Environment and Ecology 40 (2) : 395—403, April—June 2022
ISSN 0970-0420

Green Synthesis and Characterization of Zinc Oxide Nanoparticles from Leaf Extracts of *Hibiscus rosa-sinensis* and *Azadirachta indica*

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Received 27 September 2021, Accepted 24 March 2022, Published on 29 April 2021

ABSTRACT

Green synthesis of zinc oxide nanoparticles (ZnO NPs) was achieved by utilizing the reducing and capping potential of leaf of *Azadirachta indica* compared to *Hibiscus rosa-sinensis*. The bioreduced ZnO-NPs were characterized using ultraviolet–visible spectroscopy (UV–Vis spectroscopy), particle size analyzer (PSA), scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDX). The UV–Vis spectral analysis confirmed that the maximum absorption at 362 nm range corresponds to the intrinsic band gap of ZnO NPs. Particle size analyzer confirmed synthesized particles are of nanoscale i.e., below 100 nm. SEM images confirmed synthesized nanoparticles were found to be relatively rod shaped and EDX confirmed peak is of ZnO nanoparticles. This is a simple and effective method which could be an alternative for chemical and physical methods for the large-scale production of ZnO-NPs.

Keywords EDX, Nanoparticles, PSA, SEM, UV–Vis spectroscopy.

INTRODUCTION

A nanoparticle is a microscopic particle with at least one dimension less than 100 nm (Laurent *et al.* 2008). Nanoparticles are of great scientific interest due to their extremely small size and large surface to volume ratio, which leads to both chemical and physical differences in their properties (Khan *et al.* 2019). Man engineered nano materials have received a particular attention because of their positive impact on improving many sectors of economy, including consumer products, pharmaceuticals, cosmetics, transportation, energy, agriculture and the list goes on (Gubala *et al.* 2018). Nanotechnology is a promising field of interdisciplinary research which opens up wide array of opportunities in various agricultural fields.

There are several methods available for synthesis of nanoparticles, such as chemical route, hydrothermal route, sol-gel template process, photoluminescence emission technique, microwave-assisted hydrothermal and decomposition, aerosol process, sonochemical synthesis, laser ablation, microemul-

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sion method, precipitation method, hydrolyzed in polar organic solvents, solid-state thermal decomposition, microwave synthesis (Ghidan *et al.* 2017). These routes have many disadvantages due to difficulty of scale up the synthesis process, separation and purification of nanoparticles from surfactants, co-surfactants, organic solvents, high energy consumption and toxic by-products. Recently increasing awareness towards green chemistry and other biological processes have led to the development of an eco-friendly approach for the synthesis of nanoparticles (Rosi and Mirkin 2005). This biological approach appears to be a cost effective alternative to conventional physical and chemical methods of synthesis.

Green nanotechnology is a flourishing field generating interest among researchers towards eco-friendly biosynthesis of nanoparticles and having a huge potential to bring benefits in areas of interfacing physical, chemical, biological, agricultural and environmental sciences with myriad applications (Ghidan *et al.* 2017). Eco-friendly methods of green mediated synthesis of nanoparticles are the present research in the limb of nanotechnology. Green technology sustains by minimizing harmful polluting substances in the synthesis of nanoparticles, eliminating pollutants in current chemical processes. Green synthesis method is beneficial over other methods which are implemented for the synthesis of nanoparticles. Plant extracts are used for bio reduction of metal ions to form nanoparticles. It has been demonstrated that plant metabolites like sugars, terpenoids, polyphenols, alkaloids, phenolic acids and proteins play an important role in reduction of metals ions into nanoparticles and support their subsequent stability (Makarov *et al.* 2014). Synthesis of nanoparticles from plant extracts is safe to the environment and the user. Further, they are easily available and possess a broad range of metabolites.

Azadirachta indica (L.) is a tropical ever green native tree to India and is also found in other southeast countries. In India, neem is known as "the village pharmacy" because of its healing versatility and it has been used in Ayurvedic medicine for more than 4,000 years due to its medicinal properties. *Azadirachta indica* (leaf, bark and seed) contains antibacterial, antifungal and antiviral activity (Reddy *et al.* 2013). Raphael (2012) reported phytochemical present in the neem plant such as alkaloids, steroids, flavonoids, carbohydrates, glycosides and terpenoids compounds. They are responsible for their medicinal uses.

