GREEN SYNTHESIS AND CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES (ZnO NPs) FROM CAMELLIA SINENSIS LEAF EXTRACT AND ITS POTENTIAL OF ANTIBACTERIAL ACTIVITY AND ACETYL CHOLINESTERASE INHIBITORY ACTIVITIES

Abstract

Nanoparticles are widely used in the biotechnology and biomedicalfield. Green synthetic methods of nanoparticles are a simple and environmentallybenign process that declines the demerits of conventional chemical andphysical methods. The synthesis of semiconductor and metal nanoparticles is a risingdo research part, due to the possible applications in the progress of new technologies.The present research information the synthesis and characterization of zinc oxide nanoparticles (ZnO NPs) using *C. Sinensis* leaf extract. The findings of these studies show the green synthesized ZnO NPs are effective, safe, and eco-friendly as they arestable and have abundant flower shapes with maximum particles in size ranging from 100 nm indiameter. The synthesized ZnO NPs have been tested against the pathogenic microorganismsand showed an excellent zone of inhibition. DPPH radical scavenging activity of synthesized ZnO NPs expressed DPPH free radicals as a percentage of inhibition andIC50value of 70.37%. Acetylcholine esterase (AChE) inhibitory activity possesses the maximum amount of the synthesized ZnO NPs. Since the results ofthe current work, it was concluded that the synthesized ZnO NPs exhibited significant antibacterial and acetylcholine esterase inhibitory activities. Hence it can beused as a drug with multifunction in treating Alzheimer's disease (AD).

Keywords

ZnO NPs, antibacterial, FTIR, SEM, *C. sinensis,* AChE

1. Introduction

Nanotechnology contains the use of materials having nanoscale size in the range of 1to100 nm. In use by nanomaterials have acceptable researchers to have a better understanding of natural science **(Santhoshkumar*et al.*, 2017).** Nanoscience has been creating a new chemical and biological nanostructure, understanding and uncovering their novel properties and organizing these new structures into larger and more complex functional structures and devices (Prakash and Thiagarajan, 2012). The inorganic nanoparticles (NPs) like gold, silver, copper, TiO2, CuO and ZnO have deep antibacterial activities. Among the

inorganic NPs, ZnO NPs are of particular interest, because they can be prepared easily, inexpensive and safe material for human beings and animals. Nanoparticles can be synthesized by various methods like physical (evaporation, condensation and laser ablation), chemical and biological method. Different metal nanoparticles have been synthesized previously using the evaporation-condensation process Deka *et al.*, (2021).They are also generally used in the formulation of health care products. ZnO NPs has entered the scientific spotlight for its high catalytic UV filtering properties, wound healing, anti-bacterial, anti-fungal activity semiconducting properties and photochemical activity (Elumalai and Velmurugan, 2015).

Pharmacology, biotechnology, nanogenerators, biosensors, gas sensors, varistors, solar cells, photo catalysts and photo detectors are capable candidates for a variety of applications ZnO NPs (Talam *et al.*, 2012). ZnO NPsbroad applications in pharmacological biological fields.Mainly, metal oxide nanoparticles and ZnO NPs efficiently protects over a broader UV range than any of the molecular UV-absorbers. The non-toxic nature of ZnO NPs makes it fitting in drug research. ZnO NPs are also use as anti-microbials in numerous drugs medicines and formulations (Firdhouse *et al.*, 2015). Toxicological impact of nanomaterials are predictable to relate with the materials of biological components to produce important impacts on the properties and behaviour of body, organs, tissues, cells and macromolecules, cells, tissues, organs and body(Diab *et al.*, 2018). On the increasesupport of oxidative stress-mediated damages to the lipids, protein and DNA during Alzheimer's disease prompted for the detection of original molecules that play ansignificant role in cholinesterase inhibitors and scavenging the free radical produced during the pathogenesis of the ailment(Malar *et al.*, 2017).

In this study, the ZnO NPs are synthesized using simple, eco-friendly and cost effective method. The present work is aimed at synthesizing ZnO NPs from leaf extract of *C. sinensis*. The synthesized ZnONP's were characterized by Ultra Visible spectroscopy, Scanning electron microscopy, X-Ray Diffraction, FTIR, Zeta potential and also acetylcholine esterase inhibitory and antibacterial activities of prepared ZnO NPs and tested against different human pathogenic microorganisms (bacteria).

2.Materials and Methods

2.1. Materials

Zinc acetate dihydrate$Zn(CH_3COO)_2.2H_2O$(M.W 219.49g/mol) were purchased from Isochem Laboratories, Angamaly, Cochin. Catechin (M.W 290.26g/mol) and AChE were

obtained from Sigma-Aldrich Chemical ATCI (M.W 289.18g/mol) and 5,DTNB were obtained from Himedia and all other chemicals were obtained from Himedia which is of analytical grade. Bacterial strains were collected from Microbiological Laboratory Coimbatore, Tamil Nadu.

