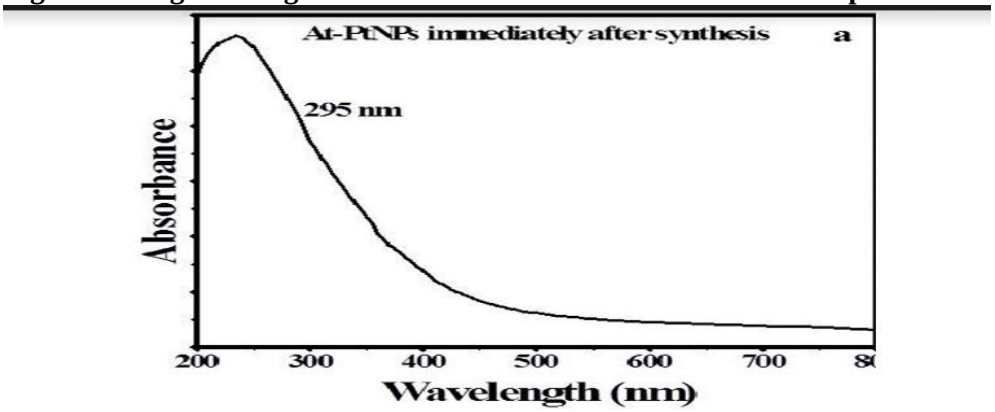

Fig. 2 UV Spectra of Biologically Synthesized Platinum nanoparticles

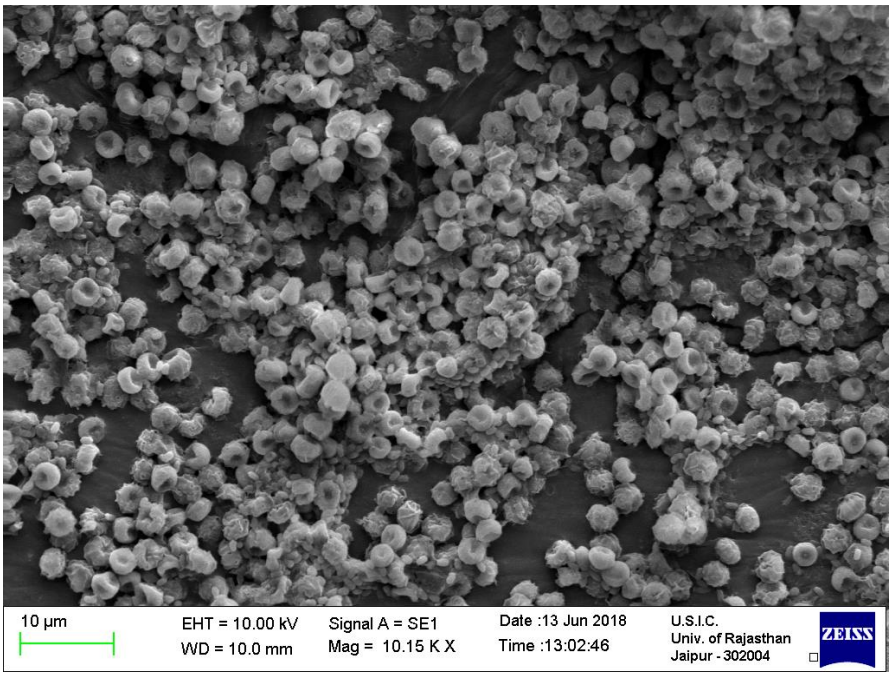

Fig. 3SEM showing spherical shape of Nanoparticles

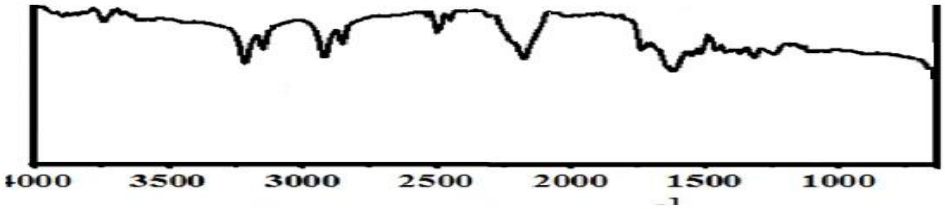

Fig. 5 FT-IR of Biologically Synthesized nanoparticles

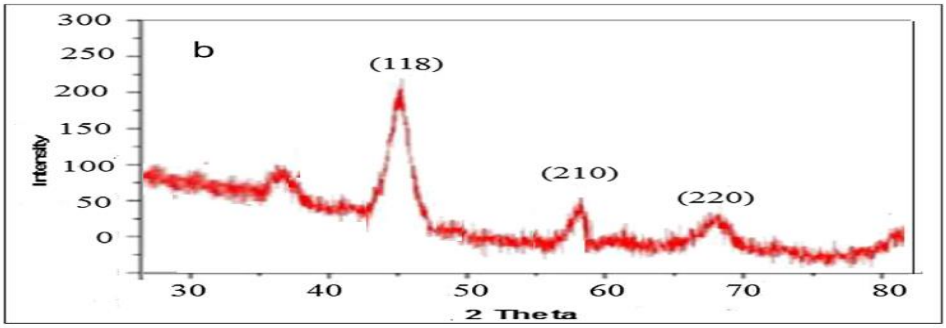

Fig. 6 XRD of Biologically Synthesized nanoparticles

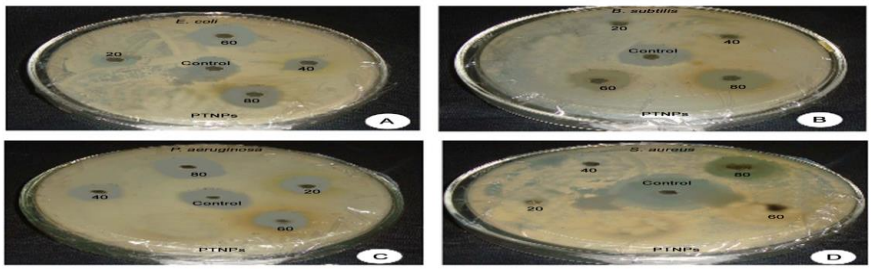

Fig. 7 Antibacterial Activity of Platinum Nanoparticles

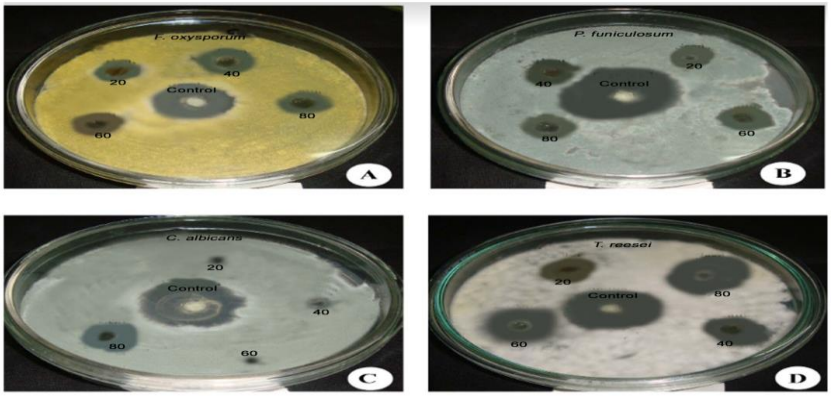

Fig. 8 Antifungal activity of Platinum Nanoparticles