Original Research Article

GREEN SYNTHESIS, CHARACTERIZATION, ANTIBACTERIAL AND ACETYL CHOLINESTERASE INHIBITORY ACTIVITIES OF ZINC OXIDE NANOPARTICLES (ZnONPs) FROM THE LEAF OF *Camellia sinensis*

Abstract

Nanoparticles are widely used in biotechnology and biomedical field. Green synthetic methods of nanoparticles are simple and environmentally benign process which declines the demerits of conventional physical and chemical method. The synthesis of metal and semiconductor nanoparticles is an increasing research area, due to the potential applications in the development of novel technologies. The present research reports the synthesis and characterization of zinc oxide nanoparticles (ZnONPs) from the leaf extract of *C. sinensis.* The findings of these studies shows, the green synthesized ZnONPs is effective, safe and eco-friendly as they are stable and have abundant flower shaped with maximum particles in size ranging from 100 nm in diameter. The synthesized ZnONPs have been tested against the pathogenic bacteria and showed a good zone of inhibition. DPPH radical scavenging activity of synthesized ZnONPs expressed as percentage of DPPH radicals inhibition and IC_{50}value of 70.37%. Acetylcholinesterase (AChE) inhibitory activity possesses the maximum amount of the synthesized ZnONPs. From the results of the present study, it was concluded that the synthesized ZnONPs exhibited significant antibacterial and acetylcholinesterase inhibitory activities. Hence it can be used as a drug with multifunction in treating Alzheimer's disease (AD).

Keywords

ZnONPs, antibacterial, FTIR, SEM, *C. sinensis*, AChE

1. Introduction

Nanotechnology contains the use of materials having nanoscale dimensions in the range of 1–100 nm. Operating with nanomaterials has allowed researchers to have a better understanding of biology **(Santhoshkumar *et al.,* 2017).** Nanoscience has been creating a new biological and chemical 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, ZnONPs are of particular interest, because they can be prepared easily, inexpensive and safe material for human beings and animals. They are also extensively used in the formulation of health care products. ZnONPs has entered the scientific spotlight for its semiconducting properties, photochemical activity, high catalytic UV filtering properties, wound healing, anti-bacterial and anti-fungal 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 ZnONPs (Talam *et al.*, 2012). ZnONPs find extensive applications in biological and pharmacological fields. Particularly, metal oxide nanoparticles and ZnONPs efficiently protects over a broader UV range than any of the molecular UV-absorbers. The non-toxic nature of ZnONPs makes it fitting in drug research. ZnONPs are also used as anti-microbials in several formulations, drugs and medicines (Firdhouse *et al.,* 2015). Toxicological impact of nanomaterials are predictable to interact with the materials of biological components to produce significant impacts on the properties and behaviour of macromolecules, cells, tissues, organs and body (Diab *et al.,* 2018). The growing evidence of oxidative stress-mediated damages to the DNA, protein and lipids during Alzheimer's disease prompted for the identification of new molecules that play an important role in cholinesterase inhibitors and

scavenging the free radical formed during the pathogenesis of the disease (Malar *et al.*, 2017).

In this study, the ZnONPs are synthesized using simple, eco-friendly and cost effective method. The present work is aimed at synthesizing ZnONPs from leaf extract of *C. sinensis*. The synthesized ZnONP's were characterized by UV-Visible spectroscopy, SEM, XRD, FTIR and Zeta potential and also acetylcholine esterase inhibitory and antibacterial activities of prepared ZnONPs 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 ZnONPs