In this work, the ZnO nanoparticles are synthesized using the cost competitive, simple and eco-friendly method. The eco-friendly method with the large scale production and without unwanted impurities is desirable for the cost-effective preparation of ZnO nanoparticles. As a consequence, the low cost precursors such as zinc sulfate heptahydrate and natural product such as neem leaves are used to synthesize the ZnO nanoparticles through a simple green route.

MATERIALS AND METHODS

Cleaning of glassware's and sterilization

Glassware's were kept in cleaning solution, prepared by dissolving 60 g of potassium dichromate ($K_2Cr_2O_7$), 60 ml of concentrated sulfuric acid (H_2SO_4) in one liter of water. Each of these chemicals were dissolved separately in 500 mL of water and finally mixed. The glassware was later cleaned by washing with detergent solution followed by tap water and finally rinsed in distilled water and kept for drying in hot air oven at 100 °C for one hour.

Preparation of leaf aqueous extracts

Fresh leaves of *Azadirachta indica* were collected in the UAS Dharwad campus and were washed several times using deionized distilled water, air-dried and chopped finely into small pieces. Twenty grams of chopped leaves were taken in pestle and mortar and finely ground by adding 100 ml of deionized distilled water. The extract mixture was then filtered using Whatman filter paper No. 1 and the filtered mixture was stored in refrigerator at 4 °C for further utilization in the experiments.

Preparation of 1000 ml aqueous $ZnSO_4$ (2000 ppm) stock solution

Zinc sulfate ($ZnSO_4.7H_2O$) procured from the firm

Himedia was used as a precursor in the synthesis of zinc nanoparticles. 2.0 g of $ZnSO_4.7H_2O$ was dissolved in 1000 ml of deionized water and stored in bottle for use in further experiments.

Standardization of the protocol for green synthesis of magnesium oxide nanoparticles

The bio-active molecules present in the plant extracts of *Amaranthus retroflexus* and *Azadirachta indica* were being tried for green synthesis of nanoparticles with different methods viz., hot plate heating method, heating with stirring method and autoclave method. Though several methods were tried, based on the size (1-100 nm) of the ZnO nanoparticles, one method (lowest size) was selected for further synthesis of ZnO nanoparticles.

Hot plate heating method

The different combinations of precursor ($ZnSO_4$) and plant extract (neem) were kept on the hot plate @ 60°C for 30 and 60 minutes and particle size analyzer observations were recorded.

Heating with stirring method

The different combinations of precursor ($ZnSO_4$) and plant extract (neem) were kept on the hot plate @ 60°C along with stirring for 500 rpm for 30 and 60 minutes and particle size analyzer observations were recorded.

Autoclave method

The different combinations of precursor ($ZnSO_4$) and plant extract (neem) were kept in autoclave (121°C temperature and pressure of 15 pounds per square inch) for 15 and 30 minutes and particle size analyzer observations were recorded.

Characterization of synthesized zinc oxide nanoparticles

The biosynthesized nanoparticles were characterized in order to know the shape, size and other parameters by using UV-Visible spectrophotometer (UV-Vis), Particle Size Analyzer (PSA) and Scanning Electron Microscope (SEM) with Energy dispersive X-ray (EDX).

UV-Visible spectroscopy

Reduction of Zn^{+2} ions to ZnO NPs were confirmed by UV–Vis spectrophotometer (SP-UV 500DB, Germany) at scanning wavelengths of 200–700 nm based on their optical absorbance peak. As the size of the nanoparticle decreases, the band gap increases and thus, the optical absorbance increases as compared to that of the bulk particles and therefore their color changes. The synthesized ZnO NPs were first sonicated using solid probe Ultra sonicator (inkarp 20 kHz ±50Hz. Sonics Vibracell) for uniform distribution of nanoparticles. An aliquot of extract was used as control and the absorbance maximum was recorded at room temperature.

Particle size analyzer (PSA)

The nanoparticles were further subjected to dynamic light scattering (DLS) to measure average particle size, in a high-performance particle-size analyzer (model Z3000, Nicomp, USA). The sample was placed in disposable polystyrene cuvettes and the analysis was carried out at room temperature in triplicate with a scattering angle of 90º.

