Plant Mediated Synthesis of Zinc Oxide Nanoparticles and Evaluation of Its Anti - microbial and Anti - oxidant Activities

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Abstract-In this research paper we have reported the single pot synthesis of zinc oxide nanoparticles (ZnONPs) for the first time by utilisation of leaves extract of Himalayan medicinal plant *Artemisia roxburghiana*. The principle of green chemistry was utilised at maximum possible level to make the synthesis not only environmentally compatible but also cost effective. The obtained nanoparticles are of good shape and size as confirmed by the instrumental techniques such as Powder XRD, HR-TEM, HR-SEM and FT-IR. The average size of the synthesized nanoparticles was between 26 to 35 nm. These nanoparticles then screened for the anti - microbial assay in which it has shown positive activity against *E. coli*, *A. Tereus* and *C. falcatum*. The second application of the synthesized nanoparticles is estimation of anti - oxidant activity against the DPPH. The IC_{50} value of the nanoparticles is formed to be 53 in EtoH while that of the standard, ascorbic acid was 26 in the same solvent.

Keywords:*Artemisia roxburghiana*, *Asteraceae*, ZnONPs, Antimicrobial activity and Antioxidant activity

Introduction

Material synthesis by green chemistry route has advantage over traditional methods of synthesis as it produces lesser environmental degradation. Now a days, environmental challenges are intrinsic part of every consideration for the developments from all sphere of mankind walk. Further the term "Sustainable development" is coined for it and came into consideration of human activity. In the synthesis of materials, it is equally important to have deep concern about the environmental health. As we deal with chemicals, sometime very hazardous one, the chances of polluting the nature with hazardous chemicals are high. Although, the materials synthesized that rely purely on chemicals have some disadvantage due to not only for their environmental impact but also for their cost at the same time, green synthesis or plant mediated synthesis is free from all these shortcomings. The materials made in this way should be of highly useful objectives.

The plant mediated synthesis of the nanomaterials is gaining interest in the researchers as it is not only environment friendly but also cost effective. There are several regions on planet which are rich in the plant diversity but due to inconvenience, such plants have not been utilised much for the synthesis of the nanomaterials. One such region of the planet earth has unique place on the global map by having the tallest mountain ranges with the ecosystem. The plants of Garhwal region of the great Himalaya have been in use since very long time by local inhabitants. Several diseases have been cured simply by using decoction of the plants in that ecosystems. There is a list of flora that has its root only in the Himalayan region due to its unique geographical condition.

Apart from this, there are several plants that may have cosmopolitan origin but they differ from one another in their phytoconstituents due to the geographical conditions. One such plant is *Artemisia roxburghiana*, which belongs to family *Asteraceae or compositae* and is perennial, pubescent undershrub, fairly common on open dry localities or terraces of crop fields, most abundantly found in submontane to montane zones of Garhwal Himalaya upto the altitude of 1100 ft. The plant *Artemisia roxburghiana* is rich with phytoconstituents, Artimisin as indicated by the isolation work performed on it. The plant *Artemisia roxburghiana* is in use by local inhabitants as antipyretic or tonic and also used to cure skin and cold related body ailments.The unique research work that has been reported in this paper is the development of the nanoparticles that can have applications from medicinal to core physical.

