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Green Synthesis and Characterization Of Zinc Oxide Nanoparticles Using Seaweed Sulphated Polysaccharide and their Anti-Bacterial Properties

S.Bhuvaneshwari[1], Dr.K.Padmalochana[2], S.Savetha[3], Dr.A.Natarajan[4]
1Assistant Professor, Department Of Biochemistry, KingNandhivarman College Of Arts And science, Thellar, Vandhavasi TK-604 406, Tamil Nadu
2Head Of The Department, Department Of Biochemistry, SriAkilandewari Women's College, TindivanamHighway,Vandavasi-604408,Tamilnadu
3Assistant Professor, Department Of Biochemistry, King Nandhivarman College Of Arts And Science, Thellar, Vandhavasi TK-604 406, Tamil Nadu
4Assistant Professor Department Of Biochemistry, KingNandhivarman College Of Arts And science, Thellar, Vandhavasi TK-604 406, Tamil Nadu

ABSTRACT
Nanotechnology is an area of science and engineering that deals with the Nan scales, which range from 1 to 100 nanometers. Zinc oxide nanoparticles are widely employed in a variety of industries, including solar cells, ultraviolet light emitting devices, gas sensors, photocatalysts, pharmaceuticals, and cosmetics. The current study focuses primarily on the synthesis of Zinc oxide nanoparticles from seaweed sulphated polysaccharides. Aim of the study isisolation and characterization of sulphated polysaccharide using seaweed *Turbinariaornata*. To Synthesis zinc oxide nanoparticles using sulphated polysaccharides and their physico-chemical characterization and study the antibacterial activity of green synthesized zinc oxide nanoparticles.

keywords: TurbinariaOrnate, ZincOxide Nanoparticles, SulphatedPolysaccharides

INTRODUCTION

Nanotechnology is an area of science and engineering that deals with the Nan scales, which range from 1 to 100 nanometers. Various nanoparticles such as Fe, Ni, Co, Mn, Zn, and others are widely used as magnetic materials in a variety of applications including electronic ignition systems, generators, vending machines, medical implants, wrist watches, inductor core, transformer circuits, magnetic sensors and recording equipment, telecommunications, magnetic fluids, microwave absorbers, and so on. They can be used in a variety of high-frequency applications (Willard et al., 2004). Drug delivery, imaging, and diagnosis are just a few of the many medical applications of nanoparticles. Nanoparticles have a high surface-to-volume ratio due to their small size, which provides them unique characteristics. Zinc oxide nanoparticles have been shown to have antifungal and antibacterial properties in numerous studies. Brown seaweed, in particular, has a wide range of bioactivities, including antioxidant, antibacterial, anticoagulant, antihypertensive, and anticancer properties (Heo et al., 2005; Mayer and Hamann, 2004). Sulfated polysaccharides and other natural chemicals from brown seaweeds have been shown to have a variety of bioactivities in recent years, including neuroprotective, antiviral, anti-cancer, anti-inflammatory, and anti-coagulant properties (Ina et al., 2007; Chen et al., 2012).However, because marine bioresources are rich in bioactive compounds such as polysaccharides, flavonoids, polyphenols, and terpenoids, they have proven to

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be the most important sources for nanoparticle synthesis. As a result, the current study focuses primarily on the synthesis of Zinc oxide nanoparticles from seaweed*(Turbinaria ornate*) sulphated polysaccharides.

MATERIALS AND METHODS

1. Collection of seaweed

Turbinariaornata, a marine brown algae, was collected in February 2022 from the intertidal region of the Gulf of Mannar (Latitude 9°16'56" N; Longitude 79°11'27" E) in Tamil Nadu, India. The seaweed was recognised using Umamaheswara Rao's standard systematic key reference (1987). The seaweed was gathered and cleansed with tap water to remove pollutants, salts, and sand from the algal surface, then rinsed with distilled water and dried in the shade. The dried seaweed was ground and stored in an airtight container using a mixer grinder.

