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Research Article

GREEN SYNTHESIS, CHARACTERIZATION, AND ANTIBACTERIAL ACTIVITY OF ZINC OXIDE NANOPARTICLE

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ABSTRACT

Objectives: Zinc oxide (ZnO) nanoparticles have received considerable attention due to their antimicrobial, UV blocking, and high catalytic and photochemical activities. Hence, an investigation has been carried out to synthesize the ZnO nanoparticle using aqueous *Phyllanthus niruri* (Keezhanelli) leaf extract. Aims and objectives of the present study are to synthesize using Keezhanelli (*P. niruri*) leaf extract, to study its characterization, and to determine its antibacterial activity.

Methods: Green synthesized ZnO nanoparticle was characterized by Fourier transform infrared (FTIR), scanning electron microscope (SEM), and transmission electron microscope (TEM) analysis. Antimicrobial activity of ZnO nanoparticle was carried out using agar well diffusion method.

Results: The result of the synthesized ZnO nanoparticle using Keezhanelli (*P. niruri*) leaf extract showed the change of color from pale white to brown color. The result of FTIR analysis of green synthesized ZnO nanoparticle revealed the presence of biomolecules such as polyphenols, flavonoids, alkaloids, polysaccharide, amino acid, and proteins. The result of the SEM studies showed that the green synthesized ZnO nanoparticle was spherical and cylindrical in shape. The size of the ZnO nanoparticle was recorded to be 5 µm. The result of TEM studies of ZnO nanoparticle showed that majority of the particles were spherical in shape with the size of 2 µm. The result of antibacterial activity against four bacterial species showed that green synthesized ZnO nanoparticle was found to be efficient in inhibiting the growth of the bacterial isolates. Maximum zone formation was exhibited against *Staphylococcus saprophyticus.*

Conclusion: Thus, from the results of the present study, it can be concluded that synthesis of ZnO nanoparticle using leaf extract of Keezhanelli (*P. niruri*) has several advantages such as simple, cost-effective, time consuming, safe, and eco-friendly compared to other methods of nanoparticle synthesis as evidenced in the present study.

Keywords: *Phyllanthus niruri* (Keezhanelli) leaf extract, Zinc oxide nanoparticle, Fourier transform infrared, Scanning electron microscope, Transmission electron microscope, Antibacterial activity.

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INTRODUCTION

Nanotechnology is the production and use of materials at the smallest possible scale [1]. In nanotechnology, a nanoparticle is defined as a small object that behaves as a whole unit in terms of its transport and properties [2]. In the current situation, one of the most promising and therapeutic agents is nanoparticles. Nanoparticles are being viewed as the fundamental building blocks of nanotechnology [3].

In general, metal oxide nanoparticles are inorganic. Various nanoparticles such as Fe, Ni, Co, Mn, and Zn are known as the enormously accepted magnetic materials for a wide range of applications such as various electronic ignition systems, generators, vending machines, medical implants, wrist watches, inductor core, transformer circuits, magnetic sensors and recording equipment, telecommunications, magnetic fluids, and microwave absorbers [4]. Metal-based nanoparticles constitute an effective antimicrobial agent against common pathogenic microorganisms. A multiple of research have established that zinc oxide (ZnO) nanoparticle has antifungal and antibacterial activity.

ZnO is an inorganic compound with the formula ZnO. It usually appears as a white powder, nearly insoluble in water. The powder is widely used as an additive in numerous materials and products including plastics, ceramics, glass, cement, rubber (e.g., car tyres), lubricants, paints, ointments, adhesives, pigments, foods (source of Zn nutrient), batteries, ferrites, and fire retardants. ZnO is present in the earth crust as a mineral zincite; however, most ZnO used commercially is produced synthetically [5].

