

Biological Synthesis of Zinc Oxide Nanoparticles from Leaf Extract of *Curcuma neilgherrensis* Wight

G. Parthasarathy*

*P.hD, Research Scholar, Department of Electronics,
Erode Arts and Science College (Autonomous),
Erode-638 009, TamilNadu, India.*

Dr. M. Saroja

*Associate Professor of Electronics, Department of Electronics,
Erode Arts and Science College (Autonomous),
Erode-638 009, TamilNadu, India.*

Dr. M.Venkatachalam

*Head & Associate Professor of Electronics, Department of Electronics,
Erode Arts and Science College (Autonomous),
Erode-638 009, Tamil Nadu, India.*

Dr. V.K. Evanjelene

*R and D Manager,
Alpha Omega Hi Tech Bio Research centre,
Salem-636 008, TamilNadu, India.*

Abstract

Biologically synthesized nanoparticles have been broadly using in the field of medicine. Research in nanotechnology highlights the possibility of green chemistry pathways to produce technologically important nanomaterials. In the present study, the objective was to study the synthesis and analyse the Zinc Oxide Nanoparticles (ZnO NPs) from *Curcuma neilgherrensis* leaf extract. The samples were characterized by Ultra-Violet Spectroscopy (UV), Fourier transform infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM), X-ray diffraction (XRD). These ZnO NPs were

evaluated for antibacterial activity. The maximum diameter of inhibition zones around the disk used for *Pseudomonas aeruginosa* indicates the resistance to ZnO NPs followed by *Escherichia coli*. Among the five bacterial species tested, the *Pseudomonas aeruginosa* is more susceptible when compared with other three species. It is concluded that the biological synthesis of ZnO NPs is very rapid, effortless, cost effective and eco-friendly and without any side effects and ZnO NPs may be used for the preparation of antibacterial formulations against *Pseudomonas aeruginosa*. Different functional groups were found to be present signifying the presence of different compounds in the extract.

Keywords: *Curcuma neilgherrensis*, Zinc Oxide Nanoparticles, Antibacterial activity, FTIR, SEM, UV, XRD.

INTRODUCTION

Nanotechnology is an imperative division in the most important fields of biology, chemistry, physics and material sciences. Nanoparticles possess a wide array of application in the different fields' viz., drug, electronics, and therapeutics and as analytical agents. The nanomaterials can be synthesized by different methods including chemical, physical, irradiation and biological methods. The improvement of new chemical or physical methods has resulted in environmental contaminations, since the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous byproducts. Thus, there is a need of "green synthesis" that includes a clean, safe, eco-friendly and environmentally nontoxic method of nanoparticle synthesis. Moreover in this process there is no need to use high pressure, energy, temperature and toxic chemicals.

Among the metal oxide nanoparticles, zinc oxide is attention-grabbing because it has enormous applications in various areas such as optical, piezoelectric, magnetic, and gas sensing. Besides these properties, ZnO nanostructure exhibits high catalytic efficiency, strong adsorption capability and are used more and more frequently in the manufacture of sunscreens, ceramics and rubber processing, wastewater treatment, and as a fungicide.

In modern years ZnO NPs have drawn attention of many researchers for their distinctive optical and chemical behaviours which can be easily tuned by changing the morphology. Within the large family of metal oxide NPs, ZnO NPs have been used in diverse cutting edge applications like electronics, communication, sensors, cosmetics, environmental protection, biology and the medicinal industry. Moreover, ZnO NPs has a tremendous potential in biological applications like biological sensing, biological labelling, gene delivery, drug delivery and nanomedicine^{5–8} along with its

antibacterial, antifungal, acaricidal, pediculocidal, larvicidal and anti-diabetic activities.

The major response concerned in the biosynthesis of ZnO nanoparticles mediated by the leaf extract of *Curcuma neilgherrensis* is reduction/oxidation reaction where the phytochemicals and enzymes present in biological resources take part for the translation of metal compounds in to specific nanoparticles.