2. Isolation of sulphated polysaccharides

The isolation of sulphated polysaccharideswas carried out following the method as described by (Yang et al., 2008). In a beaker, 100 g of seaweed powder was treated with 1 L of ethanol, and stirred continuously for about 12 h at 37 ºC in order to eliminate pigments followed by centrifugation at 2000 rpm for 10 min. The biomass was dried and dissolved in 1 L of 0.05 M HCl with continuous stirring at 37 ºC for 24 h. This procedure was repeated twice and the resultant supernatant was precipitated with 2% $CaCl_2$ and kept at 4 ºC overnight which was subsequently centrifuged at 15,000 rpm for 10 min. To the supernatant, two volumes of absolute ethanol were added and the solution was kept at 4 ºC overnight. The resultant solution was then centrifuged at 15,000 rpm for 10 min and the precipitate was collected. The collected polysaccharide was dialyzed with a membrane (MW CO 14000, Hi Media, Mumbai, India) at 4 ºC for 48 h and lyophilized for further studies. Sulphated polysaccharides yield (%) was calculated using following equation.

$$Yield\ (\%) = \frac{DW}{AW} \times 100$$

Where, DW is the dry weight of sample collected through ethanol precipitation; AW is the algal weight used in this experiment.

3. Thermal gravimetric analysis of Sulphated polysaccharides

Thermal gravimetric analysis was performed using STA 6000 system (Perkin Elmer, US) in a nitrogen atmosphere. Samples of 8-10 mg were analyzed from 37 ºC to 600 ºC at a rate of 10 ºC min^{-1}.

4. Synthesis of ZnO Nanoparticles

About 400 mL of 0.1 M Zinc acetate was added to 80 mL of sulphated polysaccharide (1 gm) and the mixture was stirred on a magnetic stirrer. Further, 400 mL of 0.2 M NaOH was added under vigorous stirring conditions for 3 h at 60 °C. After 3 h, the appearance of white precipitates indicated the formation of ZnO nanoparticles. The precipitates were then collected, centrifuged and dried using hot air oven. The dried ZnO nanoparticle powder is subjected for 4 h at 400 °C using a muffle furnace.

5.Physico-chemical Characterization

Optical absorbance of biogenic ZnO nanoparticles was determined using UV–Visible spectroscopy (Shimadzu UV-1800) in the range of 200–600 nm. The surface morphology, shape and size details of the samples were examined by high-resolution scanning electron microscopy (HR-SEM) equipped with EDAX analysis (Supra 55-Carl Zeiss, Germany).

6. Microorganism and media

The strain used in this research was luminescence *Vibrio harveyi*, which had been isolated from diseased shrimp. All culture medium contained Mueller-Hinton Broth (MHB), Mueller-Hinton agar (MHA), Nutrient broth (NB), Tryptic soy agar (TSA) and Tryptic soy broth (TSB) and

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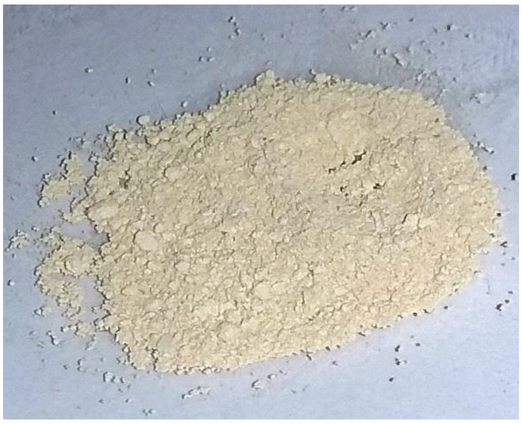
sodium chloride were purchased from Himedia (Mumbai).

7. Zone of inhibition (ZOI) assay

The antibacterial activity of green synthesized zinc oxide nanoparticles was assayed against *Vibrio harveyi* by using agar well diffusion method (Ahmad and Beg, 2001). In brief, Mueller-Hinton Agar (MHA) plates were inoculated uniformly with 1.5 × 10^8 CFU/mL (0.5 McFarland Standard) of bacterial suspension, using sterile swabs in triplicates. Wells of 6 mm size were created with sterile cork borer in the agar plates containing the bacterial inoculum. Then, 50 µL of different concentration of zinc oxide nanoparticles (10, 25, 50, 75 and 100 µg/mL) were introduced into each of the wells and allowed to diffuse at 4 °C for 2 h. Finally, the plates were incubated at 30 °C for 24 h.