2.2.Plant sample

C. sinensis leaves were collected from Aruvankadu in the Distirct of The Niligri's, Tamil Nadu, and taxonomic identification of the plant was confirmed by Botanical Survey of India, Coimbatore (Authentication No: BSI/SRC/5/23/2019/Tech./18). The collected plant leaves were kept in shade 10 days for at room temperature for complete drying. The plant material was pulverized and used for further investigations.

2.3.Preparation of the plant extract

Aqueous extract: Aqueous extract of *C. sinensis* was prepared according to the method of Kumar*et al.*(2012). 10 g of thoroughly dried leaves powder were immersed in 100 ml of double distilled water at 60°C for 15 minutes. The extract was filtered using Whatman filter paper and stored at 4°C for further use.

2.4. Synthesis and optimization parameters for ZnO NPs

The method proposed by Gnanasangeetha and Thambavani, (2013) was used for the synthesis of ZnO NPs in the selected medicinal plant and the detailed procedure was given in Figure1.

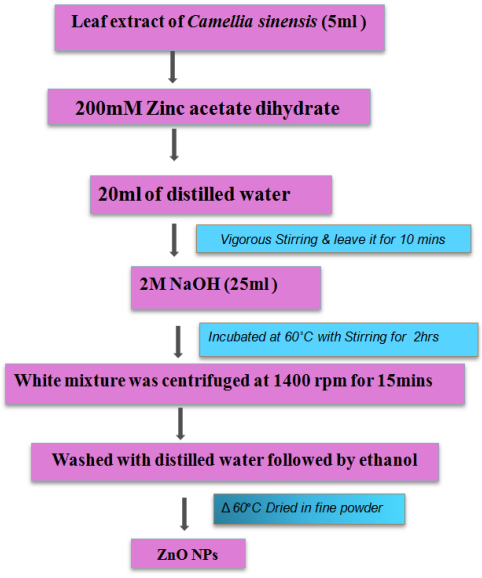


Figure 1: Synthesis of Nanoparticles

Zinc oxide nanoparticles (ZnO NPs) were synthesized using zinc acetate dehydrate $Zn(CH_3COO)_2$. $2H_2O$. (modified)briefly, 5ml of leaf extract was taken and 20 ml of 200mM solution of zinc acetate was added. The solution was stirred continuously for 1 hourand then

added 2M NaOH solution (pH 12.0). The mixture was incubated at 60˚C with stirring for 2 hours. A white precipitate was centrifuged at 1400 rpm for 5 min and washed twice with sterile distilled water followed by ethanol. Complete conversion to ZnO NPs was then dried to fine powder at 60˚C.

2.5. Characterization of ZnO NPs

The changed pH of reaction mixture was recorded using digital pH meter during the synthesis of ZnO NPs. UV-Vis spectral analysis was performed using Shimadzu 1800 spectrophotometer, whereas the morphology of the ZnO NPs was observed by SEM with EDAX, Structure and composition of ZnO NPs were analysed by XRD. Further characterization was accomplished by FTIR 2800 Shimadzu and Zeta potential

2.6. DPPH radical scavenging activity of synthesized ZnO NPs

The determination of DPPH scavenging activity of the plant extract was done by the method of Kikuzaki and Nakatani (2003). The sample (25µl) and 0.48ml of methanol were added to 0.5ml of methanolic solution of DPPH. The mixture was allowed to react at room temperature for 30 minutes. Methanol alone served as blank and DPPH in methanol, without the plant extracts, served as positive control. After 30minutes of incubation, the discolouration of the purple colour was measured at 518nm. Ascorbic acid was used as a positive control.

The radical scavenging activity was calculated as follows

$$\textbf{Scavenging activity (\%)} = \frac{\textbf{A518 (sample) – A518 (control)}}{\textbf{A518 (control)}} \textbf{ X 100}$$

2.7. Antibacterial activity of synthesized ZnO NPs

The antibacterial activity of synthesized ZnO NPs was performed by agar disc diffusion method against four bacterial strains (Gram-negative bacteria: *Escherichia coli*MG1655and *Staphylococcus aureus*, MH605510 Gram-positive bacteria: *Klebsiella oxytoca*11492-1 and *Bacillus subtilis.* MG859252). All the tested strains were reference strains. The bacterial cultures were grown on nutrient agar medium (Himedia, pH 7.4) at 37ºC. The cultures were maintained at 4°C for further use.

The antibacterial activities of synthesized ZnO NPs were screened by agar well diffusion method Tiwari *et al.*(2016). The antimicrobial compounds present in the plant extract are allowed to diffuse out into the medium and allowed to interact in a plate seeded with the test organisms. The diameter of zone of inhibition can be measured in millimetres.

