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SHORT COMMUNICATION

Synthesis of Zinc Oxide Nanoparticles Using Stem Extract of *Citrullus colocynthis,* Characterization and Evaluation of its Antibacterial Activity

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Abstract

In the current investigation, an attempt is made to synthesize and characterize the *Citrullus colocynthis* stem extract loaded Zinc Oxide Nano Particles (ZnONP-Cc) and to evaluate the cytotoxicity and antimicrobial activities of the same. The synthesis of ZnONP-Cc was carried out and the characterization in terms of UV-Spectrum, Fourier Transform Infra-Red (FT-IR) spectrum, Transmission Electron Microscope (TEM), X-Ray Diffraction (XRD) and Scanning Electron Microscope (SEM) analysis were done. The antimicrobial and cytotoxic activities of the ZnONP-Cc were evaluated using the established protocol. In the UV-Spectrum significant SPR peak was observed at 350 nm and FT-IR spectrum exhibited several biomolecules with their chemical entities. Through XRD method, the zinc oxide nanoparticles Face-Centered Cubic (FCC) structure was confirmed. The SEM and TEM confirm the synthesized nanoparticles were round and oval shape with size ranging from 10 - 20 nm. The ZnONP-Cc possess significant antimicrobial activity against wound pathogens and it was found to be non-toxic to the Brine shrimp nauplii. In conclusion, the synthesized ZnONP-Cc can be exploited in nanomedicine since it has antimicrobial activity without any toxicity.

Keywords: Antimicrobial Activity, Characterization, *Citrullus colocynthis,* Cytotoxicity, Zinc Oxide Nanoparticles

1. Introduction

Nanotechnology has wide practical applications in all domains of science such as medicine, environment, agriculture, and engineering. Chemically organic, inorganic as well as hybrid materials are utilized in nanotechnology[1]. Nanoparticles in the field of medicine provide greater benefits as anticancer anti-diabetic and antimicrobial agents due to their compatibility. They are also used in immunotherapy, bio-catalysis, gene delivery, bio-imaging, tissue engineering and biosensor[2]. By using several physical, chemical and biological methods, these nanoparticles can be synthesized in ordinary laboratory condition[3]. Due to the usage of low toxic chemicals and the low cost, the synthesis of nanoparticles using biological methods is considered greater effective than other methods. At present, Zinc Oxide nanoparticles were gained exceptional interest worldwide due to their high therapeutic role as anti-bacterial, antioxidant, anti-viral, anti-malarial, anti-cancer and wound healing potential[4]. Currently, plant extract-based Zinc nanoparticle biosynthesis has gained attention worldwide in drug delivery systems as well as cosmetic industries due to the increased bioavailability when compared to conventional peparations[5].

The plant *Citrullus colocynthis* belongs to the family Cucurbitaceae which occurs in Tunisian arid regions and the Mediterranean region, is serving as an antioxidant, antibacterial, antidiabetic anti-inflammatory and anticancer agent is considered a common traditional healer herb for centuries[6]. The bioactive phyto chemical content present in the fruits includes phenolic acids, alkali, flavonoids, cucurbitacin, fatty acids, quinolone-type

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alkaloids and chemical group chains containing alcoholic and ketonic groups with volatile nature[7]. The decoction of the whole plant is effective against many gram-positive micro-organisms[8]. The leaves are used in the treatment of jaundice, asthma and are diuretic. The roots are used to cure, amenorrhea inflammation of the breasts, and rheumatism joint pains and are usually used externally in uterine pains and ophthalmia[9,10]. The present study is aimed to prepare and characterize the *C. colocynthis* stem extract loaded Zinc Oxide nanoparticles and to study the antimicrobial and cytotoxic activities of the same.

2. Materials and Methods

2.1 Collection of Stems

The *C. colocynthis* stem was collected in Madurai. The appropriate quantity of the stem was cleaned 2 - 3 times with distilled water and shade dried for 7 – 15 days which was grind into fine powder. The stem powder was stored in a sterile container for further use.

