BIOGENIC SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING THE ROOTS OF MIRABILIS JALAPA TO DEPRIVE ELUENT, AS AN ANTI-OXIDANT AND ITS ANTI-BACTERIAL ASSAY ISOLATED FROM LESION

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Abstract: *Lesions also known as wounds, lesion/wound infections occur when the skin got cut and the microorganisms like bacteria enter and contaminate and multiplied due topathogenicity. It is therefore necessary for curing infection and preventing damage to the tissues. Infected wound enhances the rate of contamination. Antibiotics are miracle drugs to cure diseases unfortunately, the overuse of antibiotics against harmful pathogens had led to the increase of multidrug resistance in microorganisms.Zinc oxide nanoparticles are inorganic nanoparticles that showed remarkable antibacterial properties against multidrug-resistant bacteria. In this study, zinc oxide nanoparticles are synthesized by roots of mirabilis Jalapa and characterized by UV-Visible spectrometer, SEM, EDX, TEM, XRD, and FTIR respectively to know the morphology, size, and shape. They also act as anti-oxidants and pollution deprivers.*

Keywords: *FT-IR, SEM, EDX, TEM, XRD, anti-bacterial, anti-oxidant, eluent depriver.*

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INTRODUCTION

Wound/lesion infections occur when the skin got cut or injured, when pathogenic microorganisms fed on it and contaminate the wound, and cause infection. They increase the infection and may delay the healing process; heavy dosages are given to heal the wound infections [1-2]. Though antibiotics are miracle drugs for curing diseases overdosage may lead to harmful pathogenic effects and may damage the tissues[3-4]. Bacterial infections caused due to wounds may lead to adverse effects as they are mortal and morbid. The infected lesion can exhibit bacterial colonization, as itcan merge with other intermediates and seize the host immune system. The inappropriate dosage of antibiotics also causes severe damage to human health. Biogenic synthesized ZnONPs can be replaced in place of antibiotics as they embrace the use of toxic solvents[6].

Dyes play a prominent role in different pharmaceuticals, paper, plastics, leather, and textileindustries and these industries are situated near water bodies as a large amount of water is utilized for manufacturing goods due to which the discharge gets mixed into water bodies and in agricultural fields and disturb the aquatic life, food chain through irrigation. Dyes produce various types of waste in the form of solids, and liquids and some are hazardous as they may contain heavy metals which contaminate the living system[7-9]. The exposure time of the dye and its concentration is high which leads to acute or adverse effects. In the present study zinc oxide nanoparticles were used as a pollution depriverto remove Malachite Green dye and as an antioxidantagent. The biogenic synthesis of ZnO nanoparticles using different parts of plant

material has huge importance towards the ecosystem and empathy for various applications as they are non-toxic and less expensive [10].

MJ is widely used in tropical America and India as an ornamental plant. MJ is used as an inflammatory, painful disease, and skin disease [11]. The present study was carried out using the Mirabilis Jalapa root. MJR is used as a pharmacological, ethnobotanical activity such as anti-bacterial, anti-microbial, anti-fungal, anti-virus, and anti-diabetic. The root contains alkaloids, flavonoids, phenols, amino acids, glycosides, tannins, and saponins. MJ root extract acts as a reducing agent for the reduction of $ZnSO_4$ into nanoparticles[12].

Fig 1: a). Mirabilis Jalapa plant, b). Mirabilis Jalapa Root, c). Dried root d). Dried root powder, e). Root extract, f). Various concentrations of M.J. root extract.

EXPERIMENTAL

(i) Material and Methodology

Fresh plant of Mirabilis Jalapa was collected from local areas of Visakhapatnam. The root of Mirabilis Jalapa was separated from the plant and thoroughly washed with 2D water and soaked for half an hour to remove dust particles. The skin of the root was peeled and cut into small pieces and dried under shade for 10-15 days. The dried root was then ground and made into fine powder. The powder was stored in an airtight container and used for further analysis.

About 25 g of MJ root was weighed and 500 ml of 2D water is added and boiled to 60^0c for one hour and filtered thrice using Wittmann filter paper1. The extract was stored at 4^0c under the refrigerator and used for further analysis.

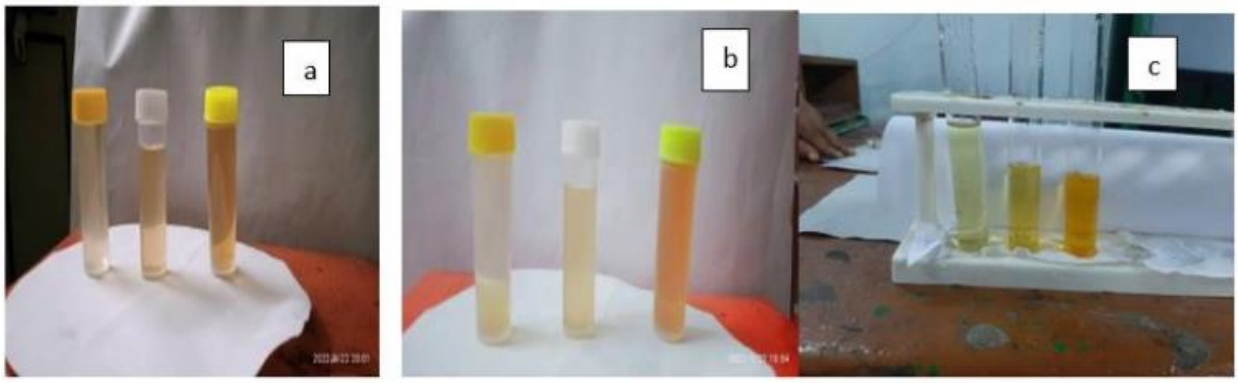


Fig 2: a. Different percentages of MJ Root extract (1%.3%,5%), b. ZnONP 's preparationusing different concentrations of $ZnSO_4$ solutions (1mM,3Mm, 5mM) c. ZnONP's preparation using different pH (5,6,10).

