Plant Archives Volume 20 No. 1, 2020 pp. 1568-1572 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING FRUIT EXTRACT OF *CUPRESSUS SEMPERVIRENS* AND ANALYSIS OF THEIR ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL

Bhawna Pawar and Devendra Singh Negi*

Department of Chemistry, Hemvati Nandan Bahuguna Garhwal (A Central) University, Srinagar-246174, India.

Abstract

The synthesis of metal nanoparticles by chemical methods commonly involved toxic chemicals so it is crucial to develop eco-friendly methods for the benefit of environment. The present study involves eco friendly green synthesis of ZnO nanoparticles using aqueous fruit extract of *Cupressus sempervirens* without using any external reducing or capping agent. (The structural and morphological properties have been characterized via XRD, SEM, EDX and TEM analysis). Using XRD, SEM, EDX, and TEM techniques we performed morphological and structural analysis of synthesized nanoparticles. The XRD patterns indicated the formation of crystalline Zinc Oxide nanoparticles. Further using XRD obtained hexagonal wurtzite geometry with an average size 27.10nm of synthesized nanoparticles. The spherical nature of synthesized nanoparticles was confirmed by SEM and TEM analysis. The synthesized nanoparticles showed considerable antioxidant activities.

Key words : *Cupressus sempervirens*, Zinc oxide nanoparticles, Antimicrobial and antioxidant potential.

Introduction

The development of innovative green synthesis methods for metal nanoparticles are key to sustainable advancements in nanotechnology by efficient use of natural resources. Using a green chemistry approach for the synthesis of nanoparticles is competitively and environmentally a better solution than using harmful chemicals as reducing agents. Therefore, for the development of nanoparticles various biological organisms such as yeast, algae, bacteria, mold and plant extracts are used (Kaviya *et al.,* 2011). Generally the synthesis of nanoparticles using methods involving plants and its extracts are preferred over biological synthesis processes as they includes complicated process of maintaining microbial cultures (Sastry *et al.,* 2003). Application of green nanomaterials has importance in medicinal and technological aspects (Mondal *et al.,* 2011; Begum *et al.,* 2009). Metabolites and metal nanoparticles produced by plant based products are generated at a much faster rate and are much more stable than ones synthesized using microorganisms.

***Author for correspondence :** E-mail: devendra_negi@yahoo.com

Different kinds of metal nanoparticles such as Ag, Au, Fe, Pd, Ru, PbS, CdS, CuO, CeO_2, TiO_2, and ZnO are synthesized by using plant materials. Among these, ZnO-NPs are used in the reduction of toxic chemicals from the resources of water such as sulfur and arsenic. This is achieved due to very large surface area to volume ratio (Bhumi *et al.,* 2014). They have large number of applications in very wide field of science such as bio-molecular detection, diagnostic, drug delivery, micro-electronics, antimicrobial and antioxidant activity etc (Santoshkumar *et al.,* 2017). There is no use of adding any stabilizing and capping agent from outside because reducing agent and other constituents present in the plant cell provides stabilizing and capping effect (Srikar *et al.,* 2016).

Cupressus sempervirens belongs to the family *cupressaceae*, comprising twelve species, is native to the North America, the eastern Mediterranean region and subtropical Asia at high altitudes (Rawat *et al.,* 2010). It is an evergreen monoecious tree, with a height of 20-30 m. A wide range of pharmacological activities including antiseptic, aroma therapeutic, astringent, balsamic antiinflammatory activities, antispasmodic, astringent, deodorant, and diuretic effects from the various part of the plant have been showed (Khan *et al.*, 2017).

In the area of nanotechnology Bhawna *et al.*, reported the silver nanoparticles synthesis via green approach using *Cupressus sempervirens* fruit extract with their antibacterial properties.

In this article we reported the synthesis of zinc oxide nanoparticles via green approach using fruit extract of *Cupressus sempervirens* with their antioxidant activity. To the best of our knowledge, in this study the synthesis of zinc oxide nanoparticles via green approach using fruit extract of *C. sempervirens* is being firstly reported.

Materials and Methods

Materials

Fresh fruits of *C. sempervirens* were aquired from Dehradun, Uttarakhand. After washing with distilled water, drying and then grinded to form powder. 10 gm of powdered fruit added in 100 ml of distilled water and heated at 60-70^0 C. then it was filtered with filter paper. Zinc nitrate hexahydrate with purity 99% is purchased from Sigma-Aldrich and NaOH from Fisher scientific.

