SYNTHESIS AND CHARACTERIZATION OF ZnONANOPARTICLES USING PEEL EXTRACT OF Luffa acutangula AND ITS ANTIBACTERIAL ACTIVITY AGAINST Staphylococcus aureus

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Abstract

Green nanotechnology has goals to fabricate nanomaterials and products without harming the nature or human health and producing nanoproducts that provide solutions to environmental troubles. The development of green chemistry in the production of nanoparticles has wrapped up a massive consideration because traces of chemicals left unreached in the chemical synthesis method can be unstable. The present study was focused on the synthesis and characterization of Zinc oxide nanoparticles (ZnO NPs) from the aqueous extracts of Luffa acutangula and to evaluate their antibacterial efficacy against the Gram positive coagulase positive cocci Staphylococcus aureus. Biological synthesis of Zinc oxide nanoparticles is reasonably safe compared to chemical synthesis. The biosynthesized nanoparticles were characterized by UV-Vis spectroscopy, FTIR (Fourier Transformed Infrared Spectroscopy), XRD (X-ray Diffraction) and SEM (Scanning Electron Microscope) for its optical property, crystallinity, size and morphology. The environmental analysis was performed for the synthesis and characterization of zinc oxide nanoparticles and showed that it was possible to identify the more environmentally well-suited process even at laboratory scale research. Thus, from this present study it was concluded that the ridge gourd peel extracts can be effectively used for synthesizing the zinc oxide nanoparticles. This approach offers environmentally advantageous alternatives to more hazardous chemicals and processes and promotes pollution prevention by the production of nanoparticle in their natural surroundings. This present research also suggests that the synthesized zinc oxide nanoparticles can be used as an antibacterial agent against pathogenic bacteria Staphylococcus aureus.

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1. Introduction

Nanotechnology is a brisk, upcoming area in material science research which deals with controlling and manipulating of materials at the nanoscale (10⁻⁹). Over last decades, nanotechnology has established as the great innovation of science and technology. In nanotechnology, ananoparticle is defined as a tiny object that behaves as a whole unit in terms of its transport and properties (Prathnaet al., 2012). Metal nanoparticles have attained an immense importance due to their unique feature such as...
catalytic, magnetic, optical and electrical properties. They have distinct or completely novel properties when compared to their bulk counterpart, which draws the entire scientific researcher to focus on their synthesis. Numerous metal nanoparticles such as silver, copper, gold, iron etc., has been explored so far (Noorjahan et al., 2015).

In recent years, Zinc oxide (ZnO) nanoparticles gain more attention by the researchers because of its wide application in various fields such as pharmaceuticals, cosmetics, agro chemicals, ceramics, optical and piezoelectric field. Zinc oxide is an inorganic compound which is in a crystalline nature and also called as zincite. Zinc nanoparticles are mostly used for the molecular diagnostics, target delivery of drugs and developing new pharmaceutical arrangements. It is also used to treat leukemia and carcinoma cancer cell (Bogutska et al., 2013). Zinc oxide is considered as multi-task metal oxide which can be used as nanoscale due to its unique physical, chemical and biological properties. Zinc oxide nanoparticles has a wide range of application in various fields such as optical, piezoelectric, mechanical, thermodynamic, electrodynamic, electromagnetic and gas sensing (Anandraj and Jayalakshmy, 2015).

Ridge (Luffa acutangula L.) gourd is a popular vegetable consumed as common vegetable in daily Indian diet and it is widely growing vegetative climber and traditionally used in folk medicines for many ailments including jaundice, diabetes, liver diseases, skin diseases, wounds, etc. In Ayurveda, ridge gourd has recognized with a number of health benefits which current clinical research is supporting as well. These plants are used in safeguard from jaundice when taken in the form of very fine powder through nose while the seeds possess emetic, expectorant and demulcent property (Anitha and Miruthula, 2014). The present study was planned to synthesize the zinc oxide nanoparticles from Luffa acutangula peel aqueous extract and its antibacterial activity was studied against pathogenic Gram positive cocci Staphylococcus aureus. In this study, we reported the biosynthesis of zinc oxide nanoparticles for the first time through a new, rapid, cost effective and eco-friendly technique.

2. Materials and Methods
Collection of plant material
The peel of Luffa acutangula used in this study was collected from the field of Kurisilapattu village, Vellore district, Tamil Nadu, India.

Preparation of aqueous peel extract
The peel were separated from the Luffa acutangula and washed several times with normal water and with double distilled water to remove the dust particles and then kept in sun shade dried to eliminate the residual moisture. The aqueous extract of Luffa acutangula was prepared by placing 50 g of dried peel powder with one liter of sterile double distilled water and then boiled for 20 mins at 60 °C. This extract was cooled to room temperature and filtered by using Whatman filter paper No.1. The filtered aqueous extract was stored in a refrigerator at 4 °C for further
experiments. The filtered aqueous extract was used as reducing agent.