(ii) Green synthesis of nanoparticles

Zinc sulfate was thought to be the best precursor when compared to other bio-synthesized processes, and this study attempted to reduce the complexity of the processes carried out in the current study, the number of reagents used, and the amount of time needed to complete the analysis without impairing the quality of the biosynthesized nanoparticle. Sigma Aldrich was used to purchase the zinc sulfate and 4.03g of it was dissolved in 250 ml to create a 100 mM zinc sulfate solution. Nitric acid is used to completely clean the glassware, and it is then allowed to dry in the oven. To this, 10 ml of a 100 mM stock zinc sulfatesolution was added along with 75 ml of 2-D water and 10 ml of the Mirabilis Jalapa root extract, pH and temperature were maintained. The acquired resulting solution is centrifuged at 20,000 rpm after being incubated for three to four hours. A 700°C oven was used to collect and dry the material. Using a mortar and pestle, the sample was taken, thoroughly ground into a fine powder, stored, and used in the manufacture of ZnONP The sample was characterized using UV-Visible spectroscopy for identifying the formation of ZnONPs formation. The formation of ZnONPs was confirmed by the SPR surface plasmon resonance band at 399 nm.

The samples have undergone various measurements and evaluations for UV-VISIBLE spectrophotometer, FT-IR, SEM, TEM, EDX, and XRD to confirm the obtained ZnO nanoparticles are of nano size. Temperature, and P^H, played an important role in the biosynthesis of ZnONPs in which 80^0c temperature and P^H 12 were found to be the best

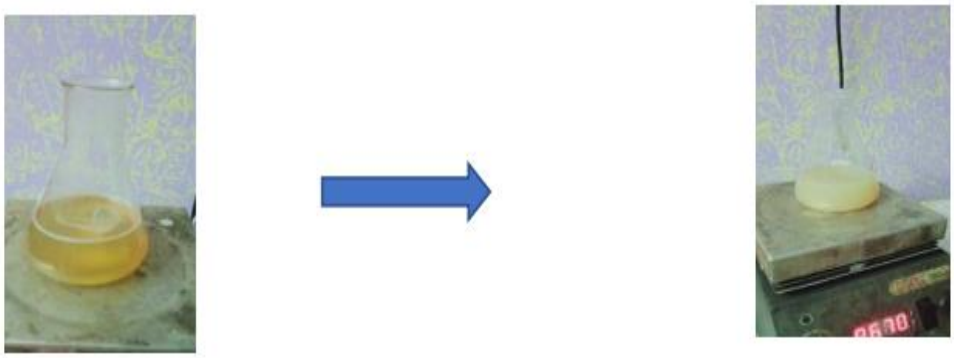


Fig 3: Preparation of Zinc oxide nanoparticles.

Optimization condition for ZnONPs

To enhance better yields ZnONPs were undergone several optimization conditions by changing the concentration of $ZnS0_4$, the concentration of root extract, pH, and temperature.

3.1. Optimisation of $ZnSO_4$ Concentrations

Various concentrations of $ZnSO_4$ solution of 1mM, 3mM, and 5mM were prepared and used for the study of the effect of $ZnSO_4$ concentration and to enhance the production of ZnONPs. This effect was monitored by using a UV-Visible spectrophotometer with an increase in absorption maximum of SPR band in the range of 384-392 nm which was shown in fig [4(a)] indicating a complete reduction of Zn^{2+} ions.

3.2. Optimisation of MJ Root extract

Different concentrations of MJ Root extracts (1%, 3%,5%) were prepared and used for the synthesis of ZnONPs preparation and subjected to UV-visible spectrophotometer yielding difference absorbance in the range of 389nm-395 nm shown in fig [4(b)]. It was observed that the increase in the percentage of root extract increased the absorbance as well as the yield as a greater number of zinc ions are being reduced. 5% root extract was found to be favorable for the synthesis of ZnONPs.

3.3. Effect of pH

pHplayed an effective role in synthesizing Zn NP's preparation. To 90 ml of 10mM zinc sulfate solution, 10 ml of 5% root extract was added by varyingpH (5, 6, 10,12).pH was adjusted by using 0.2M Hydrochloric acid and Sodium hydroxide solution. The different values of absorbance obtained at different pH value thatis shown in Fig[4(c)] as the pH of the sample increases there is an increase in absorbance due to an increase in the more reducing environment or conditions provided by the alkaline medium and pH 12 is approved to be good for synthesizing ZnONPs.

3.4. Optimisation of reaction time

Reaction time had a great effect on the synthesis of Zn NPs. The optimal duration for the reaction mixture is required the for stabilization of ZnONPs. The reaction was carried out for different time intervals like 45 min, 90 mins, and 120 mins. As the reaction time increased the color of the reaction mixture changed from pale yellow to yellow which was shown in Fig [4(d)]. The yield was obtained maximum at 120 mins.

3.5. Optimisation of temperature

To acquire optimal temperature 5ml of 10Mm zinc sulfate solution and 80 ml of deionized water were added,and 5ml of 5% root extract are added,pH 12 was maintained then incubated at different temperature conditions such as 40^0c, 60^0c, and 80^0c. The SPR band of Zn NPs exhibited absorbance as shown in Fig [4(e)] at 388nm – 399 nm.