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

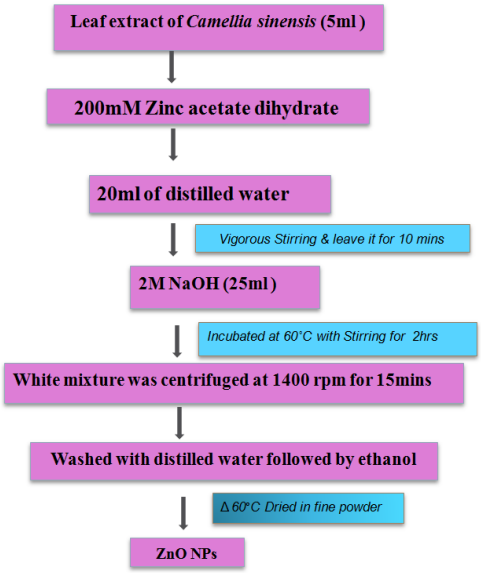


Figure 1: Synthesis of Nanoparticles

Zinc oxide nanoparticles (ZnONPs) were synthesized using zinc acetate dihydrate $Zn(CH_3COO)_2$. $2H_2O$. (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 hour and 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 ZnONPs was then dried to fine powder at 60˚C.

2.5. Characterization of ZnONPs

The changed pH of reaction mixture was recorded using digital pH meter during the synthesis of ZnONPs. UV-Vis spectral analysis was performed using Shimadzu 1800

spectrophotometer, whereas the morphology of the ZnONPs was observed by SEM with EDAX, Structure and composition of ZnONPs were analysed by XRD. Further characterization was accomplished by FTIR 2800 Shimadzu and Zeta potential

2.6. DPPH radical scavenging activity of synthesized ZnONPs

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

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

2.7. Antibacterial activity of synthesized ZnONPs

The antibacterial activity of synthesized ZnONPs 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 ZnONPs 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 dragendorf reagents [14] Dewanjee *et al*., 2015.

2.10. Acetyl cholinesterase inhibition activity

The acetyl cholinesterase inhibition activity was measured using the method described by Ingkaninan *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:

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

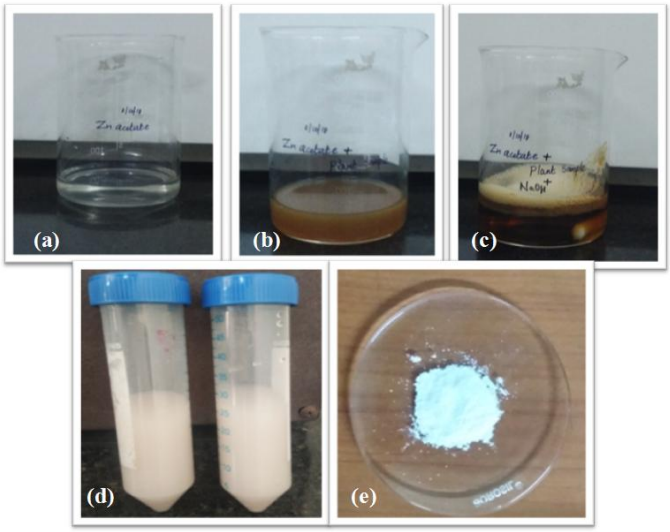
Where, E is the activity of the enzyme without test sample and S is the activity of the enzyme with test sample.

3. Results and Discussion

3.1. Color change

Reduction of zinc is confirmed by color change of the reaction mixture from pale yellow to white and shown in Figure 2 and Table 1.