Scanning Electron Microscope (SEM)

The magnesium and zinc oxide nanoparticles were then subjected to surface morphology analysis by scanning electron microscopy (SEM, Carl Zeiss, Germany). A pinch of powder sample obtained from muffle furnace was uniformly placed on an aluminum stub with carbon adhesive tape and coated with gold in a sputter coater and SEM images were captured with an EVO 18 microscope operated with smart SEM software and examined at a working distance between 8 - 9 mm and different voltage of 5 – 10 kV.

Energy dispersive X-ray (EDX)

The presence of Mg and Zn ions were further confirmed by energy-dispersive X-ray spectroscopy

Table 1. Standardization of the protocol for green synthesis of zinc oxide nanoparticles.

$ZnSO_4$ 2000 ppm (ml)	Method employed (min)	Hibiscus leaf extract (ml)	PSA (nm)	Neem leaf extract (ml)	PSA (nm)
	Heating @ 80°C				
10	30	1	620.6	1	325.3
10	30	2	642.4	2	326.4
10	30	3	667.6	3	341.6
10	30	4	685.6	4	367.5
10	30	5	693.0	5	430.3
10	60	1	614.6	1	307.5
10	60	2	627.1	2	350.7
10	60	3	643.8	3	351.7
10	60	4	650.7	4	455.1
10	60	5	675.3	5	475.2
	Heating @ 80°C with stirring @ 500 rpm				
10	30	1	319.9	1	287.4
10	30	2	352.8	2	265.4
10	30	3	362.8	3	255.7
10	30	4	372.7	4	268.4
10	30	5	385.9	5	249.3
10	60	1	297.4	1	189.5
10	60	2	310.8	2	184.7
10	60	3	311.2	3	190.4
10	60	4	322.5	4	201.3
10	60	5	323.3	5	216.9
	Autoclave method				
10	15	1	345.4	1	216.3
10	15	2	391.1	2	202.3
10	15	3	362.6	3	179.3
10	15	4	328.9	4	183.8
10	15	5	365.3	5	198.7
10	30	1	375.2	1	168.4
10	30	2	356.4	2	135.2
10	30	3	344.1	3	60.9
10	30	4	324.6	4	99.9
10	30	5	321.5	5	106.7

(EDX), attached to the EVO 18 scanning electron microscope.

RESULTS AND DISCUSSION

Green synthesis of zinc oxide nanoparticles from leaf extracts of hibiscus and neem

Hot plate heating method

The varied quantities of *Hibiscus rosa-sinensis* and *Azadirachta indica* leaf extract were used for the synthesis of ZnO nanoparticles with zinc sulfate (2000 ppm) as a precursor solution through hot plate heating method at 80°C . Hot plate heating at 80°C for 30 and 60 minutes, PSA results showed that, particle size ranged between 693.0 to 614.6 nm for *Hibiscus rosa-sinensis* and 307.5 to 475.2 nm for *Azadirachta indica* (Table 1). The size of the particles were increasing with the increasing the quantity of plant extract and decreasing with heating at 60°C compared to 30°C, but the size of the synthesized particles were not in the nano range i.e., below 100 nm.

Hot plate heating with stirring method

The varied quantities of *Hibiscus rosa-sinensis* and *Azadirachta indica* leaf extract were used for the synthesis of ZnO nanoparticles with zinc sulfate (2000 ppm) as a precursor solution through hot plate heating at 80°C along with stirring at 500 rpm. Hot plate heating at 80°C with stirring at 500 rpm for 30 and 60 minutes, PSA results showed that, particle size ranged between 297.4 to 385.9 nm for *Hibiscus rosa-sinensis* and 189.5 to 268.4 nm for *Azadirachta indica* (Table 1). The size of the particles were increasing with increasing the quantity of plant extract and decreasing with heating at 80°C along with stirring at 500 rpm for 60 minutes compared to 30 minutes, but the size of the synthesized particles were not in the nano range i.e., below 100 nm.

Autoclave method

The varied quantities of *Hibiscus rosa-sinensis* and *Azadirachta indica* leaf extract were used for the synthesis of ZnO nanoparticles with zinc sulfate (2000 ppm) as a precursor solution through autoclave method. After reaching 121°C temperature and 15 psi pressure in autoclave, kept for 15 and 30 minutes, PSA results showed that, particle size ranged between 321.5 to 391.1 nm for *Hibiscus rosa-sinensis* and 60.9 to 216.3 nm for *Azadirachta indica* (Table 1). The size of the synthesized particles was not in the nano range i.e., below 100 nm for autoclave period of 15 and 30 minutes for *Hibiscus rosa-sinensis* leaf extract. But, for the leaf extract of *Azadirachta indica* autoclaving for 30 minutes successfully synthesized particles in the nano range i.e., 60.9 nm.

