UTTAR PRADESH JOURNAL OF ZOOLOGY

43(24): 476-483, 2022
ISSN: 0256-971X (P)

SYNTHESIS AND CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES USING AQUEOUS EXTRACT OF *Illicium verum* AND ITS ANTIMICROBIAL APPLICATION

L. F. A. ANAND RAJ [a*], A. SATHYA [a], B. CELSIA ELIZABETH [a] AND SNEHA KARTHIKEYAN [a]

[a] Department of Biotechnology, St. Joseph's College of Engineering, OMR, Chennai, Tamil Nadu, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2022/v43i243349

Editor(s):
(1) Dr. Angelo Mark P. Walag, University of Science and Technology of Southern Philippines, Philippines.
Reviewers:
(1) P. Aji Udhaya, India.
(2) Anil R. Shet, KLE Technological University, India.

Received: 20 October 2022
Accepted: 28 December 2022
Published: 30 December 2022

Original Research Article

ABSTRACT

The study mainly focuses on the preparation and characterization of Zinc oxide nanoparticles using an aqueous fruit extract of *Illicium verum*. Zinc Oxide nanoparticles were synthesized by a biological method using the fruit extract of *Illicium verum* using Zinc acetate dehydrate in the presence of Sodium hydroxide. The synthesized Zinc Oxide nanoparticles were characterized using Scanning Electron Microscope (SEM), Energy Dispersive X-ray analysis (EDX) and Fourier Transform Infrared Spectroscopy (FTIR). Further applications of its antimicrobial activity were studied. SEM results reveal that the shape of Zinc oxide nanoparticles was spherical and the average size ranges from 20-40 nm. EDX analysis provides the elemental composition of Zinc and oxygen present in the ZnO nanoparticles. The weight percentage of Zinc and oxygen was found to be 62.59 and 37.41 respectively. Chemical bond formations were confirmed by using FTIR analysis. The antibacterial activity was performed against gram negative (*Escherichia coli, Klebsiella pneumonia*) and gram positive (*Staphylococcus aureus, Bacillus subtilus*) bacteria. Finally, the study concluded that can be used for the synthesis of ZnO nanoparticles in a simple, cost effective and also the zinc oxide nanoparticles demonstrated riveting antimicrobial activity with both gram-positive and gram-negative bacteria.

Keywords: Green synthesis; zinc oxide nanoparticles; *Illicium verum*; antibacterial activity.

**Corresponding author;*

1. INTRODUCTION

The most well-known technology in contemporary science is nanotechnology. Nowadays, application of nanoscale materials in the field of science and technology become inevitable due to its wide range of application. Several preparation methods have been applied to synthesis the nanoparticles, including physical, chemical, and biological processes [1]. Among these, physical and chemicals processes were highly expensive, toxic and bring deleterious effect to the environment. Biological processes were generally preferred as they have tremendous applications in the field of cytotoxicity [2], wound healing [3], waste water treatment [4], seed germination [5], etc.

In recent days, dealing with resistance against microbial pathogenicity using metal nanoparticles has caught the attention of researchers around the globe [6]. Among the various metal nanoparticles, Zinc oxide was quoted to have good antibacterial property and non-toxicity [7] and this is due to its distinct mechanisms like reactive oxygen species (ROS) production [8,9,10] and intrinsic antimicrobial properties [8,11,12]. ZnO nanoparticles were also found to exhibit membrane destabilization upon direct contact with microbial cell wall [8,13,14]. They can potentially invade the microbial cell membrane and rupture the integrity of the phospholipid bilayer, which will further make the cells to ooze out its cellular components like lipids, polysaccharides, ATP, proteins and nucleic acids [15]. Various biomedical applications, including anticancer, antibacterial, antioxidant, as well as drug transport and bioimaging applications have caused great interest towards ZnONPs [16-18]. The experiment focuses on the environmentally-friendly synthesis of ZnONPs, their characterization, and can be used as an antibacterial agent while maintaining consideration for global solid waste management and an environmentally favorable approach that is required [19].

