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Green Synthesis of Zincoxide Nanoparticles from *Peganum harmala*, and its biological potential against bacteria

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Abstract

Green synthesis of the nanoparticle is becoming popular due to its eco-friendliness, cost effectivity, and possibilities of large scale production. In the present study, *Peganum harmala* is investigated for their potential to synthesize Zinc oxide nanoparticles. Zinc acetate dihydrate was used as a metal solution and it was mixed with plant extracts under vigorous stirring to obtain NPs. Synthesis of ZnO NPs was confirmed by change in color of reactant solution from yellow to white. For further confirmation, various characterization techniques were performed to indicate the bonding composition, size, and morphology of as prepared samples. The experimental results indicated that samples were pure ZnO NPs in nature which was further employed as an antibacterial agent against clinical strains of *Listeria monocytogenes, Escherichia coli, Listeria monocytogenes* and *Salmonella typhi*. The analysis to study antibacterial potential was carried out by using well diffusion method which indicated that ZnO NPs exhibit good potential as a bactericidal.

Introduction

Nanotechnology is incipient as a rapidly emergent field with its synthesis, application, and characterization in science and technology at the nanoscale level for the purpose of manufacturing new materials [1]. Even though there are many directions available for the nanoparticle's synthesis, there is an accumulative need to develop non-toxic, low cost, high-yield and ecologically friendly procedures. So, the biological methodology for the synthesis of nanoparticles becomes vital [2]. Environmentally friendly nanoparticles synthesis procedures need low energy and time expenditure and do not use any toxic chemicals in the synthesis protocols. In these aspects, synthetic methods based on naturally occurring biomaterials offer an alternative means for obtaining these nanoparticles.

Biological molecules have attracted great attention by which they can be controlled and hierarchical assembly and makes them appropriate for the expansion of a consistent and eco-friendly process for synthesis of metal nanoparticle [3].

Biosynthetic approaches are simple and sustainable substitute to attain nanomaterials employing either plants extract or biological microorganisms [4-8]. The combination of nanotechnology and herbal plants gives concept of plant mediated nanoparticle bases drugs. Diverse categories of nanomaterials like copper, titanium [9], magnesium, gold [10], alginate [11] and silver have been reported. It has been reported that angiosperms have the potential for metallic nanoparticles synthesis [12].

Biosynthesized Ag NPs are nowadays utilized in surface Plasmon resonance studies [13,14], antiviral, and anti-HIV studies [15], label-free colorimetric assay to detect enzymatic reactions [16] and antimicrobial materials [17].

In contemporary years for the nanoscale metal synthesis, the biosynthetic method consuming plant extracts have received more attention than the other physical and chemical methods and even than the use of microorganisms, due to the lack of any requirement to maintain disinfected environment. The use of plant extract for the synthesis of nanoparticles could be profitable above other environmentally benign biological processes as it eradicates the intricate process of sustaining cell cultures. A number of plants have been effectively used and reported recently for efficient and rapid extracellular synthesis of different kinds of metallic nanoparticles such as *Eclipta prostrata* [18], *broth extracts of neem* [19], *Pongamia pinnata* [20], *Ocimum sanctum* [21] and *Annona squamosal* [22].

Peganum harmala which is commonly called harmal belongs to *Zygophyllaceae* family [23]. This plant is medicinally important and traditionally has been used for the treatment of various health issues. The main secondary metabolite, which is responsible for many pharmacological potential of *P. harmala* belongs to alkaloid class of compounds [24]. The plant is found to have potential to fight against cancer [25], inflammation [26], bacteria [27] and microbes [28]. Beside many biological activities such as anti-oxidant [29], anti-plasmodium [30], hallucinogenesis [31], anti-tumor [32], anti-virial [33] and antinociceptive potential [34] have also been reported.

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ZnO Nps are consider to be least toxic [35,36]. These particles have been reported with anti-cancer potential [37]. These particles have capacity to fight against germ positive and germ negative bacterial strain [38]. Thus, ZnO Nps can act as anti-cancer and anti-bacterial agents and can be millstone for advance drug synthesis with great efficiency. In the present investigation, we report the easy synthesis of Zinc oxide nanoparticles by an environmental friendly procedure involving the in situ reduction of Zn by *P. harmala* extracts and the evaluation of their biological potential as an antioxidant and antibacterial activity

Experimental Section

Collection and identification of plant

The whole Plant and seeds of *Peganum harmala* were collected from Main Gundi, Balochistan region of Pakistan in 2016. Collected samples of the plant were identified by taxonomist, Dr. Rasool Baksh tareen, Dean of Botany Department, University of Balochistan. 10g of sample was deposited in chemistry Lab of Sardar Bahadur Khan Women University Quetta. They were surface cleaned with running tap water, air dried and subjected to electrical grinder. The resulting powder was boiled with distilled water with ration 10:100 at 60°C for 20-25 min. This extract was filtered through nylon mesh, followed by Millipore filter (0.45µm) were placed in microwave oven for 2 to 3 min., the resulting extract was stored in glass bottles and used for further experiments.