Preparation of ZnO-NPs

20 ml of plant extract is heated at 60^0 C for 10 min under magnetic stirring and after that 50 ml solution of Zn $(NO_3)_2$.6H2O (91 Mm) in double distilled water was added drop to drop to the plant extract. The mixture is heated and continuously stirred for 15 to 20 minutes and added the few drops of NaOH for retain the PH of solution. The reaction mixture is centrifuged at 3500 rpm for precipitation and then pale white precipitate is obtained. It allowed drying at room temperature. After drying the precipitate pale white nanoparticles are washed with methanol to eliminate the unreacted material and used for further characterization (Bala *et al.*, 2015; Ghorbani *et al.*, 2015).

Characterization

For XRD analysis, X-Ray diffractometer (Shimadzu XRD-6000/6100 models) of wavelength (λ) 1.54 A^0 at 2 θ angles with CuK radians used for obtaining the crystalline size and purity of synthesized ZnO NPs.

The morphology of the ZnO-NPs was shown by scanning electron microscopy (SEM, ZEISS EVO 18) followed by EDX for the determination of elemental composition. Further, from Transmission electron microscopy (TEM, Tecnai, by Tecnai G2 F30 S-Twin (FEI) machine, operated at an accelerated voltage of 300 kV) analysis it has been confirmed the synthesized nanoparticles are in nano dimension range.

Antioxidant activity

Antioxidant activity of synthesized ZnO nanoparticles was done by DPPH free radical Scavenging method. The detail procedure for DPPH Free Radical Scavenging Activity (Mir *et al.*, 2016) is given below:

Preparation of stock solution of the sample

100 mg of sample was dissolved in 100 ml of DMSO to get 1000 μg/ml solution. Dilution of test solution: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μg/ml solution of test were prepared from stock solution.

Preparation of DPPH solution

15 mg for DPPH was dissolved in 10 ml of methanol. The resulting solution was covered with aluminium foil to protect from light.

Estimation of DPPH scavenging activity

75 μl of DPPH solution was taken and the final volume was adjusted to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 75 μl of DPPH and 100 μl of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Absorbance at zero time was taken in UV-Visible at 517 nm for each concentration. Final

Decrease in absorbance of DPPH with sample of different concentration was measured after 15 minute at 517 nm. Percentage inhibitions of DPPH radical by test compound were determined by the following formula.

% Reduction = Control absorbance – Test absorbance/ Control absorbance x 100

Antibacterial activity

The antibacterial activity was assessed by agar well diffusion method. The antibacterial activity of ZnO nanoparticles was determined against the human pathogenic bacteria *viz. B. cereus, K. pneumonia, P. vulgaris*, and *S. aurens* using a Muller-Hinton agar culture medium (Hi-media). The standard cultures were inoculated 108 CFU/ml in petri dishes with MH agar medium and then paper disks of 8mm diameter were laid on the inoculated standard culture, which was instilled with nanoparticles neat solution in DMSO. Petri dishes were incubated at 37^0 C for 24 hrs. Antimicrobial activity was determined by measuring the zone of inhibition around the disk.

Results and Discussion

X-Ray diffraction analysis

The XRD pattern of ZnO-NPs from fruit extract of

C. sempervirens has been shown in fig. 1. The crystalline size and nature of zinc oxide nanoparticles are clearly indicated by the intense diffraction peaks. The diffraction peaks appear at 2θ of 31.77, 34.41, 36.26, 47.53, 56.59, 62.81, 67.94, and 68.13 (deg). The peak intensity profiles were established the hexagonal wurtzite structure. The average size of crystallite was calculated by using Scherer's equation

$$D = K\lambda / \beta\cos\theta$$

Where D = average crystallite size, K= shape factor, λ= wavelength of X-Ray, θ= Bragg angle,

β= Full width at half maxima in radian (FWHM)

The average powder particle size was found to be 27.10 nm.

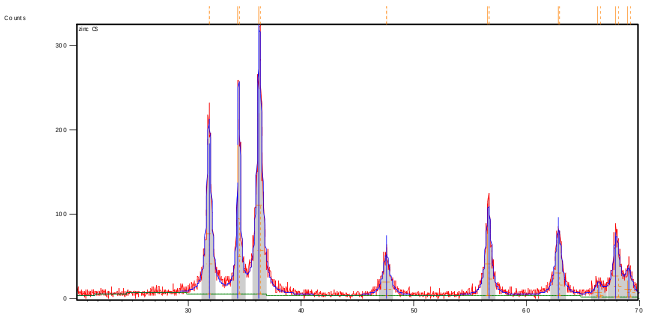


Fig. 1: XRD pattern of ZnO-NPs.