Nanoparticles were conventionally synthesized by chemical methods like, coprecipitation, hydrothermal synthesis, sono-chemical etc. But these methods were susceptible to side effects [20]. Also, since natural resources are getting diminished day by day, scientists were moving towards finding alternative biological methods that had no side effects, environmentally friendly and does not involve loss of resources [21]. That's how green synthesis of metal nanoparticles came into light. Green synthesis is a biological method, which involves the synthesis of nanoparticles based on plant, plant extracts and microorganisms [22].

For over a thousand years, medicinal plants were known for their predominant role in Ayurveda for treating human diseases. The isolated plant compounds were used as a therapeutic agent either in their natural or semi-synthetic form. The present findings and research demonstrated that the antimicrobial activity and inhibition of the enzymes varied with various components of the plant species. Medicinal plants, including water extracts of C. giganteash components, is used as bioreduction tools for the development of Zinc nanoparticles [22]. Among various medicinal plants, *Illicium verum* is one of the most widely used plant species in many countries as it is grown as an aromatic evergreen tree [23]. It is found in southern China and Vietnam [24]. Recent studies state that *Illicium verum* can effectively fight against various microorganisms like bacteria and fungi as well as show anti-inflammatory and antioxidant properties [25]. The plant extracts were also used in Ayurveda for treating rheumatoid disorders (Peng, Molecular characteristics of Illicium verum extractives to activate acquired immune response, 2015), gastro intestinal disorders, lung associated diseases, paralysis, etc. [26]. The current study is based on the green synthesis of zinc oxide nanoparticles using aqueous fruit extract of *illicium verum* in a simple, cost-effective manner and the determination of its antimicrobial activity against both gram-positive and gram-negative bacteria.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The dried fruit of *Illicium verum* was collected from the local market near Porur, Tamilnadu, India. It was soaked in water to remove the dirt present on its skin. The fruit was air dried completely to remove the moisture content and ground into fine powder [27].

2.2 Preparation of Fruit Extracts of *Illicium verum*

5g of dried fine powder was weighed and boiled for around 10 minutes (60-90°C) [22] using double distilled water (100ml). After 10 min, the extract was filtered using what man No.1 filter paper and stored at 4°C for further purposes [28,29].

2.3 Biosynthesis of ZnO NPs

Zinc acetate di-hydrate ($Zn(CH_3COOH)_2.H_2O$), Sodium hydroxide (NaOH), and prepared fruit extracts were used for the synthesis of Zinc Oxide nanoparticles [30].

0.025M of zinc acetate di-hydrate ($Zn(CH_3COOH)_2.H2O$) was prepared in 100 ml of deionized water. This was magnetically stirred for 10 minutes until the salt gets dissolved completely. To this, 500µl of the fruit extract of *illicium verum* was added slowly under magnetic stirring. 2.0 M of Sodium hydroxide (NaOH) was prepared and then added drop wise to the mixture containing the $Zn(CH_3COOH)_2.H2O$ and the fruit extract were under continuous stirring conditions kept in a magnetic stirrer. The p^H was monitored simultaneously and the addition of sodium hydroxide continued till the solution reached P^H of 12 [31].

The addition was stopped immediately when the solution reached P^H of 12 and the solution was left in the same stirring condition for 2 hours. After 2 hours, a white precipitate settles down in the solution which was centrifuged at 10,000 rpm for 10 minutes. The Supernatant was discarded and the precipitate was washed over and over again with deionized water, followed by ethanol. After this, the precipitates were kept in a hot air oven at 80°C for drying and left overnight on the Petri plate. Drying yielded a White powder which was carefully collected in a microfuge tube for Characterization [30].

2.4 Characterization of Biosynthesized ZnO NPs

The primary conformation of biologically synthesized zinc oxide nanoparticles was analyzed using UV absorption spectra with wavelength ranges from 300-400 nm [32]. The morphology characteristics of ZnO NPs were analyzed using Scanning Electron Microscopy (SEM). The Energy Dispersive X-ray spectroscopy (EDX) analyses were carried out to study the elemental compositions and purity of synthesized ZnO nanoparticles. The Fourier Transforms Infra-red (FT-IR) were determined to identify the presence of functional groups involved in the synthesized nanoparticles. The spectrum was monitored in the wave number range from 400-4000 cm^{-1} and analyzed [33]. The crystalline nature of nanoparticles was characterized by X-ray diffraction using 2ϴ values [34].