Biosynthesis of ZnO nanoparticles

For synthesis of Zinc oxide nanoparticles, aqueous solution (0.02 M) of Zinc acetate dihydrate solution was prepared and used for the synthesis of Zinc nanoparticles. (10 mL) of *P. harmala* extract was added into (500 mL) of aqueous solution of 0.02M zinc acetate at constant stirring for 2h at (60 °C temp) for reduction into Zn^{+2} ions. The pH of the solution was kept at 12. The Reduction of zinc acetate to zinc ions was confirmed by the colour change from yellow to white. White precipitate appeared which indicated synthesis on ZnO Nps. White Nps were washed with distilled water and methanol solution for purification. The formation of zinc nanoparticles was also confirmed by spectrophotometric determination.

Characterization of ZnO nanoparticles

To check phase formation and purity of green synthesized ZnO Nps, XRD patterns were recorded using powder X-ray diffractometer (Shimadzu-Model, XRD 6000). The analysis was made on scanning mode. Powedered sample of Nps was placed in instrument operating at 40 kV with 30 mA current. Debye–Scherrer equation was utilized to calculate size of synthesized Nps.

$D = k\lambda / \beta\, \theta\, cos$ Where λ is x ray wavelength which was 1.5418 Å.

Sample of ZnO Nps was placed on specimen stubs. To prevent charging, sample was coated in a sputter coater. Sample was examined under SEM (HITACH, Model S-3400N). Reaction mixture of green synthesis of Nps was placed in centrifuge at speed 45 rmp for 40 mins. Centrifuge enable the separation of solids pellets of Nps which was dissolved in distilled water followed by filtration. Small quantity of this dissolved Nps filtrate was used for SEM and EDX analysis.

Structural features of plant mediated nanoparticles were characterized by advance techniques such as FTIR, EDX, XRD and SEM. All analysis was made in triplicates. Obtained data were analyzed by origin software.

Bacterial Culture preparation

Bacterial species *Listeria monocytogenes, Escherichia coli, Listeria monocytogenes and Salmonella typhi* were collected USA. Microbiology laboratory Quaid-e-Azam University Islamabad Pakistan. All bacterial strain were provided with ager solution along 6 g of Trypton Soy Broth at 121 °C for 20 minute and then for incubation period of 24 hours.

Antibacterial assays

The antibacterial assays were done on human pathogenic *Escherichia coli* and *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella typhi* by standard well diffusion method. Antibacterial activities of ZnO Nps were evaluated against highly multiple drug resistant bacterial strains. Well diffusion method was carried out to evaluate the antibacterial activity of ZnO Nps. For this (10 mg/ml) ZnO Nps dissolved in DMSO. The inoculums of bacterial strains were prepared by growing a single colony for overnight in nutrient broth. Petri plates containing media were swabbed with bacterial strains by using cotton buds wells were made in nutrient agar 0.1M ZnS and aqueous plant extract (positive control), DMSO (negative control) and 20ml of each synthesized- ZnO Nps were added in wells. Petri plates were incubated for 24 hours at 37 °C and the inhibition zone was measured. This experiment repeated thrice.

Antioxidant activity

The DPPH free radical scavenging assay was conducted based on the method of Samiullah, et al. [38] with slight modification. The procedure involves mixing of each sample with DPPH solution with different concentration followed by spectrophotometric analysis. The solution of crude methanolic extract of varying concentration 0.02, 0.04, 0.06, 0.08, and 0.1 mg/ml was prepared in solvent. Similarly, all sample of varying concentration with NPs were prepared. Freshly prepared 1 ml of 0.1 mM DPPH solution was added with 1.5 ml of all samples including crude methanolic extract of plant and ZnO Nps. Each sample mixture was kept in dark for 25 minutes at 37 °C followed by UV. V is absorbance at 517 nm. Solution of Ascorbic acid of same concentration ranging from (0.02-0.1 mg/ml) was use as control. The percentage of inhibition or scavenging of free radicals was determined by the following formula:

Percentage of radical capturing activity = $(Abs_{(control)} - Abs_{(sample)})/ Abs_{(control)} \times 100\ \%$

Results and discussion

Plants with herbal potential are safer mean for treatment. Different phytochemicals which are present in plants concentrated on extraction, so plant extract efficiency increases and can lead for new effective drugs. The detailed study on biosynthesis of zinc oxide nanoparticles by natural plants extract of *Peganum harmala* were carried out and documented in this work. The aqueous zinc ions were reduced to zinc oxide nanoparticles when added to natural plant extract of *Peganum harmala*. It was observed that the color of the solution turned from (yellow to white during 1-48 h). of the reaction, which showed the formation of ZnO nanoparticles.

FTIR spectroscopy was used to confirm the surface groups of the nanoparticles qualitatively with spectra recorded by a Perkin-Elmer model FTIR spectrophotometer. The mean particle diameter of zinc nanoparticles was calculated from the XRD pattern rendering to the line width of the plane, refraction peak using Debye–Scherrer equation. SEM analysis was carried out to understand the topology and the

size of the ZnO-NPs, which showed the synthesis of higher density polydispersed spherical ZnO-NPs of spherical shape of 39.94 nm sizes. While the presence of metals in the sample was analysed by EDX.

FTIR analysis of P. harmala mediated ZnO Nps

The FTIR spectra of aqueous zinc oxide nanoparticles prepared from the *P. harmala* extract (Figure 1) show transmittance peaks at 3450 cm^{-1}. This peak corresponds to stretching bands for -OH group. A peak was observed at 3000 cm^{-1} which indicate the presence of C-H bond. Stretching vibration for C-C bond was observed with peak 1350 cm^{-1}. Another peak was also notice which was at 1010 cm^{-1}. This peak was due to C-O functional group.

XRD analysis of *P. harmala* mediated ZnO Nps

XRD analysis showed distinct diffraction peaks at 31.75, 34.54, 36.21, 47.67, 56.66, 62.76 and 68.16 corresponding to (100), (002), (101), (102), (110), (103), (200), (112) and (201).

The structure of ZnO Nps suggested by these observation is hexagonal wurtzite. The obtained data was matched with the database of Joint Committee on Powder Diffraction Standards (JCPDS) file No. 36-1451. The average grain size of the zinc oxide nanoparticles formed in the bio reduction method was determined to be 39.94 nm by using $D = k\lambda / \beta\, \theta\, cos$ *equation* (Figure 2)

FESEM of the synthesized ZnO Nps

SEM analysis of the synthesized ZnO Nps were carried out to determine the morphology of synthesized ZnO nanoparticles. For the SEM studies, dried sample of reaction mixture was used. A representative SEM micrograph (Figure 3) of synthesized nanoparticles showed that they have spherical structures with a size of 39.94 nm

EDX result for ZnO Nps synthesized by extract of *P. harmala*

The presence of metals in the sample was analyzed by EDS.The energy-dispersive X-ray (XRD) attachment present with the SEM was known to provide information on the chemical analysis of the fields being investigated or the composition at specific locations (spot EDX). Figure 4 shows the spectra obtained from ZnO Nps synthesized from *P. harmala* plant. The spectra shows Zn has absorption peaks approximately at 1.32 keV, 8.92 keV and 9.5 keV while O has optical absorption peaks at 0.59 keV.

The data obtained for ZnO Nps synthesized by plant extract of *P. harmala* is shown in Table 1, which shows presences of 29.39% oxygen element and 70.61% Zinc element (Figure 5 and Figure 6).

Phytochemical analysis

Phytochemical studies was carried out with aim to point out total phenolic content (TPC), total flavonoids content (TFC) and total alcoholic content (TAC). Analysis was made on crude extract as well as on plant mediated zinc oxide Nps. Higher content of flavonoids, alkaloids and phenol was observed in ZnO Nps as compare to crude extract (Table 2). Graphical representation shows flavonoids are present in high concentration as compare to alcoholic and phenolic contents.

Antioxidant analysis

Anti-oxidant analysis was conducted by total radical scavenging potential (TRP) and DPPH assay. Plant extract and plant mediated Nps both have anti-oxidant potential but crude extract is found to be more effective as compare to Nps derived from it (Table 3).