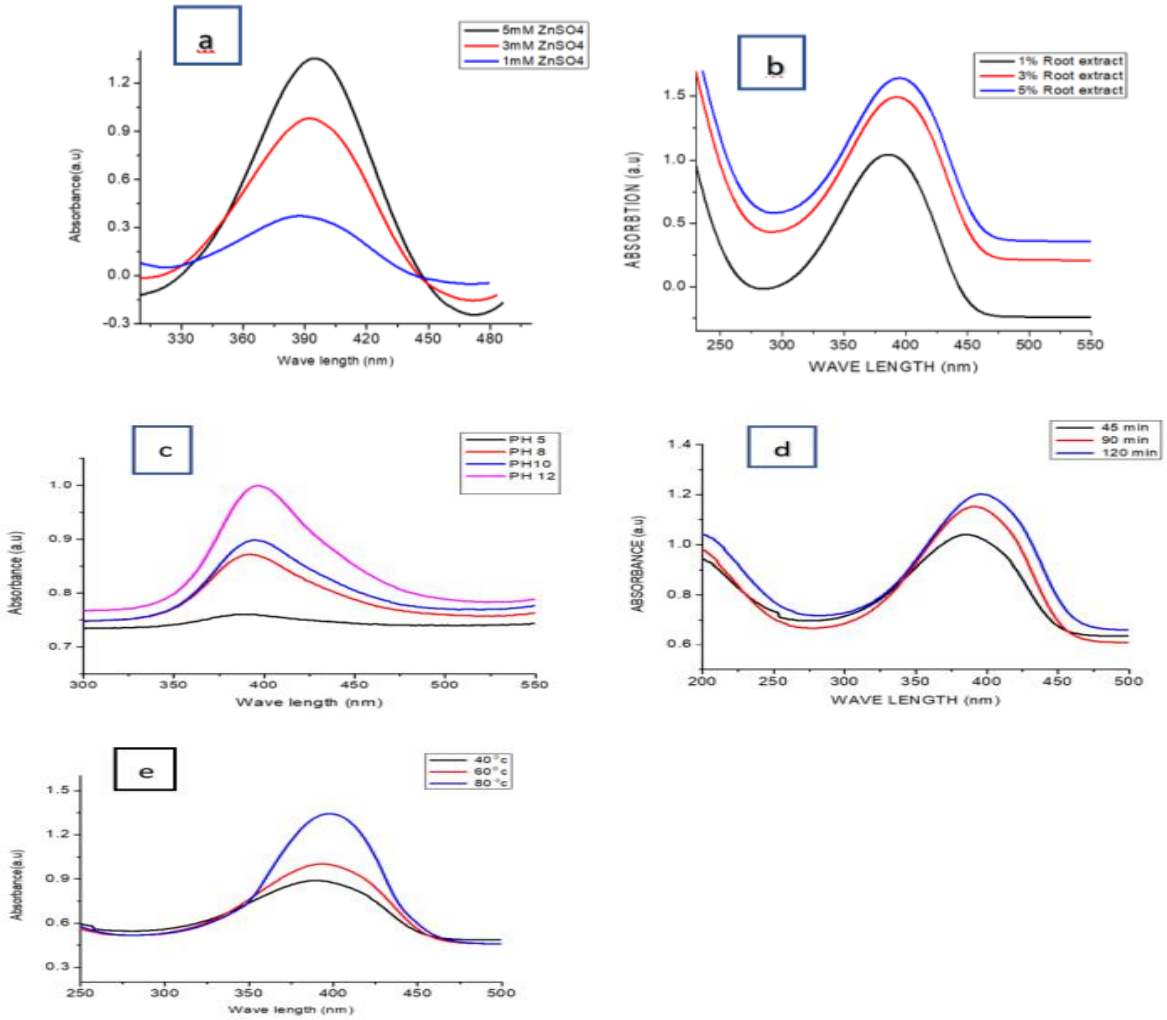


Fig 4. a) Preparation of ZnONPs using different ppm's of $ZnSO_4$ solutions, 4. b) Preparation of ZnONPs using different percentages of Mirabilli's Jalapa root extract, 4. c) Preparation of ZnONPs at different pH, 4.d) Preparation of ZnONPs at different reaction times, 4. e) Preparation of ZnONPs at different temperatures.

3. RESULTS AND DISCUSSION

(i) FT-IR analysis

The Fourier transform infrared (FT-IR) analysis of ZnONPs was done using Prestige 21 Shimadzu by the green synthesis which was required in the range of 400-4000 cm^{-1}. FT-IR spectroscopy helps to identify various Phyto-chemicals that are involved in the reduction as well as stabilization of the ZnONPsas shown in Fig (5).

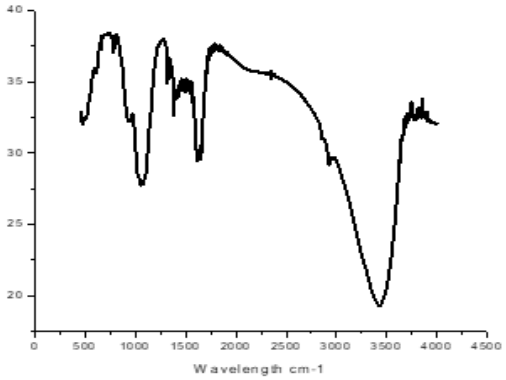
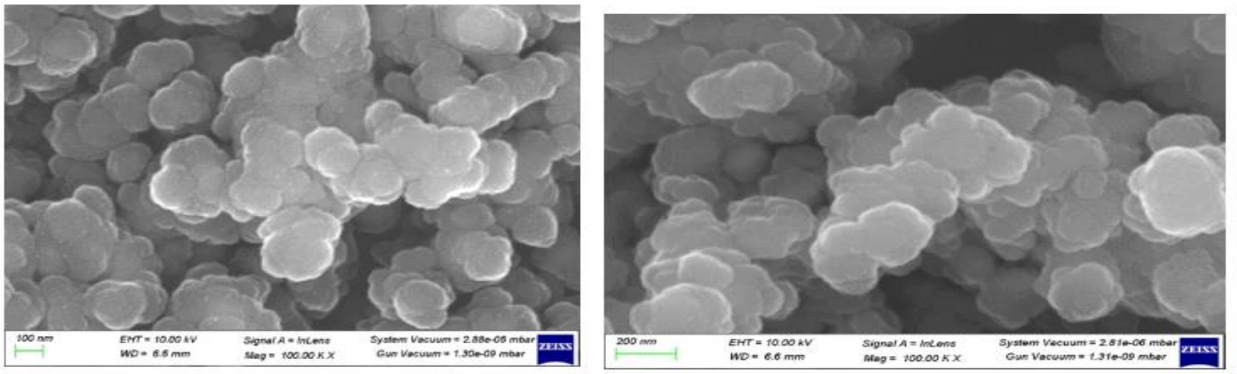


Fig 5: FT-IR spectrum of ZnONPs using M.J Root extract.

FTIR spectrum gives the sense of some functional groups. The peaks were observed at 1085cm^{-1}corresponds to strong C-O stretching having primary alcohols,1610 cm^{-1} corresponds to strong C=C stretching having α, β unsaturated ketones, 1710 cm^{-1} corresponding to C = O stretching having conjugated acids and 3546 cm^{-1} corresponding to strong broad O-H stretching having alcohols.

(ii). Sem & Edx Analysis

The SEM analysis which is also known as a Scanning electron microscope used to determine the surface morphology, size, and shape of ZnONPs. The ZnONPs morphology was assessed by using SEM (ZEISS GEMINI SEM) which was shown in Figure 6. The synthesized ZnONPs were found to be spherical in shape and have an average particle size of 73.02 nm has been shown in the histogram (7. a) EDX is used to determine the chemical composition present in ZnONP. The EDX exhibits a high signal for Zinc and Oxygen and this data enables the presence of nanoparticles in the form of Zinc Oxide. This composition of each element was composed of the analyte which was revealed from the EDX data and the peak obtained for zinc is 51.8 % at 1ev whereas for Oxygen it is 26.9% at 0.5ev [13-14]and been shown in fig (7.b).