2.8. Broth microdilution method

The broth microdilution method was carried out in a 96-well microtiter plate to determine the minimum inhibitory concentration (MIC). The different concentrations of compounds (1, 0.5, 0.25 and 0.125mg/ml) were diluted in Mueller Hinton broth and the final volume was maintained at 100 µl. The final concentration of DMSO was less than 1%. From the overnight grown bacterial culture 5 µl was added to the test medium to bring the final inoculums size to 1x105 cfu/ml. The agar plates were incubated at 37˚ for 16 hour and the absorbance was read at 600 nm. The lowest concentration of the compound that inhibits the complete growth of the bacterium was determined as MIC NCCLS, 2000.The percent growth inhibition was calculated by comparison with a control using the formula indicated below;

$$\%\ \text{of growth inhibition} = \frac{\text{control} - \text{test}}{\text{control}} \text{x } 100$$

2.9. Thin layer chromatography (TLC) with bioassay detection for AChE inhibition

The TLC with bioassay detection for AChE inhibition was modified from the study of Rhee*et al*, (2001). A 2.5mm silica gel plate F254 no. 5554 was used as a stationary phase. Two mobile phases, i.e. dichloromethane: ethanol: water 4:4:0.5 (v/v/v) were used. Three microliter of plant extracts dissolved in methanol at concentration of 5 mg/ml was applied to the plate. After the plate had been developed, it was dried at room temperature and then sprayed with 30mM ATCI followed by 20mM DTNB. The plate was dried at room temperature for 45 min, and sprayed with 10.17 U/ml AChE. After 20 min, the plate was observed under visible light. A positive spot indicating AChE inhibitor was a colourless spot on the yellow background. The result was compared to that from the TLC analysis of the same sample after spraying with Anisaldehyde and Dragendorff reagents [14] Dewanjee *et al*., 2015.

2.10. Acetyl cholinesterase inhibition activity

The acetyl cholinesterase inhibition activity was measured using the method described byingkaninan *et al.*(2003). 3 ml of 50mM Tris–HCl buffer (pH 8.0), 100 µl of sample solution at different concentrations (3 mg/ml, 1.5 mg/ml, 0.75 mg/ml) and 20µl AChE (6 U/mL) solution were mixed and incubated for 15 min at 30ºC, a 50 µl volume of 3 mM 5, 50-dithiobis-(2-nitrobenzoic acid) (DTNB) was added to this mixture. The reaction was then initiated by the addition of 50 µl of 15 mM acetylthiocholine iodide (AChl). The hydrolysis of this substrate was monitored at precisely 405 nm wavelength. At this wavelength the formation of yellow 5-thio-2-nitrobenzoate anion was noticed as the result of the reaction of

DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide. The enzymatic activity was calculated as a percentage of the velocities compared to that of the assay using buffer instead of inhibitor (extract), based on the formula:

$$\textbf{Enzyme Activity (EA)} = \frac{E-S}{E} \textbf{ X 100}$$

Where, E is the activity of the enzyme without test sample and S is the activity of the enzyme with test sample.

2.11. Statistical analysis

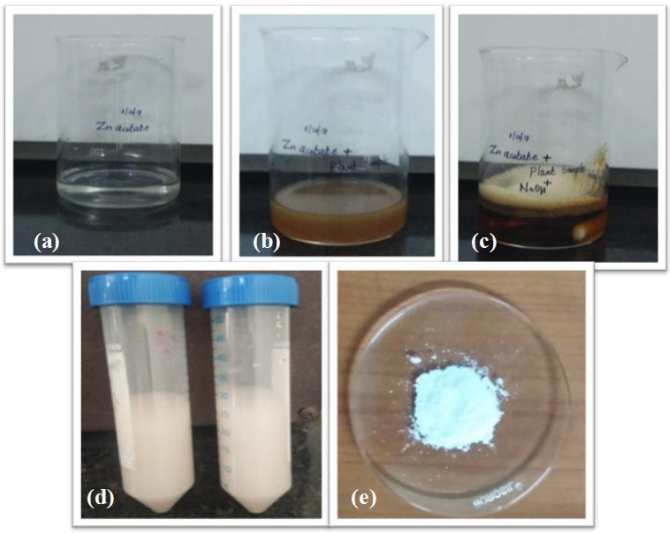
The parameters analyzed of the study were subjected to statistical treatment using SigmaStat Statistical package. All measurements were expressed as mean ± standard deviation. Statistical significance was determined by one-way ANOVA. Values of $p<0.05$ were considered significant.

3. Results and Discussion

3.1. Color change

Decrease of zinc is established by color alteration of the reaction mixture from pale yellow to white and shown in Figure 2 andTable 1.

