Research Article

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Investigation on *Trifolium prantese* Capped Zinc oxide Nanoparticles for Cancer Applications

K. Sumithra, V. Ramya*, V. Kalaiselvi, N. Vidhya, K. Surya
Department of Physics, Navarasam Arts and Science College for Women, Erode, TN, India
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*ramyathinakar@gmail.com

ABSTRACT

In the present study, zinc oxide nanoparticles which are mostly applied in the fields of medicine, electronic devices, biosensors and anti-microbial agents. were synthesized with and without *Trifolium prantese* flower extract by Sol-gel-assisted microwave irradiation method. The synthesized nanoparticles were characterized with various techniques such as XRD, FTIR, SEM and EDAX. The formation of zinc oxide nanoparticles has been characterized by X-ray Diffraction (XRD). The elemental composition of the prepared samples was analyzed using Energy Dispersive X-ray analysis (EDAX). The surface morphological structure of zinc oxide nanoparticles was obtained using Scanning Electron Microscope (SEM). The functional groups were investigated through Fourier Transform Infrared spectroscopy (FTIR).

Keywords: Green Synthesis; Microwave irradiation method; *Trifolium prantese*; Zinc oxide.

1. INTRODUCTION

Nanotechnology is increasingly gaining its application in the fields of medicine, agriculture, physics, etc. The synthesis of nanoparticles (NPs) for the development of new smart materials with unusual properties at the nanoscale has increased dramatically in recent years (Albrecht *et al*. 2006; Singh *et al*. 2016). Metal NP synthesis is a common topic in nanoscience these days. Several scientific groups have been interested in metal NPs such as iron oxide, silver nitrate, copper oxide and zinc oxide over the last few decades (Rouhi *et al*. 2013; Tiwari *et al*. 2018). Metal NPs are synthesized using a variety of processes, including the sol-gel process, thermal decomposition, hydrothermal and microwave irradiation, among many others (Kolekar *et al*. 2013). However, owing to the production of a large volume of secondary waste materials as a result of the addition of chemical agents for the reduction process, these chemical and physical synthesis approaches are time-consuming, expensive and harmful.

As a result, biological NP synthesis would be a viable alternative for reducing toxicity, cost and time. In comparison to physical and chemical approaches, biological synthesis of NPs from plant extracts has been identified as a superior option. Biologically synthesized NPs are biocompatible and non-toxic, and they are used as drug carriers and fillers in medical products (Rosi and Mirkin, 2005). Several studies were done based on green synthesis of ZnO NPs of various plants extract exist, such as *Cassia tora* L. (Manokari and Shekhawat, 2017), *Sageretia thea* (Khalil *et al.* 2017), *Calotropis gigantean* (Chaudhuri and Malodia, 2017), *Azadirachat indica* (Bhuyan *et al.* 2015), *Hibiscus rosa-sinensis* (Bala *et al.* 2015), *Ocimum basilicum* L. *var. Purpurascens* (Bi *et al.* 2017), *Corymbia citriodora* (Zheng *et al*. 2015), *Zingiber officinale* (Raj and Jayalakshmy, 2015) and *Anisochilus carnosus* (Anbuvannan *et al.* 2015).

Trifolium prantese, also known as red clover, is a flowering plant of Indian origin which belongs to the Fabaceae tribe. It is a well-known medicinal plant with a high concentration of secondary metabolites, with over 200 terpenoid-based indole alkaloids found in different plant parts such as leaf, stem, vine and root. These alkaloids are responsible for anticancer, astringent, antibacterial, anti-diabetic, anti-fungal and anti-malarial effects, among others. (Noble, 1990). The alkaloid content of *C. roseus* varies considerably in various parts, the maximum being in the root bark, which ranges from 0.15 to 1.34% and even up to 1.79% in some strains. The plant contains about 130 alkaloids of the indole group, out of which 25 are dimeric. Two of the dimeric alkaloids vinblastine and vincristine, mainly present in the aerial parts, have found extensive application in the treatment of human neoplasma. The reports published on the green synthesis of ZnO NPs using flower extract of *T. prantese* are very few (Bhumi and Savithramma, 2014; Kalaiselvi *et al.* 2016). Green ZnO NPs were synthesized, characterized, and their antimicrobial activities were assessed in this report. During the research, the synthesized NPs exhibited a synergistic interaction with pre-existing antibiotics.