Green synthesis of nanoparticles has gained significant importance in recent years and has become one of the most preferred methods of the synthesis of nanoparticle. Green synthesis of nanoparticle aims to protect the environment not only by cleaning up but also by inventing new chemical process that does not defile [6]. The uses of plant extracts are far more advantageous than that of other biological methods of synthesis since this process eliminates the tedious task of maintaining microbial medium and cultures [5]. Based on literature survey, ZnO nanoparticle was synthesized using *Phyllanthus niruri* (Keezhanelli) leaves which is distributed throughout the tropical and subtropical regions of both hemispheres. Thus, the aims and objectives of the present study are to green synthesize ZnO nanoparticle using Keezhanelli (*P. niruri*) leaf extract, to characterize green synthesized ZnO nanoparticle using Fourier transform infrared (FTIR), scanning electron microscope (SEM), and transmission electron microscope (TEM) analysis and also to determine the antibacterial activity of green synthesized ZnO nanoparticle using agar well diffusion method.

METHODS

Preparation of zinc acetate dihydrate solution for the synthesis of ZnO nanoparticle

Zinc acetate dihydrate with 90% purity was obtained from Hi-Media and distilled water was used throughout the experiments for the synthesis of ZnO nanoparticle.

Selection of plant

Fresh and healthy Keezhanelli (*P. niruri*) leaves were collected from the tree. The leaves were ensured that they were healthy, uninfected, thoroughly washed, and rinsed with sterile distilled water.

Authentication of plant material

The fresh leaves of Keezhanelli (*P. niruri*) collected and it was identified and authenticated by Prof. P. Jayaraman, Taxonomist, Plant Anatomy Research Centre (PARC), Chennai (Certificate No. PARC/2018/3882).

Collection of bacterial isolates for antibacterial activity of ZnO nanoparticle

Clinical isolates such as *Bacillus* sp., *Staphylococcus epidermidis, Staphylococcus saprophyticus,* and *Escherichia coli* were collected from a tertiary hospital. Samples were transported to the laboratory for further processing in an ice box.

Green synthesis of ZnO nanoparticle using Keezhanelli leaf extract

Preparation of zinc acetate dihydrate solution for the synthesis of ZnO nanoparticle was carried out by following the procedure of Senthilkumar *et al*. [7]. 0.2 M of zinc acetate dihydrate was dissolved in 70 ml of distilled water and stirred for few minutes. Preparation of leaf extract from Keezhanelli (*P. niruri*) plant was carried out by following the procedure of Kanthimathi and Soranam [8]. 10 g of the fresh green leaves of Keezhanelli (*P. niruri*) was added to 100 ml of distilled water and magnetically stirred for 2 h at 80°C. After cooling to room temperature and filtering through Whatman No. 1 paper, 30 ml of this Keezhanelli leaf extract was mixed homogeneously with the already prepared zinc acetate solution. The reacted solution was dried at 60°C overnight to yield pale white color ZnO nanoparticle, which was finally calcined at 100°C for 1 h and preserved in airtight vials for further studies.

Characterization of ZnO nanoparticle

FTIR spectroscopy

The FTIR spectra of ZnO nanoparticle of *P. niruri* powder were recorded in SHIMADZU-8400 spectrometer using KBr pellet method.

SEM

In the present work, SEM machine was employed to study the morphology of green synthesized nanoparticle. The experiment was performed at an accelerating voltage of 20 kV. The slide was coated with platinum, and after the platinum coating, the SEM image was taken.

TEM

TEM analysis of green synthesized ZnO nanoparticle was done using Philips (Technai 10). Thin films of sample were prepared on a carbon-coated copper grid by just dropping a very small amount of sample on the grid, extra solution was removed using a blotting paper, and then, the film on the TEM grid was allowed to dry by putting it under incubator. The images were obtained by Technai, Twin 200 KV, and a bias voltage of 200 kV was used to analyze sample.

Antibacterial activity of green synthesized ZnO nanoparticle using agar well diffusion method

The effect of Keezhanelli (*P. niruri*) leaves extract on the test organisms was assayed by agar well diffusion method. Mueller-Hinton agar medium was poured into the Petri plates aseptically and was allowed to solidify. The lawns of the test bacterial strains were done with the help of sterile cotton swab. Wells were made with the help of sterile cork borer (6 mm), and the cut agar discs were removed aseptically with sterile forceps. 100 µl of green synthesized ZnO nanoparticle was added into the wells. The test plates were incubated aerobically at 37°C for 24 h. After incubation, the results were recorded based on the presence or absence of inhibition zone. The antibacterial activity of green synthesized ZnO nanoparticle was assayed by measuring the diameter of the inhibition zone formed around the wells.