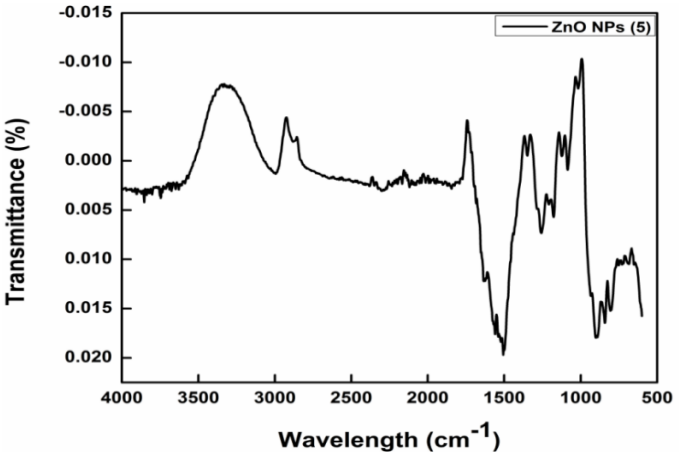

Figure 1. FTR spectrum of the Nps

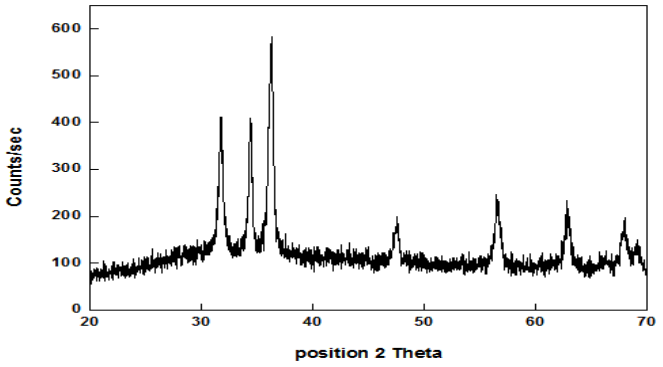

Figure 2. XRD spectra of plant mediated ZnO Nps

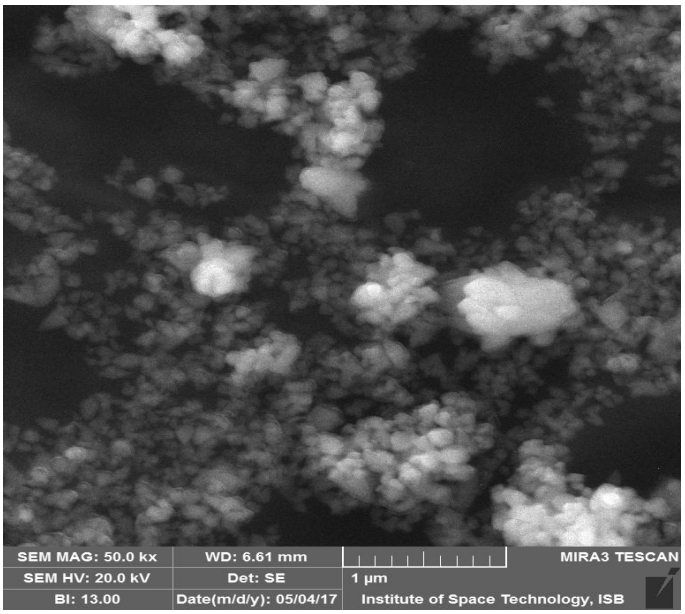

Figure 3. SEM result for green synthesized Nps

Antibacterial analysis

Antibacterial activity of zinc nanoparticles were investigated against four bacterial strain *S. aureous, E. coli, L. monocytogenes* and *S. typhi* expressed in Table 4. Plant mediated ZnO Nps are found to be more