2.5 Antimicrobial Activity

The antimicrobial activity of the biosynthesized ZnO nanoparticles was visualized against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus,* and *salmonella mutants* by using the agar disc diffusion method (Debjani, 2013). 25 ml of prepared media was poured into Petri plates and allowed to solidify. 100 µl of each strain from overnight culture samples was inoculated on the individual plates and left for a few minutes to dry. After drying, the diameter of a 5mm well was made on the plate and the test sample of different concentrations was added the plates were incubated overnight (24 hours) at an appropriate temperature of 37°C [35] After a period of incubation, the zone of inhibition of different levels formed was measured [36].

3. RESULTS AND DISCUSSION

3.1 UV-Visible Spectroscopic Analysis

The UV-Vis analysis is the primary confirmation of synthesized nanoparticles. The biosynthesized ZnO nanoparticles were analyzed in aqueous suspension using Double beam UV-Visible spectrophotometer 2202. Fig. 1 shows that the obtained ZnO nanoparticles exhibit the absorption peak at 380 nm [37].

3.2 SEM with EDX Analysis

The size and the morphological characteristics of the synthesized Zinc Oxide Nanoparticles were studied using High-resolution Scanning Electron Microscopy [38]. It also determined the structure of the obtained ZnO nanoparticles. SEM image of ZnO nanoparticles synthesized by aqueous fruit extract of *Illicium verum* was shown in Fig. 2. The figure, clearly shows the synthesized ZnO nanoparticles were spherical shaped and the average size of the obtained zinc oxide nanoparticles was found to be 27.56 nm. The energy dispersive X-ray diffractive studies were done to know the elemental composition of the synthesized Zinc Oxide nanoparticles. Fig. 3, shows the elemental composition of obtained nanoparticle was found to be 62. 59% of Zinc and 37.41% of Oxide. Thus, it confirmed the obtained nanoparticle was Zinc oxide.

3.3 FT-IR Analysis

The Fourier transform infrared spectroscopy analysis was studied for synthesized nanoparticles (Fig. 4) and it ranges from 400 – 4000 cm^{-1}. A strong absorption peak of the zinc oxide nanoparticle was seen at 2925 cm^{-1}. This range confirms the presence of O-H bonds. The C=C bonds were seen at the range of 1630 and 1406 cm-1, the N-H bond stretching at 3436cm-1 and also the peak at 1029, 894, and 566 cm-1 which corresponds to C=O, C=H, C-Br bond stretching respectively [39].

3.4 XRD Analysis

The X-ray diffraction analysis was carried out to study the nature of crystalline structure and size of

ZnO nanoparticle for 2ϴ values ranging from 10 to 80° using CuKα radiation at λ = 1.5406 Å. In biologically synthesized ZnO nanoparticles (Fig. 5), the 2ϴ values with hkl plane correspond to 31.5° (100), 36.1° (101), 47.4° (102), 56.5° (110), 62.8° (103), 68.9°(112) and 67.8°(200) were observed. The XRD spectrum confirms the obtained zinc oxide nanoparticle was found to be a hexagonal wurtzite structure and hence the average particle size was calculated by using the Scherrer formula. The calculated value of the nanoparticle was found to be 18.737 nm [40].

3.5 Antimicrobial Studies

The synthesized zinc oxide nanoparticles using an aqueous extract of *illicium verum* showed the activity against Gram-positive bacteria (*S. aureus* and *B. subtilis*) and gram-negative bacteria (*E. coli* and *S. mutans*). Varying concentration was used for the antibacterial activity such as 25%, 50%, 75%, and 100%. The zone of inhibition was found to be increased with an increase in the concentration of obtained ZnO NPs. The zone of inhibition in gram-negative bacteria was found to be