Statistical analysis

The data obtained from the experiments were analyzed and expressed as mean and standard deviation.

$$\text{S.D} = \sqrt{\frac{1}{N-1}\sum x^2 - \frac{(\sum x^2)}{N}}$$

Where, N=Number of individual observation.
$\sum x^2$=Sum of square of individual observation.
$(\sum x^2)$=Square of the total individual observation.

$$\text{Chi - square test} = \chi^2 = \Sigma \frac{(O-E)^2}{E}$$

Where, O=observed values.
E=expected values.

RESULTS

Green synthesis of ZnO nanoparticle using Keezhanelli (*P. niruri*) leaf extract

The results of the green synthesis of ZnO nanoparticle using Keezhanelli (*P. niruri*) leaves extract are shown in plate 1. The results of the study revealed that the color of the sample changed from pale white to brown color which was due to the reduction of zinc ions into zinc nanoparticle that occurred due to the surface plasmon resonance phenomenon. The metal nanoparticle has free electrons, which helps in the formation of the surface plasmon resonance absorption band.

characterization of green synthesized ZnO nanoparticle

Further studies were extended to characterize the green synthesized nanoparticle using FTIR spectroscopy, SEM, and TEM analysis.

The results of FTIR absorption spectra of green synthesized ZnO nanoparticle are presented in Fig. 1. The FTIR spectroscopy analysis of ZnO nanoparticle has revealed the presence of different peaks pertaining to various functional groups.

The results of FTIR study showed that the peaks at 3104 cm^{-1}–3433 cm^{-1} were due to stretching vibration of N-H groups in amines. C=O stretching in polyphenols and carboxyl was observed at 1498–1565 cm^{-1}, bands at 1190–1421 cm^{-1} were attributed to the C=C stretching in aromatic ring. C-O stretching in amino acids showed a band at 923–932 cm^{-1}. Bands at 632–680 cm^{-1} were due to C-H binding in aliphatic region. In addition to the absorption bands of these biomolecules, two new peaks appear at 615–618 cm^{-1}, 505–510 cm^{-1}, and 490–494 cm^{-1} in the IR spectrum of the ZnO nanoparticles which are the characteristic peaks of ZnO nanoparticle.

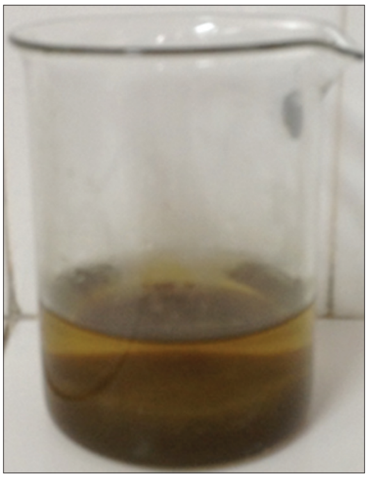
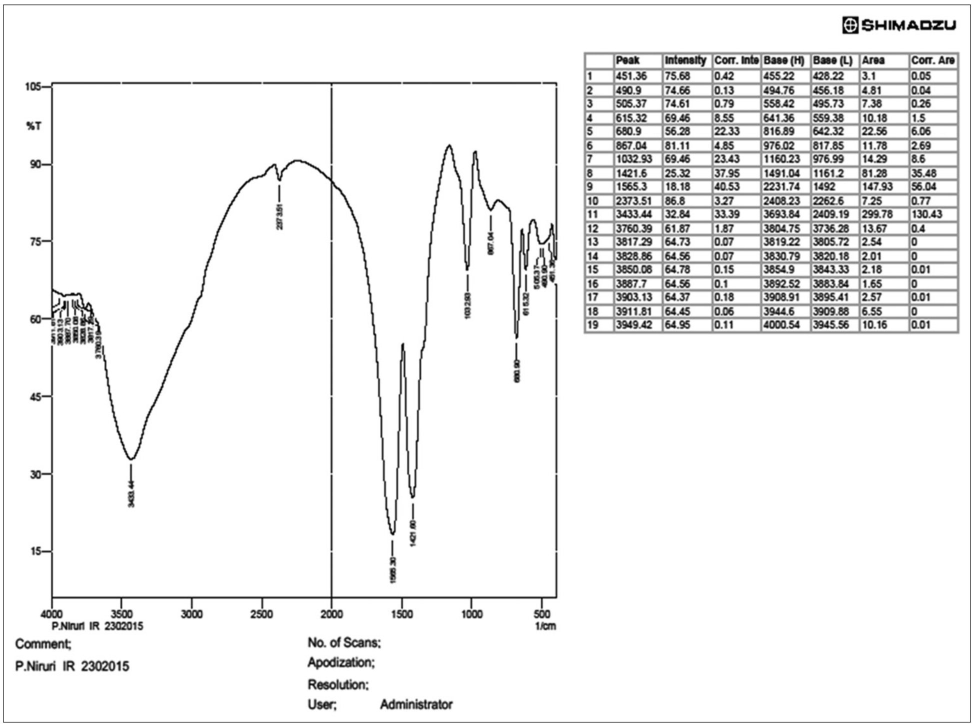