Standardized protocol for green synthesis of zinc oxide nanoparticles by using leaf extract of neem (*Azadirachta indica*)

The zinc oxide nanoparticles were successfully synthesized from 10 ml of zinc sulfate solution and 3.0 ml of neem leaf aqueous extract which was autoclaved. After 30 minutes of autoclave, maximum number of Zn^{+2} ions to zinc nanoparticles which was confirmed by change in color from initial yellowish to dark brown, indicating the reduction of Zn^{+2} to ZnO in the $ZnSO_4$ solution (Plate 1). Previous studies suggested that the aldehyde groups are responsible for reduction of zinc sulfate to zinc oxide nanoparticles (Nath and Banerjee 2013). The stability of ZnO nanoparticles may be due to the free amino and carboxylic groups that have interacted with the zinc surface. The bonds of functional groups such as –CO–C–, –C–O– and –C=C– are derived from heterocyclic compounds

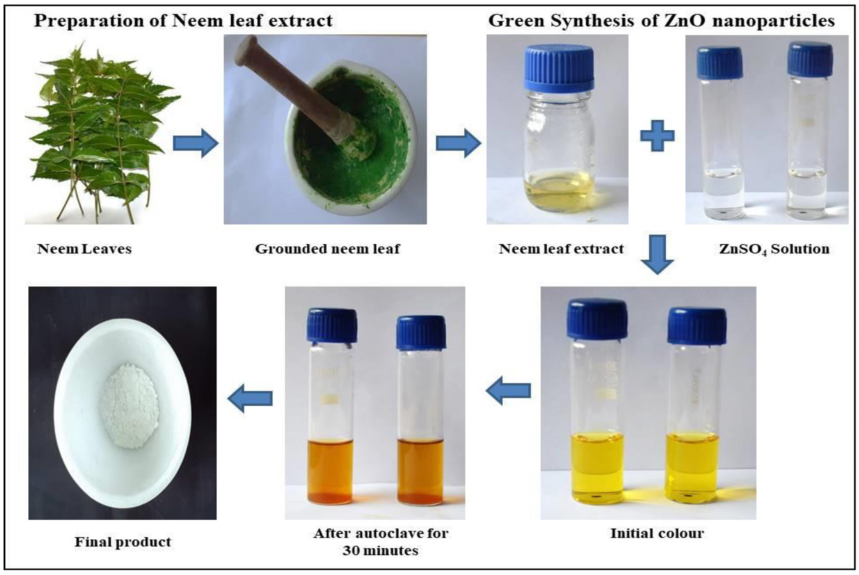

Plate 1. Standardized protocol for synthesis of ZnO nanoparticles using neem leaf extract.

and the amide bands derived from the proteins are present in the leaf extract and they are the capping ligands of the nanoparticles. The natural products, namely flavanones, terpenoids and reducing sugars are the main constituents of the neem leaf extract that acts as stabilizing agents (Nath and Banerjee 2013). Moreover the proteins present in the medium prevent agglomeration and aids in the stabilization by forming a coat, covering the metal oxide nanoparticles (Peletiri *et al.* 2012). Thus, it is concluded that the neem leaf extract acts as reducing and stabilizing agents for the formation of zinc oxide nanoparticles.

Characterization of synthesized ZnO nanoparticles

UV-Visible spectrophotometer

UV-Visible spectroscopy is sufficiently effective as a preliminary analysis for the confirmation of nanoparticle formation. According to Mie theory, light absorbance is directly proportional to the particulate size of metal nanoparticles, as metal nanoparticles are conductors and possess surface plasmon resonance (Kelly *et al.* 2003). In this study, a strong and broad surface plasmon resonance peak at 362 nm for ZnO, was obtained confirming the successful synthesis of nanoparticles (Plate 2). Previous studies reported that a surface plasmon resonance peak located between 350 to 400 nm for ZnO nanoparticles synthesized through different plant extracts as reported by several researchers Ajayan and Hebsur (2020), Awwad *et al.* (2020), Jayappa *et al.* (2020), Selim *et al.* (2020), Fakhari *et al.* (2019), Datta *et al.* (2017).