Figure2. Color change- synthesized ZnONPs



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

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

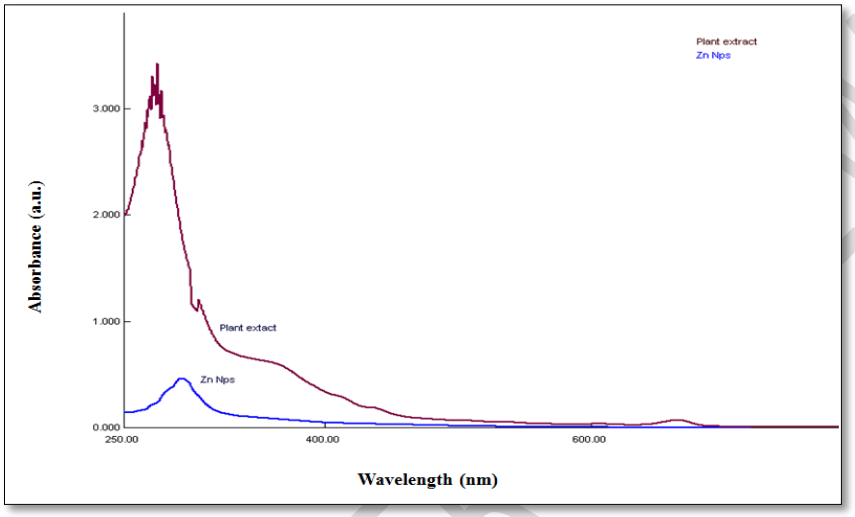
Solution	**Before reduction**	**After reduction**	**Color intensity**	**Time**
Camellia sinensis Extract	Light brown	-	-	-
200 mM zinc acetate	Transparent	Pale yellow	+	Immediate
		Yellowish white	++	After 6 h
		White	+++	After 24 h

Visual colour change is the preliminary test for nanoparticles synthesis. During synthesis, the change in color of the solution and formation 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 ZnONPs was synthesized using freshly prepared *Passiflora caerulea* fresh leaf extract.

3.2. UV-Vis spectrum of synthesized ZnONPs

UV-Vis spectroscopy is an important tool for the determination of formation, shape and stability of nanoparticles. The UV-spectrum of the synthesized ZnONPs is shown in Figure 3.

Figure 3: The UV- Vis spectrum of the ZnONPs



A. Standard **B. ZnONPs**

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

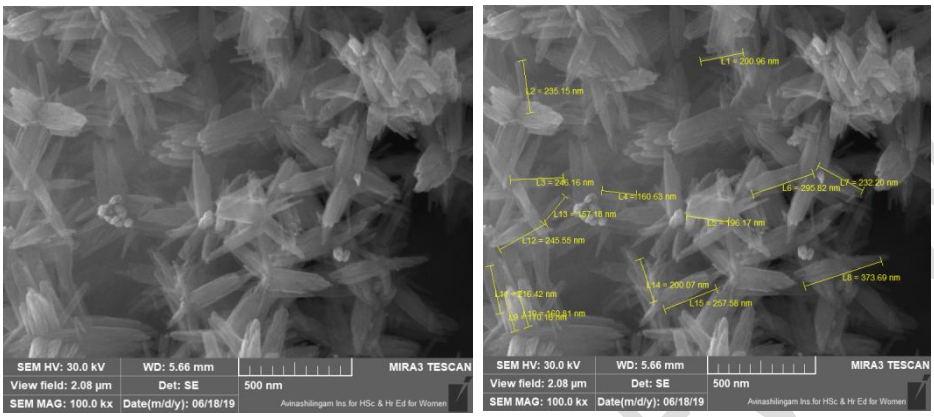
$$E = {}^{hc}/_{\lambda}$$

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 synthesized ZnONPs was found to be 3.38eV. Green synthesis of ZnONPs was obtained using *Pongamia pinnata* (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 synthesized Zinc oxide Nanoparticles

SEM analysis is done to visualize shape and size of the nanoparticle. The surface morphology of the resulting synthesized ZnONPs is examined in Figure 4.

Figure 4: SEM image of synthesized ZnONPs



SEM images were seen in different magnification ranges such as 2µm-200µm which clearly demonstrated the presence of distinctive and abundant flower shaped ZnONPs.

