



GREEN SYNTHESIS OF ZNO NANOPARTICLES FROM THE METHANOLIC EXTRACT OF *EMBELIA RIBES* AND THEIR CHARACTERIZATION BY UV-VISIBLE, FTIR, XRD AND SEM ANALYSIS

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ABSTRACT

Green synthesis of nanomaterials has become a prominent branch of nanotechnology. In the current study we have reported a cost effective and ecofriendly process for synthesis of Zinc oxide nanoparticles from *E. ribes* seeds extract. The resultant nanoparticles were characterized using UV-visible spectroscopy, Fourier Transform Infrared Spectroscopy, X-ray diffraction, and Scanning Electron Microscopy. UV-Vis analysis of these microwave assisted ZnO NPs have shown change in color with the time interval of microwave irradiation, which became stable after 8 min of microwave irradiation, hence we can conclude that the reaction was complete. FTIR data reveals the presence of biomolecules which could have reduced the Zn ions to ZnO as well capping and stabilizing agent. Resultant ZnO NPs were found hexagonal in structure, and of dimension 57.57 nm. SEM results found were in accordance with the XRD data obtained from ZnO NPs. Thus, zinc oxide nanoparticles with well defined dimensions could be synthesized from the methanol extract of *E. ribes* seeds.

KEY WORDS

Zinc oxide nanoparticles; green synthesis; *Embelia ribes*; seeds; UV-Vis; FTIR; XRD; SEM

1. INTRODUCTION

Particles of matter with diameters principally ranging from 1 nanometer to 100 nanometers have characteristic properties. Such a particle can be made from a single crystal, an aggregate of crystals, or be completely non-crystalline; it can be a metal, a semiconductor, or an insulator. Innovative techniques are being refined for inquisition of atoms and molecules. Nanoparticles of 10^{-9} m is defined by nanotechnology as structures small enough to behave different as a unit, this property is very useful to interaction with other matters [1].

In our present study we have synthesized ZnO nanoparticles using methanol extract of the seeds of *Embelia ribes*, which is commonly known as false black pepper. It is one of the most significant plants in Indian traditional medicine. Vaibidang is a Sanskrit name of *E. ribes*, which belongs to family Myrsinaceae. It is a large scandent, struggling shrub with a long slender brittle stem, it is a climbing creeper shrub, flexible, and terete branches, bark studded with lenticels. Though different parts of this plant can be used to prepare their extracts, but only seed extract have been investigated and reported extensively in literature. In fact, use of this plant for synthesis of metal nanoparticles is rare, there are very few studies found on biosynthesis of

nanoparticles from *E. ribes*. The only study which is reported in recent years is formation of gold and silver nanoparticles from the seeds of *E. ribes* was conducted by Dhayalan *et al.* [2]. They have studied *invitro* antimicrobial, antioxidant, cytotoxic potential of silver and gold nanoparticles which were synthesized using *E. ribes* seeds. It was reported in this study that the biosynthesized nanoparticles of gold and silver were found to be crystalline, uniform and spherical, when characterized using various techniques with 20–30 nm of average particles size. Both gold and silver nanoparticle showed significant amount of antioxidant property which was evaluated by both DPPH and PMA assay. Both silver and gold nanoparticles can inhibit the growth of gram positive (*S. aureus*) and gram negative (*E. coli*) as both agents have good antibacterial activities. They found to be profoundly effective for gram negative bacteria.

Although we have not found the studies in which synthesis of ZnO was reported from extract of *E. ribes* but synthesis of ZnO from various other plants has been well documented in literature.

Metallic nanoparticle synthesis via green route is charming the scientist over a decade and is now extensively being used in engineering and biomedical sciences. They are of immense interest due to their huge potential. Various processes have been designed by nature for desirable condition for making micro and nano scaled inorganic materials, which are contributing to new evolution of unexplored area of research, which is based on the grounds of biosynthesis of nanomaterials [3]. Bottom up approach have been used for biosynthesis of nanoparticles, in which dominating reactions are reduction/oxidation. The plant phytochemicals or microbial enzymes with reducing properties or anti-oxidant potential is usually consequential for reduction of metal compounds into their respective nanoparticles.