Schematic diagram of green synthesis of zinc oxide nanoparticles using sulphated polysaccharide from seaweed *Turbinariaornata*

Turbinariaornata **Powder of*Turbinariaornata***



Zinc oxide nanoparticles Sulphated polysaccharide

RESULTS AND DISCUSSION

Nanoscience and nanotechnology provide a wide range of instruments for use in several sectors of science. Numerous biomimetic approaches have been developed for the advancement of nanomaterials, thanks to the ever-growing technology at the nanoscale. As a result, this "green chemistry," which employs biological materials such as plant biomass or plant extracts, could be a viable alternative to traditional chemical and physical processes for creating nanomaterials that are

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less toxic, biocompatible, and cost-effective. Green production of zinc oxide nanoparticles and their antibacterial properties were proven in this study.

UV-VISISBLESPECTROSCOPYAND THERMAL ANALYSIS OFSULPHATED POLYSACCHARIDES

Figure.1, shows the distinctive UV-vis spectrums of sulphated polysaccharide. Sulphated polysaccharide with a prominent distinctive peak at 292 nanometers. In Fig. 2, the TGA curve of sulphated polysaccharides was interpreted in three steps. The initial step of the TGA curve, which occurred between 40 and 138 ^{o}C, revealed weight loss in the sample due to the dehydration process. The material was then subjected to pyrolysis processes at 140 degrees Celsius. The second stage, which took place between 215 and 400 ^{o}C, showed the evolution of the volatile matter, indicating that the sample had devolatilized. The final stage started at around 450 degrees Fahrenheit and went up to 600 degrees Fahrenheit. The ash content is visible in the residual sample taken at the end of the process. The remaining mass is most likely made up of sulphates, phosphates, and carbonates, all of which are frequent in sulfated polysaccharides (Rodriguez-Jasso et al., 2011).

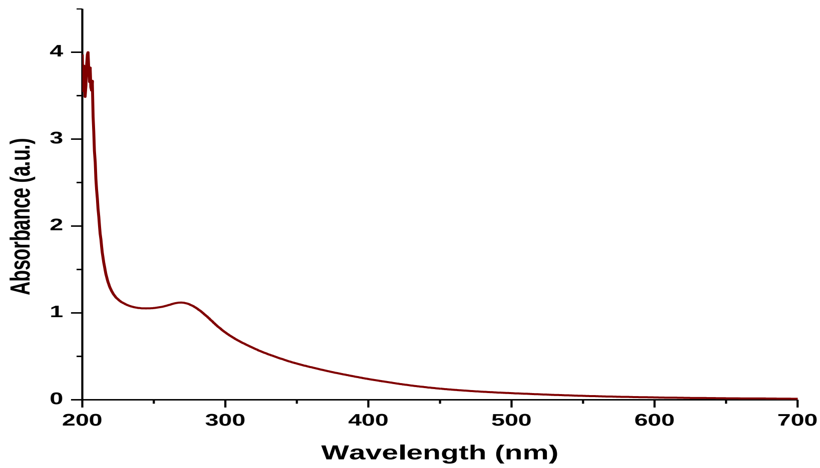

Fig.1. UV–Vis absorption spectra of sulphated polysaccharides isolated from seaweed