2.2 Preparation of Stem Extract and Synthesis of Zinc Oxide loaded Nanoparticles of Cc (ZnONP-Cc)

The *C. colocynthis* stem extract loaded Zinc Oxide nanoparticles were prepared according to the method of Rajeshkumar *et al*[11]. One gram of stem powder of *C. colocynthis* was boiled for 15 minutes in 100 ml of distilled water followed by filtration through Whatman No. 1 filter paper. The 100 ml of 0.594 mM Zinc nitrate solution was added to 10 ml of the filtrate and treated in the magnetic stirrer at 30˚C for 4h. The resultant solution was centrifuged to obtain the pellets of Zinc Oxide nanoparticles reinforced with *C. colocynthis* stem.

2.3 Characterization of ZnONP-Cc

Initial characterization of the ZnONP-Cc was done using UV-visible Spectroscopy (Perkin-Elmer MA, USA) at the range of 350 - 750 nm to obtain the Surface Plasmon Resonance (SRP) absorption bands. In the range of 4000 - 400 cm^{-1} the Fourier Transform Infrared (FTIR Perkin-Elmer, MA, USA) spectra were performed to trace the possible functional groups present in the ZnONP-Cc. The Scanning Electron Microscope (SEM) was performed to identify the size and shape of the nanoparticles. By using the X-Ray Diffraction pattern (XRD-Bruker AXS, Inc., Madison, USA) the biosynthesized Zinc oxide nanoparticles crystal structure was confirmed.

2.4 Antimicrobial Activity of ZnONP-Cc

The agar well diffusion method of Shuang, *et al.*,[12] was followed to determine the ZnONP-Cc antimicrobial activity of wound pathogens. The organisms such as *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis* and *Streptococcus mutans* were grown in Luria Bertani broth and 24 hrs grown culture organisms were swabbed on Muller Hinton Agar which was freshly prepared. Using the gel puncture, 4 wells with a 6 mm diameter were made and the biosynthesized Zinc oxide nanoparticles at the concentration of 25 µL, 50 µL and 100 µL and 25 µL of antibiotic were added in the wells and the treated plates were incubated for 24 hrs at 37°C. The zone of inhibition is measured in millimetre as diameter to detect the growth inhibitory effect of ZnONP-Cc against the studied pathogens.

2.5 Cytotoxicity Effect of ZnONP-Cc

The Zinc oxide reinforced *C. colocynthis* stem extracts cytotoxicity effect was evaluated using Brine shrimp lethality assay[13]. To the 6 wells containing ELISA plate 10 nauplii were added in saltwater in respective wells. Aliquots of different concentrations of ZnONP-Cc were added in the already nauplii-added wells and allowed to stand for 24 hrs in room temperature. At the end of 24 hrs of incubation, the number of nauplii which are alive and dead in each well is calculated and the mortality rate due to the treatment of ZnONP-Cc was evaluated using the formula to calculate the percentage toxicity of ZnONP-Cc.

% death = Number of dead nauplii − number of live nauplii × 100

3. Results and Discussion

3.1.Physical Observations of ZnONP-Cc

The zinc ions present in the solution were reduced to nanoparticles of zinc oxide by the secondary metabolites present in the plant[14]. Figure 1 visually confirms this reduction by producing a yellow colour solution after 4 hrs of reaction from the initial colourless solution. The change in colour is progressive starting from the 30 minutes of heating and followed by the rapid colour change which indicates the synthesis of ZnONP-Cc by the reduction of zinc by the plant biomolecules. The plant extract not only acts as a reducing agents but as stabilizing agent as well. This was confirmed by taking the UV–visible spectrum analysis in the range of 250 – 750 nm.

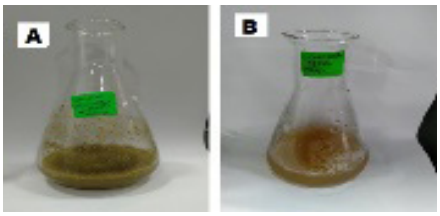

Figure 1. Visual observation of ZnONP-Cc. **(A).** Initial colour and **(B).** Final colour.