Zinc (Zn) being the foremost nutrient needed for all living organisms represent the 23rd most abundant element on earth [4] and the 2nd most abundant transition metal, successive to iron [5]. Also, ZnO has high optical absorption in UVA (315–400 nm) and UVB (280–315 nm) regions which enables it to have a broad range of applications in cancer therapy, antibacterial and antifungal agent, biosensing, drug/ gene delivery, nanomachines that can act as biological mimetic and biomaterials for tissue engineering, shape memory

polymers can act as molecular switches. Considering these broad range of applications of ZnO nanoparticles, various types of ZnO nanostructures have been manufactured, including nanoparticles, nanorods, nanobelts [6]. Due to ZnO NPs varying morphologies it shows significant antibacterial activity over a wide range of bacteria as reported by various researchers over the globe [7].

ZnO on the other hand has an array of applications and considered as functional, encouraging as well as a versatile inorganic material. ZnO contains unique properties such as piezoelectric properties and chemical sensing, optical semiconducting properties [8]. ZnO is characterized by high excitonic binding energy (60 meV) and of wide band gap (3.3 meV) at room temperature in the near UV spectrum [9]. Its wide band gap bestows some eminent impression on its characteristics, likewise optical absorption and electrical conductivity. ZnO has subtle covalent character, but it shows very sturdy ionic bonding in the Zn–O. The heat resistance property as well as longer durability and higher selectivity antecedent ZnO over other inorganic and organic materials. Oxidative stress mediated cell death has been reported by zinc ions released from ZnO materials [10], [11]. ZnO nanoparticles also have an innate property of selective cytotoxicity against cancer cells when assessed *invitro*. Further surface engineering is required to increase its selective cytotoxicity over a wide range of cancer cells [12]. Zn deficiency might lead to cancer progression.

For the present work we have synthesized the extract from seeds of *E. ribes* utilized it for the reduction of zinc acetate to obtain ZnO nanoparticles.

2. EXPERIMENTAL PROCEDURE

Preparation of extract

Extraction from *E. ribes* seeds is conventionally done by traditional distillation process which requires high amount of energy, as well as a huge amount of solvents. Apart from that, these methods are time consuming. Therefore, we aim towards the use of new 'green technique' for extraction with minimum energy requirement and less use of solvents, additionally less time is required. Hence, in the present work we have used microwave assisted extraction method for getting methanol extract from *E. ribes* seeds. All the reagents used for reaction were of analytical grade and used without further purification. Redistilled deionized water

was used for sample preparation. Seeds of *E. ribes* were purchased from Indore. The seeds were dried in shade at room temperature; ground to a coarse powder in a mechanical blender, a measured amount of methanol was added to the crushed sample. The mixture was kept in microwave for different intervals.

Vidanga (*E. ribes*) is a large scandant, struggling shrub with a long slender brittle stem, it is a climbing creeper shrub, flexible, and has terete branches, bark studded with lenticels. *E. ribes* has a long history of use in ayurvedic system of medicine in various forms like churna, asava, arishta, lauha and taila. Embelin which is main constituent of *E. ribes* is found to be in golden yellow needles, which is soluble in alcohol, chloroform and benzene but insoluble in water. Christembine, quercitol, vilangin and resinoid are few other components [13]. The plant is not only traditionally believed to possess medicinal values but has already been reported scientifically for several activities like Hepatoprotective, Neuroprotective, Antibacterial, Antifungal, Analgesic, Hypoglycemic, Antioxidant, Anticancer, Anthelmintic, Anticonvulsant, Wound healing, Antifertility, Adaptogenic, Cardioprotective etc. The operating frequency of microwave ovens are around 2.45 GHz. Both Electric and magnetic fields are possessed by Microwaves which are perpendicular to each other. Microwave energy is a non-ionizing radiation. This radiation governs magnitude scale from 300 MHz to 300 GHz. The electrical field and magnetic fields are two oscillating perpendicular fields. They can be used both as energy vectors or information carriers. The first application is the direct action of waves on a material which is able to absorb a part of the electromagnetic energy and to transform it into heat [14].