In this paper, we are reporting the utilisation of *Artemisia roxburghiana* leaves extract for the synthesis of the zinc oxide nanoparticles. The zinc oxide being building material is present in abundance and has been highly utilised in the several applications i.e. electric insulators etc. The plant mediated synthesis of the nanomaterials is not only compatible with the coagulation method but also is one of the most important methods for the synthesis of the nanomaterials at micro to tonnage level. The higher aspect ratio[1,2] of the nanoparticles made it very important and interesting material for different applications. Synthesis of nanoparticles by advanced techniques such as laser ablation method[3,4], chemical reduction[5] method and green chemistry routes[6,7]make it a versatile materials that has more than one synthetic methods. Green chemistry provides an opportunity for the better utilisation of the raw materials with minimum or no waste production at all. The green chemistry route is one of the most important method for the synthesis of, not only nanoparticles but also other chemically derived products. The plant or micro-organism mediated green synthesis of metallic nanoparticles has some advantages over traditional chemical methods as it is cost effective due to availability of plant materials, Further the ease associated with the synthesis. The environmental compatibility is another factor because the solvents used in the green synthesis of metallic nanoparticles does not harm environment so badly as traditional solvents such as benzene. Several metallic nanoparticles have been synthesized and well documented. The silver[8], gold[9], palladium[10] copper[11] and various other metallic nanoparticles have been synthesized and screened for different properties for example magnetic[12], electric[13], biological[14] and in sensing[15]. Zinc in free or combined form is actively involved in many natural or manmade processes i.e. in trace amount, it is present in human body and in different concentration it has been utilised to make circuits, batteries, solar cells etc.

There are large number of infectious diseases that must be checked before its outbreak. A large number of scientists are interested in the development of nanomaterials that can be used to counter such type of diseases. The high surface area to volume ratio of nanoparticles are one of the factors that make it hope against many pathogens. ZnO nanoparticles provide a promising antibacterial and antifungal activity against many pathogens.

The present paper deals with synthesis and characterisation of zinc oxide nanoparticles from the leaves extract of *Artemisia roxburghiana*. The synthesized ZnO nanoparticles were characterised by several techniques such as FT-IR, Powder XRD, HR-SEM and HR-TEM. This confirms that the synthesised nanoparticles are in the range of 46 nm to 50 nm. These nanoparticles were screened for biological activities against common pathogens.

Material and Methods

Synthesis of nanoparticles

Chemicals Used: Zinc acetate pentahydrates ($Zn(CH_3COO)_2.5H_2O$) and sodium hydroxide (NaOH) flakes were the chemicals used, purchased from Sigma-Aldrich, India and Millipore water was the medium of synthesis.

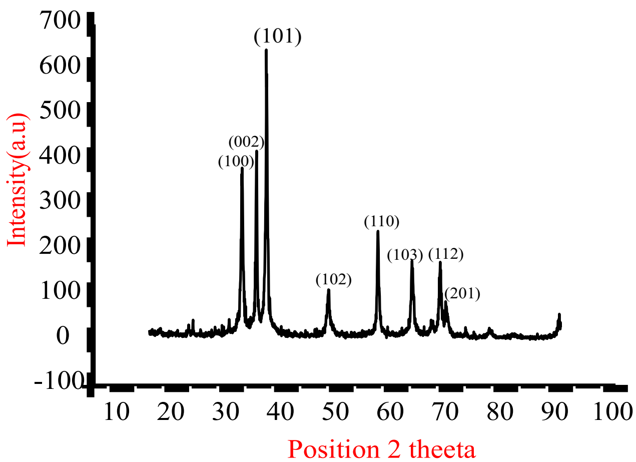
Collection of Plant Materials: The selected plant, *Artemisia roxburghiana* is highly abundant in Himalayan region and still in traditional uses in different region of the Garhwal Himalaya. The plant material used for study were collected from Ghasiaya Mahadev, Srinagar, Garhwal, Uttarakhand, India at the altitude of 1100 meter and identified by Taxonomist, Department of Botany, HNB Garhwal University, where the Voucher Specimen (GUH6391) was deposited .

Experimental The well washed and dried leaves of *Artemisia roxburghiana* dipped in 100 ml double distilled water in the Erlenmeyer flask, the Erlenmeyer flask was put in the heating mantle for 10 min at 60^0C. The colour change from transparent to light brown takes place during extraction, the extract is allowed to cool at room temperature. Then it was filtered off twice a time with Whattman filter paper. The filtrate was stored in refrigerator for further experimentation.