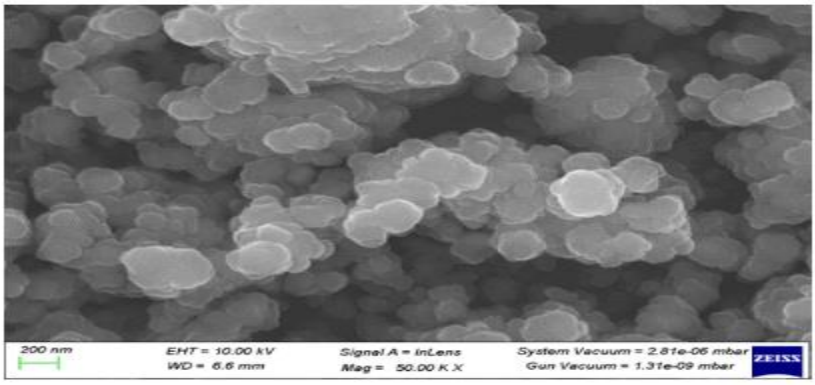


Fig 6. S.E.M images of Zinc oxide nanoparticles at different magnifications.

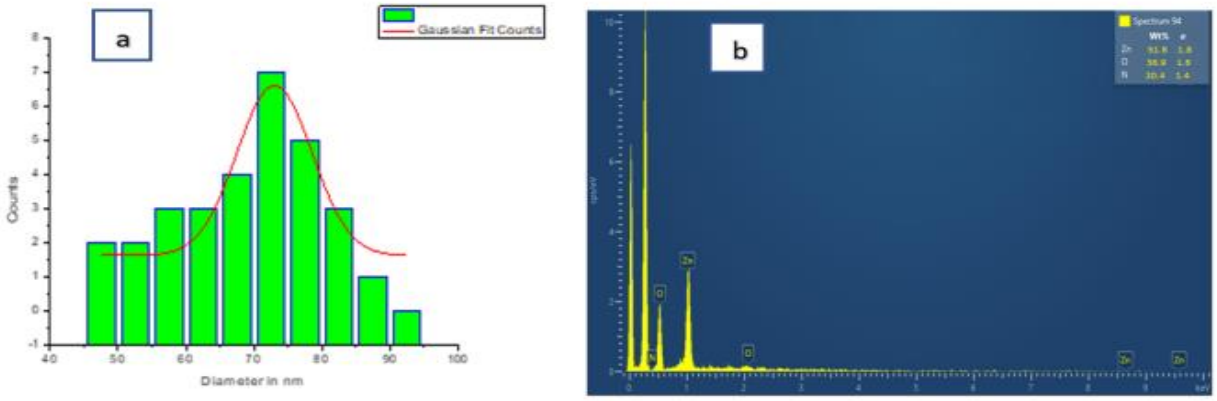


Fig 7. a) Gaussian distribution curve for S.E.M analysis. 7. b) E.D.X spectrum and its components present in bio-synthesis of ZnONPs.

(iii). Transmission electron microscopy (TEM)

ZnONPs were analyzed by TEM using FEI Tecnai G2 20 S-Twin. The TEM images were studied with the SAED pattern also known as selected area electron diffraction and images of synthesized ZnONPs are been given in the following Fig. The shape of obtained nanoparticles was spherical and was found to be crystalline. The particle size of ZnONPs was measured to be 50.12 nm. The histogram was shown in Fig (8.a)

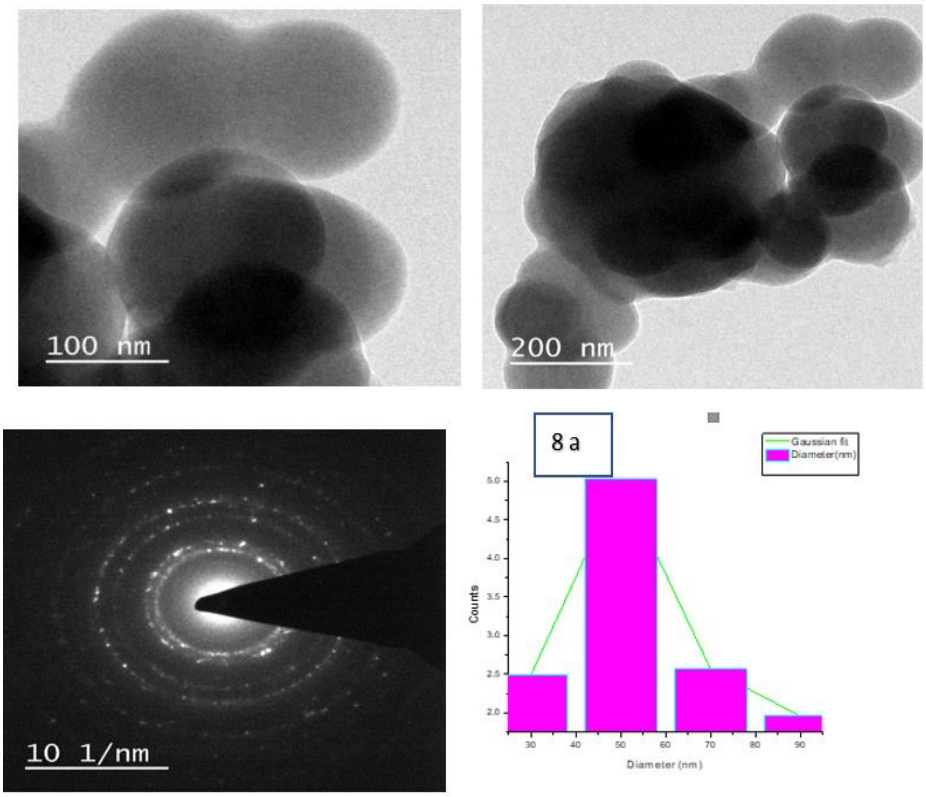


Fig. 8: Images of T.E.M analysis of various magnifications and S.A.E.D pattern 8. a) Histogram for particle distribution.

