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Short Communication

Green Synthesis of Zinc Oxide Nanoparticles Using Fresh Peels Extract of *Punica granatum* and its Antimicrobial Activities

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ABSTRACT

Wide application of nanoparticles stimulates the need for synthesizing them But, the conventional methods are usually hazardous and energy consuming. This lead to focus on "green synthesis"of nanoparticles which seems to be easy efficient and ecofriendly approach. In this study the green synthesis of zinc oxide nanoparticles was carried out using peels extract of *Punica granatum* as a reducing agent and their antimicrobial activity. The nano synthesis was monitored under different range of temperatures, radiations, time periods and concentration of peels extract. The optimized nano zinc thus obtained was quantified and characterized using UV-Visible Spectroscopy, Scanning Electron Microscopy. Stability of reduced zinc oxide nanoparticles was analyzed using UV Visible spectra shown that the absorption peak, occurring due to surface Plasmon resonance, exists at 364nm and their antimicrobial activity was screened against microbial culture. Zone of inhibition exhibited by aqueous solution of zinc oxide nanoparticles materials and standard antibiotic used at the concentration of 50µg/ml. Zinc oxide nanoparticles showed greater effect for fungal culture of *Aspergillus niger* as compare to bacterial culture of *Proteus vulgaris*. The result emphasized the potent application of *Punica granatum* peels in the synthesis of zinc oxide nanoparticles with economic viability and easy in scaling up for mass production and it is proposed that nanoscale size zinc ions in the presence of antimicrobial bioactive compounds in synthesis of particles combine to promote this biocidal property. Test for protein and nucleic acid leakage were performed to study the biocidal.

Keywords: Nanoparticles, green synthesis, *Punica granatum*, SEM, Antimicrobial activity, *Aspergillus niger*

1. INTRODUCTION

Term Nanomaterials are synthesized from the novel ecofriendly and sustainable biological and engineering process[1]. In the [21]th century, the nanotechnology has emerged as an interdisciplinary field with the

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biosynthesis of metal nanoparticles. Nanotechnology is gaining importance in various fields such as health care, food & feed, cosmetics environmental health biomedical science chemical industries drug and gene delivery, energy science, electronics mechanics and space industries[2]. In recent year, different synthetic routes have followed to prepare non material such as chemical method microwave and laser ablation method[3]. Scientists show high interest for magnetic nanomaterials due to its potential application; also bulk materials could show a high super metallic or metallic resistance, when prepared in nanoscale[4]. Nanoparticles present a higher surface area to volume ratio with decrease in the size, distribution and morphology of the particles[5]. The growing need of environmental friendly nanoparticles, researchers are using green method for the synthesis of various metal nanoparticles for pharmaceutical applications. Biological approaches using microorganisms and plant or plant extracts for metal nanoparticles have been suggested as valuable alternatives to chemical methods. Several biological systems including bacteria, fungi, and yeast have been used in synthesis of nanoparticles[6]. Synthesis of nanoparticles using microorganisms involves elaborate process of maintaining cell cultures, intracellular synthesis and multiple purification steps. In this regard using green methods in the synthesis of zinc oxide nanoparticles has increasingly become a topic of interest as conventional chemical methods are expensive and require the use of chemical compounds/ organic solvents as reducing agents[7]. Green method using peels aqueous extract of *Punica granatum* has been used for the first time as a reducing material as well as surface stabilizing agent of the synthesis of spherical shaped zinc oxide nanoparticles. The structure, phase and morphology of synthesized product were investigated by the standard characterization techniques.

2. MATERIALS AND METHODS

2.1 Plant material and preparation of the extract

Punica granatum were used to make the aqueous extract. *Punica granatum* peels powder weighing 5gm were thoroughly washed in distilled water, and mixed in to 100ml sterile distilled water and filtered through Whatman No.1 filter paper. The filtrate aqueous extract use as reducing agent.

2.2 Synthesis of silver nanoparticles

For the synthesis of nanoparticles 50ml of *Punica granatum* peels aqueous extract was taken and boiled to 60-80˚C using a stirrer heater. 5 grams of zinc nitrate was added to the solution as the temperatures reached 60˚C. this mixture is then boiled until it reduced to a deep brownish yellow colored paste. This paste was collected in a ceramic crucible and heated in an oven at 90˚C for 8 hours. Turmeric yellow colored powder was obtained and this was carefully collected and packed for characterization purpose. The material was mashed in a mortar-pestle so as to get a fine nature for characterization.

2.3 UV-Vis spectroscopy analysis

The reduction of pure zinc ions was monitored by measuring the UV-Vis spectroscopy of the reaction medium at 2 hours after diluting small aliquot of the sample in to distilled water. UV-Vis spectral analysis was done done by using UV-Vis spectroscopy (Shimadzu).