3.2. UV Visible Spectroscopy Analysis of ZnONP-Cc

The synthesis of ZnONP-Cc is further confirmed by UV spectroscopy which is considered one of the analytical tools to confirm any nanoparticle synthesis. The *C. Colosynthsis* Zinc Oxide nanoparticles were synthesized within 4h under warm conditions. The synthesized ZnONP-Cc were analysed periodically in UV visible Spectroscopy at 250 - 700 nm wavelength. The colour change in the reaction mixture is due to the excitation of SPR vibration surrounding the metal nanoparticles. According to Sanaz Alamdari *et al.,*[15] the presence of bioactive molecules in the stem extract of *C. colocynthis* influences the dimensions, shapes and composition of nanoparticles synthesized and thereby its SPR peaks. The maximum Surface Resonance Peak (SPR) was found at 350 nm (Figure 2). By the above analysis the synthesis of Zinc Oxide nanoparticles from aqueous extract of *C. colocynthis* stem is confirmed which was reported by Jayachandran, *et al.,* also[16]. Authors like Hassan, *et al.,*[17] also reported the same SPR peaks for the ZnONP prepared from *sativum* leaf extract and *Lippiaadenosis* leaf extract.

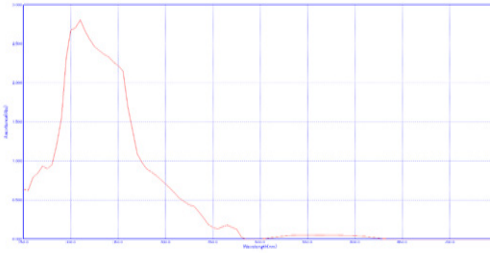

Figure 2. UV-Vis analysis of zinc oxide nanoparticles mediated *C. clocynthis* (ZnONP-Cc).

3.3. FTIR Analysis of ZnONP-Cc

The FTIR analysis helps to identify the phytochemicals present in the Zinc Oxide nanoparticles synthesized from *C. colocynthis* stem extract. Figure 5 signified the spectra of the synthesized Zinc Oxide mediated stem extract of *C. colocynthis* nanoparticles (ZnONP-Cc) resulted in peaks at 1374, 1637, 2326, and 3290 cm^{-1}. The *Cayratia pedata* leaf extract mediated Zinc Oxide nanoparticles prepared by Zhang *et al.,*[18] also showed the similar observation. The peak which shows in 1374 cm^{-1} corresponding to O-H group of alcohol. Similar observation is reported in synthesized Zinc Oxide nanoparticles using *Sesbania grandiflora* leaf by Sharmila, *et al*[19]. The well denoted peak at 1637 cm^{-1} shows the halo compounds (C-Cl or C-Br stretching) vibrations. The C-C=C symmetric stretching of conjugated alkene groups (1- Propane) was represented by the medium peak 1637 cm^{-1}. The peak at 1500 cm^{-1} region is due to the vibration due to the N=C=S stretching of isothiocyanate group. The presence of alkynes group of propyne (HC Ξ C-CH_3) as well as O=C=O stretching of carbonic dioxide, in the FTIR analysis of ZnONP-Cc were confirmed by the strong peak at 2326 cm^{-1}. The peak observed in 3290 cm^{-1} confirms the presence of N-H stretching due to primary and secondary amines groups. The presence of alcohol group is seen by O-H vibration at 3500 cm^{-1}.

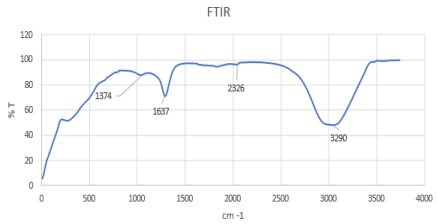

Figure 3. FTIR analysis of ZnONP-Cc.