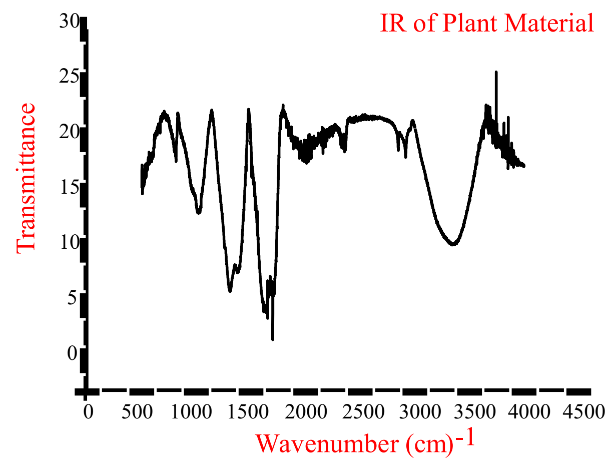
Synthesis of Nanoparticles[16-18] 1 molar solution of NaOH (sodium hydroxide) was prepared by the dissolving it in deionised water (50 ml). 2 mM solution of zinc acetate was also prepared in 500 ml beaker. The beaker containing zinc acetate solution was kept on the magnetic stirrer at the temperature of 120^0C in which plant extract was added drop by drop and the pH of the solution was maintained around 10 and monitored by pH meter. After adding whole plant material the solution was removed from magnetic stirrer and allowed to cool at room temperature. A precipitate of pale yellow colour was formed. The synthesized nanoparticles then centrifuged at 5000 rpm for 20 minutes . After the recovery of sample it was washed twice a time with the deionised water. The synthesized nanoparticles was kept in oven for 48 hours for the complete conversion of $Zn(OH)_2$ (zinc hydroxide) into ZnO (zinc oxide.

Characterisation The synthesized ZnO nanoparticles were characterised by several techniques such as FT-IR, Powder XRD, HR-SEM and HR-TEM

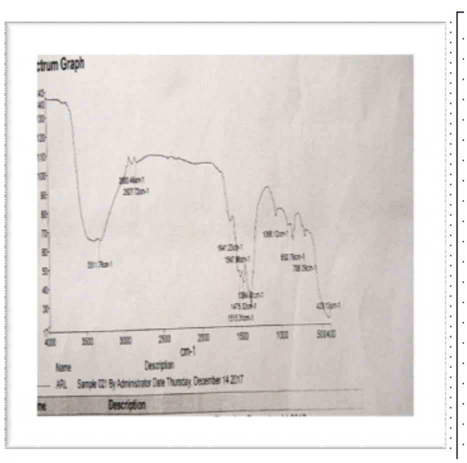
FT-IR The FT-IR of the plant extract and synthesized nanoparticle was carried out by FT-IR spectrophotometer (Agilent Technologies Model: L1600312 spectrum TWOLITA/ZnSe) for determination of functional group present in the plant material and synthesized nanoparticles. It has been found that the synthesized NPs were encapsulated by the compounds which have hydroxyl group at 3500 cm-1, N-H bond peak at 3400 cm-1, C-H bond peak at 2840 cm-1 and a 1717 cm-1 peak for carbonyl bond (C=O). All the mentioned functional groups are also present in the plant extract from which the encapsulating compounds are derived. Apart from those common peaks, there are some additional peaks in the plant extract such as peak for the carbon-carbon triple bond at 2167 cm-1. The change in the absorption pattern from plant material to synthesized zinc oxide nanoparticles indicate that the formation nanomaterials has taken place. So it can be concluded that nearly all functional groups present in the plant have been transferred to synthesized NPs.Powder XRDFor the crystallographic study the powder XRD was done by using PANalytical XPERT-PRO D3663 diffractometer. The average crystallite size of synthesized nanoparticles are of 23.2 nm with hexagonal crystal system. The synthesized nanoparticles have value of coefficient value of a=b=3.2417 and c=5.1876. The diffraction planes of synthesized nanoparticles are indexed as (100), (002), (101),(102),(110),(103)(121) and (201) which indicate the Wurtzite structure of synthesized ZN-NPs. The XRD data has been obtained from the International Diffraction Data Card with reference code 01-079-0205.