2.4 Scanning electron microscopy analysis

Scanning electron microscopy analysis was done using ZESS EVO-50 SEM machine. Thin films of the sample on the were prepared on a carbon coated tape by just place a very small amount of the sample on the grid, extra sample removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 minutes. The

SEM analysis was used to determine the structure of the reaction products that were formed. SEM image has showed individual zinc particles as well as a number of aggregates.

2.5 Antimicrobial assays

The antimicrobial assays were done on human pathogenic culture *Proteus vulgaris* and *Aspergillus niger* by standard disc diffusion method. Briefly nutrient broth/ agar medium bacterial culture and Sabauroud Dextrose Agar medium were used to cultivate fungal culture. Fresh overnight cultures of inoculums 100µl of each culture were spread on to nutrient agar medium plates. Sterile paper discs of 6mm diameter containing 50µg/ml zinc oxide nanoparticles along with each bacterial and fungal standard antibiotic containing discs were placed in each plate.

3. RESULT AND DISCUSSION

Plants are easily available safe and nontoxics in most cases have a broad variety of metabolites. That can aid in reduction of metallic ions, and are quicker than microbes in the synthesis of particles. That is the major advantage of using plant extract for nanoparticles synthesis. The main mechanism considered for the process is plant, assisted reduction due to phytochemical[8]. The metallic nanoparticles are traditionally synthesized by using chemical, biological methods and they required high pressure energy, temperature and toxic chemicals in this regards biomaterial based synthesis of particles from plant part extract[9], bacteria[10], papaya fruit[11], and fungus[12] have been found to be cost effective, easiest method. Rapid synthesis and environment friendly approach[13]. Several approaches have been employed to obtain a better synthesis of zinc oxide nanoparticles such as chemical and biological methods. Recently, synthesis of metallic nanoparticles using plant extracts getting more popular[14]. *Punica granatum* aqueous extract of peels to zinc nitrate solution, the color of the reaction medium changed rapidly from colorless to brownish yellow paste equal ratio (Fig. 1). Similar results are reported by earlier worker[15] that the color indicated surface plasmon vibration typical of zinc oxide nanoparticles. While no absorbance peaks was observed in control a characteristic surface Plasmon absorption peak at 364nm was observed 24 hours. That attained the maximum intensity after 48 hours in intensity at 364nm was observed indicating complete reduction of zinc ions[16] also stated the some findings of nanoparticles.

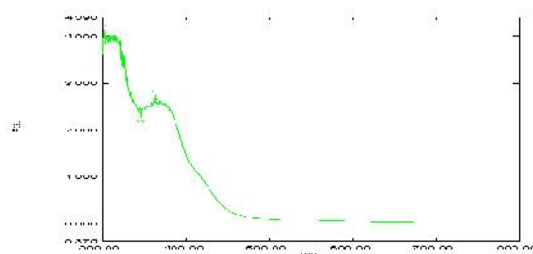

Fig 1: UV-Visible absorption spectra of zinc oxide nanoparticles synthesized by exposure of Punica granatum peels broth with zinc nitrate of equal concentration.

S. No.	Wavelengths	Observation
1.	364nm	2.613
2.	301nm	4.000
3.	346nm	2.405

Fig. 2 shows the scanning electron micrograph of the *Punica granatum* peels aqueous extract treated with 5gm zinc nitrate are seen clearly at a high magnification in zinc nitrate treated *Punica granautm* peels extract. Spherical and square like structure was observed formed with diameter range 50-100nm and similar phenomenon was reported by[17] the biologically synthesized nanoparticles were stable for more than six months and showed very little aggregation[18]. Besides, the Plasmon bands were broadened during absorption in responsible wave length which may exhibits the size, shape and their distribution of the similar[19] particles in the aqueous suspension.

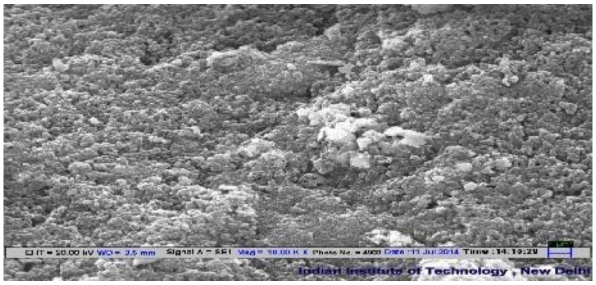

Fig 2: Scanning electron microscopy image of synthesis of zinc oxide nanoparticles by exposure of *Punica granatum* peels aqueous extract.

The results of antimicrobial activity of zinc oxide nanoparticles synthesis by green route are found highly toxic against multidrug resistant human pathogenic bacterial and fungal culture. Zinc oxide nanoparticles exhibited antimicrobial activity against *Proteus vulgaris* and *Aspergillus niger* as it showed a clear inhibition zone at the concentration of 50µg/ml .Similar results were also reported by[20] at the same concentration and the standard antibiotic like streptomycin sulphate .Antimicrobial effects of zinc oxide nanoparticles obeyed a dual action mechanism of antimicrobial activity i.e, the bacterial and fungicidal effect of zinc ions membrane disrupting effect of the polymer subunits[21]. The microbial activity of particles depends on the stability in the cultured medium too. Hence to use zinc in various fields against microorganism, it is need to prepared the zinc particles with cost effective methods and to find out the mechanism of antimicrobial activity there are alarming reported of opportunistic fungal infection. The result confirmed that the treated microbial cells were damaged showing leakage of proteins and nucleic acid in to nutrient agar media. These particles which can be prepared in a simple, rapid and cost effective manner are suitable for the formulation of new types of microbial materials[22].