Curcuma neilgherrensis Wight (Family: Zingiberaceae); well-known as “Kattukalvazhai” in Tamil is a folklore medicine widely used by the tribes of Western Ghats for the management of diabetes mellitus. The traditional medicinal practitioners of Kodagu District of Karnataka have recognized its usage in diabetes mellitus, even so the Palliayar tribes of Tamilnadu are using its tuber for edible purposes. The leaf is considered as the constructive part for counteracting the ailing effects of diabetes mellitus. In spite of its reputation, it has not yet been investigated scientifically and hence its leaves were contemplation worth to study in detail. The existing textual information about the herb is very minimum and inadequate. *C. neilgherrensis* is a herb with small conical rhizomes inside whitish in color, ending in root tubers, fusiform. Leaves green, lanceolate/oblong - lanceolate in shape, 25cm in length and 8 cm wide. Inflorescence there in both lateral and central, long with a distinct coma. Coma bracts are oblong- lanceolate, fused only at base, light to dark pink or violet in colour. Fertile bracts are combined about lower 1/3, slightly curved, margin wavy, green, green with a pink or violet spot at the tip, and densely pubescent. The bracteoles are triangular in shape. Flowers are longer than the bracts, 3-4 in each bract, and light yellow in colour. Calyx three lobed at apex, violet dotted, and densely pubescent. Corolla tube light yellow in colour, lobes unequal, pubescent, hooded at tip. Labellum shows a median cleft, yellow with a deep yellow median band.

MATERIALS AND METHODS

Extraction of the plant material

The fresh plant materials were washed with running tap water and shade dried. The leaves of *Curcuma neilgherrensis* were crushed to coarsely powdered by grinder. These coarse powders (25g) were then subjected to successive extraction in 250ml of each solvent (methanol) by using Soxhlet apparatus. The collected extracts were stored and then taken up for further investigations.

Phytochemical analysis

Preliminary phytochemicals analysis was carried out for all the *Curcuma neilgherrensis* extracts as per standard methods described by Brain and Turner 1975 and Evans 1996.

Detection of alkaloids

Curcuma neilgherrensis extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

Detection of Flavonoids

- a) Lead acetate test: Extracts were treated among few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.
- b) H₂SO₄ test: Extracts were treated with few drops of H₂SO₄. Formation of orange colour indicate the presence of flavonoids.

Detection of Steroids

2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H₂ SO₄. The colour changed from violet to blue or green in some samples indicate the presence of steroids.

Detection of Terpenoids**Salkowski's test**

0.2g of the extract of the whole plant sample be assorted with 2ml of chloroform and concentrated H₂SO₄ (3ml) was carefully added to form a layer. A reddish brown coloration of the inner face was indicates the presence of terpenoids.

Detection of Anthraquinones

About 0.2g of the leaf extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl₃ was added to the filtrate. Few drops of 10% NH₃ were added to the mixture and heated. Formation of pink colour indicates the presence anthraquinones.

Detection of Phenols

- a) Ferric chloride test: Leaf Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

- b) Lead acetate test: Leaf Extracts was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

Detection of Saponins

About 0.2g of the leaf extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy mass of small bubbles) shows the presence of saponins.

Detection of Tannins

A small quantity of extract was assorted with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

Detection of Carbohydrates

Extracts were dissolved separately in 5ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

Detection of Oils and Resins

Test solution was applied on filter paper. It develops a clear appearance on the filter paper. It indicates the presence of oils and resins.