Figure2. Color change- synthesized ZnO NPs



a)200 mM zinc acetate (transparent) solution, b) 35 ml of zinc acetate (yellowish white) solution + 15 ml of leaf extract, c) after 6 hours (pale yellow color), d) after 24 hours (white color intensity increased) e) SynthesizedZnO NPs

Table 1: Change in color of solution during formation of synthesized ZnO NPs using leaf extract of *Camellia sinensis*

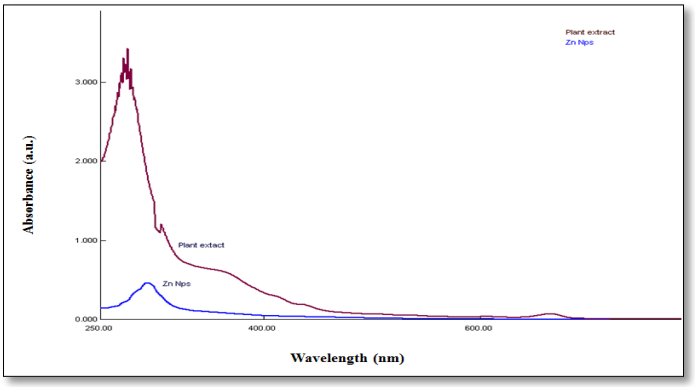
Solution	Reduction		Color intensity	Time
	Before	After		
Camellia sinensis Extract	Light brown	-	-	-
200 mM zinc acetate	Transparent	Pale yellow	+	Immediate
		Yellowish white	++	After 6 h
		White	+++	After 24 h

Visual colour change is the preliminary test for nanoparticles synthesis.For the duration of synthesis, the transform in color of the solution and development of a yellowish-white precipitate was an indication that zinc acetate had been reduced. Similar results was observed by Santhoshkumar*et al.* (2017),who stated that the ZnO NPs was synthesized using freshly prepared leaf extract*of Passifloracaerulea.*

3.2. UV-Vis spectrum of synthesizedZnO NPs

UV-Vis spectroscopy is asignificant tool for the determination of development shape and stability of nanoparticles. The UV-spectrum of the synthesizedZnO NPs is shown in Figure 3.

Figure 3: The UV- Vis spectrum of the ZnO NPs



A. Standard B. ZnO NPs

The spectrum reveals a broad peak which was obtained at wavelength 318nm which can be assigned to the intrinsic band gap absorption of ZnO NPs due to the electron transition from the valence band to the conduction band. The band gap energy ofsynthesizedZnO NPs is calculated by using formula;

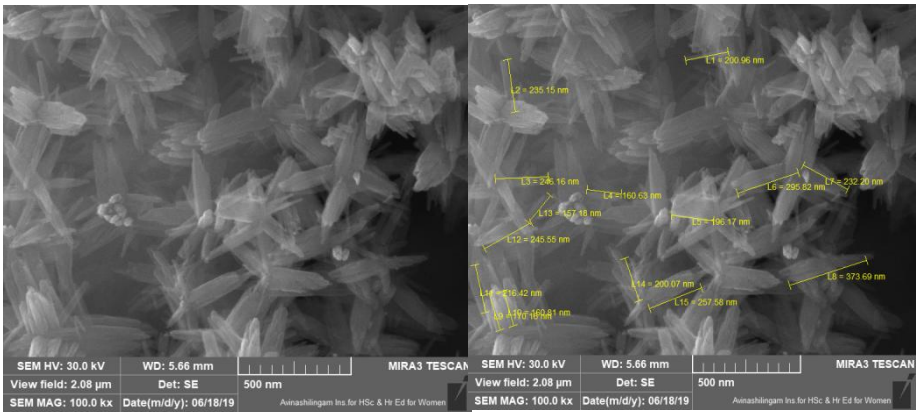
$$E = {}^{hc}/_{\lambda}$$

Where h (6.626x 10 -34 JS) is planks constant c (3x10 8 m/s) is the velocity of light λ (318 nm) wavelength. The band energy of synthesizedZnO NPs was found to be 3.38eV.Green synthesis of ZnO NPs was obtained using *Pongamiapinnata*(Suresh *et al.*2015). The results was in accordance with the Rajeshkumar*et al.*(2018) and confirmed the absorption peak at 380nm by UV spectrum

3.3. SEM analysis of synthesizedZinc oxide Nanoparticles

SEM analysis is through to imagine shape and size of the nanoparticle. The surface morphology of the resulting synthesizedZnO NPs is examined in Figure4.

Figure 4: SEM image of synthesizedZnO NPs



SEM images were seen in different magnification ranges such as 2µm-200µm which obviouslyconfirmed the incidence of distinctive and abundant flower shaped ZnO NPs.

Figure 5: EDAX spectrum of synthesizedZnO NPs

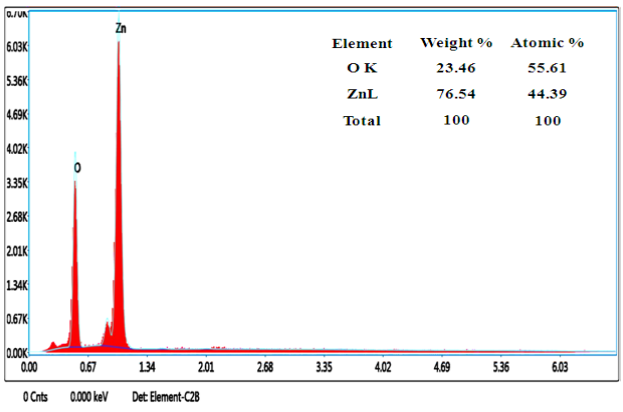


Figure 5 showthe EDAX spectrum of synthesized ZnO NPs. The composition showed from EDAX analysis reveals zinc 76.54% and oxygen 23.36%.