T. prantese flower extract was used in this analysis to biosynthesize ZnO NPs under various physical conditions. SEM (Scanning Electron Microscopy), EDAX (Energy Dispersive X-ray Analysis), FTIR (Fourier Transform Infrared Spectroscopy), and XRD were used to classify the synthesized ZnO NPs (X-Ray Diffraction).

2. MATERIALS & METHODS

2.1 Green Synthesis of ZnO

Zinc can be found in ocean water, and the air and sodium hydroxide (NaOH) were received from India. *Trifolium prantese* flower (Fig. 1) was collected from the surrounding area and washed several times using running tap water and then again washed double distilled water to remove dust particle and then dried to remove residual moisture. 50 g of the flower was mixed with 150 ml of distilled water and boiled at 75 ºC for 30 minutes. The extract was filtered using Whatmann No. 1 filter paper to get a clear solution. In this method, 10.10 g of zinc acetate was dissolved with 100 ml of distilled water, and it was stirred for about 30 minutes, then, 10 ml of flower extract was added drop-wise into the above solution. A pale green color solution was obtained. The mixture was stirred for 45 minutes. During this process, the NaOH solution was added drop-wise to maintain the pH at 12. The gelatinous precipitate was continuously stirred for 1 hour. It was aged for 24 hours at room temperature. Then the precipitate was washed once with distilled water to remove impurities. To attain the minimum time consumption microwave oven was used to dry the precipitate at 70 W for 35 minutes. Finally, the dried sample was ground using mortar to get *Trifolium prantese* flower-capped ZnO (GZnO) nanoparticles. Further, zinc acetate with no flower extract was also synthesized (PZnO).

2.2 Characterization Techniques

2.2.1 FTIR

The functional groups of prepared samples were identified using Fourier transform spectroscopy analysis. The spectrum was recorded in 4000 - 400 cm^{-1} region.

2.2.2 XRD

The prepared samples were analyzed using XRD (X-ray Diffraction) technique. This XRD patterns predicted the lattice parameters (a and c), unit cell volume and crystalline size of the samples. The XRD pattern of prepared samples well-matched with JCPDS Card No.: 09-0432 (corresponding to hexagonal phase). The lattice parameters of the samples were calculated using the equation:

$$1/d^2 = (4(h^2 + hk + k^2) / 3a^2) + (1^2/c^2)$$

where, d is the spacing between the planes and a and c are the lattice parameters. The unit cell volume (V) of the samples were found using the equation:

$$V= (\sqrt{3}/2) + a^2 + c^2$$

The average crystalline size of the sample was determined by using Debye-Scherrer's formula.

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

where, D denotes the average crystalline size of the sample, K represents the broadening constant, λ denotes the wavelength of CuKλ radiation source (1.54Å), β represents the full width at half-maximum and θ denotes the angle of diffraction.

Fig. 1: *Trifolium prantese* flower.

2.2.3 SEM & EDAX

The surface morphologies of synthesized ZnO samples were analyzed using Scanning Electron Microscopic analysis (SEM). Energy Dispersive spectroscopy was used to identify the elemental compositions of the samples.