Antimicrobial activity

The disc diffusion method (Bauer *et al.*, 1966) was used to display the antimicrobial activity. *In vitro* antimicrobial activity was screened by via Muller Hinton Agar (MHA) obtained from Hi-media (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterilized petriplates. The plates were allowed to coagulate for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums be allowed to dry for 5 minutes. The concentration of extracts is 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was positioned on the surface of medium and the extract was allowed to spread for 5 minutes and the plates were kept for incubation at 37⁰C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Synthesis of ZnO Nano Particles

Preparation of zinc oxide NPs, for the synthesis of NPs, 50 ml of plant leaves extract was taken and boiled at 60⁰–80⁰C by using a stirrer-heater. Then, 5 g of zinc nitrate was added to the solution as the temperature reached at 60⁰C. This mixture was then boiled until it transformed to a deep yellow coloured suspension. This paste was then collected in a ceramic crucible and heated in an air heated furnace at 60⁰C for 2 h. A light white coloured powder was obtained and this powder was carefully collected and sent for different characterizations. The material was powered by a mortar and pestle so, that got a fine powder, which is easy for further characterizations.

FT-IR Spectroscopy

Infrared light from suitable source passes through a scanning Michelson interferometer and Fourier Transformation gives a plot of intensity versus frequency. When a powdered plant sample is placed in the beam, it absorbs particular frequencies, so that their intensities are reduced in the interferogram and the ensuing Fourier transform is the infrared absorption spectrum of the sample.

Ultra- Violet Spectroscopy

The UV spectrum provides a useful means of detecting conjugated unsaturated chromophores within a molecule such as polyenes, α , β -unsaturated ketones and aromatic compounds. This can be particularly helpful in the identification of chromophores and flavones. The UV spectrum may be caused by the summation of chromophores from different parts of a polyfunctional molecule, and this should be considered in the light of deduction drawn from other spectroscopic methods and chemical degradation.

SEM analysis

Scanning electron microscopic (SEM) analysis was performed with the Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by simply dropping a very minute amount of the sample on the grid, with excess solution being removed using blotting paper. The film on the SEM grid was then allowed to dry by putting the grids under a mercury lamp for 5 min.

XRD Analysis

ZnO nanoparticles were examined by X-ray diffractometer. The powdered metal was stucked in the cubes of XRD and then the result was taken in the XRD equipment.

RESULTS AND DISCUSSION

Qualitative Phytochemical Analysis

The qualitative phytochemical analysis of the leaf methanol extract of *Curcuma neilgherrensis* was done to test for presence of various phytochemicals (**Table 1**). The plant *Curcuma neilgherrensis* shows more phytochemical constituents like alkaloids, flavonoids, steroids, phenols, tannin and carbohydrates. Terpenoids, Anthroquinone, saponins, oils and resins were absent in *Curcuma neilgherrensis*.

Table 1: Qualitative phytochemical analysis of *Curcuma neilgherrensis*

Phytochemicals	Observations	Sample CN
Alkaloids	Cream colour	+
Mayer's test	Reddish brown solution/ precipitate	+
Wagner's test		
Flavonoids	Yellow orange	+
Lead acetate test	Reddish brown / Orange colour precipitate	+
H ₂ SO ₄ test		
Steroids	Violet to blue or Green colour formation	+
Liebermann-Burchard test		
Terpenoids		-
Salkowski test	Reddish brown precipitate	
Anthroquinone		-
Borntrager's test	Pink colour	
Phenols		+
Ferric chloride test	Deep blue to Black colour formation	+
Lead acetate test	White precipitate	
Saponin	Stable persistent	-
Tannin	Brownish green / Blue black	+
Carbohydrates	Yellow / brownish / blue / green colour	+
Oil and Resin	Filter paper test	-

Antimicrobial activity of *Curcuma neilgherrensis*

The antimicrobial activity of *Curcuma neilgherrensis* methanol leaf extract was studied at concentrations of 20, 30, 40 and 50 μ l against the organisms *S.typhi*, *S.aureus*, *B.subtilis*, *E.coli* and *P.aeruginosa* shows better activity next to that is *E.coli* (**Table 2**). *In vitro* antimicrobial activity of methanolic extract of *Curcuma angustifolia* was studied against six pathogenic bacteria; on the basis of zone of inhibition it was reveal that this extract is quite effective against both gram positive and gram negative bacteria. Our results (**Fig. 1**) showed that extract has more effective towards gram positive i.e. *Staphylococcus epidermis*, *Staphylococcus aureus* than gram negative bacteria. Antimicrobial properties are very important tool for control of harmful microbial strains especially in the treatment of various infectious diseases. The methanolic extract rich in several bioactive compounds like phenol, ascorbic acid, flavonoids are the potent inhibitors against several harmful microorganisms. The previous report showed that plant rich in bioactive components are known to be antibacterial, antifungal, and thus conferring antimicrobial activities (Aboaba *et al.*, 2006). Early in 1992, Ferreira reported that arturmerone which is the main constitute of *Curcuma angustifolia* had antibacterial activity.