Particle size analyzer

The particle size distribution analysis revealed the average diameter and per cent distribution in relation to intensity-weight, volume-weight and number weight of nanoparticles. This analysis yielded a diameter of 60.9 nm for ZnO nanoparticles (plate 3). These values given by the Gaussian distribution confirmed by particle size distribution analysis at 100 per cent intensity single peaks. These results are in agreement with the previous report given by Ajayan and Hebsur (2020), and Elumalai and Velmurugan (2015) they synthesized Zinc oxide nanoparticle using the leaf extract of neem and the particle size were 101.6 and 40 nm.

Scanning electron microscope (SEM) and energy dispersive X-ray spectroscopy (EDX)

SEM images of the ZnO NPs synthesized using the *Azadiractha indica* leaf extract where the scale

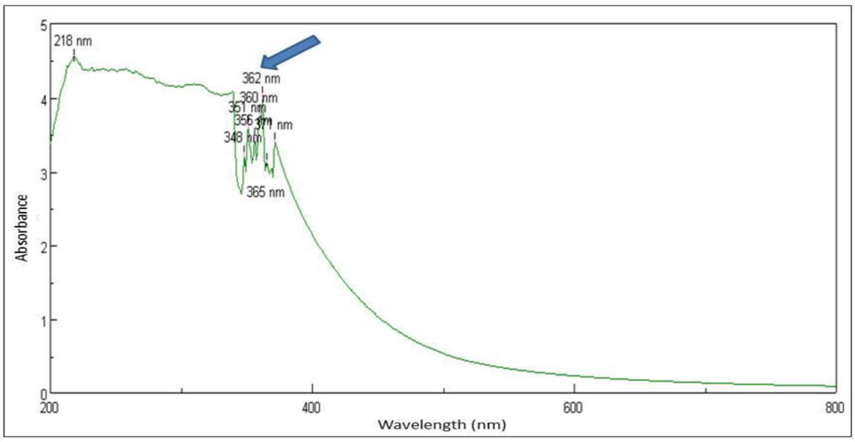

Plate 2. Characterization of synthesized ZnO nanoparticles from neem leaf extract by UV-Visible spectrophotometerc.

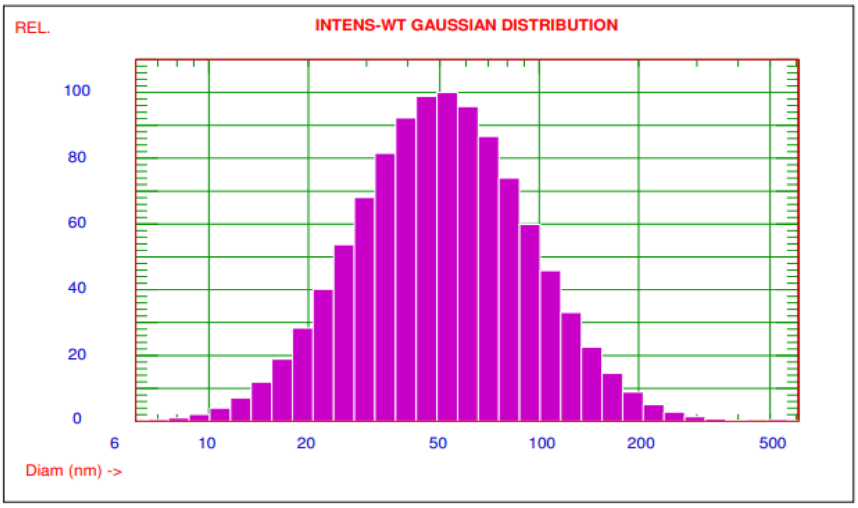

Plate 3. Characterization of synthesized ZnO nanoparticles from neem leaf extract by particle size analyzer (Nicomp).

bar is 200 nm. Figure X (a) shows the size of ZnO nanoparticles were below 100 nm. Furthermore, synthesized nanoparticles of various sizes were located with smooth shiny surfaces and were rod shaped (Plate 4). Elumalai and Velmurugan (2015) obtained spherical shaped ZnO nanoparticles. Further analysis of the ZnO NPs by EDX profile confirmed the signal characteristic of zinc and oxygen. EDS profile is the additional evidence of the formation of pure MgO nanoparticles. Plate 5 presents peaks between 0.5 and 1.2 keV, which indicate the presence of zinc and oxygen. Plate 5 shows the zinc content at the level of 82.6 %, and the oxygen content at the level of 17.3 %. Several reports have been found that the peak of Zn in EDX spectra appears to be around the same region (Joel and Badhusha 2016).