SEM with EDX analysis

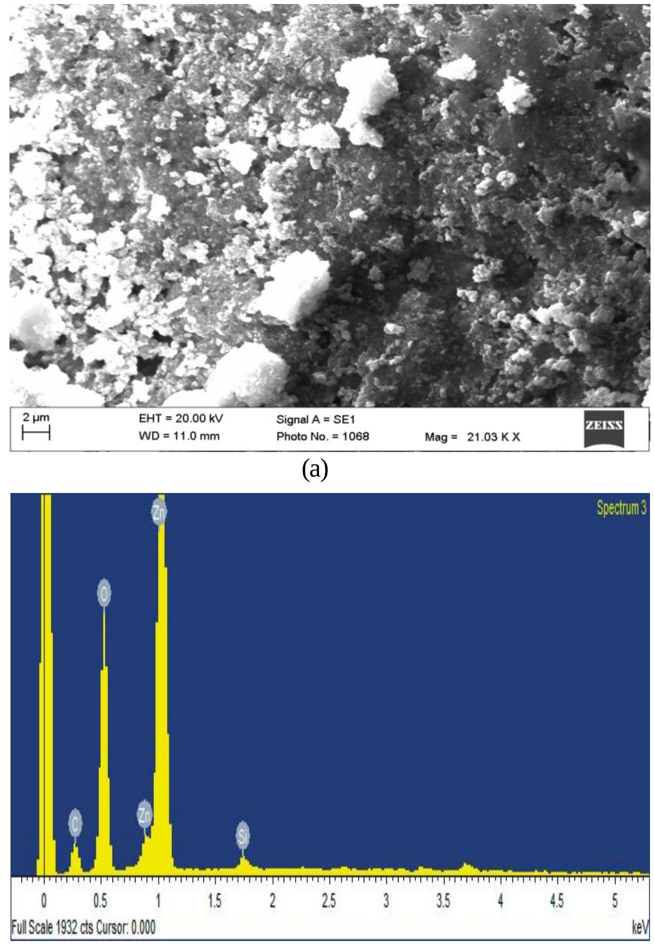
The shape and morphology of synthesized ZnO-NPs was confirmed by SEM image as show in Fig. 2a, the image indicate that ZnO-NPs have nano sized range with nearly spherical shape particles. The presence of elemental Zn and oxygen signals of ZnO-NPs is confirmed via Energy dispersive X-ray analysis as shown in Fig. 2b.

TEM analysis

The shape and average size of bioreduced ZnO-NPs further confirmed with the help of TEM analysis. HRTEM images of ZnO-NPs resulting from the extract of *C. sempervirens* are shown in Fig. 3. TEM images of ZnO-NPs confirms the spherical shape of nanoparticles with most clearly apparent average size of 28.14 nm which is very close to the particle size from XRD analysis. And approximately the synthesized ZnO-NPs are homogeneous in nature.

Antioxidant activity

Antioxidant activity of silver nanoparticles was analyzed by DPPH scavenging assay with ascorbic acid as a standard or positive control and also observed in another study (Gomma, 2017). As the concentration of ZnO nanoparticles was increased, the antioxidant activity tested by DPPH was also found to be increase. ZnO nanoparticles from fruit of *C. sempervirens* at the concentration of 10µg/ml showed 31.01% reduction. On parallel examination for standard compound Ascorbic acid it was found to be 40.47 %. However, upon increasing the concentration up to 100µg/ml, the free radical scavenging potential was also increased. ZnO nanoparticles from fruit of *C. sempervirens* and standard ascorbic acid at 100µg/ml showed percent inhibition of 81.6 % and 75.36 % respectively. This noticeably indicates that antioxidant activity is concentration dependent. The efficiency of the antioxidant activity can be expressed by evaluated IC_{50} value. The IC_{50} value is the concentration (µg/ml) of extract/standard necessary to reduce the absorbance of DPPH by 50% compared to the control. The IC_{50} value decreases as a function of increasing antioxidant activity of samples. These IC_{50} values can be calculated from absorbance value. The IC_{50} value of standard ascorbic acid was found to be 28.7µg/ml shown in Fig. 4b, while that of nanoparticles was observed 33.8µg/ml Fig. 4a which was very near to the IC_{50} Value of standard.



(a)

(b)

Fig. 2: (a) SEM image of ZnO-NPs (b) EDX spectrum.