Figure 5: EDAX spectrum of synthesized ZnONPs

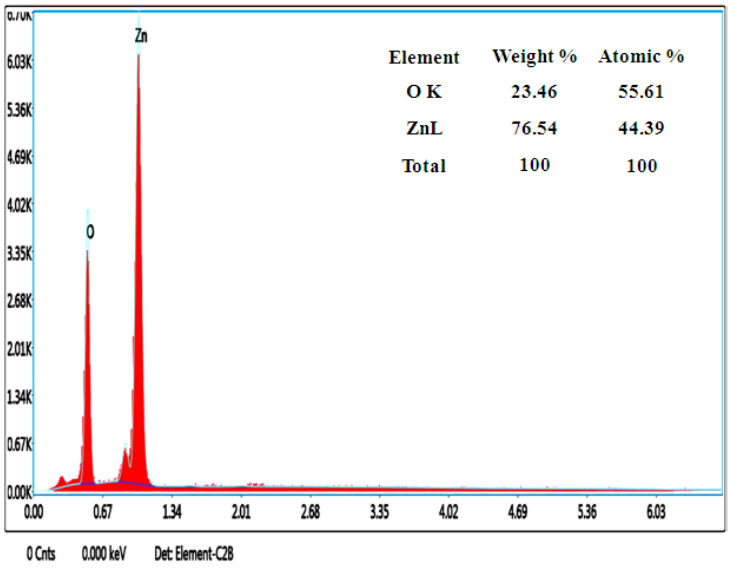


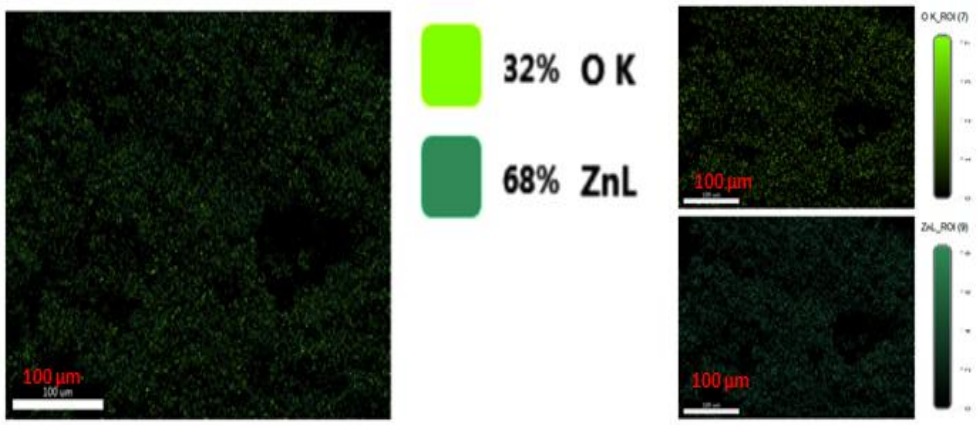
Figure 5 show the EDAX spectrum of synthesized ZnONPs. The composition obtained from EDAX analysis reveals zinc 76.54% and oxygen 23.36%.

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 ZnONPs. The results of Rajeshkumar *et al.*, (2018) showed average size of nanoparticles 80 nm with some agglomerate structures. The synthesized ZnONPs are in accordance with root extract of *Zingiber officinale* Raja and Jayalakshmy, (2015).

3.4. Elemental Mapping analysis of synthesized ZnONPs

The EDAX spectrum Figure 6 shows only the peaks of zinc and oxygen elements which confirm synthesized ZnONPs.

Figure 6: Elemental Mapping analysis of synthesized ZnONPs



Synthesized ZnONPs 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 ZnONPs

The crystalline size and structure properties of synthesized ZnONPs are revealed using X-ray diffractions analysis. The XRD carried out with Cu – κα radiation (κ – 0.154nm) and 2 theta range from 20˚ to 80˚. The XRD image of synthesized ZnONPs 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