3.4. XRD Analysis of ZnONP-Cc

The powder X-ray diffraction analysis proves the crystalline nature of Zinc oxide nanoparticles derived from *C. colocynthis* stem. The XRD results of biosynthesized Zinc oxide nanoparticles was shown in Figure 7. Based on the analysis, Zinc oxide nanoparticles synthesized from *C. colocynthis* stem clearly showed Face-Centered Cubic (FCC) structure and diffraction patterns was showed 2Θ value of 23.53, 29.33, 33.84, 38.88, 49.70 which were well coordinated with their corresponding reflection patterns (100), (002), (101), (102), (110), (103), (200), (112) and (201) respectively. The measurement of zinc oxide nanoparticles was corresponded with the JCPDS File No. 0361451 and declared the results of *C. colocynthis* synthesized Zinc oxide mediated plant was crystalline condition. The XRD pattern of Zinc oxide nanoparticles showed diffraction peaks agreeing to (110), (123), (111) and (231) respectively. These peaks representing the crystalline nature of our synthesized Zinc oxide nanoparticles. In the present investigation the occurrence

of additional intense peaks at 2Θ angle for Zinc oxide nanoparticles mediated of *C. colocynthis* (ZnNOP-Cc) is due to the immersion of biomolecules present in the stem extract which reacted with Zinc nitrate solution during the ZnONP synthesis as stated by Sandhiya, *et al*[20].

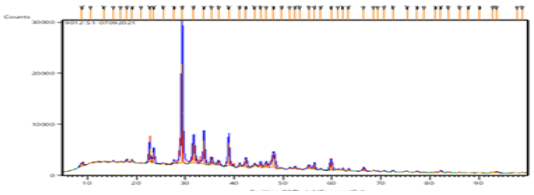

Figure 4. XRD Analysis of ZnONP-Cc.

3.5. SEM and TEM Electron Microscopic Analysis of ZnONP-Cc

The scanning electron microscope image exhibits the well dispersed nature of synthesized ZnNOP-Cc particles. It also shows the particle size at the range of 10 – 25 nm. The size of the particles was observed in the range of 10 - 25nm. The Figures 5(a) and 5(b) shows the biosynthesized zinc oxide nanoparticles mediated *C. colocynthis* stem which were found mostly rectangular shape. As per Naseer, *et al.,*[21] the presence of some secondary metabolites in the *C. colocynthis* is likelihood to cover or decrease the size of metal nanoparticles. The same effect has been observed in our present investigation where the mean size of the nanoparticles ranges from 10 – 25 nm indicating that our stem extract was quite efficient in transforming Zinc Oxide solution into nanoparticles. Previously similar result was found by Degefa, *et al.,*[22] in Zinc Oxide nanoparticle synthesized using pomegranate (*Punica granatum*) leaf extract. With the help of TEM analysis the shape and distributions of nanoparticles were clearly analysed. Figures 5(a) and 5(b) exhibits the TEM images of Zinc Oxide nanoparticles synthesized by using *C. colocynthis* stem. The shapes of the nanoparticles were clearly observed and majority of the particles seem to be spherical with occasional round particles.

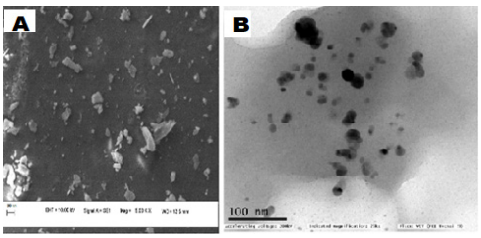

Figure 5. **(a).** SEM image of ZnONP-Cc. **(b).** TEM image of ZnONP-Cc.

3.6 Antimicrobial Activity of ZnONP-Cc

Chaudhary, *et al.,*[23] states that biosynthesized Zinc Oxide nanoparticles possess great antimicrobial activities. The antibacterial activity of synthesized Zinc Oxide nanoparticles reinforced with *Citrullus colocynthis* stem extract was performed using the following microorganisms isolated from wounds such as *Staphylococcus mutans, Staphylococcus aureus* (Gram positive bacteria) and *Enterococcus faecalis,* (Gram negative bacteria) at different concentration (Figure 6). Among the four samples, significant antimicrobial activity was observed for *S. mutans* (30 mm) when compared to *E. faecalis* (25 mm) and *S. aureus* (27 mm). The researchers Keshari, *et al.,*[24] has also performed the similar study using *C. colocynthis* stem mediated silver nanoparticles (AgNp-Cc) and reported maximum zone of inhibition for the microbes, indicating the efficiency of our synthesized zinc oxide nanoparticles mediated *C. colocynthis* has excellent antimicrobial activity against the pathogens isolated from wounds (Figure 7). Among the above pathogens, gram positive bacteria presented higher zone of inhibition compared to gram negative bacteria. Similar results were reported by Guntur, *et al.,*[25] for the Zinc Oxide nanoparticles synthesized using *Lippi adenosis.* This is attributed to the tendency of Zinc Oxide nanoparticles to adhere on the bacterial cell membranes which are rich in sulphur, containing protein which makes the nanoparticles easily to enter into the cell wall and causing damage to the bacterial cells. According to the authors Sorna Prema Rajendran and Kandasamy Sengodan[26], the efficiency of ZnONP adhesion may differ on the cell wall composition of different bacterial species.