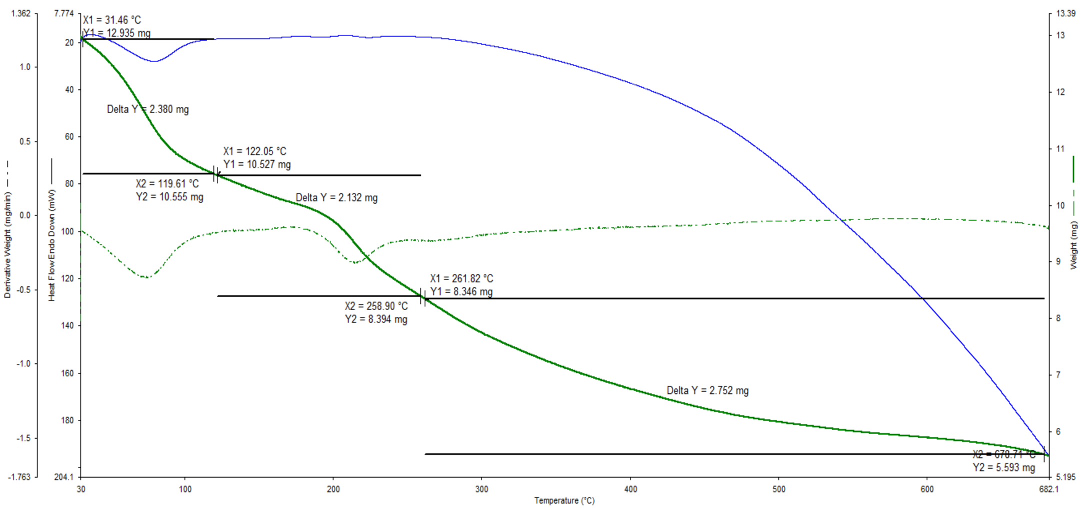

Fig. 2. TGA characteristics of sulphated polysaccharides isolated from seaweed

UV–VISIBLE SPECTROSCOPY OF ZINC OXIDE NANOPARTICLES

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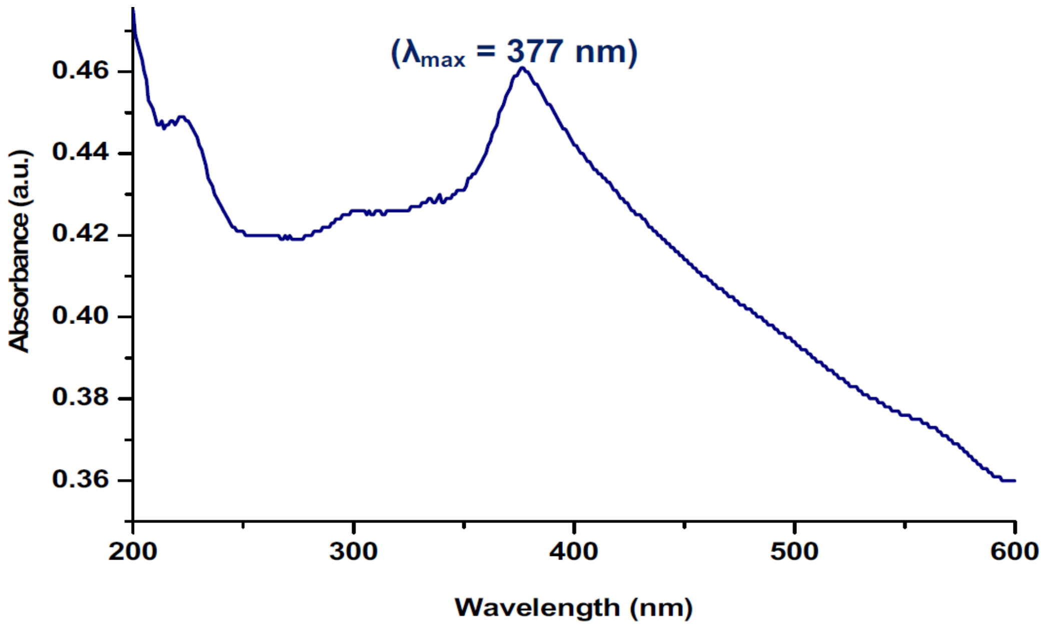
The technique of UV–Vis absorption spectroscopy is commonly utilised to examine the optical characteristics of nanoscale materials. The UV–Vis spectroscopy of ZnO nanoparticles is shown in Figure 3.3, with a prominent distinctive peak at 377 nm.



FE-SEM WITH EDAX STUDIES
FE-SEM micrograph is used to predict the surface morphology of the seaweed *T. ornata*-mediated synthesis of ZnOnanoparticles. Figure .4 showed that most of the ZnO nanoparticles were spherical and some of them were also rod shaped with average size of 15 nm. Further, EDAX analysis of the ZnO nanoparticles (Fig. 5) confirmed the signal characteristic of Zn and O only confirms the high purity of ZnO nanoparticles formed.
WELL DIFFUSION STUDIES

Besides antibacterial activity of zinc oxide nanoparticles was assessed using the well diffusion technique. The zinc oxide nanoparticles exhibited excellent inhibitory effects against *V. harveyi* (Fig. 6). And zone of inhibition for sizes of 2, 6, 9, 12, 14 mm were observed. ZnO is described as a functional, strategic, promising, and versatile inorganic material with a broad range of applications. It is characterized by a direct wide band gap (3.3 eV) in the near-UV spectrum, a high excitonic binding energy (60 meV) at room temperature (Wang, 2004; Wang and Song, 2006; Janotti and Van de Walle, 2009; Zhang et al., 2012;). The synthesis of nano-sizedZnO has led to the investigation of its use as new antibacterial agent. In addition to its unique antibacterial and antifungal properties, ZnO-NPs possess high catalytic and high photochemical activities. ZnO possesses high optical absorption in the UVA (315–400 nm) and UVB (280–315 nm) regions which is beneficial in antibacterial response and used as a UV protector in cosmetics (Song et al., 2011).