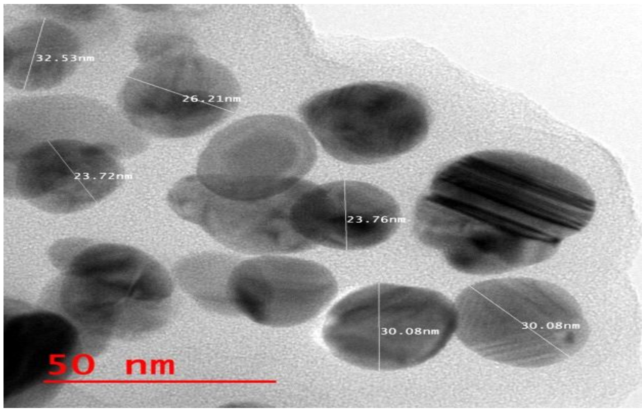
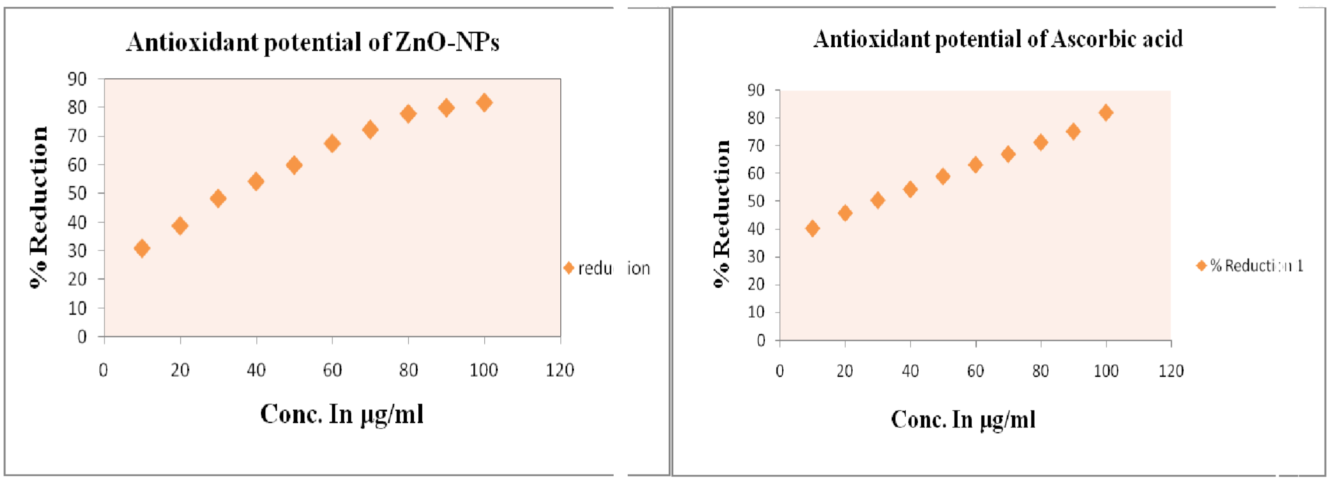


Fig. 3: TEM images of synthesized ZnO-NPs.

ZnO-NPs against *B. cereus* as compared to streptomycin and also has considerable effect against other two bacterial strains.

Conclusion

Green synthesis of silver nanoparticles is advantageous over other methods of synthesizing nanoparticles. In the present study ZnO nanoparticles are synthesized using *C. sempervirens* fruit extract without using synthetic reagents. Here we investigated an eco-friendly, nontoxic, cost effective and convenient green method for the synthesis ZnO NPs. The synthesized nanoparticles were characterized by different methods,



(a) (b)

Fig. 4: (a) Antioxidant activity of synthesized ZnO-NPs, (b) Antioxidant activity of DPPH.

Table 1: Zone of inhibition against bacteria pathogens of synthesized ZnO-NPs.