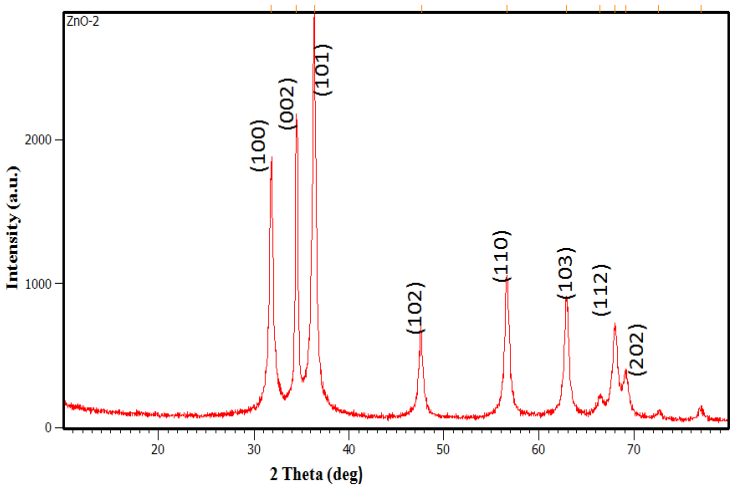
No: 36-1451 (Aneesh *et al.*,2007) and with JCPDS card No: 89-7102 (Rajiv *et al.*,2013 and Slevarajan & Mohanasrinivasan , 2013).

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 ZnONPs (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 ZnONPs diffraction peaks were observed at 2^{θ}. The peaks have been attributed to hexagonal phases of ZnONPs. The value of particle size was found to be 16.58 nm.

Figure7: XRD image of synthesized ZnONPs

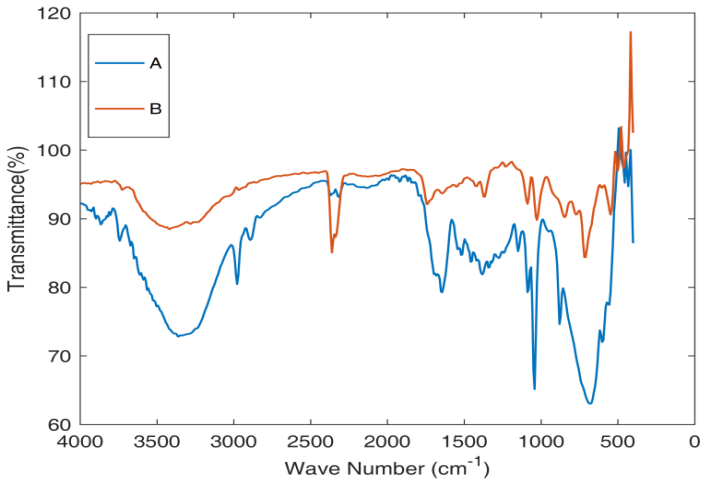


3.6. FTIR Spectra of synthesized ZnONPs analysis

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 acid stretching 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 ZnONPs, 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 ZnONPs.

Figure 8: The FT-IR spectra of synthesized ZnONPs



A.Plant extract **B. ZnONPs**

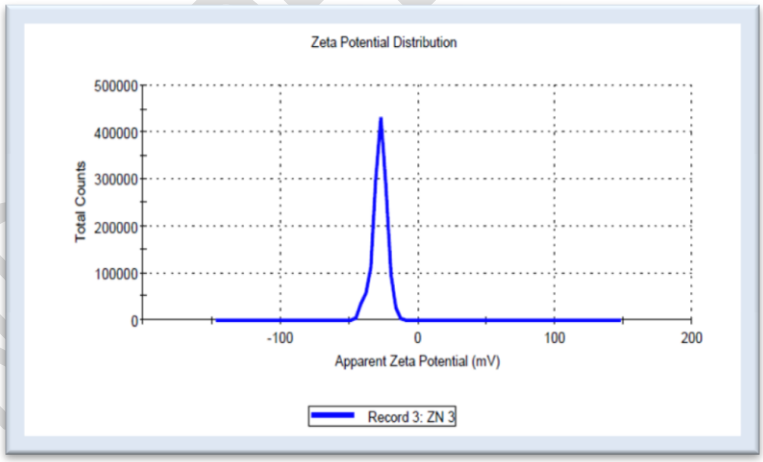
Yedurkar *et al.* (2016) demonstrated the presence of C-Alkyl chloride and hexagonal phase ZnO and show peaks at 650.01 and 532.35. Spectroscopy Tutorial 2016 revealed that the 1362.5 peak results from aromatic amines and the 1040 and 1026.65 results from C-N stretch of aliphatic amines.746.25 and 620.65 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 ZnONPs