(iv). X-ray diffraction analysis:

X-ray diffraction (XRD) of ZnONPs has been analyzed using Bruker's AXS D8 at a wavelength of 1.5406 A˚. XRD analysis was performed in the 2θ range of 20 to 90 degrees at 40 KV and 40 mA with a divergence slit of 10 mm in 2 θ / θ in continuous scanning mode 38. The peak position with 2Ɵ values are 31.78, 47.9, 57.04, 63.3, and 66.68 are indexed as (1,0,0), (1,0,2), (1,1,0), (1,0,3), (2,0,0) planes using JCPDS card number (JCPDS) (NO 36-1451) [15].The shape of nanoparticles was found to be spherical.
The average particle size of the Zinc oxide nanoparticle was measured using Scherrer's formula.
$D = (K.\lambda / \beta \cos \theta)\ ^0A$
K is called the Scherrer's constant or shape factor = 0.9560
λ is the wavelength of the X-rays used=1.5406 ^{0}A
β is F.W.H.M also called Full width at half maximum
θ is Bragg's diffraction angle
D = Size of the particle / Crystallinity

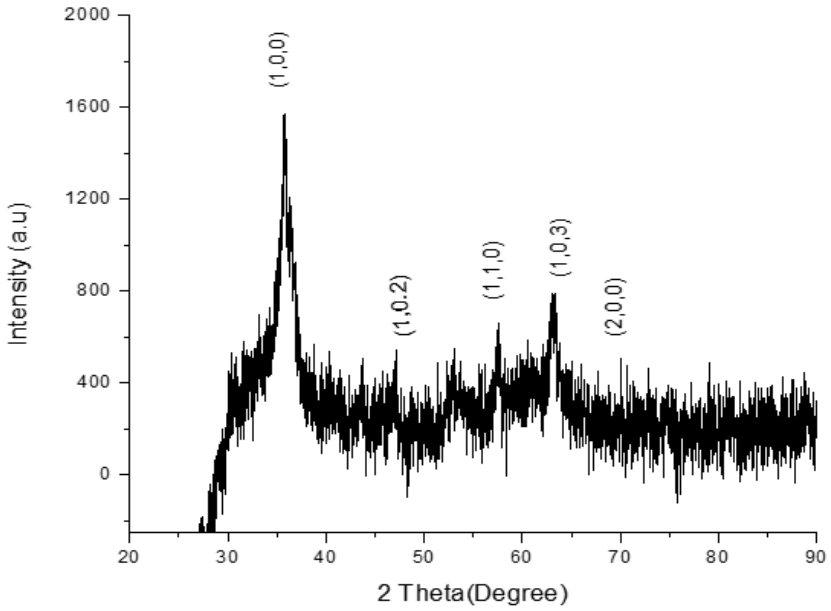


Fig 9: XRD of ZnONPs

4. APPLICATIONS

(i).Photocatalytic activity of ZnONPs using malachite green dye:

Dyes play a prominent role in different pharmaceuticals, paper,plastics, jute, cotton, leather, and textiles industries and these industries are situated near the water bodies as a large amount of water is used for manufacturing goods due to which the discharge gets mixed into water bodies and in agricultural fields and disturb the aquatic life, food chain through irrigation. Humans are attracted bycolors and a variety of dyes are used in textile industries which leads to a crucial role in depriving the environment. Dyes produce various types of waste in the form of solids, and liquids and some are hazardous as they may contain heavy metals which contaminate the living system. The exposure time of the dye and its concentration is high which leads to acute or adverse effects. Malachite green (MG) is used as an anti-fungal agent to cure protozoan infections, bactericidal efficacy, and parasiticide in agriculture and also in some industries it is found to be carcinogenic, mutagenic, and chromosomal. Hence M.G. dyes are not only toxic to flora and fauna but also to humans and they can cause severe damage to human organs such as kidneys and liver. Various methods were employedfor the removal of dyes they are photodegradation, oxidation, ion exchange, and adsorption methods the adsorption method is found to be much more effective and low-cost. Investigations were carried out for the efficient removal of dyes with zinc oxide nanoparticles. M.G. is highly light-resistant and a good oxidizing agent

The photocatalytic activity of ZnONPs with Malachite green dye (M.G) was done using a UV-Visible spectrophotometer. In this study, it was observed that as the Malachite Green dye's concentration was increased the removal time /contact time also increased. M.G which is an eluent is deprived in the presence of sunlight using biogenically synthesizedZnONPssynthesized using Mirabilis Jalapa root extract. Malachite green dye which is an organic dye and also a cationic dye. Different ppm'sof MG solutions such as 5ppm, 10ppm, and 15ppm solutions were prepared in 100 ml each in which 5ppm solution exhibited 92% degradation and the highest absorption peak was observed at 618nm.

Effect of contact time for removal of M.G

The efficiency of ZnONPs for the degradation of Malachite Green dye was increased with increasing contact time. To 5ppm MG solution about 3 mg of ZnONPs was added and kept under sunlight. The

complete degradation of MG dye was observed for 180 minutes which was shown in Fig (10. a) 180 minutes was found to be the optimum contact time for the complete removal of MG and the maximum absorption peak was observed at 618 nm. This indicates that as the contact time increases the efficiency for the removal of MG also increases [16-18].

Effect of concentration of MG

Initially, 3 mg of ZnONPs and 180 minutes of irradiation were kept constant. Various ppm's of MG solutions were prepared such as 5ppm, 10ppm, and 15ppm. Maximum degradation of 92% of MG dye was observed for 5ppm solution whereas for 10ppm and 15ppm solutions the degradation percentage was found to be 62% and 41%. The percentage removal of MG dye was calculated using the formula and was shown in Fig (8. d, 8. e, 8. f).

The plot of time Vs (C/C_0) vs time andtime Vs ln (C/C_0) vs time was shown in fig (8. b, 8. c,). The rate constant calculated the slope, intercept and R^2 were found to be -0.01115, -0,08477, 0.98187.

% Removal of MG = $(C_0 - C) / C_0] \times 100$

C_0 = Initial concentration of M.G dye before adding the catalyst and irradiating (mg L^{-1})

C = Concentration of M.G dye after time 't' and irradiation.

The kinetic study MG dye using ZnONPswas found to be pseudo-first ordered and the rate constant was given by the following equation.