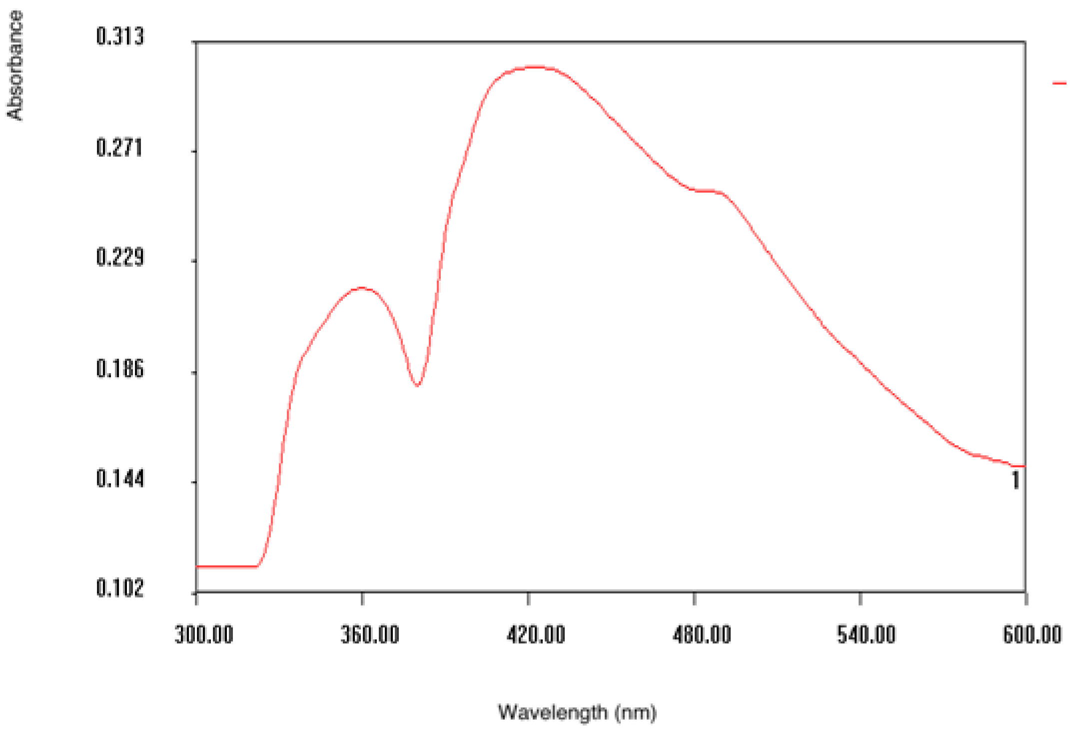


Fig. 1. UV-Vis spectra of synthesized ZnO nanoparticles

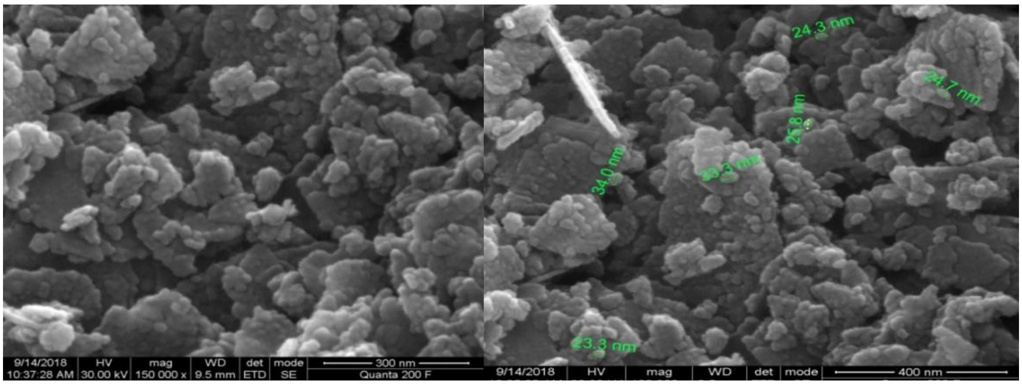
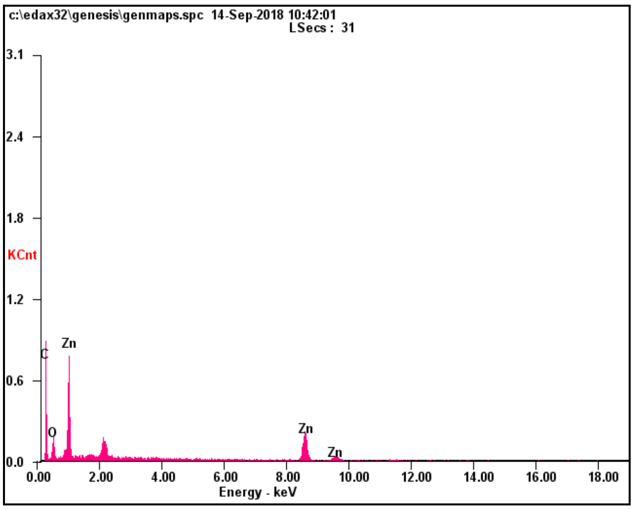