Plate 1: Green synthesis of zinc oxide nanoparticle using Keezhanelli (*Phyllanthus niruri*) leaves extract.



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	451.36	75.68	0.42	455.22	428.22	3.1	0.05
2	490.9	74.66	0.13	494.76	456.18	4.81	0.04
3	505.37	74.61	0.79	558.42	495.73	7.38	0.26
4	615.32	69.46	8.55	641.36	559.38	10.18	1.5
5	680.9	56.28	22.33	816.89	642.32	22.56	6.06
6	867.04	81.11	4.85	976.02	817.85	11.78	2.69
7	1032.93	69.46	23.43	1160.23	976.99	14.29	8.6
8	1421.6	25.32	37.95	1491.04	1161.2	81.28	35.48
9	1565.3	18.18	40.53	2231.74	1492	147.93	56.04
10	2373.51	86.8	3.27	2408.23	2262.6	7.25	0.77
11	3433.44	32.84	33.39	3693.84	2409.19	299.78	130.43
12	3760.39	61.87	1.87	3804.75	3736.28	13.67	0.4
13	3817.29	64.73	0.07	3819.22	3805.72	2.54	0
14	3828.86	64.56	0.07	3830.79	3820.18	2.01	0
15	3850.08	64.78	0.15	3854.9	3843.33	2.18	0.01
16	3887.7	64.56	0.1	3892.52	3883.84	1.65	0
17	3903.13	64.37	0.18	3908.91	3895.41	2.57	0.01
18	3911.81	64.45	0.06	3944.6	3909.88	6.55	0
19	3949.42	64.95	0.11	4000.54	3945.56	10.16	0.01

Fig. 1: Fourier transform infrared analysis of green synthesized zinc oxide nanoparticle using leaves extract of Keezhanelli (*Phyllanthus niruri*)

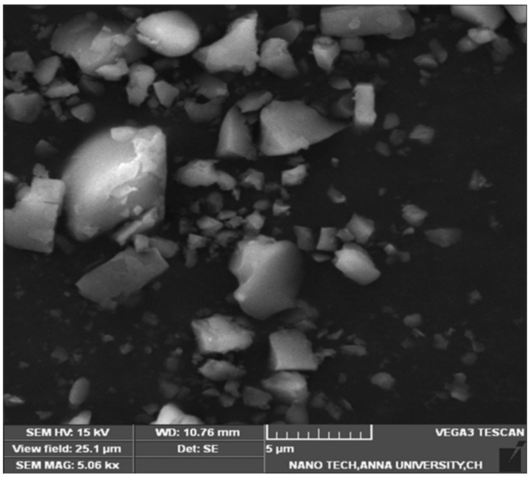


Plate 2: Scanning electron microscope analysis of green synthesized zinc oxide nanoparticle using leaves extract of Keezhanelli (*Phyllanthus niruri*)