$\ln (C/C_0) = -kt$

Effect of pH

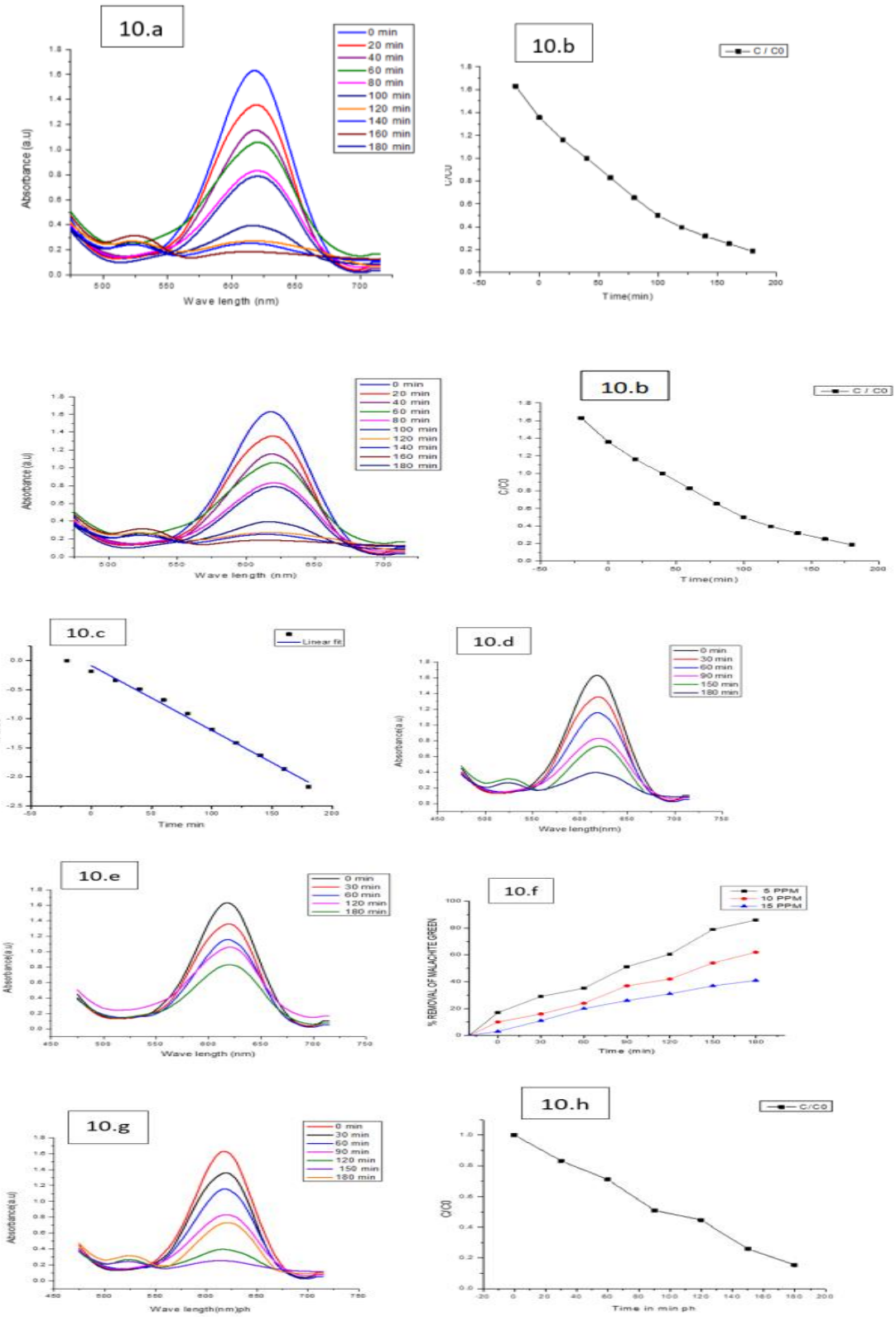
pH plays a major role in the removal of MG dye. Initially, MG dye has a pHin the range of 5 keeping 5ppm MG dye solution, 3 mg of ZnONPs, and contact time was kept constant. pH in the range of 4-9 was prepared by using 0.2 M HCl and 0.2 M NaOH. As the pHrange was increasing from 4 -9 the % removal of M.G was also increased [19]-[21]. pH 9 was found to be good for the removal of MG dye which was shown in fig (10. g). The removal of M.G dye was high in the basic medium at pH9. The percentage removal of M.G dye at various pHwas shown in Fig (10. h). The plot of (C/C_0) was shown in fig (10. h) and the plot of ln (C/C_0) was shown in fig (10. i) and the complete removal of MG using 10ppm and 15 ppm was shown in fig (10. j). The value of R^2 was found to be 0.98772.

Effect of photocatalyst dose

As the contact time was set to 180 minutes contact and 5ppm of MG solution and pH 9 constant. Different mg of ZnONPs dosage was taken in the range of 1-5 mg i.e.,1mg, 3mg, and 5mg. Despite using an excess catalyst, it is necessary to optimize the catalytic dosage for the removal of M.G dye[22]-[24]. Hence the amount of catalytic dosage was varied from 1mg to 5 mg. As the amount of photocatalyst increases the MG dye removal percentage also increases by 35% as shown in Fig (10. k). The plot of (C/C_0) was shown in Fig (10. l) and the plot of ln (C/C_0) was shown in Fig (10. m). The percentage removal of M.G dye at a different dosage of ZnONPs was shown in Fig (10. n). 5mg of ZnONP's found to be best and further increase showed light scattering and may decrease the consumption of light [23-24]. The R^2 value was found to be 0.99385.

CONCLUSION

ZnONPs played an important role in the removal of malachite green dye at 180 min contact time taking 5ppm M.G solution at pH 9 where the maximum percent of M.G dye was removed using 5mg of ZnONPs as a photocatalyst.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Time in min ph