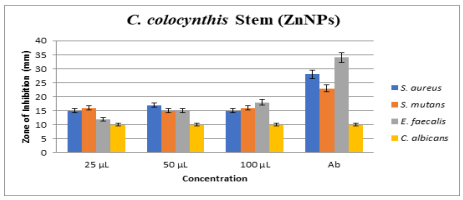

Figure 6. Antimicrobial activity of ZnONP-Cc against wound pathogens.

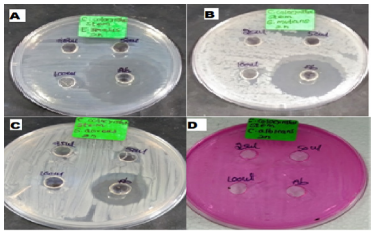

Figure 7. Antibacterial activity of ZnONP-Cc against wound pathogens.

3.7 Cytotoxicity activity of ZnONP-Cc

The toxic nature of any compound may be studied by using the Brine shrimp lethality assay since it is a useful assay to evaluate the cytotoxic nature of any compound to cells. In the present study, to analyse the toxic nature of ZnONP-Cc, Brine shrimp nauplii were used and Figures 8 and 9 show the nature of Zinc Oxide nanoparticle that are synthesized from *C. colocynthis* stem (ZnONP-Cc). In the present investigation, it is observed at all concentrations, understudy 100% the nauplii were alive indicating that the synthesized nanoparticles are nontoxic in nature. Similar findings were reported by Chithralekha, *et al.*,[27] using *Aloe vera* and *neem* herbal formulations assisted silver nanoparticles.

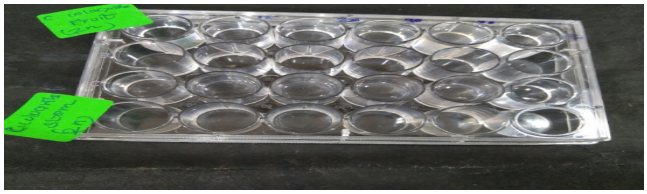

Figure 8. Cytotoxic activity of ZnONP-Cc on Brine shrimp nauplii.

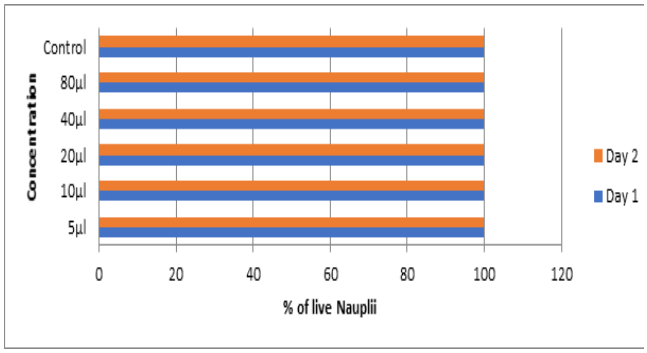

Figure 9. Cytotoxicity activity ZnONP-Cc on Brine shrimp nauplii.

4. Conclusion

The present investigation explains the rapid and green synthesis of Zinc Oxide nanoparticles loaded with *C. colocynthis* stem extract and their characterization by UV-spectroscopy, XRD, FT-IR, SEM and TEM. The biosynthesized ZnONP-Cc showed potential antimicrobial activity against wound pathogens and exhibited less toxicity in brine shrimp nauplii. Hence, our finding confirmed that the synthesized Zinc Oxide nanoparticles can be further investigated for various pharmacological activities.

5. Acknowledgement

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6. References

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