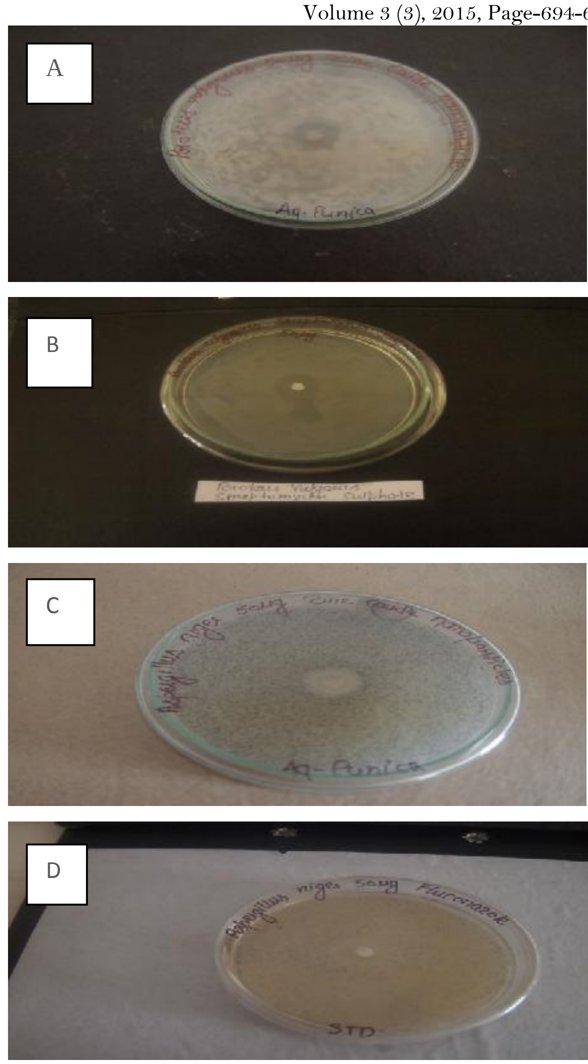

Fig 3: Antimicrobial activity of 50µg/ml (Bacteria and Fungi) zinc oxide nanoparticles (A) *Proteus vulgaris* (C) *Aspergillus niger* and standard antibiotic (B) *Proteus vulgaris* (D) *Aspergillus niger* comparative representation of inhibition zone.

Table 1: Zone of inhibition by disc diffusion method

S.No.	Culture name	Zinc oxide nanoparticles 50µg/ml	Antibiotics 50µg/ml
1.	*Proteus vulgaris*	15mm	13mm
2.	*Aspergillus niger*	19mm	16mm

The infection caused by opportunistic fungi is included under new spectrum of fungal pathogens. The result suggests zinc oxide nanoparticles may have excreted antifungal activity by disrupting the structure of cell membrane and inhibiting the normal budding process due to the destruction of membrane integrity. Recently nanoparticles particularly Fe_3O_4, ZrO_2 and MgO show antimicrobial activities against ophthalmic pathogens. Generally, the nanoparticles bind with the thiol (-SH) groups of protein that destroy the cell wall. But in the case of resistance bacteria, the possible mechanism of activity is, the MgO nanoparticles might inhibit the production of β-lactamase enzyme which involved in the drug deactivation process or the particles block the efflux pump pathway which involved drug elimination process.

4. CONCLUSIONS

The bio-reduction of aqueous zinc ions by the peels extract of the *Punica granatum* plant has been demonstrated. The reduction of metal ions through peels extracts leading to the formation of zinc oxide nanoparticles of fairly well defined dimensions. But the capability of the other plant part such as peels as a capping and reducing agent is not tested and not well defined. In the present study we found that peels can be also good source for the synthesis of zinc oxide nanoparticles. This green chemistry approach toward the synthesis of zinc oxide nanoparticles has many advantages such as, ease with which the process can be scaled up. Economic viability, etc. application of such ecofriendly nanoparticles in bactericidal, wound healing and other medical and electronic applications make this method potentially exciting for the large scale synthesis of other inorganic materials. Toxicity studies of zinc oxide nanoparticles on human pathogen opens a door for a new range of antimicrobial agent. The antimicrobial activity test examined by disc diffusion method showed that at 50µg/ml concentration, zinc oxide nanoparticles have better antimicrobial properties. However, at high concentration of antimicrobial activities of zinc oxide nanoparticles and zinc nitrate were relatively close to each other.

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