Fig. 2. SEM image shows the ZnO nanoparticles



Element	Wt%
Zn	62.59
O	37.41

Fig. 3. EDX Spectrum of ZnO NPs

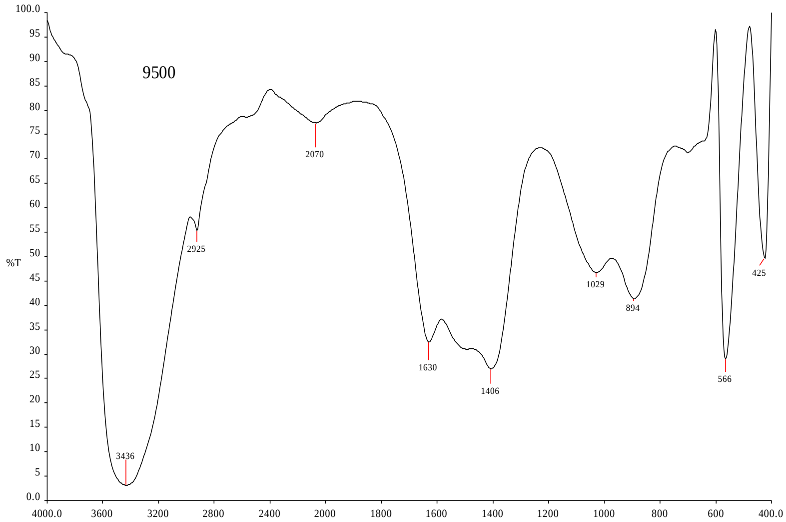


Fig. 4. FT-IR analysis of an aqueous extract of ZnO NPs

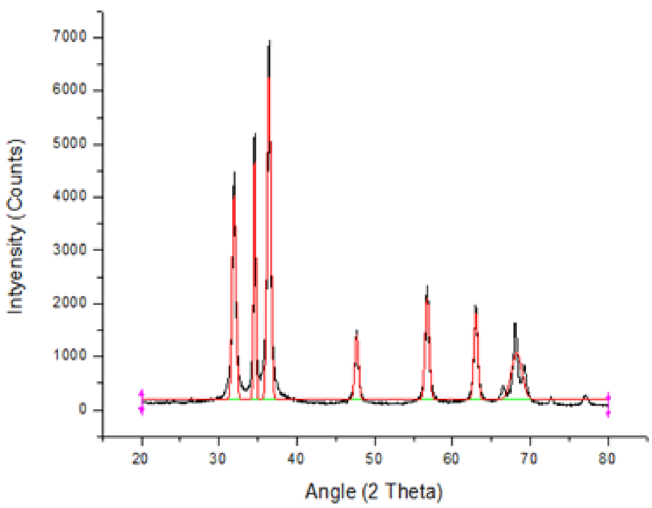
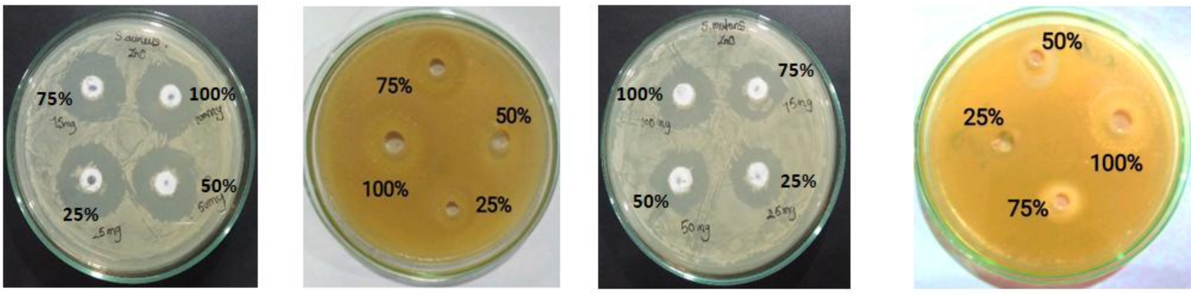