ZnO-NPs antibacterial activity directly correlates with their concentration as reported by several studies, likewise, the activity is size dependent, however, this dependency is also influenced by concentration of NPs. Larger surface area and higher concentration are accountable for ZnO-NPs antibacterial activity (Zhang et al., 2007; Peng et al., 2011). ZnO-NPs of smaller sizes can easily penetrate into bacterial membranes due to their large interfacial area, thus enhancing their

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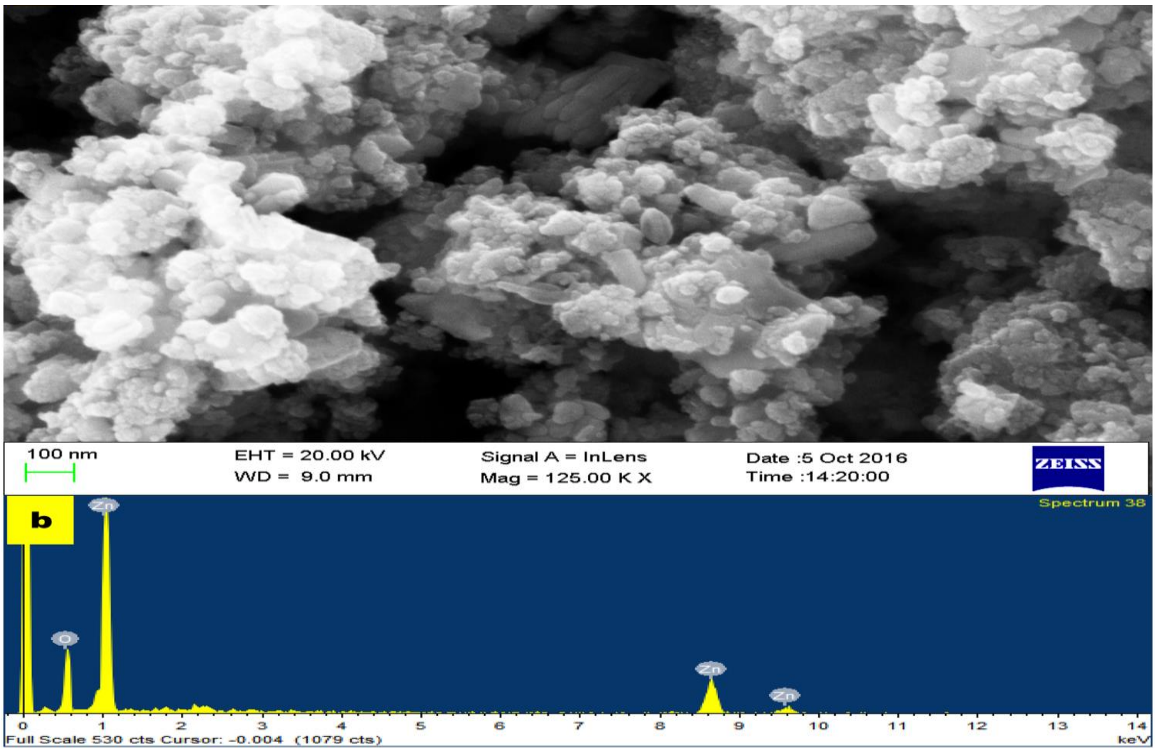
antibacterial efficiency. A large number of studies investigated on the considerable impact of particle size on the antibacterial activity, and the researchers found that controlling ZnO-NPs size was crucial to achieve best bactericidal response, and ZnO-NPs with smaller size (higher specific