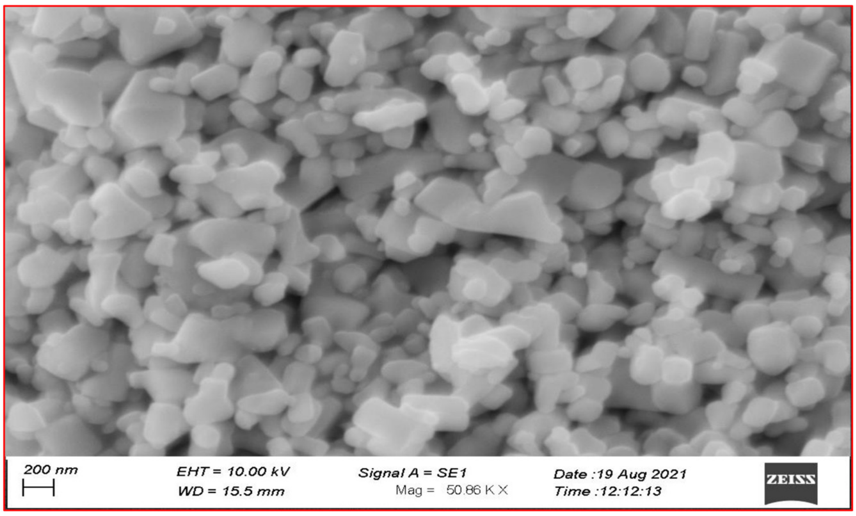

Plate 4. Surface morphology of ZnO nanoparticles by SEM.

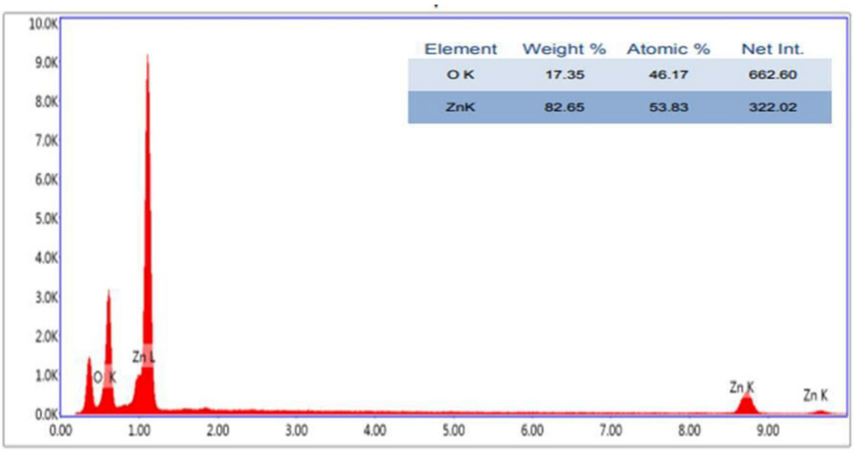

Plate 5. Elemental peaks and distribution of ZnO nanoparticles with EDX.

CONCLUSION

Azadirachta indica (neem) leaves aqueous extract can be used as an efficient reducing and capping agent for green synthesis of ZnO nanoparticles compared to *Hibiscus rosa-sinensis*. The UV–Vis spectral analysis confirmed that the maximum absorption at 362 nm range corresponds to the intrinsic band gap of ZnO-NPs. Particle size analyzer confirmed synthesized particles are of nanoscale. SEM images confirmed zinc oxide nanoparticles synthesized were found to be relatively rod shaped and EDX confirmed peak is of ZnO nanoparticles. This is a simple and effective method which could be an alternative for chemical and physical methods for the large-scale production of ZnO-NPs.

REFERENCES

Ajayan AS, Hebsur NS (2020) Green synthesis of zinc oxide nanoparticles using neem (*Azadirachta indica*) tulasi (*Ocimum tenuiflorum*) leaf extract their characterization. *Int J Curr Microbiol Appl Sci* 9(2): 277-285.

Awwad AM, Amer MW, Salem NM, Abdeen AO (2020) Green synthesis of zinc oxide nanoparticles (ZnO-NPs) using *Ailanthus altissima* fruit extracts antibacterial activity. *Chem Int* 6(3): 151-159.