Fig. 5. XRD spectrum of ZnO NPs



S. aureus ***B. subtilis*** ***S. mutans*** ***E. coli***

Fig. 6. Antimicrobial activity of both Gram-negative and Gram-positive Bacteria

25mm (50 mg/ml)(50%) in *S. aureus*, 28.5mm (75 mg/ml)(75%) in *B. subtilis*, and gram-positive bacteria to be 19mm (25 mg/ml)(25%) in *E. coli*, 21mm (75 mg/ml)(75%) in *S. mutants* (Fig. 6).

According to Raj et al. [30], the concentration of nanoparticles increases with an increase in antimicrobial activity due to the presence of synthesized ZnO nanoparticles and showed that the destructive action of ZnO nanoparticles was more against gram-negative bacterial strains than the gram-positive bacterial strains.

4. CONCLUSION

Thus, it was found that the Synthesis of zinc oxide nanoparticles from aqueous fruit extract of *illicium verum* is a simple, eco-friendly and inexpensive process. The characterization studies demonstrated the synthesized products were spherical shaped and hexagonal in structure which is evident for SEM and XRD. The EDX confirmed the presence of zinc and oxygen in the nanoparticle. The FTIR demonstrated the presence of chemical bonds that are involved in the incorporation of *illicium verum* in the production of ZnO nanoparticles. The synthesized nanoparticle was monitored for antibacterial activity and concluded that the activity increases with an increase in concentration. Hence, it is approached for the green synthesis method for obtaining ZnO NPs can have great potential in fewer toxins.

ACKNOWLEDGEMENT

The authors extend their deepest gratitude to the management and staff of St. Joseph's College of Engineering for providing the facilities in carrying out the work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gupta K, Chundawat TS. Zinc oxide nanoparticles synthesized using *Fusarium oxysporum* to enhance bioethanol production from rice-straw. Biomass and Bioenergy. 2020 Dec 1;143:105840.
2. Kolahalam LA, Prasad KR, Krishna PM, Supraja N. Saussurea lappa plant rhizome extract-based zinc oxide nanoparticles: synthesis, characterization and its antibacterial, antifungal activities and cytotoxic studies against Chinese Hamster Ovary (CHO) cell lines. Heliyon. 2021 Jun 1;7(6):e07265.
3. Melnikova N, Knyazev A, Nikolskiy V, Peretyagin P, Belyaeva K, Nazarova N, Liyaskina E, Malygina D, Revin V. Wound healing composite materials of bacterial cellulose and zinc oxide nanoparticles with immobilized betulin diphosphate. Nanomaterials. 2021 Mar 12;11(3): 713.
4. Tatagar AM, Moodi JI, Abbar JC, Phaniband MA. Photocatalytic activity and anti-microbial application of synthesized Zinc oxide nanoparticles (ZnO Nps) towards remediation of hospital waste water (HWW). Materials Today: Proceedings. 2022 Jan 1;49:699- 02.
5. Chaudhuri SK, Malodia L. Biosynthesis of zinc oxide nanoparticles using leaf extract of Calotropis gigantea: characterization and its evaluation on tree seedling growth in nursery stage. Applied Nanoscience. 2017 Nov;7(8):501-12.
6. Jomehzadeh N, Koolivand Z, Dahdouh E, Akbari A, Zahedi A, Chamkouri N. Investigating in-vitro antimicrobial activity, biosynthesis, and characterization of silver nanoparticles, zinc oxide nanoparticles, and silver-zinc oxide nanocomposites using Pistacia Atlantica Resin. Materials Today Communications. 2021 Jun 1;27:102457.