Table 2: Antimicrobial activity of *Curcuma neilgherrensis*

S. NO.	Organisms	Zone Of Inhibition (mm)				
		Control	Concentration of Sample 20 μ l	Concentration of Sample 30 μ l	Concentration of Sample 40 μ l	Concentration of Sample 50 μ l
1	<i>E.coli</i>	23mm	20mm	21mm	25mm	27mm
2	<i>P.aeruginosa</i>	26mm	15mm	18mm	19mm	28mm
3	<i>S.aureus</i>	23mm	12mm	16mm	20mm	24mm
4	<i>B.subtilis</i>	24mm	16mm	17mm	21mm	26mm
5	<i>S.typhi</i>	26mm	14mm	16mm	21mm	23mm

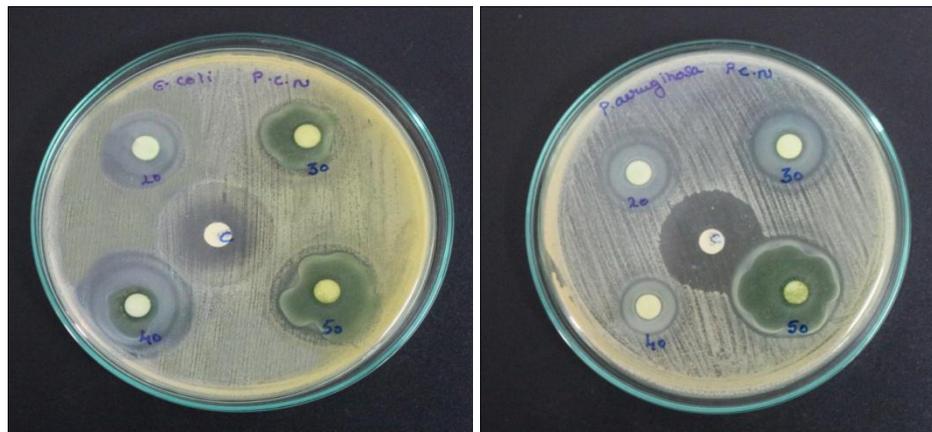


Fig.1 (a)

Fig.1 (b)

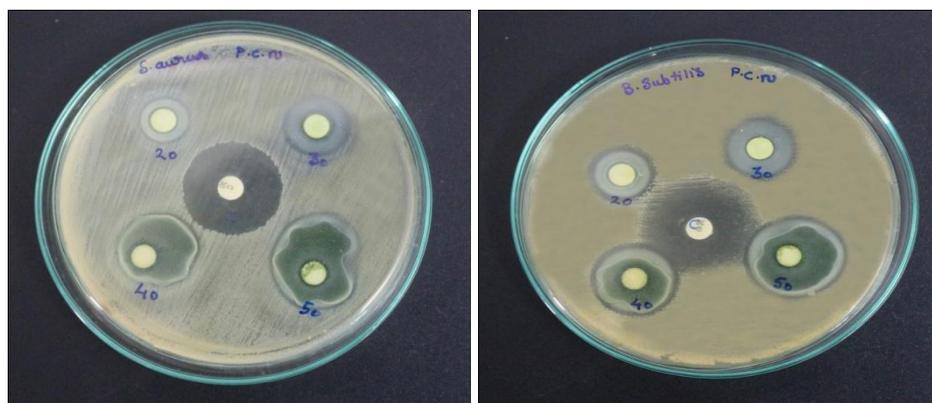


Fig.1 (c)