Synthesis of nanoparticles

In the present study, we have applied microwave assisted extraction. Extract was concentrated by Buchi rotavaporator. To 10 ml of this concentrated extract 90 ml of 1mM aqueous solution of Zinc acetate ($\text{Zn}(\text{O}_2\text{CCH}_3)_2$) was added. The reaction mixture is irradiated in microwave at different interval of time from 0 to 8 mins. Color change has been observed with each irradiation which became stable at the end of 8th min suggesting reduction of Zn^{+} ions from zinc acetate to ZnO by the extract. A sufficient amount of pale-white precipitate was observed and it was separated by high speed centrifugation. The separated solid mass was

washed with double distilled water thrice for removing impurities. After complete washing the solid mass was oven dried. The color change occurred was compared with seed extract and 1mM Zinc acetate solution as controls.

3. CHARACTERIZATION OF ZNO NPS

We have employed various methods for the characterization of ZnO NPs, mainly

UV-Vis Spectroscopic Analysis

To investigate the absorption pattern of biosynthesized ZnO NPs, the samples were periodically recorded by UV-Vis spectrophotometer (Perkin Elmer) between 200 and 800 nm using quartz cuvette with aqueous extract as baseline.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectroscopy analysis of biosynthesized ZnO NPs was performed for the identification of functional groups capping. The suspension was completely dried and analyzed by FTIR. The methanolic seed extract was also dried and subjected to FTIR analysis.

X-Ray Diffraction Studies

X-ray diffraction (XRD) measurement of the *E. ribes* reduced zinc oxide nanoparticles was carried out using powder x-ray diffractometer instrument (Bruker D8 advance) at the angle range of 300-800 operated at a voltage of 40kV and a current of 40mA with $\text{CuK}\alpha$ radiation in a θ -2 θ configuration. The crystallite domain size was calculated using Debye- Scherrer formula.

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

Scanning Electron Microscopy (SEM)

The sample was prepared by placing a drop of colloidal solution of zinc oxide nanoparticles on carbon coated copper grid and subsequently dried in air, before transferring it to the microscope operated at an accelerated voltage of 0.5-30 kV (JEOL JSM 5600).

4. RESULT AND DISCUSSION

1. UV-Vis Spectroscopy

UV-Vis spectroscopy analysis was done at 0 min, 2 min, 4 min, 6 min and 8 min of microwave irradiation of the mixture containing zinc acetate ($\text{Zn}(\text{O}_2\text{CCH}_3)_2$) solution and the plant extract. We have seen the change of color with each succeeding microwave irradiation. After 8 min the change in color of the solution was stable (Fig. 1) also the mixture became colloidal showing yellowish white color of precipitation since the ZnO NPs were

formed. The mixture was taken in quartz cuvette and exposed to UV-Visible radiation and the absorbance was measured. The absorption spectrum of ZnO NPs has shown strong absorption bands in between 330-380 nm, the data is given in the Table 1. The absorption spectrum of ZnO NPs has shown strong absorption band at 373 nm and 375 nm respectively which has been reported earlier by Sai *et al.* [15], Ali *et al.* [16]. Whereas Murali and co-workers [17] have reported an absorption

peak of ZnO NPs at 320 nm. It has been observed that each element has their specific excitation energy which is excited upon UV-Vis radiations as suggested by the earlier studies showing that ZnO possesses high optical absorption in the long wave ultraviolet region (A: 315-400 nm) and also short-wave ultraviolet region (B: 280-315 nm) [18]. The absorption spectra are shown in Fig. 2.