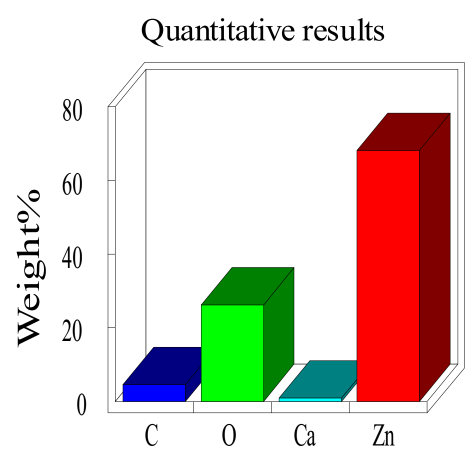
Graph-1 XRD peaks of ZnO-NPs



Graph-2 IR spectra of the plant material



Graph-3 IR spectra of ZnO-NPs



Bar Graph-1 Elemental composition

SEM analysis The scanning electron micrography and EDX data has revelded that the synthesized nanoparticles are spherical in nature with smooth sucface. The EDX (Energy Dispersive X-Ray) data has shown that the synthesized nanoparticles have zinc 68.25% by weight, further calcium is also present by 0.95% as may come from the plant materials, as it has been well established that the calcium pectate functions as adhesive for cell and various other metabolic functions.

TEM analysis The Transmission Electron Microscopy of synthesized nanoparticles was carried out by JEOL JEM 1400 at IIT Delhi. It has been found that the synthesized nanoparticles are of average size of 45 nm with almost spherical in nanture and highly aglomerated. Due agglomeration the size of synthesized nanoparticles comes out to be more than the real value. The size of synthesized nanoparticles was estimated with the help of softwares digimiser and Image.

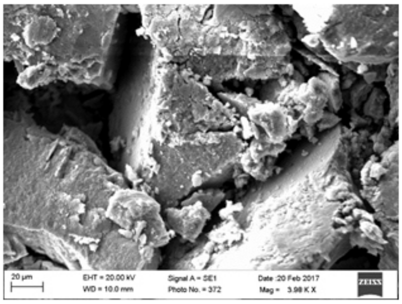
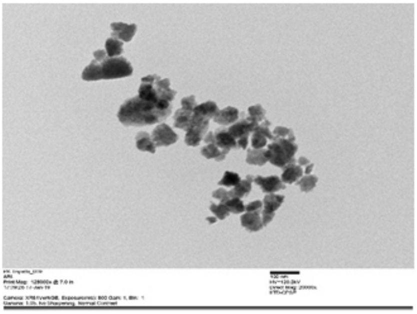
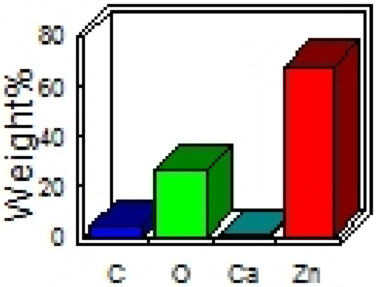


Image-1 SEM images of ZnO-NPs



Images-2 TEM image of ZnO-NPs

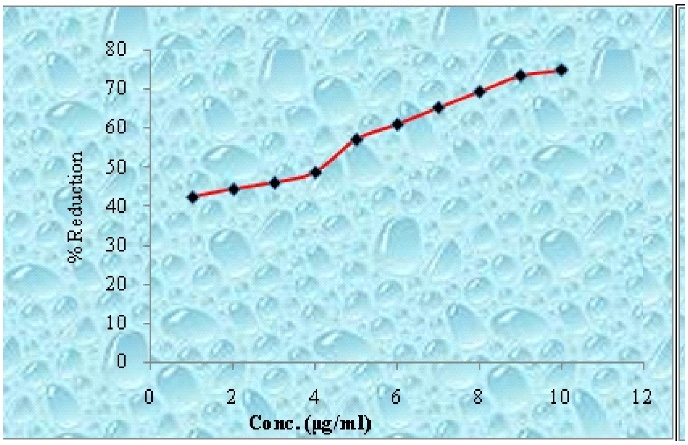


Quantitative results

Biological activity: The antioxidant activity of the synthesized nanomaterials was performed through DPPH methods. DPPH method is widely used to determine the free radical scavanging activity of natural compound or nanomaterials. This method is based on the detrmination of the scavanging ability of antioxidant materials towards stable radicals[19,20].