Fig.1 (d)



Fig.1 (e)

Fig. 1: Antibacterial activity of ZnO Nanoparticles synthesised using 50ml of *Curcuma neilgherrensis* extract against multiple pathogenic bacteria

UV Spectrum of *Curcuma neilgherrensis* extract

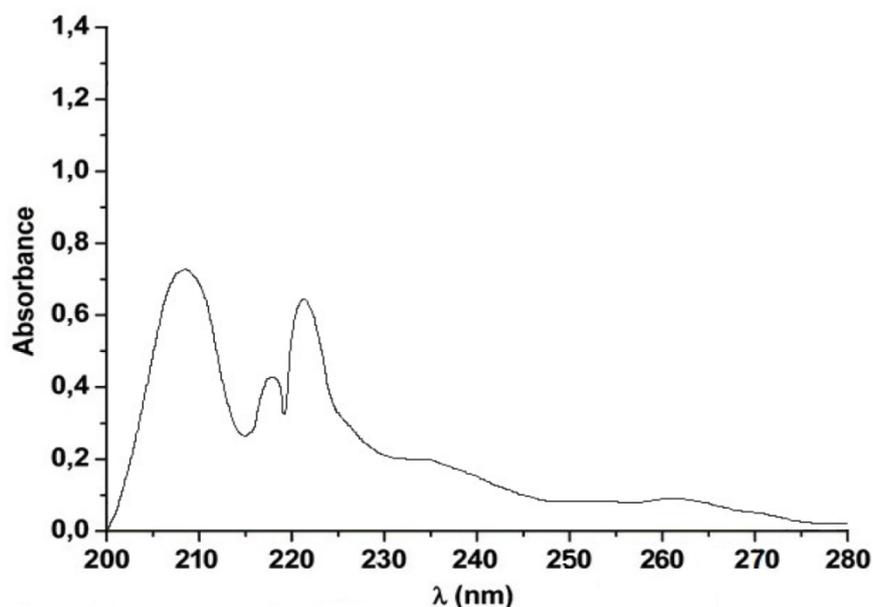


Fig. 2: UV spectra of ZnO Nanoparticles synthesised using 50ml of *Curcuma neilgherrensis* leaf extract

The UV analysis of the methanol extract synthesized zinc oxide nanoparticles of *Curcuma neilgherrensis* leaf extract (**Fig. 2**). The maximum absorption peak was obtained at 208 nm wavelength. And there are other peaks also which are 217 nm and 221 nm. The peak 208 nm is polyacetylenes which is Falcarinone, 217 nm is hydroxyl coumarins and 221 nm is Phenolic acids that are salicylic acids.

FTIR Spectrum of *Curcuma neilgherrensis* extract

The appearance of peaks at 3365.42cm^{-1} is Alcohol / Phenol O-H Stretch. The Alkyl C-H Stretch is 2930.40cm^{-1} , 14059.31cm^{-1} is Aliphatic Aldehydes (Very strong). The Aliphatic Esters (Very Strong) is 1362.96cm^{-1} . Aromatic Esters (Very strong) is 1106.20cm^{-1} . Meta di-substituted (Very strong) is 762.51cm^{-1} . Primary Amines (Medium) is 608.94cm^{-1} respectively (**Fig. 3**).