surface areas) showed highest antibacterial activity (Zhang et al., 2007; Yamamoto, 2001; Sawai et al., 1996). In general, a correspondence between NPs size and bacteria appears to be required for the bioactivity of ZnO-NPs, as well the concentration. Yamamoto (2001) examined the influence of ZnO-NPs size (100–800 nm) on the antibacterial activity, against *S. aureus* and *E. coli* by changing the electrical conductivity with bacterial growth. It was concluded that decrease in particle size will increase the antibacterial activity. Similarly, it was found that ZnO-NPs antibacterial activity toward *S. aureus* and *E. coli* increases with decreasing the size (Jones et al., 2008; Padmavathy and Vijayaraghavan, 2008; Zhang et al., 2007). Size-dependent bactericidal activity was also extensively evaluated by Raghupathi et al. (2011). The authors targeted a number of major gram-negative and gram-positive strains, and revealed that ZnO-NPs antibacterial activity was inversely proportional to the particle size. Based on the growth curves and percentage viability, their findings revealed that the activity is size dependent, where smaller sized ZnO-NPs possess best antimicrobial action under visible light. The importance and significance of ZnO-NPs in various areas has developed global interest to study their antibacterial activity. The documented antibacterial actions of ZnO-NPs have stimulated a considerable range of antimicrobial applications. When compared to organic-based disinfectants, ZnO-NPs have unique features and have a long life, which has prompted its use as an antibacterial agent. Because of their enormous surface area-to-volume ratio, they can be used as novel antibacterial agents, which have recently become a source of concern for researchers.



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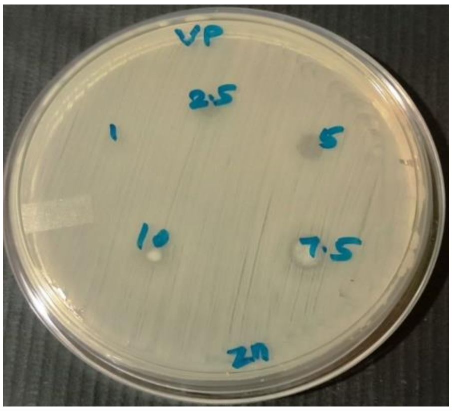

Figure 6. Zone of inhibition area caused by different concentration of zinc oxide nanoparticlesSUMMARY

- *Turbinariaornata*, a brown alga, was used to isolate the sulfated polysaccharide. TGA analysis was also used to describe the sulfated polysaccharide. The initial step of the TGA curve, which occurred between 40 and 138 oC, revealed weight loss in the sample due to the dehydration process. The material was then subjected to pyrolysis processes at 140 degrees Celsius. The second stage, which took place between 215 and 400 oC, demonstrated the progression of the volatile matter, indicating that the sample had devolatilized. The final stage started at around 450 degrees Fahrenheit and went up to 600 degrees Fahrenheit.

- Surface plasmon resonance (SPR) about 377 nm was observed in zinc oxide nanoparticles produced with "sulfated polysaccharide" from the seaweed *Turbinariaornata*. The nanoparticles were mostly spherical, with an average size of about 15 nanometers. Furthermore, EDAX examination of the ZnO nanoparticles corroborated the signal characteristic of Zn and O, indicating that the nanoparticles are of excellent purity.

- The well diffusion approach was used to test the antibacterial activity of zinc oxide nanoparticles. Against V. harveyi, the zinc oxide nanoparticles had excellent inhibitory effects.

REFERENCES

- Willard, M. A.,Kurihara, L. K., Carpenter, E. E., Calvin, S.,and Harris, V. G.Chemically prepared magnetic nanoparticles. International Materials Reviews 49 (2004):pp. 125-170
- Shankar, S. S., Rai, A., Ahmad, A., and Sastry, M. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (Azadirachtaindica) leaf broth.Journal of Colloid and Interface Science 275(2004):pp. 496–502
- DeSimone, J. M. (2002). Practical approaches to green solvents.Science 297 (2002): pp.799–803
- Raveendran,P., Fu,J.,Wallen, S.L. Completely green synthesis and stabilization of metal nanoparticles. Journal ofthe American Chemical Society 125 (2003): pp. 13940–13941