S.No.	Organism 100 µg/ml	Zone of inhibition (mm)	Positive control (Streptomycin-25µg/ml)
1	*B. cereus*	25	23
2	*K. pneumonia*	-	19
3	*P. vulgaris*	12	30
4	*S. aurens*	22	27

Antimicrobial activity

The antimicrobial activity of zinc oxide nanoparticles synthesized by fruit extract of *C. semoervirens* was investigated against bacterial pathogens such as *B. cereus*, *K. pneumonia*, *P. vulgaris*, and *S. aurens* using well diffusion method. The diameter of zone of inhibition (mm) with synthesized ZnO nanoparticles using fruit extract is represented in (Table 1). The zone of inhibition of zinc oxide nanoparticles have been found against *B. cereus* (25 mm), *P.vulgaris* (12 mm) and *S. aureus* (22 mm) and no activity has been found against *K. pneumonia*. The zone of inhibition is more for synthesized

XRD, SEM and TEM. The observations suggest that nanoparticles are crystalline, and spherical in shape with 27.1 nm size. These green synthesized nanoparticles exhibited excellent antioxidant property and antibacterial activity against three bacterial strains out of four strains. This green easy and inexpensive method can be used as alternative to chemical methods for the synthesis of ZnO nanoparticles.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

References

Begum, N.A., S. Mondal, S. Basu, R.A. Laskar and D. Mandal (2009). Biogenic synthesis of Au and Ag nanoparticles using aqueous solutions of Black Tea leaf extracts. *Colloids. Surf. B. Biointerfaces.*, **71:** 113-8.

Bhumi, G. and N. Savithramma (2014). Biological Synthesis of Zinc oxide Nanoparticles from Catharanthus roseus (l.) G. Don. Leaf extract and validation for antibacterial activity. *Int. J. Drug Dev. Res.*, **6 :** 208-214.

Ghorbani, H.R., F.P. Mehr, H. Pazoki and B.M. Rehmani (2015). Synthesis of ZnO Nanoparticles by Precipitation Method. *Orient. J. Chem.*, **31 :** 1219-1221.

Gomaa, E.Z. (2017). Antimicrobial, antioxidant and antitumor activities of silver nanoparticles synthesized by Allium cepa extract: A green approach. *J. Genet. Eng. Biotechnol*., **15 :** 49-57.

Kaviya, S., J. Santhanalakshmi, B. Viswanathan, J. Muthumary, and K. Srinivasan (2011). Biosynthesis of silver nanoparticles using citrus sinensis peel extract and its antibacterial activity. *Spectrochim. Acta A: Mol. Biomol. Spectrosc.*, **79 :** 594-98.

Khan, M.F., T. Ahamad and P. Rawat (2017). Biomedicinal and Chemical Profile of *Cupressus sempervirens*: A Mini Review. *Insights Biomed*., **2 :** 16.

Mir, M.A., S. Manzoor, R. Nazir, R. Farooq and B. Ahmad (2016). Food Value Analysis and Antioxidant Properties of Various Extracts of Rheum emodii. *Imp. J. Interdiscip. Res.*, **2 :** 1468-73.

Mondal, S., N. Roy, R.A. Laskar, I. Sk, S. Basu, D. Mandal and N.A. Begum (2011). Biogenic synthesis of Ag, Au and bimetallic Au/Ag alloy nanoparticles using aqueous extract of mahogany (Swietenia mahogani JACQ.) leaves. *Colloids. Surf.B. Biointerfaces.*, **82 :** 497-504.

Niranjan, B., S. Saha, M. Chakraborty, M. Maiti, S. Das, R. Basub and P. Nandy (2015). Green synthesis of zinc oxide nanoparticles using *Hibiscus subdariffa* leaf extract: effect of temperature on synthesis, anti-bacterial activity and anti-diabetic activity. *The Royal Society of Chemistry*, **5 :** 4993-5003.

Pawar, B. and D.S. Negi (2017). Synthesis of silver nanoparticles via green approach using fruit extract of *Cupressus sempervirens* L. and their antimicrobial evaluation. *Int. J. Pharm. Bio. Sci.*, **8 :** 156-162.

Rawat, P., M.F. Khan, M. Kumar, A.K. Tamarkar, A.K. Srivastava, K.R. Arya and R. Maurya (2010). Constituents from fruits of Cupressus sempervirens. *Fitoterapia*, **81:** 162-6.

Santhoshkumar, J., S.V. Kumar and S. Rajeshkumar (2017). Synthesis of zinc oxide nanoparticles using plant leaf extract against urinary tract infection pathogen. *Resource-Efficient Technologies*, **3 :** 459-465.

Sastry, M., A. Ahmad, M.I. Khan and R. Kumar (2003). *Biosynthesis of metal nanoparticles using fungi and actinomycete*. *Curr. Sci.*, **85 :** 162-170.

Srikar, S.K., D.D. Giri, D.B. Pal, P.K. Mishra and S.N. Upadhyay (2016). Green Synthesis of Silver Nanoparticles: A Review. *Green and Sustainable Chemistry*, **6 :** 34-56.