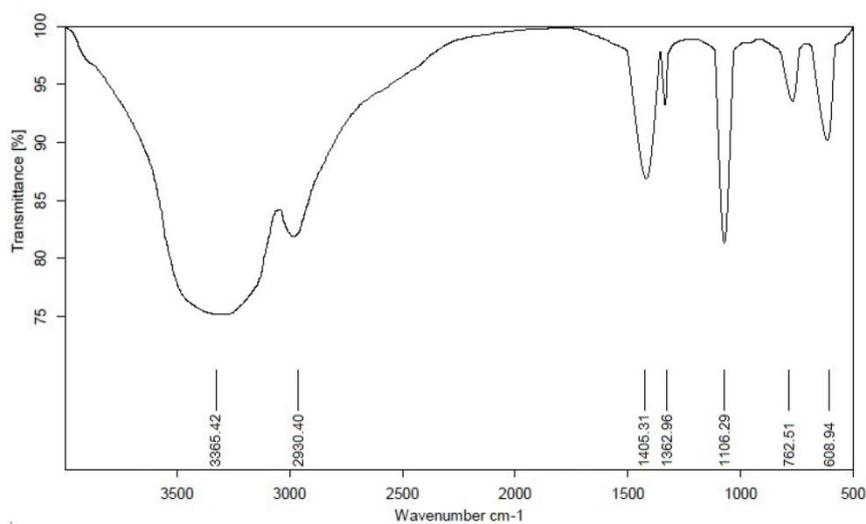


Fig. 3: FT-IR Spectra of ZnO Nanoparticles synthesised using 50ml of *Curcuma neilgherrensis* leaf extract

SEM analysis of *Curcuma neilgherrensis* extract

The SEM analysis of the zinc oxide nanoparticles revealed that their shape and sized less than 5 μm . The surface of nanoparticles were smooth (**Fig. 4**).

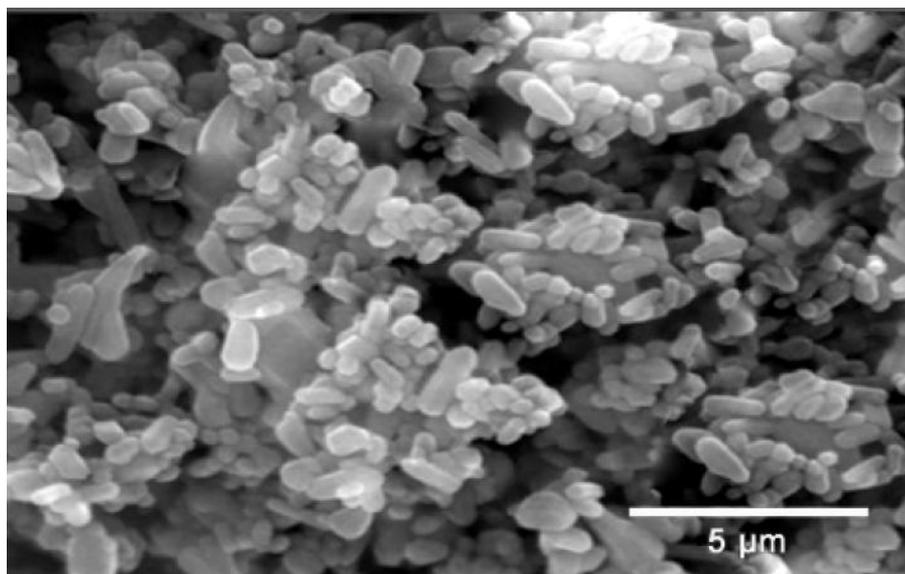


Fig. 4: SEM image of ZnO Nanoparticles synthesised using 50ml of *Curcuma neilgherrensis* leaf extract

XRD analysis of *Curcuma neilgherrensis* extract

XRD patterns of ZnO synthesized for *Curcuma neilgherrensis* leaf extract are shown in the **Fig. 5**. The diffraction peaks at $2\theta=32.16, 34.93, 36.81, 47.82, 56.97$ and 64.14 corresponding to (100), (002), (101), (102), (110), and (103) planes respectively were observed and compared with the standard powder diffraction card of JCPDS No. 36:1451.