3. RESULTS

3.1 XRD Analysis

The most prominent peaks of the ZnO samples were observed, as illustrated in Fig. 2. The XRD pattern of wurtzite hexagonal structure of ZnO nanoparticles has been verified with JCPDS file number: 36-1451 and it was well-matched. The lattice constants a and c and unit cell volume (V) were calculated using the standard equation. For PZnO the average crystalline size was 18.67 nm and for GZnO it was 15.58 nm, as shown in Table 1. It was evident that the capping of flower extract enhanced the property by decreasing the crystalline size in the sample. The diffraction peaks of the prepared PZnO and GZnO occurred at 2θ values, corresponding to (101), (103), (112) and (201) hkl planes, respectively.

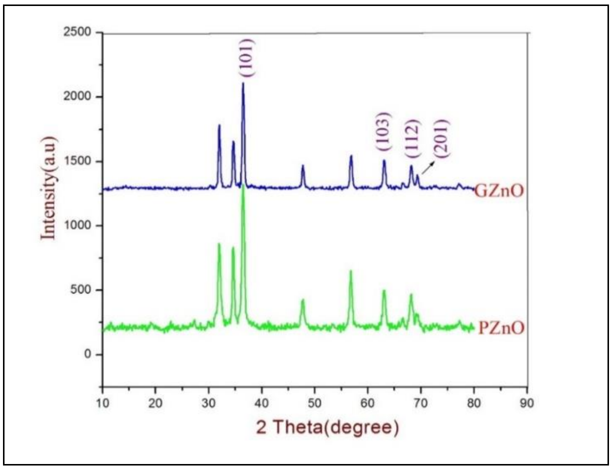

Fig. 2: Synthesized sample with XRD pattern for PZnO and GZnO.

Table 1. XRD patterns of PZnO and GZnO nanoparticles.

Sample	2θ (deg)	FWHM (deg)	d (Å)	Intensity (Counts)	Crystalline size (nm)	Average Crystalline size	hkl	Lattice constants a= b	Lattice constants c	Unit cell volume (V)
PZnO	36.33	0.4703	2.46	1195	17.78	18.67	101	3.22	5.20	PZnO
	63.03	0.5020	1.47	358	18.86		103			
	68.12	0.5205	1.37	292	18.42		112			
	69.29	0.4849	1.35	163	19.91		201			
GZnO	36.42	0.5609	2.46	739	17.57	15.58	101	3.22	5.23	46.51
	63.00	0.5807	1.47	210	14.42		103			48.39
	68.11	0.6641	1.37	177	16.09		112			46.51
	69.24	0.6759	1.35	84	14.27		201			46.80

3.2 FTIR Analysis

FTIR spectrums of the prepared ZnO samples were recognized at a wavelength range of 4000-400 cm^{-1} (Table 2). The observed peak resulted from the chemical synthesis method was at 3861.49 to 879.54 cm^{-1}, whereas from the green synthesis method, the peak was observed at 3960.65 to 570.92 cm^{-1}. The vibrations of a variety of groups were present at different wavenumbers of IR radiation. The broad peak was absorbed at 3861.49 cm^{-1} and 3960.65 cm^{-1} (alcohol), corresponding to O-H stretching band. C-H stretching was confirmed by the absorption peak of 2800.64 and 2809.44 cm^{-1} (Alkynes). N=O stretching was evident from the absorption peaks at 1450.47 cm^{-1} and 1450 cm^{-1} (nitro). The FTIR spectrum peaks at 3589.53 cm^{-1} and 3446.81 cm^{-1} were calculated with the stretching vibrations of N-H (amine) bond. Introducing a capping agent has created a minor change in the functional group analysis of the samples. The spectrum (Fig. 3) reveals the FTIR graph of PZnO and GZnO.

3.3 SEM AND EDAX

SEM analysis was performed to determine the shape and morphology of ZnO nanoparticles under different magnifications. Fig. 4 illustrated morphological descriptions and elemental composition of PZnO and GZnO, which have shown needle-shaped morphology for PZnO and spherical-shaped morphology for GZnO. The average grain size was found to be around approx. 27 to 42 nm for PZnO .and 70 to 84 nm for GZnO.