- Deng, Z., Chen, M., Gu, G., Wu, L.A facile method to fabricate ZnO hollow spheres and their photocatalytic property. Journal of Physical Chemistry B 112 (2008):pp. 16-22
- Yang, S.J., Park, Ch.R. Microwave-assisted additive free synthesis of nanocrystalline zinc oxide. Nanotechnology 19 (2008):pp. 035609.
- Krishnakumar,T., Jayaprakash, R., Pinna, N., Singh, V.N., Mehta, B.R., Phani, A.R. Triangular CdS nanocrystals: Structural and optical studies. Materials Letters 63 (2) (2008): pp. 242

DOI:10.46647/ijetms.2022.v06i04.0051 ISSN: 2581-4621

- Cao, B., Cai, W. From ZnONanorods to Nanoplates.Chemical Bath Deposition Growth and Surface-Related Emissions. J. Phys. Chem. C, 112 (3) (2008):pp.680-685
- Li, M., Bala, H., Lv, X., Ma, X., Sun, F., Tang, L., Wang, Z. Direct synthesis of monodispersedZnO nanoparticles in an aqueous solution. Materials Letters 61 (2007):pp.690-693.
- Raoufi, D. Synthesis and microstructural properties of ZnO nanoparticles prepared by precipitation method. Renewable Energy 50 (2013): pp. 932-937
- Arabi, F., Imandar, M., Negahdary, M., Imandar, M., Noughabi, M. T., Akbari-dastjerdi, H., Fazilati, M. Investigation anti-bacterial effect of zinc oxide nanoparticles upon life of Listeria monocytogenes. Scholars Research Library Annals of Biological Research 3 (7)(2012): pp. 3679-3685
- Singh, G., Joyce, E. M., Beddow, J., and Mason, T. J.Evaluation of antibacterial activity of ZnO nanoparticles coated sonochemically onto textile fabrics, Journal of Microbiology, Biotechnology and Food Sciences 2 (1) (2012): pp. 106-120
- Padmavathy, N.,Vijayaraghavan, R. Enhanced Bioactivity of ZnO Nanoparticles - an Antimicrobial Study. Science and Technology of Advanced Materials9 (2008) : pp.1468-6996
- Ahmad I, Beg AZ (2001) Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J Ethnopharmacol 74(4):113–123.
- Jones N, Ray B, Ranjit KT, Manna AC. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS Microbiol. Lett. 2008; 279(1):71–76.
- Padmavathy N, Vijayaraghavan R. Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study. Sci. Technol. Adv. Mater. 2008;9(3):035004.
- Raghupathi KR, Koodali RT, Manna AC. Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. Langmuir. 2011;27(7):4020–4028.
- Fan Z, Lu JG. Zinc oxide nanostructures: synthesis and properties. J. Nanosci. Nanotechnol. 2005;5(10):1561–1573.
- Wang ZL. Zinc oxide nanostructures: growth, properties and applications. J. Phys.: Condens. Matter. 2004;16(25):R829–R858.
- Wang ZL, Song J. Piezoelectric nanogenerators based on zinc oxide nanowire arrays. Science. 2006;312(5771):242–246.
- Janotti A, Van de Walle CG. Fundamentals of zinc oxide as a semiconductor. Rep. Prog. Phys. 2009;72(12):126501.
- Zhang Y, Ram MK, Stefanakos EK, Goswami DY. Synthesis, characterization, and applications of ZnO nanowires. J. Nanomater. 2012;2012:1–22.
- Schmidt-Mende L, MacManus-Driscoll JL. ZnO-nanostructures, defects, and devices. Mater. Today. 2007;10(5):40–48.
- Wellings J, Chaure N, Heavens S, Dharmadasa I. Growth and characterisation of electrodeposited ZnO thin films. Thin Solid Films. 2008;516(12):3893–3898.
- Song Z, Kelf TA, Sanchez WH, Roberts MS, Rička J, Frenz M, Zvyagin AV. Characterization of optical properties of ZnO nanoparticles for quantitative imaging of transdermal transport. Biomed. Opt. Express. 2011;2(12):3321–3333.
- Zhang L, Jiang Y, Ding Y, Povey M, York D. Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids) J. Nanopart. Res. 2007;9(3):479–489.
- Yamamoto O. Influence of particle size on the antibacterial activity of zinc oxide. Int. J. Inorg. Mater. 2001;3(7):643–646.
- Peng X, Palma S, Fisher NS, Wong SS. Effect of morphology of ZnO nanostructures on their toxicity to marine algae. Aquat. Toxicol. 2011; 102(3):186–196.