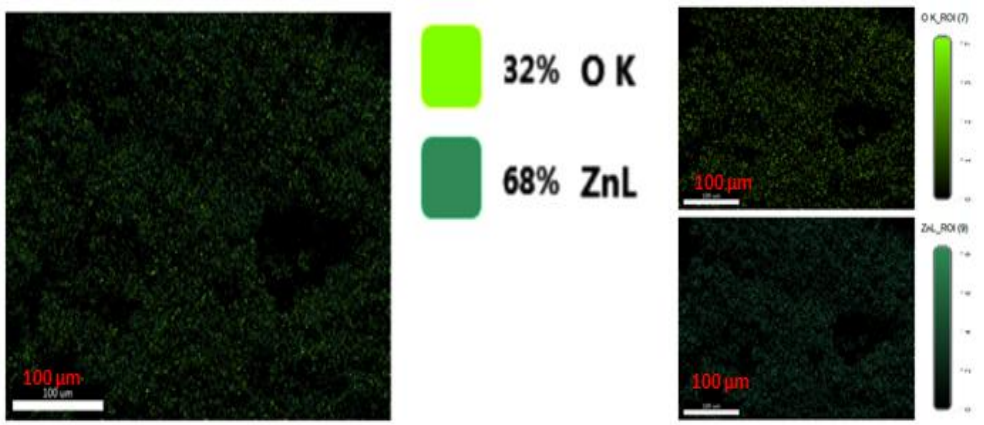
Bala *et al.* (2015) reported that the presence of carbon in trace amount indicates the involvement of plant phytochemical group in reduction and capping of the synthesized ZnO NPs. The results of Rajeshkumar *et al.*, (2018) showed average size of nanoparticles 80 nm

with some agglomerate structures. The synthesized ZnO NPs are in accordance with root extract of *Zingiber officinale* Raja and Jayalakshmy, (2015).

3.4. Elemental Mapping analysis of synthesized ZnO NPs

The EDAX spectrum Figure 6 shows only the peaks of zinc and oxygen elements which prove synthesized ZnO NPs.

Figure 6: Elemental Mapping analysis of synthesizedZnO NPs



Synthesized ZnO NPs is essentially free from impurities and it is also seen in the limit of EDAX. The Zn and O observed atomic percent value of 23.46 and 76.54 and weight percent value of 55.61 and 44.39 respectively.

3.5. XRD patterns of synthesized ZnO NPs

The crystalline size and structure properties of synthesized ZnO NPs are revealed using X-ray diffractions analysis. The XRD carried out with Cu – κα radiation (κ – 0.154nm) and 2theta range from 20˚ to 80˚. The XRD image of synthesized ZnO NPs is showed in Figure 7. The strong Bragg reflection peaks (2^{θ}= 31.8˚,34.4˚,36.3˚,47.6˚, 56.6˚,62.9˚,66.4˚ and 77.0˚) matched by their miller indicates ((100),(002),(101), (102), (110),(103),(112) and (202)) were obtained wurtizile structure (Hexagonal phase) comparison with JCPDS card No: 36-1451 (Aneesh *et al.*,2007) and with JCPDS card No: 89-7102 (Rajiv *et al.*,2013 and Slevarajan & Mohanasrinivasan , 2013).

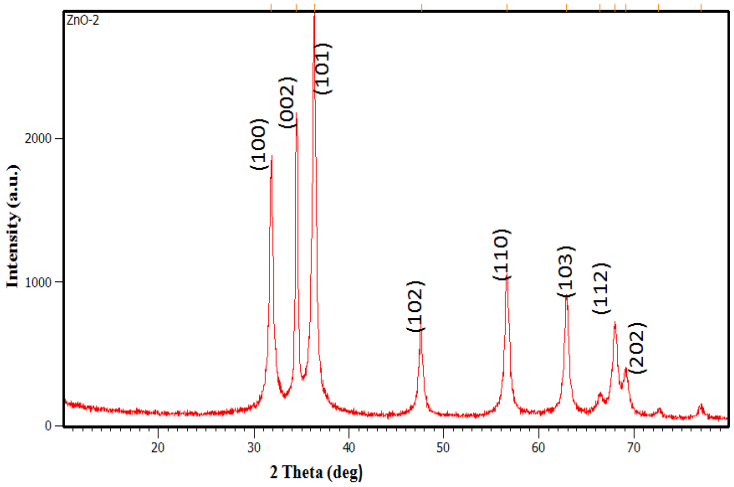
The mean crystallite size (D) of the particles was determined from the XRD line broadening measurement using Scherrer equation

$$D = {}^{0.9\lambda}/_{\beta cos^{\theta}}$$

Where λ is the wave length (Cu – κα), β is the full width and half maximum (FWHM) of the ZnO NPs (101) line and theta is the diffraction angle. The calculated crystallite size of

the powder is about 160 nm. Jamdagni *et al.*,(2016) observed that the XRD spectrum crystal lattice indices and particles size calculation of ZnO NPs diffraction peaks were observed at 2^{θ}. The peaks have been attributed to hexagonal phases of ZnO NPs. The value of particle size was found to be 16.58 nm.