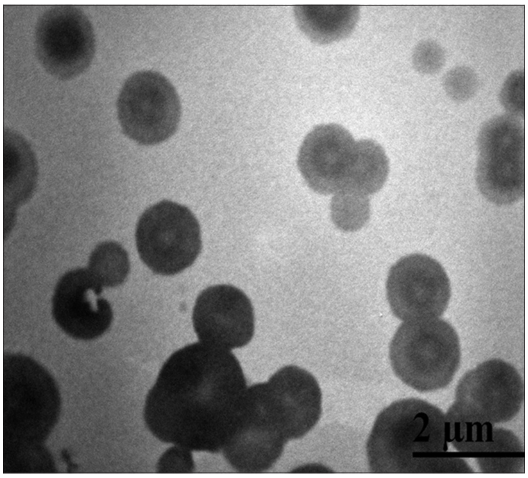


Plate 3: Transmission electron microscope analysis of green synthesized zinc oxide nanoparticle using leaves extract of Keezhanelli (*Phyllanthus niruri*)

From the results of FTIR spectrum, it can be observed that ZnO nanoparticle is rich in polyphenols, alkaloids, flavonoids, etc. The involvement of these biomolecules in the reduction and stabilization (capping actions) is clearly evident from the IR spectrum of the green synthesized ZnO nanoparticle.

The results of the SEM studies on ZnO nanoparticles using Keezhanelli (*P. niruri*) leaves extract are depicted in Plate 2. Spherical- and cylindrical-shaped nanocrystals were formed by exposing the leaf extracts of aforesaid plant to the aqueous zinc acetate dihydrate solution. The size of the ZnO nanoparticles synthesized using Keezhanelli (*P. niruri*) leaf extracts ranges from 5 µm in diameter.

The results of the TEM analysis of green synthesized ZnO nanoparticle are depicted in Plate 3. TEM micrographs provide a clear idea on the shape of nanoparticle. Majority of the particles were spherical in shape with the size of 2 µm. These nanoparticles were well distributed without much aggregation.

Antibacterial activity of green synthesized ZnO nanoparticle

The results of the antibacterial activity of green synthesized ZnO nanoparticle against the bacterial isolates are shown in Table 1. The result of the study showed that ZnO nanoparticle formed a zone whose size ranged from 16±0.8 mm to 29.6±1.2 mm in diameter. The maximum zone of 29.6±1.2 mm was exhibited toward *S. saprophyticus* and the least zone of 16±0.8 mm in diameter was shown by *S. epidermidis*. The values were significant at 0.1% level.

The result of the antibacterial activity of green synthesized nanoparticle against four bacterial species showed that green synthesized ZnO

Table 1: Statistical data of agar well diffusion method of green synthesized zinc oxide nanoparticle

S. No.	Name of the organism	Mean±standard deviation	Chi-square test
1.	*Bacillus* sp.	16.3±1.2	0.1
2.	*Escherichia coli*	18±0.81	
3.	*Staphylococcus epidermidis*	16±0.8	
4.	*Staphylococcus saprophyticus*	29.6±1.2	

±: Standard deviation, The values were significant at 0.1% level

nanoparticle was found to be efficient in inhibiting the growth of the bacterial isolates.

Statistical analysis

The results of the statistical analysis of the data obtained from the above experiments revealed that the probability was found to be significant at 0.1% level.

DISCUSSION

Nanotechnology is a multidisciplinary scientific field undergoing explosive development. Nanometer-sized particles offer novel structural, optical, and electronic properties that are not attainable with individual molecules or bulk solids [9]. The characters of metal and metal oxide nanoparticles have been of great interest due to their distinctive features such as catalytic activity, optical, magnetic, and electrical properties [10].