Table-1 Antioxidant activity

S. No.	Conc. (µg/ml)	Absorb	% Reduction	IC_{50} Value
1.	10	0.282	42.44	53
2.	20	0.272	44.48	
3.	30	0.264	46.12	
4.	40	0.251	48.77	
5.	50	0.210	57.14	
6.	60	0.191	61.02	
7.	70	0.170	65.30	
8.	80	0.150	69.38	
9.	90	0.130	73.46	
10.	100	0.123	74.89	



Graph-4 Antioxidant activity of ZnO-NPs

Antimicrobial activity[21,22] The synthesized nanoparticle were screened against two gram positive (*Staphylococcus aureus and Bacillus cereus*), two gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and two fungal (*Aspergillus terreus* and *Cyrtomium falcatum*). The methodology of screening was Petriplates containing 20ml Muller Hinton medium (for bacterial species) and 20ml Sabouraud Dextrose agar (for fungal isolates) were seeded with 24hr culture of bacterial and fungal strains respectively. Wells were cut and 20 µl of the sample diluted in distilled water for different concentrations were added. The plates were then incubated at 37°C for 24 hours. The antibiotic activity was assayed by measuring the diameter of the inhibition zone formed around the well. Oxytetracyclin (800ppm) disc was used as a positive control for bacterial strains while Fluconazole (1000ppm) was used as positive control for fungal strain.

Table-2 The antibacterial activity of ZnO-NPs against different pathogens

	Zone of Inhibition (cm)			
Pathogens Name	**Nanoparticles**			**Oxytetracyclin (800) ppm**
	25 mg/ml	**50 mg/ml**	**100 mg/ ml**	**+ve control**
E. coli	Nil	1.1	1.2	4.2
S. aureus	Nil	Nil	Nil	3.9
B. cereus	Nil	Nil	Nil	2.7
P. aeruginosa	Nil	Nil	Nil	2.6
Aspergillus terreus	0.7	1.0	1.2	1.4
C. falcatum	Nil	Nil	0.8	1.5

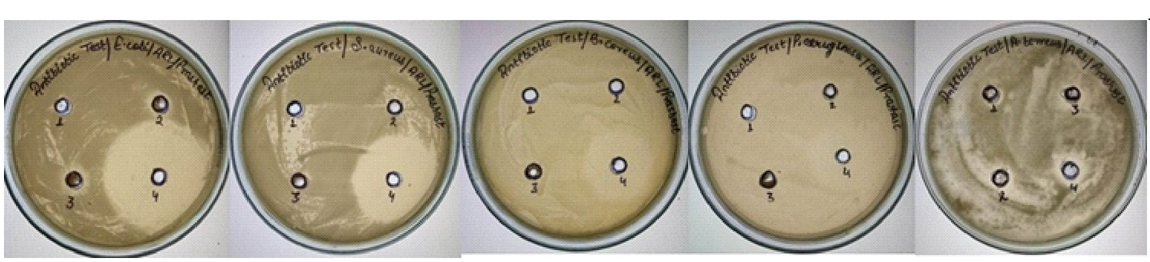


Image (a) Image (b) Image (c) Image (d) Image (e)

The images from (a) to (e) represent the MIC of the synthesized nanoparticles: image (a) E.coli (b) S. aureus (c) B. cereus (d) P. aeruginosa and (e) A. terreus

Conclusion

It has been found that the synthesized ZnO-NPs have activity against gram negative bacteria *E. coli*, fungal pathogens, *Aspergillus terreus* and *Cyrtomium falcatum* at different concentration. Further the antioxidant activity of the synthesized ZnO-NPs were found to have IC_{50} value equal to 53 which shows it has mild activity against radicals.

Acknowledgments

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