Table 1 Absorption of ZnO nanoparticles at different irradiation time

Wavelength (nm)	Absorbance at 0 min	Absorbance At 2 min	Absorbance At 4 min	Absorbance At 6 min	Absorbance At 8 min
250	0.31	0.35	0.40	0.43	0.46
275	0.37	0.45	0.54	0.65	0.77
325	0.50	0.58	0.78	0.95	1.19
350	0.43	0.50	0.70	0.83	1.10
375	0.24	0.28	0.40	0.48	0.60
400	0.18	0.20	0.24	0.25	0.30
425	0.15	0.16	0.20	0.22	0.25

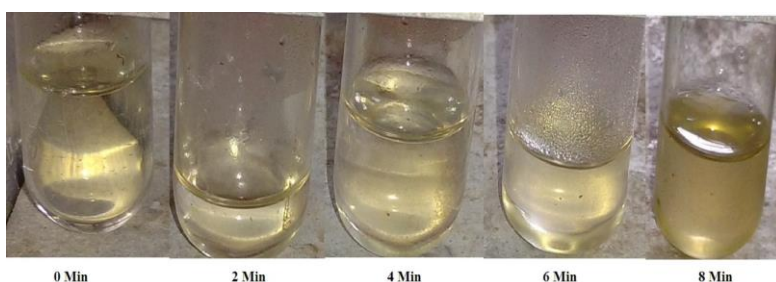


Fig. 1 Microwave assisted synthesis of ZnO NPs at different irradiation time

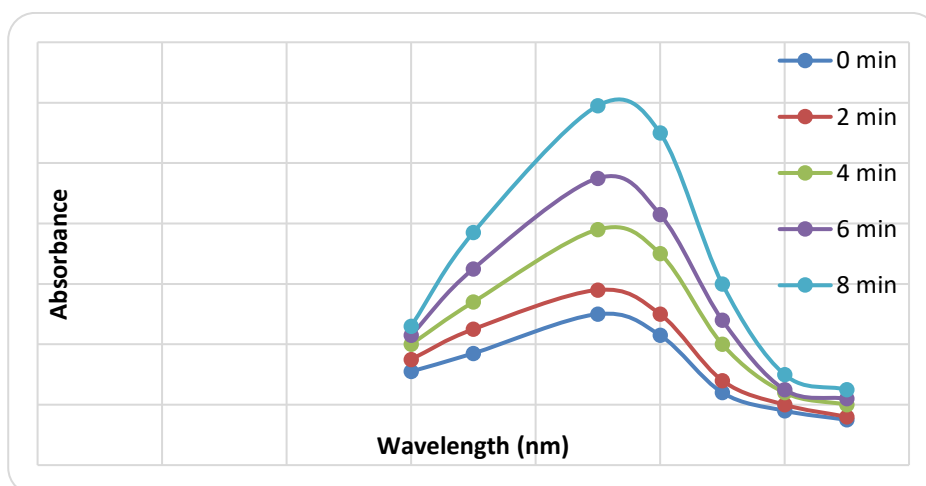


Fig. 2 UV-Visible analysis of ZnO nanoparticles synthesized from methanol extract of *E.ribes* seeds at different time intervals of microwave irradiation