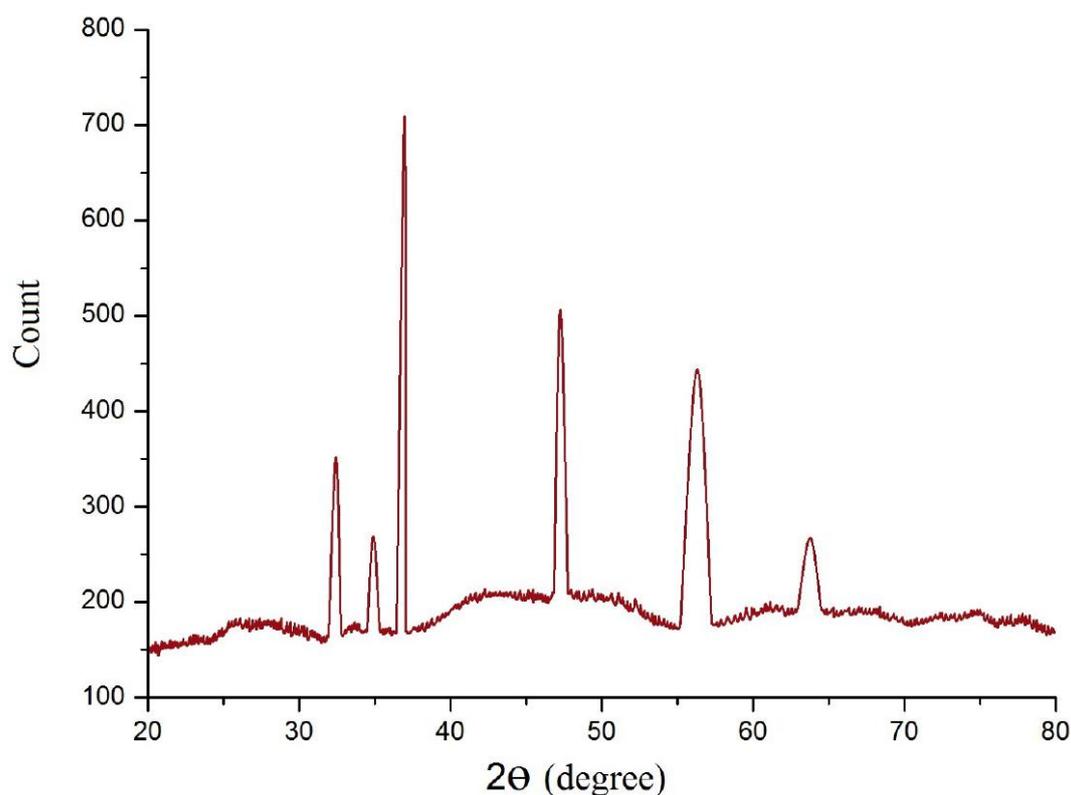


Fig. 5: XRD spectrum of ZnO Nanoparticles synthesised using 50ml of *Curcuma neilgherrensis* leaf extract

CONCLUSION

In the present study, the ZnO nanoparticles were synthesized using *Curcuma neilgherrensis* methanol leaf extract. The methanol leaf extract was found to possess alkaloids, flavonoids, steroids, phenols, tannin and carbohydrates. The nanoparticles were subjected to antibacterial study and were found to be effective. The nanoparticles were analysed using UV, FTIR, SEM and XRD. In UV analysis, the maximum absorption was at 208 nm. The FTIR analysis showed the presence of

Alcohol / Phenol O-H Stretch, Alkyl C-H Stretch, Aliphatic Aldehydes, Aliphatic Esters, Aromatic Esters, Meta di-substituted and Primary Amines (Medium). The SEM analysis revealed that the nanoparticles were of less than 5 μm shape. The size of the nanoparticles are 3.34, 3.63, 5.08, 2.27, 1.47 and 1.56. From this study, it was evident that the plant *Curcuma neilgherrensis* can be used to synthesize nanoparticles using green chemistry methods for various applications.

FINANCIAL SUPPORT AND SPONSORSHIP:

Nil.

CONFLICT OF INTEREST:

There are no conflicts of interest.

ACKNOWLEDGEMENTS

The author is sincere thanks to the Managing Director of Alpha Omega Hi-tech Bio Research Centre, Salem for supporting and providing the facility to work in the lab.

