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Preparation and Study of Zinc Oxide Nanoparticles to Evaluate Their Antioxidant Activity

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Abstract

Zinc oxide nanoparticles were prepared by chemical and mechanical reaction, which is one of the methods that does not require high temperatures during preparation. The prepared particles were subjected to Smallness varies. Separate concentrations and periods to evaluate the antioxidant performance of ZnO nanoparticles. UV-vis spectrophotometry was used to track the DPPH scavenging activity, and ZnO nanoparticles showed strong antioxidant activity.

Keywords: ZnO NPs, Antioxidants, Cytotoxicity, chemo-mechanical method.

Introduction

The production and use of nanomaterials in the past ten years is a vital part because of their special properties. There are more uses for these nanomaterials, including microelectronics, semiconductors, sensors, cosmetics, and medical applications among others [1]. We must alter the surface of these materials at a nanoscale to create products with unique properties [2,3]. Human-generated nanoparticles are currently being manufactured in large numbers. Due to their high surface-to-volume ratio and strong interaction, nanomaterials have been found to have different toxicity profiles than larger particles. Direct or indirect interaction with these produced nanomaterials has unknown toxicological and environmental effects [4,5]. Evaluation of the antioxidant activity of nanomaterials is one of the most important basic research in pharmaceutical sciences, nanoscience, and technology. All vital systems depend heavily on

antioxidants to function properly. In biological systems, Free radicals are formed as a result of interactions between biomolecules and an oxygen molecule [6,7].

The deterioration of biomolecules is brought on by these free radicals. Food discoloration and nutritional quality decline are also caused by oxidation [8,9]. Consuming oxidized food consequences in the production of fat peroxides and low molecular weight chemicals, which can lead to catastrophic illnesses like hepatomegaly or the necrosis of epithelial cells. Antioxidants are very necessary in biological systems to get rid of these dangerous free radicals [10,11]. To date, much research has been done to stop these oxidation reactions and a wide range of natural and synthetic antioxidants have been researched to stop it. Fe3O4 and NiO nanoparticles exhibit strong antioxidant activity, according to Saikia et al. According to Banerjee et al. [12,13], Due to the large interaction between matter and the environment, has led to nanostructured materials having better functions in eliminating free radicals compared to their bulk counterparts. The high "surface-to-volume" ratio of nanostructures is responsible for this property. The "DPPH" scanning assay is believed to be one of the most common analysis methods for the antioxidant capacity of a substance. With this approach, we can measure the effectiveness of antioxidants at room temperature, Numerous natural and synthetic substances have been studied for their antioxidant properties by various researchers [14,15].

Hemolysis is the visible process in a mammalian system where red blood cells (RBC) are destroyed and hemoglobin is released into the surrounding fluid. Spectroscopic analysis can be used to distinguish this hemoglobin release. Significant hemolysis can result in pathological situations that are harmful. Therefore, the hemolytic characteristics of all biomedical items intended for intravenous administration should be evaluated [16].

Mohanan and colleagues manufactured zinc oxide nanoparticles and studied their impact on antioxidant defense mechanisms and cellular oxidative stress in mouse liver. They found that injection of zinc oxide nanoparticles into homogenized liver tissue resulted in a concentration-dependent decrease in cell viability and an increase in zinc oxide nanoparticle levels. enzymatic antioxidants [17, 18].

Wang and his colleagues combined daunorubicin, an anticancer drug, with different-sized zinc oxide nanoparticles to study the synergistic cytotoxic effect on leukemia cell lines. They found that leukemia cells were effectively suppressed through synergistic cytotoxicity [19].

In previous work, the antioxidant and antibacterial properties of copper nanoparticles were evaluated in which DPPH was used as a radical generator and CuO as a radical scavenger to determine the antioxidant activity [20,21]. Using these nanoparticles, a strong antibacterial response and free radical scavenging activity of up to 85% were obtained. CuO nanoparticles have a lethal effect, making them unusable in biological applications. This prompts researchers to search for a new class of non-toxic nanoparticles that can be used in biological applications. The extremely pure ZnO nanoparticles have been attempted to be created in this research using the thermal breakdown technique, and their antioxidant and cytotoxic properties have been tested. ZnO nanoparticles' antioxidant activity was evaluated using a free radical scavenging experiment with different nanoparticle concentrations and specific time intervals. The bio-compatibility of these ZnO nanoparticles was also tested in an in vitro hemolysis assay to confirm their prospective use in biomedicine [22].

Methodology and Materials

Zinc acetate dehydrate (Zn $(CH_3COO)_2$ $2H_2O$) of oxalic acid ($C_2H_2O_4$ $2H_2O$) were purchased from Merk, India. For washing, ethanol, and distilled water were used, all chemicals were used to prepare zinc nanoparticles (ZnO NPs), filter paper was used during the samples washing stage, and a pestle was used to grind the materials.

Preparation of ZnO nanoparticles

Zinc oxide ZnO was prepared using the chemical-mechanical reaction method. This method is easy and does not require a long preparation time .ZnO NPs were first obtained by manually mixing 35 m mol or 50.15 g of zinc acetate dehydrate ($Zn(CH_3COO)_2$ $2H_2O$) and 50 mmol 54.5 g of oxalic acid ($C_2H_2O_4$ $2H_2O$) were mixed. In a pestle or mortar at room temperature. Continue mixing and grinding until we get a homogeneous viscous mixture. We continue grinding until the removal of the smell of acetic acid (CH_3COOH). This indicates the completion of the reaction. This indicates the formation of hydrated zinc oxalate (ZnC_2O_4) left for 30 minutes. Then the mixture is washed off ethanol several times and then with distilled

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water. Then the mixture (ZnO NPs) was oven-dried at (60 °C) for 10 hours. Then the annealing stage for the dried NPs is carried out at (500 °C). For 1 hour in a temperature-controlled oven to obtain fine-crystalline pristine ZnO [23,24].