Different types of physical and chemical methods are employed for the synthesis of nanoparticles. The use of these synthetic methods requires both strong and weak chemical reducing agents and protective agents such as sodium borohydride, sodium citrate, and alcohols. These agents are mostly toxic, flammable, cannot be easily disposed off due to environmental issues and also show a low production rate [11-14]. It leads to in search of alternatives which could be ecofriendly and does not cause any harm to live organisms including human. One such method is the biological method using microbes and plants either as reducing agents or protective agents. Many biological organisms, both unicellular and multicellular, are known to produce inorganic materials either intra- or extra-cellular, often of nanoscale dimensions and exquisite morphology and hierarchical assembly. The biosynthesis of nanoparticles employs the use of biological agents such as bacteria, fungi, actinomycetes, yeast, algae, and plants [11,15]. The rate of the reduction of metal ions using biological agents is found to be much faster and also at ambient temperature and pressure conditions. Hence, the present investigation was aimed to synthesize ZnO nanoparticle using *P. niruri* leaf extract, to characterize the biosynthesized ZnO nanoparticle, and to evaluate their antibacterial efficiency against some pathogenic bacterial isolates such as *Bacillus* sp., *E. coli, S. epidermidis,* and *S. saprophyticus.*

The results of the biosynthesis of ZnO Nanoparticle using Keezhanelli (*P. niruri*) leaf extract showed the change of colour from pale white to brown colour which may be due to surface Plasmon Resonance phenomenon as ZnO Nanoparticle have free electrons which helps in the formation of the Surface Plasmon Resonance absorption band, that occurred due to the united vibration of the electrons of metal nanoparticles in resonance with light wave, which is supported by the work [7].

Further studies were extended to characterize the biosynthesized nanoparticle using FTIR spectroscopy, SEM, and TEM analysis.

The results of FTIR analysis of the biosynthesized nanoparticle revealed the presence of biomolecules such as alkaloids, flavonoids, and phenolic groups of molecules present in *P. niruri* leaf extract that is responsible for the reduction and amino acids and amide linkages in proteins were responsible for the stabilization process of the biosynthesized nanoparticle which was identified by FTIR analysis. The involvement of these biomolecules in the reduction and stabilization (capping actions) is clearly evident from the IR spectrum of the biosynthesized ZnO nanoparticle. The result of the above study was supported by the work [16].

SEM was used to determine size, location, and shape of the ZnO nanoparticle. The results of the SEM studies of biosynthesized nanoparticle showed that spherical- and cylindrical-shaped ZnO nanocrystals were formed by exposing the leaf extract of aforesaid plant to the aqueous zinc acetate dihydrate solution. The size of the ZnO nanoparticle synthesized using Keezhanelli (*P. niruri*) leaf extracts ranged from 69.71 nm to 94.36 nm in diameter [16].

TEM micrographs provide a clear idea on the shape of nanoparticles. Shape of ZnO nanoparticles of Keezhanelli leaf extract was spherical with the size of 2 µm. This nanoparticle was well distributed without any aggregations. The separation between the ZnO nanoparticles without any aggregation observed in the TEM image could be due to capping by proteins [16].

The result of the antibacterial activity of biosynthesized nanoparticles using Keezhanelli (*P. niruri*) leaf extract against four bacterial species showed that though biosynthesized ZnO nanoparticle was found to be efficient in inhibiting the growth of the bacterial isolates which may be due to the presence of different functional groups, proteins, amides, etc., present in biosynthesized ZnO nanoparticle using *P. niruri* leaf extract. This may be due to the electrostatic interaction between bacterial cell surface and nanoparticle for inhibiting the growth and also production of hydrogen peroxide from ZnO nanoparticle that leads to the entry of particle into bacterial cell membrane cause injury and finally causes the death of the bacterium [17]. Thus, the use of plant extract with known antimicrobial properties can be of great significant for therapeutic treatment. This is supported by the work [18].

Thus, the results of the above study show that the biosynthesized ZnO nanoparticle can be used in the treatment of wastewater and also in the development of antibacterial water filters for water purification as bacterial diseases are transmitted through water [19].

CONCLUSION

Thus, from the results of the above study, it can be concluded that green synthesis of ZnO nanoparticle using plants has several merits such as simple, inexpensive, good stability of nanoparticles, less time consumption, non-toxic byproducts, ecofriendly, and large-scale synthesis.

AUTHORS' CONTRIBUTIONS

All the practical and theoretical works were carried out by the author – Dr. C.M. Noorjahan herself.

CONFLICTS OF INTEREST

The author declares that there are no conflicts of interest.

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