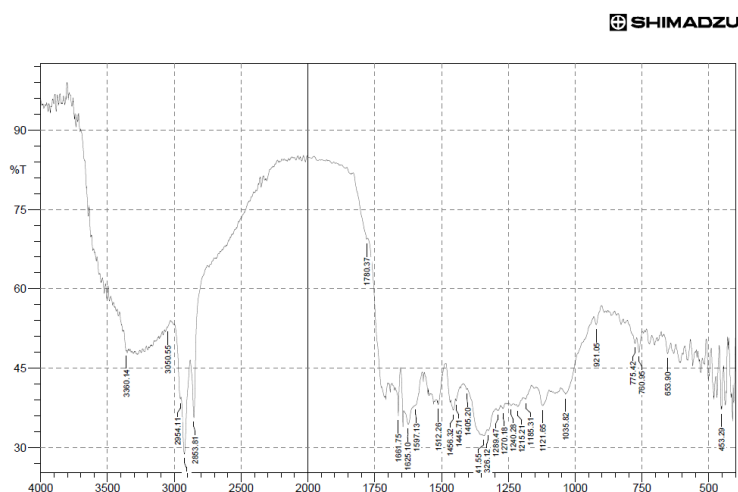


Fig. 3 FTIR spectrum of ZnO NPs synthesized from methanol extract

2. FTIR analysis

FTIR spectrum of the synthesized ZnO NPs is shown in Fig. 3 which reveals the possible biomolecules present in the methanol extract which is accountable for the reduction of zinc ions and its interaction with the ZnO NPs. The IR spectrum of ZnO NPs shows intense bands at 3360, 3050, 2954-2924, 2853, 1780, 1661-1625, 1597-1512, 1456-1405, 1341-1326, 1289-1215, 1185-1121, 1035, 921, 775-760, 653, 453 cm^{-1} . IR analysis of methanol extract was also done, and significant difference was observed between the spectral positions of IR bands in methanol extract and biosynthesized ZnO NPs due to the reduction process. The spectrum of IR band at 3360 cm^{-1} corresponds to the strong stretching vibrations of hydroxyl group ($-\text{OH}$) of phenolic compounds. Moderate band at 3050 cm^{-1} is attributed to N-H bonded and NH_3^+ stretching vibrations. The sharp two intense peaks at 2924-2924 cm^{-1} and 2853 cm^{-1} can be attributed to the C-H stretching vibrational mode, which are being shifted from 2956-2923 and 2852 present in the extract indicating the presence of aliphatic and aromatic groups of the protein and metabolites present in the methanol extract that may be involved in the reduction process. The IR spectrum of methanol extract exhibits a strong band at 1619 cm^{-1} corresponding to the C=O (amide I), C=C stretching and NH_3^+ asymmetric bending mode and this peak shifted to 1661 cm^{-1} suggesting the probable association of groups mentioned above in ZnO NPs synthesis. This amide I band indicates that proteins can bind to Zn^{2+} through carboxylate ions or free amine groups [19]. Bands at 1597-1512 and 1456-1405 cm^{-1} were mainly attributed to the amide C=O stretching and nitro N-O bending respectively present in the *E. ribes* seeds. Bands at 1341-

1326 cm^{-1} were assigned to C-O and O-H in plane bending vibrations, which must have been shifted from 1354-1326 of the extract. Bands at 1289-1215 corresponds to C-N, C-O stretching and O-H bending vibrations. Medium intensity band at 1185-1121 is assigned to C-O stretching vibrations due to presence of carboxylic acids and acid anhydrides. Strong band at 1035 cm^{-1} were due to the O-H bending, C-O stretching and C-O-C bending vibrations. Strong band at 921 cm^{-1} suggest the presence of CH_2 in plane and out of plane bending vibrations Strong band at 775-760 cm^{-1} suggest the presence of CH_2 in plane and out of plane bending vibrations and OCN deformation (Amide IV band). The band at 653 cm^{-1} is assigned to NH_3^+ and N-H out of plane bending which may be due to amine salts. Thus, from the IR spectrum, it may be assumed that these biomolecules act in the bio reduction as well as in the stabilization of biosynthesized ZnO NPs through carboxylate ions or free amine groups.

3. Scanning electron microscopy (SEM)

Scanning electron microscope (FE-SEM) is the technique used for understanding the surface morphology of Zinc nanoclusters. The sample of ZnO was analyzed for its microstructure using SEM operated at 20 kV. The specimen of SEM was mounted on a carbon tape by dispersing small amount of the powdered material. SEM images are given in Fig. 4-5. For the sample two images were produced one at magnification x1000 with scale of 10 μm and another at magnification x5000 with scale of 5 μm . All the images of SEM showed aggregates of ZnO NPs which are roughly spherical in shape with the dimension of 400-800. The SEM images are in confirmation with the form obtained by XRD analysis [21]. The SEM image of ZnO NPs was due to interactions

of hydrogen bond and electrostatic interactions between the bioorganic capping molecules bound to the ZnO NPs. The larger particles found may be due to the aggregation of the smaller ones. Moreover, there is

a point of view about nanoparticles to congregate or assemble each other at higher intensification by microscope [21].