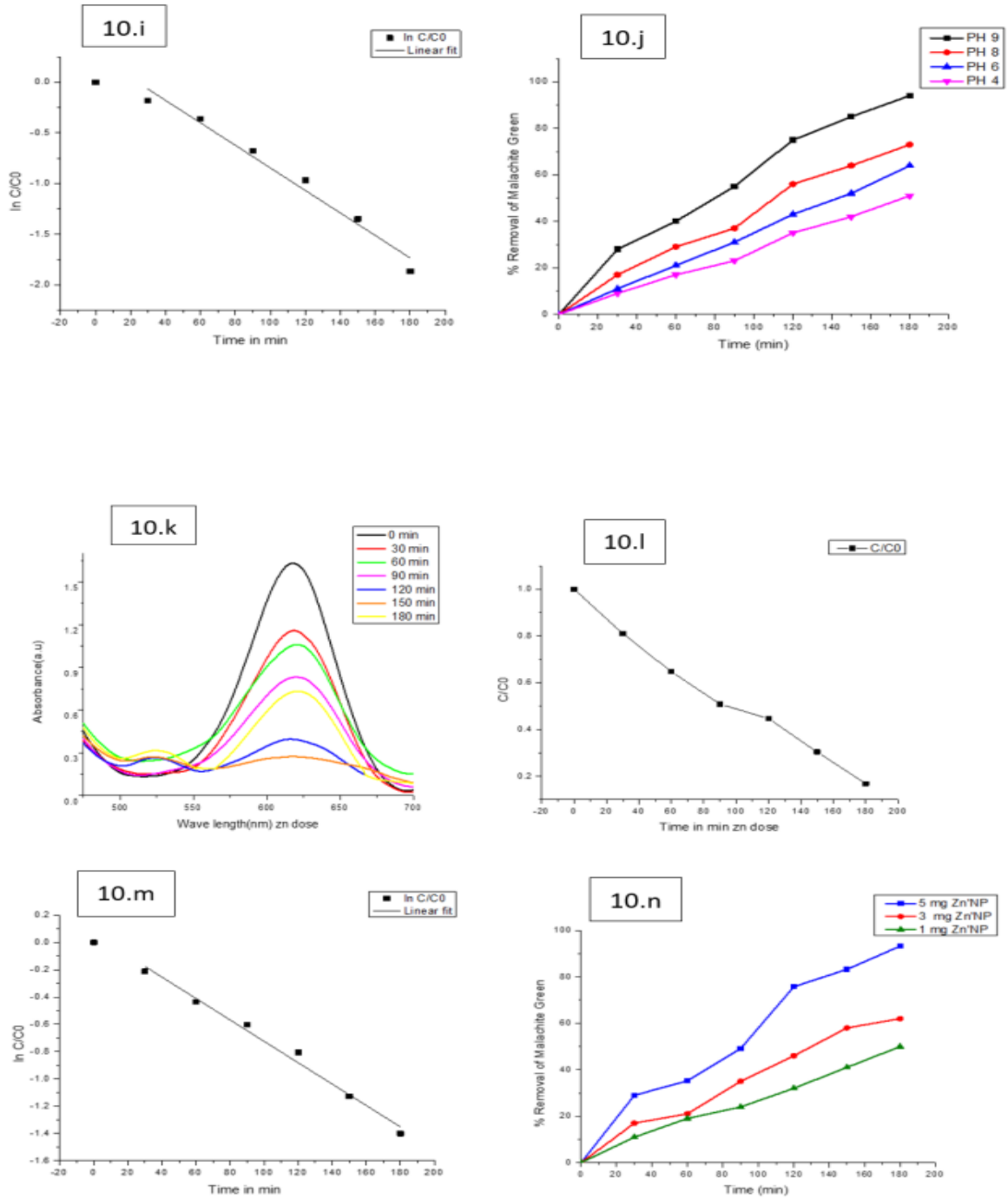


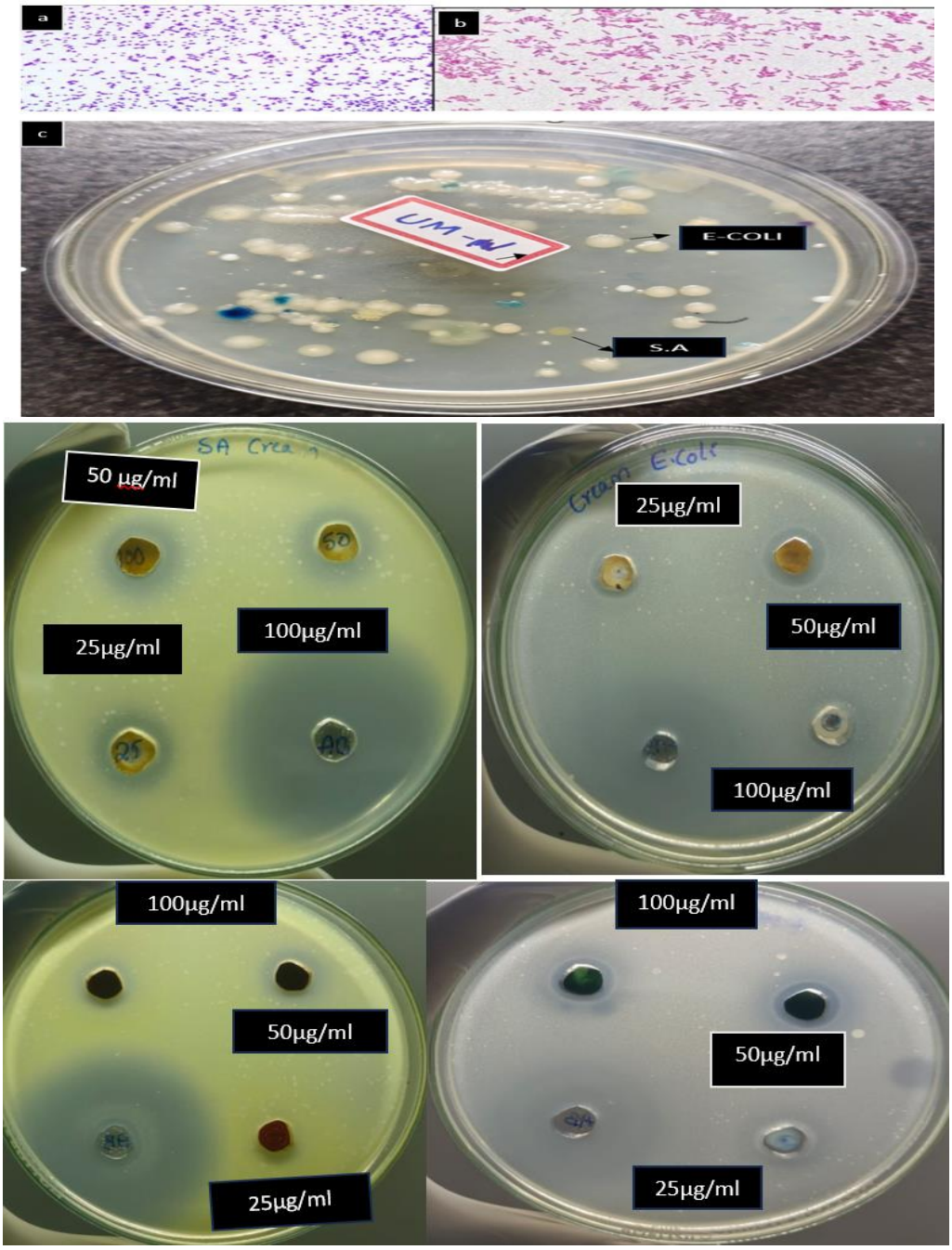
Fig 10. a). U.V- visible absorption spectra of M.G dye using 5ppm at 180 min contact time, 10. b). Time Vs C/C0 using 5ppm M.G, 10. c).Time Vs ln Ct/C0 with 5ppm M.G or first order rate kinetics of M.G 10.d).10ppm of M.G at 180 min contact time. 10. e). 15 ppm M.G at 180 min contact time. 10. f). % removal of M.G. with different ppm's. 10. g). Effect of pH 9. 10. h). Time Vs C/C0 with pH 9. 10. i). Time Vs ln Ct/C0 at pH 9 or first order rate kinetics at pH 9. 10. j). % removal of M.G at different pH. 10. k). 5mg of ZnONPs dosage for removal of M.G. 10. l). C/C0 using 5mg ZnONPs.