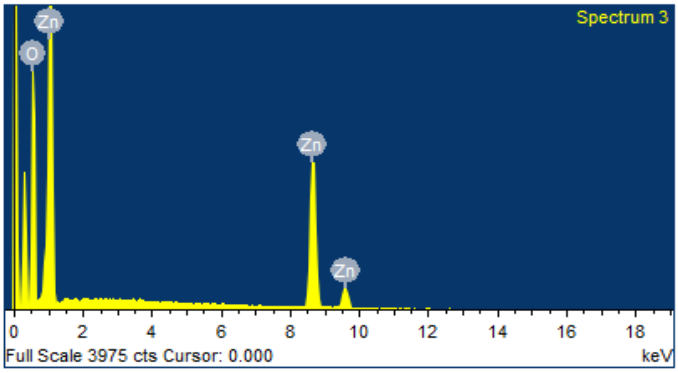

Figure 4. EXD spectra of ZnO Nps

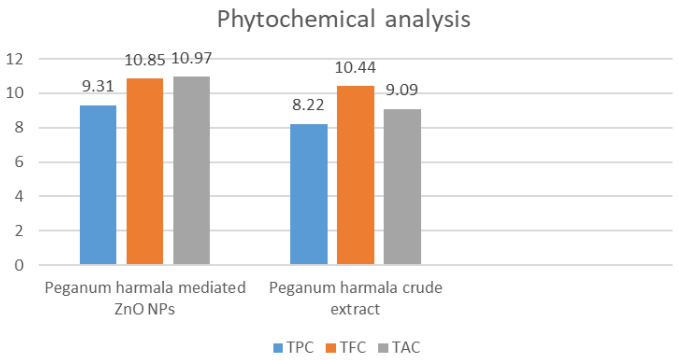

Figure 5. Comparison of phytochemical b/w ZnO Nps and crude extract

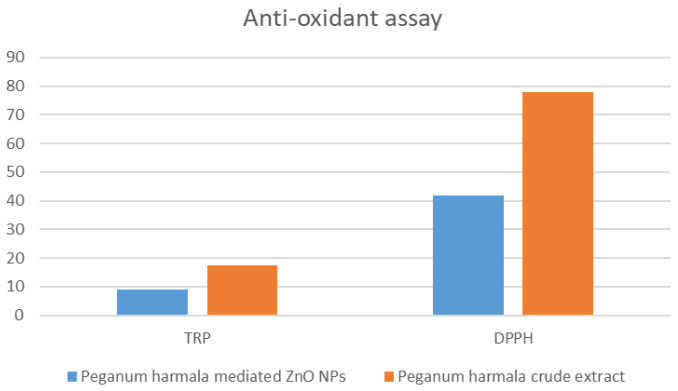

Figure 6. Graphical representation of TRP and DPPH assay of Nps and crude extract of *P. harmala*

Table 1. Data of composition for P. harmala mediated ZnO NPs

Element	Weight%	Atomic%
O K	29.39	62.98
Zn K	70.61	37.02
Totals	100.00	

Table 2. Phytochemical analysis of crude extract and Nps

Plant Name	TPC	TFC	TAC
P. harmala mediated ZnO Nps	9.31	10.85	10.97
P. harmala crude extract	8.22	10.44	9.09

Table 3. Anti-Oxidant Assay of crude extract and ZnO Nps

Plant Name	TRP	DPPH
P. harmala mediated ZnO NPs	9.09	41.91
P. harmala crude extract	17.41	77.97

Table 4. Antibacterial assay of *P.* harmala mediated ZnO Nps and its crude extract

Bacteria	ZnO Nps	Plants Extracts
Staphylococuss aureous	17mm	16mm
Listeria monocytogenes	13mm	12mm
Escherichia coli	10mm	14mm
Salmonella typhi	10mm	11mm

effective against *S. aureous* (17mm). The response of ZnO nanoparticles against *Listeria monocytogenes* (10mm) was also satisfactory. However lowest response was observed against *E. coli* (10mm) and *S. typhi* (10mm) with minimum zone of inhibition.

Conclusion

Nano-biotechnology signifies a new epoch of ground-breaking approach to develop and test modern drug formulations established on biosynthesized nanoparticles with diverse biological activities such as antioxidant, antibacterial and anticancer properties The zinc nanoparticles have been produced by *P. harmala* (plant extracts) a medicinally significant plant, which is an economical, efficient and eco-friendly process. UV-vis spectrophotometer, XRD, SEM and EXD techniques have confirmed the reduction of zinc acetate to zinc nanoparticles. Basic composition of ZnO Nps were 29.39% with regard of oxygen element and 70.61% with regard of Zinc. Comparative phytochemical study of plant mediated ZnO Nps and plant's crude extract showed higher content of TAC, TFC and TPC. The antioxidant activity was determined by DPPH assay showed good activity. The zones of inhibition were formed in the antibacterial screening test indicated, that the ZnO nanoparticles synthesized in this process has the efficient antibacterial activity against pathogenic bacteria. The biologically synthesized silver nanoparticles could be of immense use in medical field for their efficient antibacterial and antioxidant function.

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