**Preparation of zinc nanoparticles**

Zinc nitrate hexahydrate \([\text{Zn (NO}_3\text{)}_2\cdot 6\text{H}_2\text{O}]\) was used as precursor to synthesize \(\text{ZnO}\) nanoparticles using \(\text{Luffa acutangula}\). For the synthesis of zinc oxide nanoparticles, 500 ml of peel aqueous extract of \(\text{Luffa acutangula}\) was taken in a clean conical flask and 10 g of Zinc nitrate was added to the solution and mixed thoroughly and kept in shaking incubator at 150 rpm for 2 hours. After the incubation, the mixture was allowed to cool at room temperature. And then, the solution was centrifuged at 4000 rpm for 15 mins. After centrifugation, supernatant were discarded and obtained solid product were separated and kept in Hot air oven for 7 hrs at 80 °C. The resultant sample was collected and smashed in a mortar and pestle so as to get a better nature for further characterization of zinc oxide nanoparticles and stored in an air tight container.

**Characterization of Zinc oxide nanoparticles**

**UV–visible spectrum for synthesized nanoparticles**

The sample was measured for its maximum absorbance using UV–Vis spectrophotometer. The optical property of \(\text{ZnO}\) nanoparticles was analyzed via ultraviolet and visible absorption spectroscopy (UV – Vis – Varian – Cary 50 Bio) in the range of 200 – 600 nm.

**FTIR analysis for synthesized nanoparticles**

The FTIR spectrum was taken in the mid-IR region of 400 – 4000 cm\(^{-1}\). The spectrum was recorded using ATR (attenuated total reflectance) technique. The dried sample was mixed with the KBr (1:200) crystal, and the spectrum was recorded in the transmittance mode (PERKIN ELMER – Spectrum - 2).

**X-ray diffraction (XRD) analysis for synthesized nanoparticles**

The phyto-reduced zinc nanoparticles were characterized to reveal their crystal structure using X-ray diffraction technique. The XRD pattern was recorded using computer controlled XRD- system (JEOL, and Model: JPX-8030) with CuKa radiation (Ni filtered = 13418 A°) in the range of 40 kV, 20 A. The built-in software (syn master 7935) program was used for the identification of XRD peaks corresponding to the Bragg’s reflections. The estimation of the size of particles was performed by Scherrer’s formula.

**SEM analysis for synthesized nanoparticles**

Scanning Electron Microscope (SEM) analysis was done by using Hitachi S – 4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry putting it under a mercury lamp for 5 min.

**Antibacterial assay**

**Collection of \(\text{Staphylococcus aureus}\)**

The bacterial culture \(\text{Staphylococcus aureus}\) was collected from the Microbiology Laboratory, Department of Biochemistry, Sacred Heart College (Autonomous), Tirupattur, Tamil Nadu, India.

**Inoculum preparation**

Bacterial inoculum \((\text{Staphylococcus aureus})\) was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37 °C for 3 - 5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farland standards and then used for the determination of antibacterial activity.

**Preparation of test solution**

The test solution was prepared with known weight of sample dissolved in 5 % Dimethyl Sulphoxide (DMSO) (corresponding to 20, 30 and 40 \(\mu\)g/ml).

**Determination of Antibacterial activity**

The Agar Well diffusion method was followed for the determination of antibacterial activity of zinc oxide nanoparticles against \(\text{Staphylococcus aureus}\). The Mueller Hinton Agar medium was prepared, sterilized by Autoclaving at 15 lbs, poured on petriplates and allowed to solidify. After solidification, 0.1 ml of standardized microbial inoculum suspension was
poured and spread uniformly with the help of cotton swab. The excess inoculums were drained and the plates were allowed to dry for 5 min. After drying, wells of 6 mm in dm were prepared with the help of well cutter and the samples which were prepared in various concentrations were placed on the wells with the help of a sterile pipette. The antibiotic Streptomycin (5 µg) was used as the positive controls. The DMSO (5%) was used as a blind or negative control. Finally, the inoculated plates were incubated at 37°C for 24 hrs. The zone of inhibition was observed and measured in millimeters. This experiment was repeated three times for concordance.

3. Results and Discussion

UV visible Spectroscopy

Optical properties of the as-prepared zinc nanoparticles sample was revealed by UV–Vis spectrum and photoluminescence spectroscopy at room temperature, as shown in Fig - 1. It can be seen from the Fig – 2 that there was intensive absorption in the ultraviolet band of about 200-600 nm. The maximum absorption wavelength was seen at about 246 nm which shows similar to the results of Supraja et al. (2015) where they had absorbance at 240 nm. The difference in the UV absorbance may be due to the difference in the reducing activity of our leaf extract.