7. Jebel FS, Almasi H. Morphological, physical, antimicrobial and release properties of ZnO nanoparticles-loaded bacterial cellulose films. Carbohydrate polymers. 2016 Sep 20;149:8-19.
8. Pasquet J, Chevalier Y, Pelletier J, Couval E, Bouvier D, Bolzinger MA. The contribution of zinc ions to the antimicrobial activity of zinc oxide. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2014 Sep 5;457:263-74.
9. Applerot G, Lipovsky A, Dror R, Perkas N, Nitzan Y, Lubart R, Gedanken A. Enhanced antibacterial activity of nanocrystalline ZnO due to increased ROS- mediated cell injury. Advanced Functional Materials. 2009 Mar 24;19(6):842-52.
10. Lipovsky A, Nitzan Y, Gedanken A, Lubart R. Antifungal activity of ZnO nanoparticles—the role of ROS mediated cell injury. Nanotechnology. 2011 Feb 2;22(10):105101.
11. Brunner TJ, Wick P, Manser P, Spohn P, Grass RN, Limbach LK, Bruinink A, Stark WJ. In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. Environmental science & technology. 2006 Jul 15;40(14):4374-81.
12. Li M, Zhu L, Lin D. Toxicity of ZnO nanoparticles to Escherichia coli: mechanism and the influence of medium components. Environmental science & technology. 2011 Mar 1;45(5):1977-83.
13. Brayner R, Ferrari-Iliou R, Brivois N, Djediat S, Benedetti MF, Fiévet F. Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano letters. 2006 Apr 12;6(4):866-70.
14. Jiang W, Mashayekhi H, Xing B. Bacterial toxicity comparison between nano-and micro-scaled oxide particles. Environmental pollution. 2009 May 1;157(5):1619-25.
15. Rajeshkumar S, Agarwal H, Sivaperumal P, Shanmugam VK, Lakshmi T. Antimicrobial, anti-inflammatory and anticancer potential of Microbes mediated zinc oxide nanoparticles. Journal of Complementary Medicine Research. 2021 Mar 14;11(5):41.
16. Anandraj LFA, Jayalakshmy E. A biogenic approach for the synthesis and characterization of zinc oxide nanoparticles produced by Tinospora Cordifolia. International Journal of Pharmacy and Pharmaceutical Sciences. 2015;7(8):384-386.
17. Peng W, Lin Z, Wang L, Chang J, Gu F, Zhu X. Molecular characteristics of Illicium verum extractives to activate acquired immune response. Saudi Journal of Biological Sciences. 2016 May 1;23(3):348-52.
18. Nath D, Banerjee P. Green nanotechnology–a new hope for medical biology. Environmental toxicology and pharmacology. 2013 Nov 1;36(3):997-1014.
19. Al Awadh AA, Shet AR, Patil LR, Shaikh IA, Alshahrani MM, Nadaf R, Mahnashi MH, Desai SV, Muddapur UM, Achappa S, Hombalimath VS. Sustainable synthesis and characterization of zinc oxide nanoparticles using Raphanus sativus extract and its biomedical applications. Crystals. 2022 Aug 14;12(8):1142.
20. Gur T, Meydan I, Seckin H, Bekmezci M, Sen F. Green synthesis, characterization and bioactivity of biogenic zinc oxide nanoparticles. Environmental Research. 2022 Mar 1;204:111897.
21. Gonçalves RA, Toledo RP, Joshi N, Berengue OM. Green synthesis and applications of ZnO and TiO2 nanostructures. Molecules. 2021 Apr 13;26(8):2236.
22. Farooq A, Khan UA, Ali H, Sathish M, Naqvi SAH, Iqbal S, Ali H, Mubeen I, Amir MB, Mosa WFA, Baazeem A, Moustafa M, Alrumman S, Shati A, Negm S. Green Chemistry Based Synthesis of Zinc Oxide Nanoparticles Using Plant Derivatives of *Calotropis gigantea* (Giant Milkweed) and Its Biological Applications against Various Bacterial and Fungal Pathogens. Microorganisms. 2022 Nov 4;10(11):2195.
23. Peng W, Lin Z, Wang L, Chang J, Gu F, Zhu X. Molecular characteristics of Illicium verum extractives to activate acquired immune response. Saudi Journal of Biological Sciences. 2016 May 1;23(3):348-52.
24. Wang GW, Hu WT, Huang BK, Qin LP. Illicium verum: a review on its botany, traditional use, chemistry, and pharmacology. Journal of Ethnopharmacology. 2011 Jun 14;136(1):10-20.
25. Sharafan M, Jafernik K, Ekiert H, Kubica P, Kocjan R, Blicharska E, Szopa A. Illicium verum (Star Anise) and Trans-Anethole as valuable raw materials for medicinal and cosmetic applications. Molecules. 2022 Jan 19;27(3):650.
26. Patra JK, Das G, Bose S, Banerjee S, Vishnuprasad CN, del Pilar Rodriguez- Torres M, Shin HS. Star anise (Illicium verum): Chemical compounds, antiviral properties, and clinical relevance. Phytotherapy Research. 2020 Jun;34(6):1248-67.
27. Sri PU, Leelavathi V, Sree NV, Kumar MA. Antihelmenthic and antimicrobial activity of