REFERENCES

- [1] C. Dagdeviren, S. W. Hwang, Y. Su, S. Kim: Transient, Biocompatible Electronics and Energy Harvesters Based on ZnO. *Small* 2013; 9(20): 3398–3404.
- [2] L. Wang, Y. Kang, X. Liu, S. Zhang: ZnO nanorod gas sensor for ethanol detection. *Sens. Actuators B* 2012; 162(1): 237–243.
- [3] S. E. Cross, B. Innes, M. S. Roberts, T. Tsuzuki: Human skin penetration of sunscreen nanoparticles: in vitro assessment of a novel micronised zinc oxide formulation. *Skin Pharmacol. Physiol* 2007; 20(3): 148–154.
- [4] J. Zhou, N. Xu and Z. L. Wang: Dissolving behavior and stability of ZnO wires in biofluids: a study on biodegradability and biocompatibility of ZnO nanostructures. *Adv. Mater* 2006; 18(18): 2432–2435.
- [5] J. W. Rasmussen, E. Martinez, P. Louka and D. G. Wingett: Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. *Expert Opin. Drug Delivery* 2010; 7(9): 1063–1077.
- [6] G. Apperrot, A. Lipovsky, R. Dror and N. Perkas: Enhanced antibacterial activity of nanocrystalline ZnO due to increased ROS-mediated cell injury, *Adv. Funct. Mater* 2009; 19(6): 842–852.

- [7] D. Sharma, J. Rajputa, B. S. Kaitha and M. Kaurb: Synthesis of ZnO nanoparticles and study of their antibacterial and antifungal properties. *Thin Solid Films* 2010; 519(3): 1224– 1229.
- [8] S. Nair: Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. *J. Mater. Sci.: Mater. Med* 2009; 20 Suppl. 1: S235–S241.
- [9] V. Kirthi, A. A. Rahuman, G. Rajakumar, S. Marimuthu: Acaricidal, pediculocidal and larvicidal activity of synthesized ZnO nanoparticles using wet chemical route against blood feeding parasites. *Parasitol. Res* 2011; 109: 461–472.
- [10] Alkaladi, A. M. Abdelazim and M.A: Antidiabetic Activity of Zinc Oxide and Silver Nanoparticles on Streptozotocin-Induced Diabetic Rats. *Int. J. Mol. Sci* 2014; 15: 2015–2023.
- [11] R. Seshadri, in: C.N.R. Rao, A. Muller, A.K. Cheetham (Eds.): *The Chemistry of Nanomaterials*. Wiley-VCH Verlag GmbH, Weinheim 2004; 1: 94–112.
- [12] L. Theodore, *Nanotechnology: Basic Calculations for Engineers and Scientists*. Wiley, Hoboken; 2006.
- [13] X. Wang, J. Lu, M. Xu, B. Xing: Sorption of pyrene by regular and nanoscaled metal oxide particles influence of adsorbed organic matter. *Environmental Science and Technology* 2008; 42: 7267–7272.
- [14] J. Nicole, R. Binata, R.T. Koodali, M.C. Adhar: *FEMS Microbiol. Lett* 2008; 279: 71–76.
- [15] Zhang M, Liu M, Prest H and Fischer's: Nanoparticles secreted from iry rootlets for surface climbing. *Nanoletters* 2008; 8: 1277 – 1280.
- [16] Jeong S, Yeo S and Yis: Antibacterial characterization of silver nanoparticles against *E. coli* ATCC-15224. *Journal of Material Science* 2005; 40: 5407.
- [17] Savithamma N, Lingarao M, Rukmini K and Suvarnalatha DP: Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. *International Journal of Chem Tech Research* 2011; 3: 1394-1402.
- [18] Aboaba OO, Smith SI, Olude FO: Antimicrobial effect of edible Plant extract on *Escherichia coli* 0157: H7. *Pak J Nutri* 2006; 5(4): 325-327.
- [19] Negi, P.S, Jayaprakasha, G.K. Rao, L.J.M. Sakariah, K.K: Antibacterial activity of turmeric oil: A byproduct from curcumin manufacture. *J of Agri and Food Chem* 1999; 47, 4297-4300.