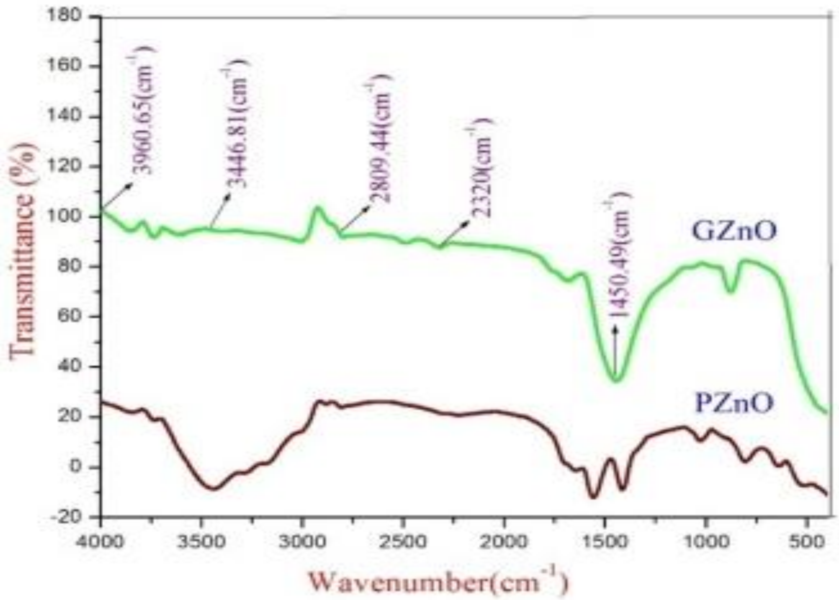

Fig 3: The FTIR spectra of PZnO and GZnO.

Table 2. Functional groups of PZnO and GZnO.

S. No.	Sample	Wave Number, cm^{-1}						
		O-H Stretching Vibration (free)	O-H Stretching Vibration (bonded)	C-H Stretching Vibration	N=O Stretching Vibration	N-H Stretching Vibration	O-H Stretching Vibration (free)	O-H Stretching Vibration (bonded)
1	GZnO	2320	3960.65	2809.44	1450.49	3446.81	2320	3960.65
2	PZnO	2318.44	3861.49	2800.64	1450.47	3589.53	2318.44	3861.49

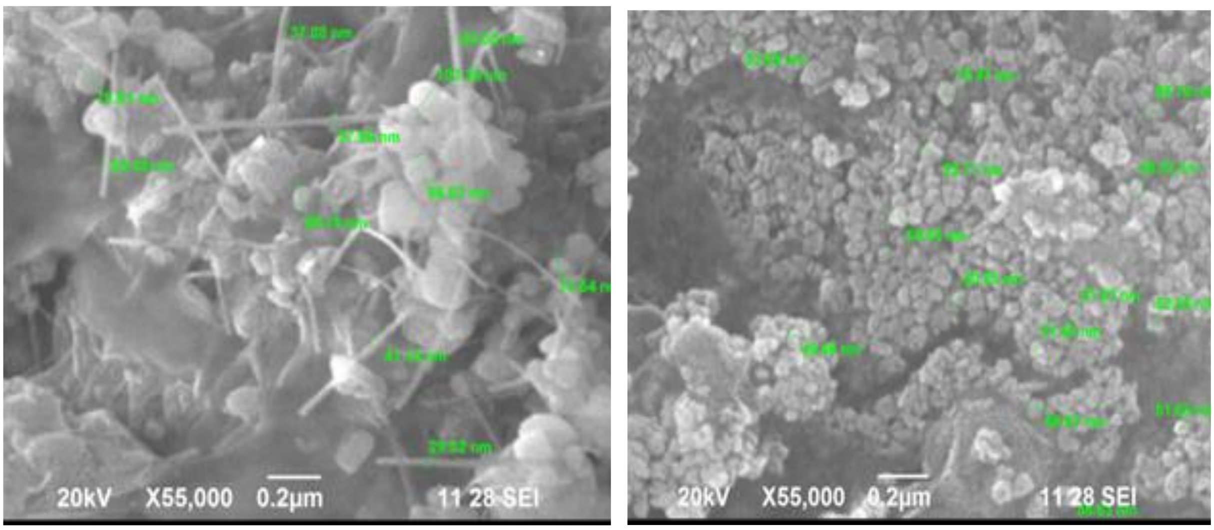

Fig. 4: SEM performances of: (a) PZnO and (b) GZnO.