green synthesized silver nanoparticles from Illicium Verum Hook. F. fruit. IOSR Journal of Pharmacy and Biological Sciences. 2015; 10(6):61-5.

28. Pinto GM, Nazareth R. Green synthesis and characterization of zinc oxide nanoparticles. Journal of Chemical and Pharmaceutical Research. 2016;6:427-32.
29. Aminuzzaman M, Ying LP, Goh WS, Watanabe A. Green synthesis of zinc oxide nanoparticles using aqueous extract of Garcinia mangostana fruit pericarp and their photocatalytic activity. Bulletin of Materials Science. 2018 Apr;41(2):1-0.
30. Raj LF, Jayalakshmy E. Biosynthesis and characterization of zinc oxide nanoparticles using root extract of *Zingiber officinale*. Orient. J. Chem. 2015 Mar;31(1):51-6.
31. Yang JF, Yang CH, Chang HW, Yang CS, Wang SM, Hsieh MC, Chuang LY. Chemical composition and antibacterial activities of Illicium verum against antibiotic-resistant pathogens. Journal of medicinal food. 2010 Oct 1;13(5):1254-62.
32. Ramesh M, Anbuvannan M, Viruthagiri GJ. Green synthesis of ZnO nanoparticles using Solanum nigrum leaf extract and their antibacterial activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015 Feb 5;136:864-70.
33. Datta A, Patra C, Bharadwaj H, Kaur S, Dimri N, Khajuria R. Green synthesis of zinc oxide nanoparticles using parthenium hysterophorus leaf extract and evaluation of their antibacterial properties. J. Biotechnol. Biomater. 2017; 7(3):271-6.
34. Fu L, Fu Z. Plectranthus amboinicus leaf extract–assisted biosynthesis of ZnO nanoparticles and their photocatalytic activity. Ceramics International. 2015 Mar 1;41(2): 2492-6.
35. Bala N, Saha S, Chakraborty M, Maiti M, Das S, Basu R, Nandy P. Green synthesis of zinc oxide nanoparticles using Hibiscus subdariffa leaf extract: effect of temperature on synthesis, anti-bacterial activity, and anti-diabetic activity. RSC Advances. 2015;5(7):4993-5003.
36. Meruvu H, Vangalapati M, Chippada SC, Bammidi SR. Synthesis and characterization of zinc oxide nanoparticles and their antimicrobial activity against Bacillus subtilis and Escherichia coli. J. Rasayan Chem. 2011;4(1):217-22.
37. Sangeetha G, Rajeshwari S, Venkatesh R. Green synthesis of zinc oxide nanoparticles by aloe barbadensis miller leaf extract: Structure and optical properties. Materials Research Bulletin. 2011 Dec 1;46(12):2560-6.
38. Shah RK, Boruah F, Parween N. Synthesis and characterization of ZnO nanoparticles using leaf extract of Camellia sinesis and evaluation of their antimicrobial efficacy. Int. J. Curr. Microbiol. App. Sci. 2015;4(8):444-50.
39. Santhoshkumar J, Kumar SV, Rajeshkumar S. Synthesis of zinc oxide nanoparticles using plant leaf extract against urinary tract infection pathogen. Resource-Efficient Technologies. 2017 Dec 1;3(4):459-65.
40. Varghese E, George M. Green synthesis of zinc oxide nanoparticles. International Journal of Advance Research in Science and Engineering. 2015;4(1):307-14.