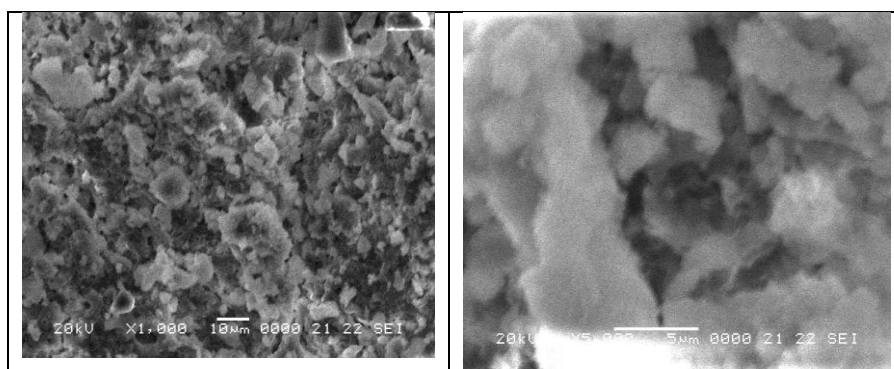


Fig. 4 and 5 SEM images of ZnO NPS synthesized from methanol extract of *E.ribes*

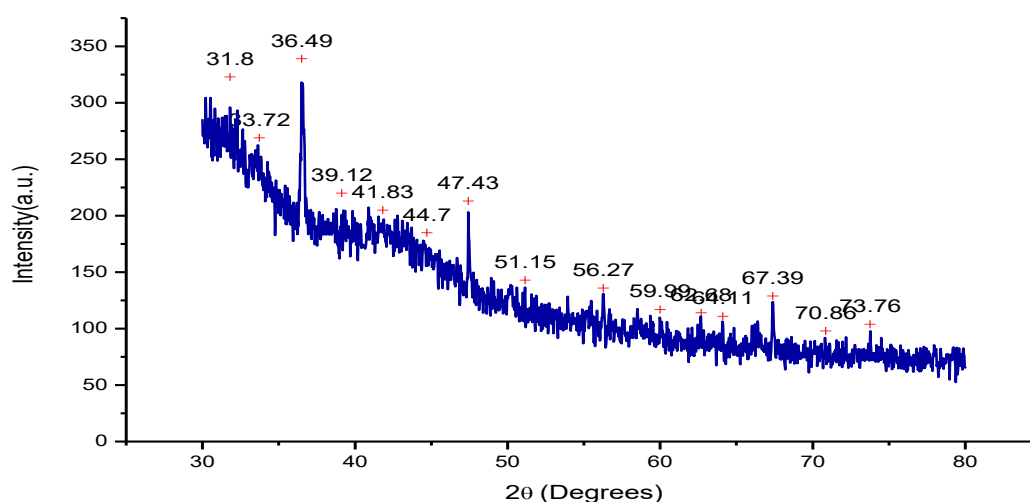


Fig. 6 X-ray diffraction pattern of ZnO NPs Synthesized from methanol extract of *E.ribes*

4. X-ray diffractometry (XRD)

ZnO NPs synthesized from methanol extract is presented in the Fig. 6. The XRD data is given in Table 2 which includes 2θ values (Degrees) corresponding to the peaks given in the diffractogram and θ , $\cos \theta$, $\sin \theta$ for further calculations. The XRD diffraction peaks at 2θ of 31.8, 33.72, 36.54, 47.45, 62.63, 67.39 which were assigned to the (100), (002), (101), (110), (103) and (112) planes, respectively. The XRD spectrum revealed that ZnO NPs have crystalline nature and hexagonal structure, which was confirmed by the International Centre of Diffraction Data Card (JCPDS NO: 00-036-1451). No other phases were observed, indicating the

purity of ZnO NPs. The average crystalline size of ZnO NPs was calculated using Debye sheerer equation as mentioned above in the text. The average size of the green synthesized ZnO NPs was found be about 57.57 nm. The XRD diffraction patterns shown in this study are in good agreement with the earlier research reported for the green synthesis of ZnO NPs [23].