Datta A, Patra C, Bharadwaj H, Kaur S, Dimri N, Khajuria R (2017) Green synthesis of zinc oxide nanoparticles using parthenium hysterophorus leaf extract evaluation of their antibacterial properties. *J Biotechnol Biomater* 7(3): 271-276.

Elumalai K, Velmurugan S (2015) Green synthesis, characterization antimicrobial activities of zinc oxide nanoparticles from the leaf extract of *Azadirachta indica* (L). *Appl Surface Sci* 345: 329-336.

Fakhari S, Jamzad M, Kabiri Fard H (2019) Green synthesis of zinc oxide nanoparticles: A comparison. Green Chem Letters Rev 12(1): 19-24.

Ghidan AY, Al-Antary TM, Salem NM, Awwad AM (2017) Facile green synthetic route to the zinc oxide (ZnO-NPs) nanoparticles: Effect on green peach aphid antibacterial activity. *J Agric Sci* 9(2): 131.

Gubala V, Johnston LJ, Liu Z, Krug H, Moore CJ, Ober CK, Schwenk M, Vert M (2018) Engineered nanomaterials human health: Part 1. Preparation, functionalization characterization. *Pure Appl Chem* 90(8): 1283-1324.

Jayappa, Manasa Dogganal, Chandrashekar Konambi Ramaiah, Masineni Allapuramaiah Pavan Kumar, Doddav enkatanna Suresh, Ashwini Prabhu, Rekha Punchappady Devasya, Sana Sheikh (2020) Green synthesis of zinc oxide nanoparticles from the leaf, stem *in vitro* grown callus of *Mussaenda frondosa* L: Characterization their applications. *App Nanosci* 10(8): 3057-3074.

Joel C, Badhusha MSM (2016) Green synthesis of ZnO nanoparticles using *Phyllanthus embilica* stem extract their antibacterial activity. *Der Pharmacia Lettrer* 8(11): 6.

Kelly K, Coronado E, Zhao Lin, Schatz G (2003)The optical properties of metal nanoparticles: The influence of size, shape, dielectric environment. *ChemInform* 34: 10-18.

Khan I, Saeed K, Khan I (2019) Nanoparticles: Properties, applications toxicities. *Arabian J Chem* 12(7): 908-931.

Laurent S, Forge D, Port M, Roch A, Robic C, Vander Elst L, Muller RN (2008) Magnetic iron oxide nanoparticles: Synthesis, stabilization, vectorization, physico-chemical characterizations, biological applications. *Chem Rev* 108(6): 2064-2110.

Makarov VV, Love AJ, Sinitsyna OV, Makarova SS, Yaminsky IV, Taliansky ME, Kalinina NO (2014) Green nanotechnol ogies: Synthesis of metal nanoparticles using plants. *Acta Naturae* 6(1): 20.

Nath D, Banerjee P (2013) Green nanotechnology–a new hope for medical biology. *Environ Toxicol Pharmacol* 36(3): 997-1014.

Peletiri C, Matur BM, Ihongbe JC, Okoye M (2012) The effect of *Azadirachta indica* (Neem Tree) on human plasmodiasis: The laboratory perspective. *Global Res J Med Sc*i 2(1): 13-17.

Raj LFA, Jayalakshmy E (2015) Biosynthesis characterization of zinc oxide nanoparticles using root extract of *Zingiber officinale. Orient J Chem* 31(1): 51-56.

Raphael E (2012) Phytochemical constituents of some leaves extract of *Aloe vera* and *Azadirachta indica* plant species. *Global Adv Res J Environ Sci Toxicol* 1(2): 14-17.

Reddy YRR, Kumari CK, Lokanatha O, Mamatha S, Reddy CD (2013) Antimicrobial activity of *Azadirachta Indica* (neem) leaf, bark seed extracts. *Int J Res Phytochem Pharmacol* 3(1): 1-4.

Rosi NL, Mirkin CA (2005) Nanostructures in biodiagnostics. *Chem Rev* 105(4): 1547-1562.

Selim YA, Azb MA, Ragab I, Abd El-Azim MH (2020) Green synthesis of zinc oxide nanoparticles using aqueous extract of *Deverra tortuosa* their cytotoxic activities. *Sci Rep* 10(1): 1-9.