According to Figure - 3, it was observed that the bands are at 2919 cm⁻¹, 1656 cm⁻¹, 1388 cm⁻¹, 637 cm⁻¹ and 441 cm⁻¹. The FTIR spectrum of ZnO nanoparticles was recorded in the range of 400 – 4000 cm⁻¹. The peaks at 2919 cm⁻¹ reveal the presence of C–H bend, indicating the alkanes and alkyls respectively. The medium band of –C–C– stretch (alkenes) was recorded at 1656 cm⁻¹. The medium bands of –C–N– stretch (alkanes and alkyls) were recorded at 1388 cm⁻¹. The band present at 637 cm⁻¹ indicates the presence of C–Br stretching vibration. The peak in the region between 400 and 600 cm⁻¹ was allotted to ZnO region (Yuvakkumar et al., 2015). So, the band located at 441 cm⁻¹ was observed due to the reduction and stabilization of metal group ZnO. Gnanasangeetha and Thambavani (2015) also got the ZnO FTIR band between 540 - 417 cm⁻¹.

FTIR Analysis

XRD Analysis

X-ray diffraction was taken to further confirm the zinc oxide phase of the nanoparticles. The XRD pattern of zinc oxide nanoparticles was shown in Figure – 4. The XRD peaks were identified as (110), (002), (101), (102) & (110) (JCPDS 36-1451). The narrow and strong diffraction peaks indicate the well crystalline nature of zinc oxide (Rajivgandhi et al., 2015). The size of ZnO nanoparticles was obtained by Debye – Scherrer’s formula given by the equation:

\[ D = \frac{K\lambda}{\beta\cos\Theta} \]

Where:
D – The crystal size,
\( \lambda \) – The wavelength of the X-ray radiation (\( k = 0.15406 \text{ nm} \)) for CuKa,
K – Usually taken as 0.89,
\( \beta \) – The line width at half-maximum height (Dobrucka and Dugaszweska, 2015).

The Scherrer’s formula was used to calculate the particle sizes and was found to be in the range of 25 nm. XRD study confirmed the presence of even smaller particles than the SEM examination. The larger nanoparticles of ZnO (about 50 nm) in the sample result from the agglomeration of smaller nanoparticles, whose presence is confirmed by X-ray diffraction (XRD). The XRD method allowed for the identification of smaller sizes of nanoparticles. The agglomeration of smaller nanoparticles occurs due to the fact that we are dealing with biological material.

**Figure – 4: XRD analysis**

**SEM Analysis**

SEM studies were revealed to visualize the size and shape of the zinc oxide nanoparticles and (Fig - 5). The synthesized ZnO nanoparticles were agglomerated with a particle size range was observed at 25 nm. In this study, obtained ZnO nanoparticles were appeared with that most of spherical in shape.

**Figure – 5: SEM analysis of Zinc oxide Nanoparticles**

**Antibacterial activity**

The results of antibacterial activity of zinc oxide nanoparticles synthesis are found highly toxic against human pathogenic bacteria. Zinc oxide nanoparticles exhibited antimicrobial activity against *Staphylococcus aureus* as it showed a clear maximum inhibition zone at the concentration of 40 μg/ml. From the results obtained due to the antimicrobial activity of ZnO nanoparticles on *Staphylococcus aureus* it was interesting to note that as the concentration of ZnO nanoparticles increases, the zone of inhibition also increases i.e. a minimum for control (almost none) to a maximum in 40 μg/ml. The bacterial activity of synthesized zinc nanoparticles depends on the stability in the cultured medium too. Hence, to use zinc oxide in various fields against microorganism, it is need to prepared the zinc oxide particles with gainful methods and to find out the mechanism of antibacterial activity there are offensive reported of opportunistic bacterial infection. This result was confirmed that the zinc oxide particles which can be prepared in easy, quick and cost effective manner are suitable for the formulation of new types of antibacterial agents. The result of Vani et al. (2011) shows the similar kind of effect against Streptococcus aureus.
Figure – 6: Antibacterial activity of biosynthesized
Zinc oxide nanoparticles

4. Conclusion
The green synthesis of ZnO nanoparticles is much safer and environmentally friendly as compared to chemical synthesis. In response to this study demonstrates *Luffa acutangula* aqueous peel extract can be used as an effective stabilizing as well as reducing agent for the synthesis of zinc oxide nanoparticles. The biosynthesized ZnO nanoparticles were characterized by UV–Vis absorption spectroscopy, X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Scanning electron microscopy (SEM). These methods confirmed the presence of the synthesized ZnO nanoparticles in the range of 25 nm. The larger nanoparticles of ZnO resulted from the agglomeration of smaller nanoparticles. These experiments clearly prove that *Luffa acutangula* mediated zinc oxide nanoparticles synthesis. Also the synthesized ZnO nanoparticles exhibit their effect against *Streptococcus aureus*.

5. Reference

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nanochains for biomedical applications. 