We have also estimated the value of interplanar spacing between the atoms, 'd' for the data sets of all the peaks which is also given in the Table 2. Hence interpretation of XRD data revealed the well-defined dimension of ZnO nanoparticles which were synthesized by reducing Zinc metal ions due to methanol extracts of *E. ribes* seeds.

Table 2: XRD data of ZnO NPs synthesized from methanol extract of *E.ribes* seeds giving value of 2θ , Braggs angle θ , $\cos \theta$, $\sin \theta$, FWHM β in degree and radian of significant peaks in diffractogram as determined from Gaussian fitting, average crystalline size 'D', interplanar spacing 'd' values of hexagonal crystal, hkl values from JCPDS file.

Peak no	2θ	Bragg angle θ	$\cos \theta$	$\sin \theta$	FWHM B degree	FWHM B radian	Crystalline size 'D' nm	Interplanar spacing 'd' A	hkl		
1	31.8	15.9	0.9617	0.2739	0.275	0.0048	29.94	2.81	1	0	0
2	33.72	16.86	0.9570	0.2898	0.1022	0.0017	80.93	2.65	0	0	2
3	36.54	18.27	0.9495	0.3134	0.2989	0.0052	27.90	2.45	1	0	1
4	47.45	23.72	0.9154	0.5023	0.1362	0.0023	63.48	1.53	1	0	2
5	56.26	28.13	0.8818	0.4714	0.1434	0.0025	62.59	1.63	1	1	0
6	62.63	31.31	0.8560	0.5197	0.1276	0.003	72.60	1.48	1	0	3
7	67.39	33.69	0.8318	0.5547	0.1452	0.0019	65.54	1.38	1	1	2

CONCLUSION

In the present study we have investigated a cost effective and nontoxic method for the synthesis of ZnO nanoparticles from methanol extract of *E. ribes* seeds. For the characterization UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM) and powder X-ray diffractometry (XRD) were used. UV-Vis spectrometry was done in the colloidal solution formed after mixing $\text{Zn}(\text{O}_2\text{CCH}_3)_2$ and plant extracts and irradiating them in the microwave for different time intervals, we have obtained broad peaks in between 330-380 which corresponds to ZnO nanoparticles on each irradiation time. The peak occurred due to surface plasma resonance (SPR) phenomena which were observed in ZnO NPs synthesized from methanol extract. We can conclude from our data that microwave assisted synthesis of nanoparticles is rapid.

FTIR spectra of the extracts as well as ZnO NPs were recorded to identify the biomolecules and functional groups responsible for ZnO NPs synthesis and its stabilization. The spectra of ZnO NPs show a lot of bands corresponding to various functional groups. The phenolics and flavonoids responsible for the reduction of $\text{Zn}(\text{O}_2\text{CCH}_3)_2$. The biomolecules present in the extract act as encapsulating as well as stabilizing agents. SEM morphology images shows microstructure of the particles which unveiled them to be roughly spherical and depicts their size in between 330-850 nm. The larger size of the particles may be due to agglomeration of smaller particles in the sample of which SEM analysis was done. XRD analysis of synthesized NPs showed hexagonal structure with dimension 57.57nm. Hence the present work shows that the reduction of Zn ions from Zinc acetate to ZnO occurs due to seed extract of

E. ribes. The obtained XRD data for 2θ positions identifies the sample as ZnO crystalline particles having (hkl) values corresponding to hexagon crystal. Thus, the present method leads to the formation of ZnO nanoparticles with well-defined dimensions.

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Received:06.08.18, Accepted: 09.09.18, Published:01.10.2018

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