Zeta potential was used to determine the surface potential of the synthesized ZnONPs Zeta potential is an essential characterization of stability for aqueous ZnONPs. A minimum of +30 mV Zeta potential is required for the indication of stable synthesized ZnONPs. For the obtained nanoparticles, zeta values were measured and found to be -11.6 mV with a peak area of 100% intensity. These values provide 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 among the particles and thereby increases in stability of the formulation (Yedurkar *et al.*, 2016).

Figure. 9:The Zeta potential of synthesized ZnONPs



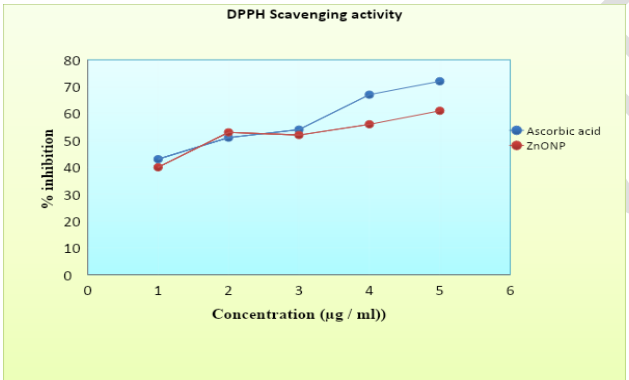
3.8. DPPH radical scavenging activity of synthesized ZnONPs

The percentage inhibition of scavenging activity of synthesized ZnONPs for DPPH was shown in Figure10. The percent inhibition of scavenging activity of the synthesized ZnONPs was increasing in dose dependent manner which was comparable to that of standard

at the same concentration. IC_{50} value of the synthesized ZnONPs was determined to be the percent inhibition was 70.37%.

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 Synthesized ZnONPs



3.9. Antibacterial activity of Synthesized ZnONPs

The antibacterial activity against ZnONPs showed zones of inhibition of bacterial growth which varied against test organisms with different concentration ranging from 20 to 80µl and it is represented in Table2 and Figure. 10.

Table 2. Antibacterial activity of synthesized ZnONPs

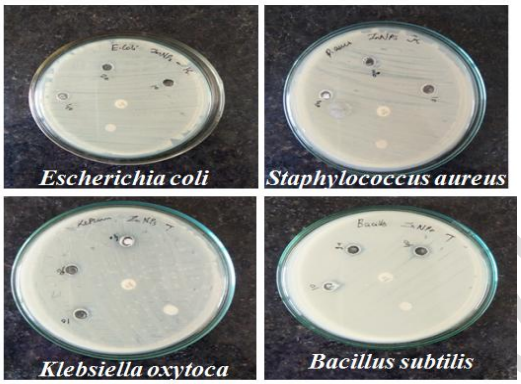
(Diameter of zone of inhibition in mm)

Name of the organism	Control	ZnONPs(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
Klebsiella oxytoca	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 ZnONPs were able to resist

against some of the bacterial species. The ZnONPs showed stronger moderate activity in 50µl concentration against *Escherichia coli* (1.2mm), *Staphylococcus aureus* (0.8mm), *Klebsiella oxytoca* (0.8mm) and *Bacillus subtilis* (0.9mm).

Figure 10: Antibacterial activity of synthesized ZnONPs



Similar study was reported by Mishra *et al.,* (2015) where the ZnONPs 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)

Minimum inhibitory concentration for selected organism using synthesized ZnONPs gave maximum zone in well diffusion method and the results are presented in Table 3.