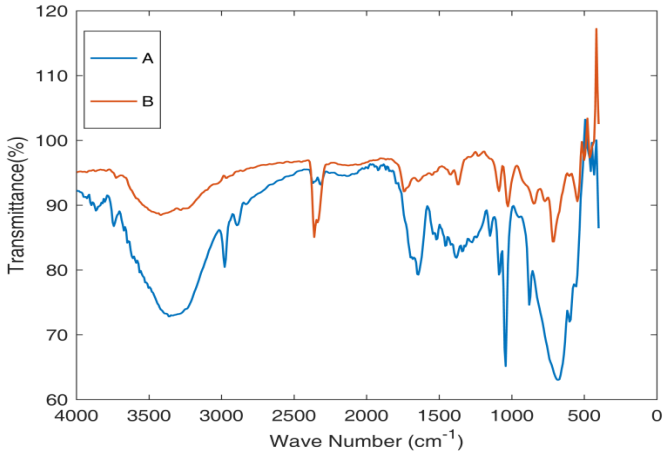
Figure7: XRD image of synthesized ZnO NPs



3.6.FTIR Spectra of synthesized ZnO NPsanalysis

The FT-IR spectra of *Camellia sinensis* leaf extract before reduction was shown in Figure 8. The absorption bands at 3390-3228 cm^{-1} representing O-H stretching alcohol and carboxylic acid. The absorption peak is located at around 2773 cm^{-1} represented –O-H stretching carboxylic acidstretching vibration present at 1643 and 1701 cm^{-1} are associated with (C=O stretch) vibration of amides and aldehydes respectively. The strong absorption peaks at 1361 and 1226cm^{-1}which are assigned to $CH(CH_3)_2$ stretching alkanes and alkyls and =C-O- symmetric and asymmetric stretch ethers. Small bands at 1504, 929 and 821cm^{-1}represents N-H stretching amides, =C-H- stretching alkanes and C-H stretching vibration of aromatic compounds respectively. After the synthesis of ZnO NPs, FT-IR spectra showed (Figure. 8) strong absorption peaks at 3441, 2300,1366,1026 and 709 cm^{-1}which were assigned to –N-H- stretching amines symmetric and asymmetric –O-H- stretching carboxylic acids, CH_3-C-H stretching alkanes and alkyls, C-O stretching alcohol and C-H stretching aromatic compounds respectively. The weaker bands at 1419, 1087,848 and 547cm^{-1}corresponds to aromatic compounds (-C-C- stretching), alcohol (C-O- stretching), alkenes (=C-H- stretching) and alkyl halides (C-Br- stretching) respectively. The band at 463cm^{-1}confirms stretching vibration of ZnO NPs.

Figure 8: The FT-IR spectra of synthesizedZnO NPs



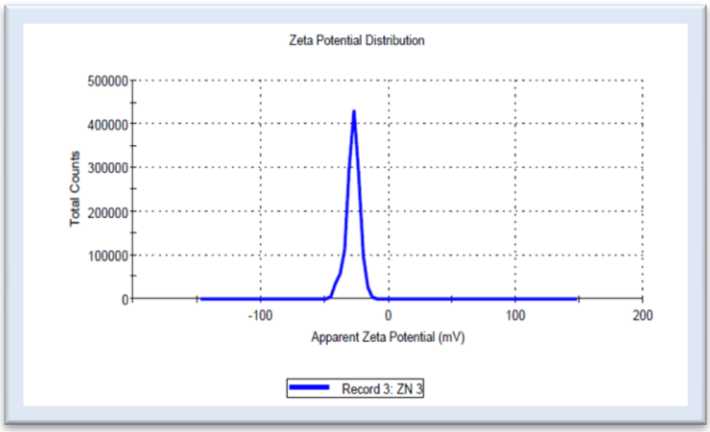
A. Plantextract B. ZnO NPs

Yedurkar *et al.*(2016) demonstrated the presence of C-Alkyl chloride and hexagonal phase ZnO and show peaks at 650.01 and 532.35.Spectroscopy Tutorial 2016 revealed that the 1362.5 peak results from aromatic amines and the 1040 and 1026.65 results from C-N stretch of aliphatic amines.746.25 and 620.65 peaks correspond to alkanes and supposedly C-H bend in alkynes respectively, hence it confirmed the functional group of zinc oxide nanoparticles.