10.m). ln Ct/C0 using 5mg of ZnONPs or first order rate kinetics of catalytic dosage using 5mg ZnONPs .8. n). % removal of M.G using different catalytic dosages of ZnONPs.

5. Anti-Bacterial Activity Assessed From Infected Wound

Bacterial pathogens that feed on wound gets infected and causesthe chronic effect of delay. As the dosage of antibiotic increase for healing causes side effects due to their morbidity and mortality[25].The wound gets contaminated when the pathogenic microorganisms adhere and damage the tissues[26-28] Various types of bacteria are present on the infected wound and overwhelm the host immune system[29-30].ZnONPs as a vast dominance in the fields of medicine that are being utilized in drug delivery due to their small size and can inhibit the growth of pathogenic microorganisms. Wound infection was collected from the government King George Hospital, Visakhapatnam. staphylococcus aureus a gram-positive bacteria and E-Coli gram-negative bacteria were isolated, the E-coli was round in shape with greyish white colorand staphylococcus aureus was in a cluster withgolden yellow color ona petri dish. The microscopic image was shown in Fig (11 a,b).The lesion sample was collected with the help of sterile swabs and was streaked on a nutrient agar plate and incubated at 30^0c for 48 hours. Various bacteria were developed on nutrient agar plates and shown in Fig (11c). Staphylococcus aureus and E-coli were identified by the gram staining process and isolateswere used for further studies. The Kirby-Bauer method also called agar well diffusionwas employed, about 20ml of nutrient agar medium was poured into a Petri dish after autoclave at 120^0c and cooled for solidification. Four holes were done with a sterile borer to create a 6mm diameter in each plate and a standard antibiotic ciprofloxacin was used as reference. Various concentrations of ZnONPs were prepared with adequate solvent and added into the wells and kept for incubation for 12 hours at 37^0c. Using aHimedia antibiotic zone scale the inhibitory zone was measuredindiameter.

Name of the bacteria	Zone of inhibition			
	Concentration of ZnONPs 25 µg/mL	Concentration of ZnONPs 50 µg/mL	Concentration of ZnONPs 100µg/mL	Antibiotic Ciprofloxacin µg/mL
E-COLI	15	16	17	30
Mean ± S.D	15.33 ± 0.57	16.66± 0.57	17.66 ± 0.57	30
Staphylococcus aureus	12	14	17	35
Mean ± S.D	12.33 ± 0.57	13.66 ± 0.57	17.33±0.57	35



Antioxidant Activity

The free radical scavenging effect was carried out using DPPH, and a 0.1 mM methanolic DPPH solution was used. Various concentrations of ZnONPs were prepared by dissolving in methanol. To 1ml of DPPH solution 3ml of methanolic ZnONPs were mixed and allowed to stand in the dark at room temperature for halfan hour. The absorbance was measured using a UV-Visible spectrophotometer at 517nm. A reference sample of Ascorbic acid was used as a control and the absorbance was measured. The color of the solution was changed from violet to pale yellow. The free radical scavenging effect increases with the low absorbance value of the reaction mixture. The scavenging effect was calculated using the formula.

% inhibition= $\{(A_C-A_S)/A_C*100\}$

A_C=Absorbance of control (Ascorbic acid)
A_S=Absorbance of the sample
The Absorbance of DPPH gradually decreases with an increase in the concentration of ZnONPs.
Mean ± SD was calculated and shown in the table below

Sample Number	Concentration of ZnONPS (µg/mL)	% of DPPH scavenging activity mean ±SD
1	50	12.42333± 0.005774
2	100	25.73333± 0.005774
3	150	44.36333± 1.737824
4	200	59.16667± 0.011547
5	250	80.44333±0.005774

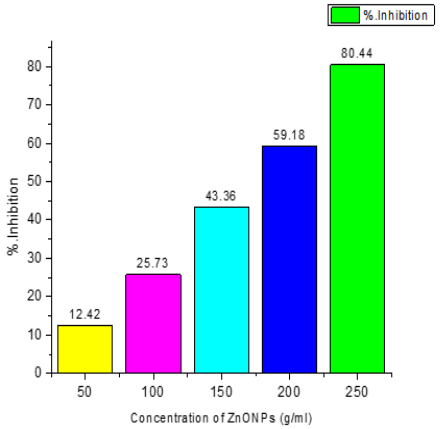
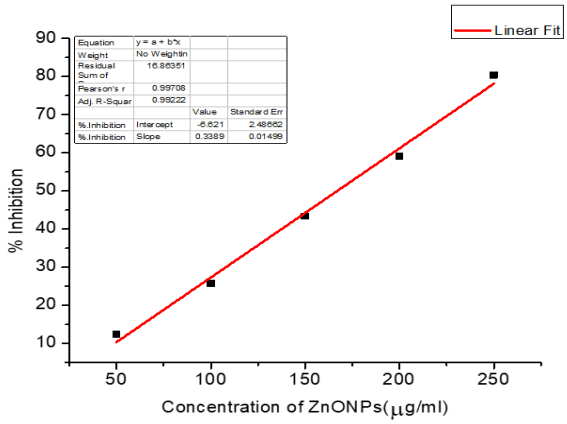


Fig 12. Antioxidant efficacy of ZnONPsagainst DPPH.



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