Table 3. Antibacterial activity of synthesized ZnONPs against organisms by minimum inhibitory concentration (MIC) method

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

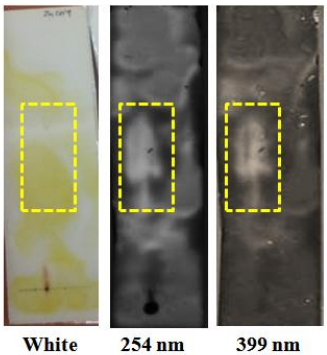
Synthesized ZnONPs were tested for *Escherichia coli, Staphylococcus aureus, Klebsiella oxytoca* and *Bacillus subtilis.* The bacterial strains were maintained on nutrient agar at 28°C. MIC value was defined, primarily based upon visual examination bacterial growth in serially diluted nanoparticles suspensions 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 ZnONPs 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 ZnONPs 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 ZnONPs were visible after 5 min apparently tested against positive enzyme and gives inhibition at concentration of 5 mg ml^{-1} in figure 11.

Figure 11. TLC qualitative acetyl cholinesterase inhibition (AChEI) assay of ZnONP's



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

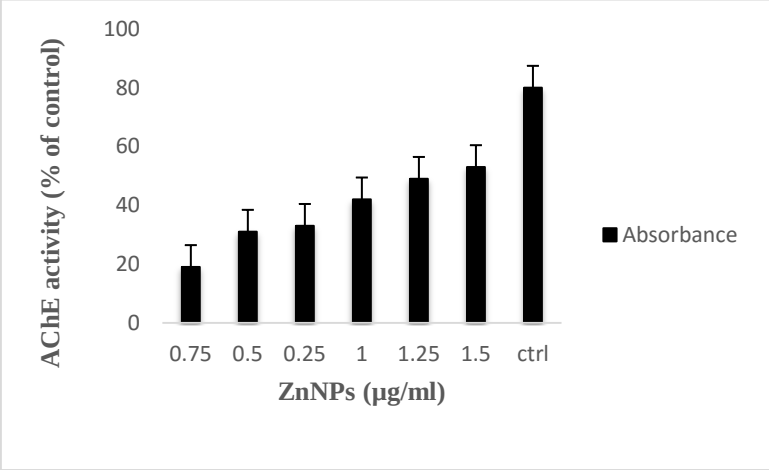
Kalanchoe brasiliensis extract of 2mg ml^{-1} concentration of TLC with white spot showed inhibitory property of cholinesterase with flavonoid compounds (Trevisan *et al.*,

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 ZnONPs

The percent inhibition data and IC_{50} value and Concentration (0.75, 0.50, 0.25, 1, 1.25, 1.5µg/ml) of synthesized ZnONPs is presented in Figure 12.

Figure 12: Acetyl cholinesterase (AChE) inhibitory activity of synthesized ZnONPs



When studied for its possible inhibitory effect in the *in vitro* analysis, synthesized ZnONPs 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 ZnONPs 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

Green synthesis of nanoparticles used in this experiment is found to be eco-friendly, non-toxic and less usage of chemicals compared to physical and chemical method. The presence of phytochemicals in the leaf extract itself helps in the synthesis of metal oxide nanoparticle by inducing oxidation and reduction reaction. The method stands out primarily

due to the fact that it is eco-friendly and shuts down the demerits of conventional physical and chemical methods. As a preliminary confirmation, the rapid synthesis of ZnONPs was measured using the UV- Visible spectroscopy at a maximum absorbance of 318 nm. Further, the XRD analysis proved the crystalline nature of the ZnONPs, while the EDAX analysis confirmed the presence of zinc and oxide ions in the nanoparticles. The SEM analysis of green synthesis of flower shaped synthesized ZnONPs of about 100nm has been achieved from leaf extract of *C. sinensis*. The anti-bacterial activity of synthesized ZnONPs can be used as potent antibacterial agent against pathogenic microorganism and acetylcholinestrase inhibitory activity of ZnONPs is proved to be a promising agent of anti-Alzheimer's activity. These particles are anticipated to have extensive applications in drug and pharmacology industries.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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