3.7. Zeta potential synthesized ZnO NPs

Zeta potential was used to conclude the surface potential of the synthesized ZnO NPs Zeta potential is an essential characterization of stability for aqueous ZnO NPs. A minimum of +30 mV Zeta potential is essential for the indication of stable synthesized ZnO NPs. For the showed nanoparticles, zeta values were measured and found to be -11.6 mV with a peak area of 100% intensity. These values present full stabilization of the nanoparticles which may be the main reason in producing particles sizes with a narrow size distribution as shown in Figure 9.The zeta potential of the synthesized zinc oxide nanoparticles was determined in water as dispersant. The high value confirms the repulsion between the particles and thereby increases in stability of the formulation(Yedurkar *et al.*, 2016).

Figure. 9:The Zeta potential of synthesized ZnO NPs

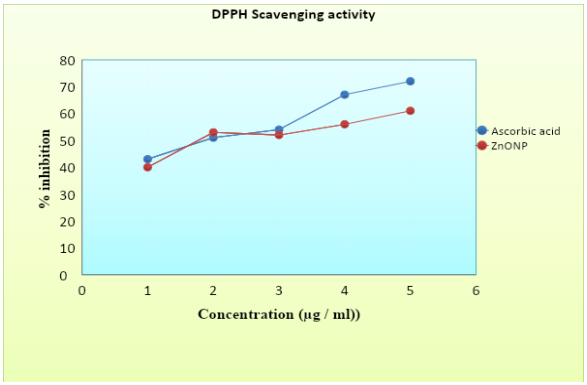


3.8. DPPH radical scavenging activity of synthesized ZnO NPs

The inhibition of scavenging activity of synthesized ZnO NPs for DPPH was shown in Figure10. The inhibition of scavenging activity of the synthesized ZnO NPs was increasing in dose dependent manner and IC_{50} value of was70.37%.

Lawrence *et al.,* (2016) observed that the DPPH activity of *Gmelina arborea* extract on radical increased with an increasing concentration of the extract. The IC_{50} value of the extract was found to be 124.39µg/ml. Similar results was reported by Mathew *et al.*(2012), showed raw curcumin showed more than 80% DPPH free radical scavenging capacity.

Figure 10: DPPH scavenging activity of SynthesizedZnO NPs



3.9. Antibacterial activity of Synthesized ZnO NPs

The antibacterial activity againstZnO NPs obtained zones of inhibition growth of the bacteria which varied against test organisms with different concentration ranging from 20 to 80µl and it is represented in Table 2 and Figure. 11.

Table 2.Antibacterial activity of synthesized ZnO NPs

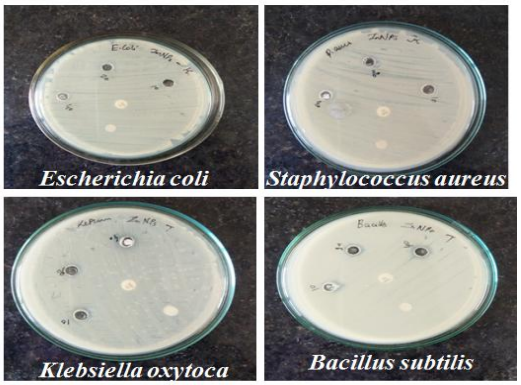
(Diameter of zone of inhibition in mm)

Name of the organism	Control	ZnO NPs(mm)

		20	40	60	80
Escherichia coli	0.9	1.6	1.5	1.8	1.5
Staphylococcus aureus	1.3	1.8	1.5	1.3	1.5
Klebsiellaoxytoca	1.5	1.2	1.1	1.2	1.3
Bacillus subtilis	1.1	1.5	1.2	1.4	1.4

The inhibition of zone produced by the sample extract compared with zones produced by ampicillin was used as a control. The results revealed that the ZnO NPswere able to resist against some of the bacterial species. The ZnO NPs showed stronger moderate activity in 50µl concentration against *Escherichia coli* (1.2mm), *Staphylococcus aureus* (0.8mm), *Klebsiellaoxytoca* (0.8mm) and *Bacillus subtilis* (0.9mm).

Figure 11: Antibacterial activity of synthesized ZnO NPs



Similar study was reported by Mishra *et al.*,(2015)where the ZnO NPs inhibit the microbial growth. Stan *et al,* (2015) have also demonstrated that the synthesized Zinc oxide nanoparticles using *Petroselinum crispum* extract showed the microbial growth inhibition which has enhanced 2-16 times when compared with the plant extract.

3.10. Determination of minimum inhibitory concentration (MIC)

MIC (Minimum inhibitory concentration) for certain bacteria using synthesized ZnO NPs gave maximum zone in well diffusion method and the results are obtainable in Table 3.