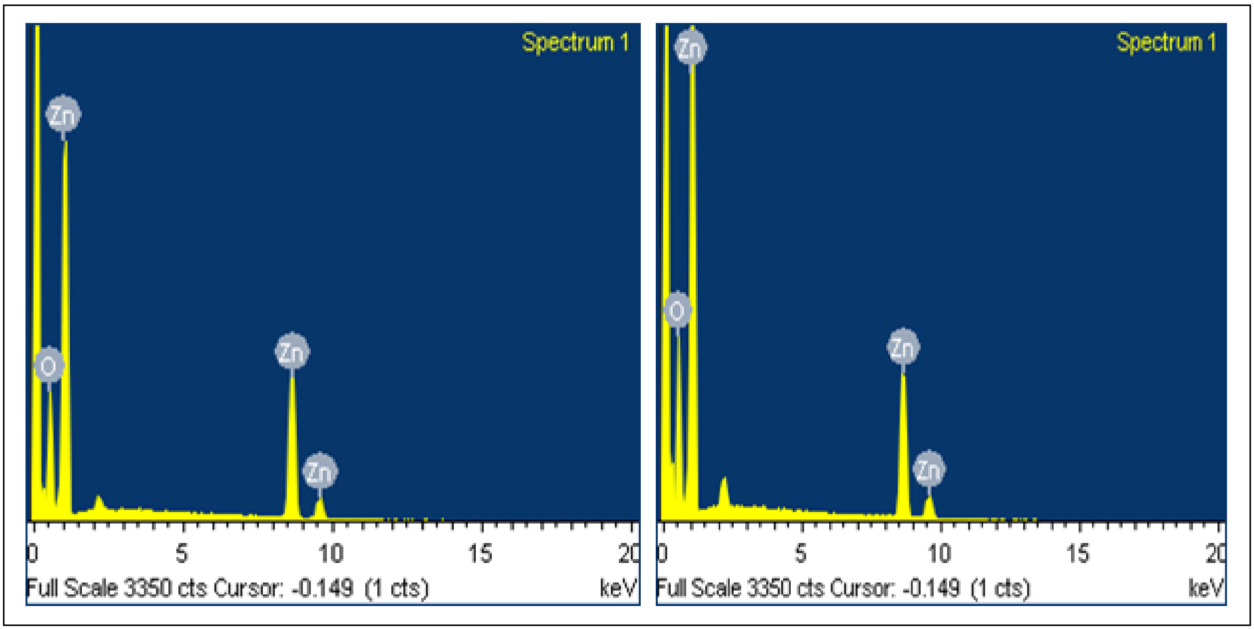

Fig. 5: EDAX performance of: (a) PZnO and (b) GZnO.

The Energy Dispersive X-Ray Spectroscopy was used to investigate the elemental composition and chemical analysis of PZnO and GZnO. The analysis observed Zn (Zinc) and O (Oxygen) for the samples (Fig. 5).

4. CONCLUSION

Zinc oxide nanoparticles were synthesized by chemical and green synthesis method. XRD analysis predicted the crystalline size, lattice parameters and unit cell volume of the sample. The morphological structure was revealed by SEM. EDAX analysis revealed the elemental composition of the sample. Based on the results obtained, it was concluded that the synthesized samples can be applied in the field of medicine for cancer treatment and as a water purifier in environmental science.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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