Table3. Antibacterial activity of synthesized ZnO NPs against organisms by minimum inhibitory concentration (MIC) method

Name of the organism	ZnO NPs(mm)			
	20	40	60	80
Escherichia coli	+	-	+	-
Klebsiella oxytoca	+	-	+	-
Staphylococcus aureus	-	-	-	+
Bacillus subtilis	+	-	+	+

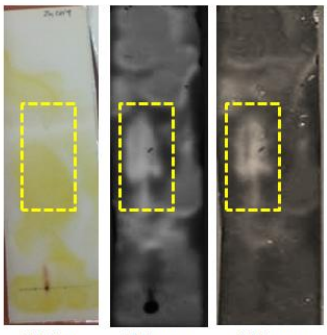
Synthesized ZnO NPs were tested for *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella oxytoca* and *Bacillus subtilis.* The bacterial strains were maintained on nutrient agar at 28°C. MIC value was defined, primarily based upon visual examination bacterial growth in serially diluted nanoparticles suspensions and absence of bacterial growth was visually checked to defined MIC values.

Similar study was reported by Jamdagni *et al.*(2016) and reported that the synthesized ZnO NPs were tested for microbial pathogens and the strains showed the absence of microbial growth was visually checked to define MIC values.

3.11. Thin layer chromatography (TLC) with bioassay detection for AChE inhibition

The qualitative results of inhibition of enzyme acetyl cholinesterase in Thin Layer Chromatography showed that synthesized ZnO NPs inhibited the enzyme by the appearance of yellow back ground with white spots was visible after about 5 min. Following are the results of the first test, yellow background with white spots for inhibiting sample and for ZnO NPs were visible after 5 min apparently tested against positive enzyme and gives inhibition at concentration of 5 mg ml^{-1} in figure 12.

Figure 12.TLC qualitative acetyl cholinesterase inhibition (AChEI) assayof ZnONP's



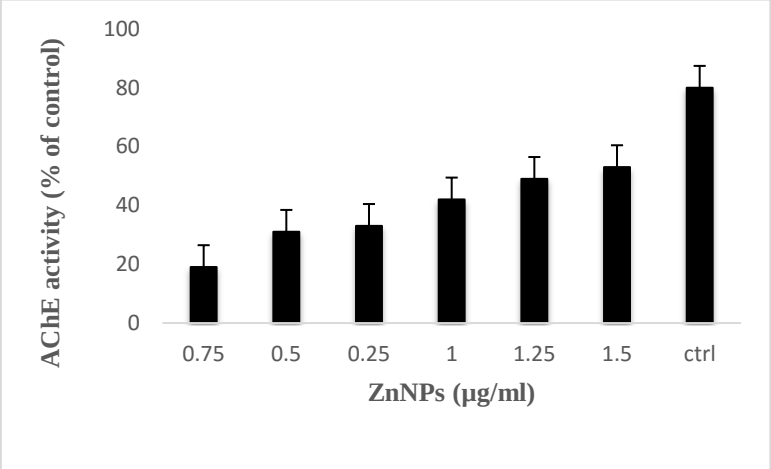
TLC elutionsystem - dichloromethane: ethanol: water (4:4:0.5 v/v/v).

Kalanchoebra siliensis extract of 2mg ml^{-1} concentration of TLC with white spot showed inhibitory property of cholinesterase with flavonoid compounds (Trevisan *et al.*, 2006). Similar results were obtained by Feitosa *et al*., (2011) and found that the acetylcholinestrase inhibitors are successfully used to treat the symptoms of Alzheimer's disease.

3.12.Acetyl cholinesterase (AChE) inhibitory activity of synthesized ZnO NPs

The percent inhibition data and IC_{50} value and Concentration (0.75, 0.50, 0.25, 1, 1.25, 1.5µg/ml) of synthesized ZnO NPs is presented in Figure 13.

Figure 13: Acetyl cholinesterase (AChE) inhibitory activity of synthesized ZnO NPs



When studied for its possible inhibitory effect in the *in vitro* analysis, synthesized ZnO NPs showed AChE inhibitory activity in a dose dependent manner with an IC_{50} value increasing the extract concentration. Concentration of 1.25 µg/ml in the synthesized ZnO NPs showed the most potent effect in AChE inhibition activity.

Rashed *et al.*,(2015) evaluated anti-alzheimers activity from the isolated compound of 80% methanolic extract of *Ampelopsis brevipedunculata* arial parts.

4. Conclusion

The green synthesis of nanoparticles used in this research is found to be non-toxic, eco-friendly, and have less usage of chemicals compared to the chemical and physical methods. The occurrence of phytochemicals in the leaf extract itself helps in the synthesis of metal oxide nanoparticles by inducing oxidation and reduction reactions. The antibacterial activity of synthesized ZnO NPs can be used as a potent antibacterial agent against pathogenic microorganisms and acetylcholinesterase inhibitory activity of ZnO NPs is proved

to be a promising agent of anti-Alzheimer's activity. ZnO NPs are anticipated to have extensive applications in the drug and pharmacology industries.

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Conflict of interest

Authors submitting a manuscript we declared that there are no conflicts of interest in the course of conducting the research. The author has the final decision regarding the manuscript and the decision to submit the findings for publication.

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