Characterization of zinc oxide particles Nanocrystalline

X-ray diffraction

The spatial properties of the crystal lattice were determined by measuring and analyzing the locations and intensities of Braak peaks, whereby the three-dimensional (3D) atomic arrangement in the bulk crystals was determined. This is the essence of the so-called "crystalline structure". [25].

Figure :1 shows the obtained theta angles corresponding to the JCPDS 36-1451 peaks at “2-theta angles” of “31.06◦, 33.83◦, 35.69◦, 47◦, 56◦, 62.3◦”, and “67.38◦ “ corresponding to their reflections. Of {(100), (002), (101), (102), (110), (103)}, and {(200)} crystals of zinc oxide, which is a typical nano-type crystal and may form the structure of pure zinc from zinc oxide particles Nanoparticles as evidenced by the presence of (100), (002) and (101) in XRD. Since there were no peaks due to unwanted material, this approach must have produced highly purified ZnO nanoparticles. This particular type of resonant appears when the incident light's wavelength is significantly greater than the particle's diameter. The strong top indicates that the size of each synthesized particle is the same. The average crystalline size was determined to be “46.49 nm” using Scherer's formula. [26-27].

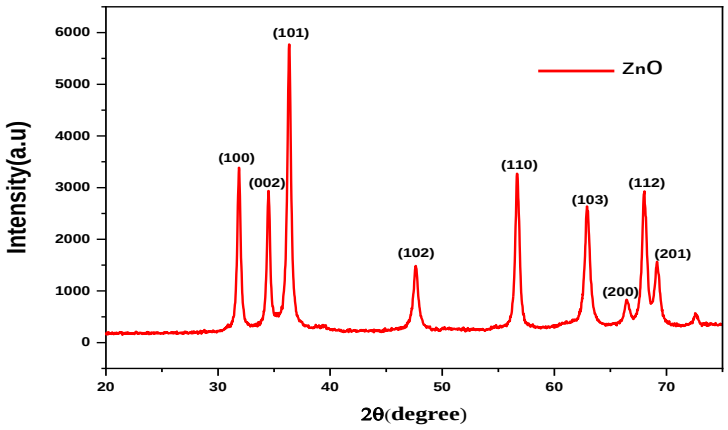

Figure 1: XRD spectra of ZnO nanoparticles.

Field emission scanning electron microscopy (FE-SEM) and EDX analysis of ZnO nanoparticles

Field emission scanning electron microscopy is one of the most important techniques for scanning the surfaces of materials with an accuracy of no more than a few nanometers, In Figure 2, the surface morphology of the prepared ZnO nanoparticle samples calcined at 500°C was observed by FE-SEM. The particle size of the prepared powders, in addition to the structural concentration, was determined using this device... In the "FESEM image", we note that the prepared nanoparticles have a diameter ranging between "40-50 nanometers" and are approximately sphere-shaped. The EDX spectrum gives the elemental composition of ZnO molecules and shows four strong peaks, which are identified as zinc and oxygen as in Figure 3 [28].

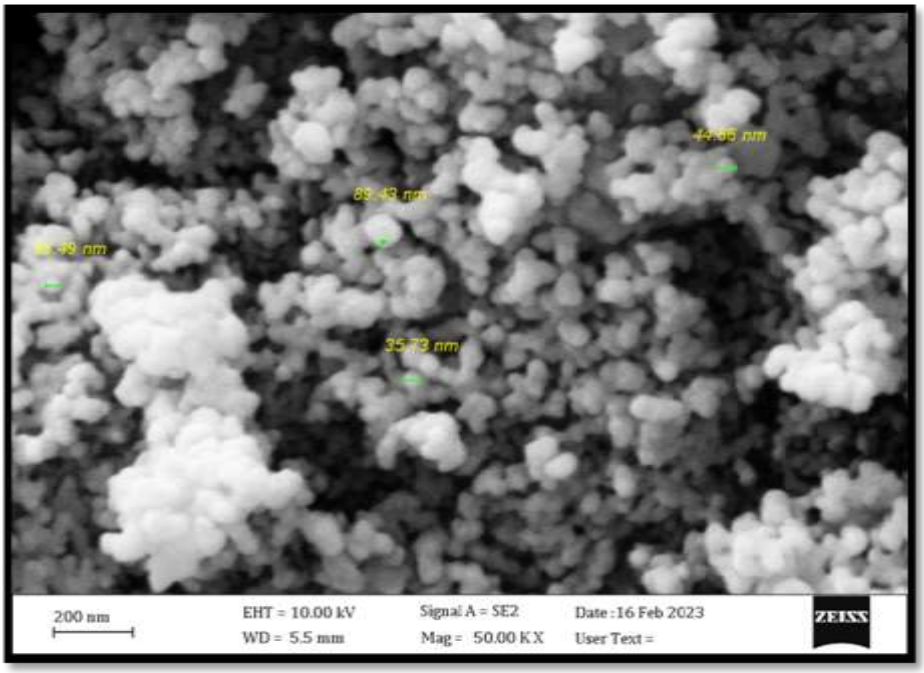

Figure 2: FESEM image of ZnO nanoparticles

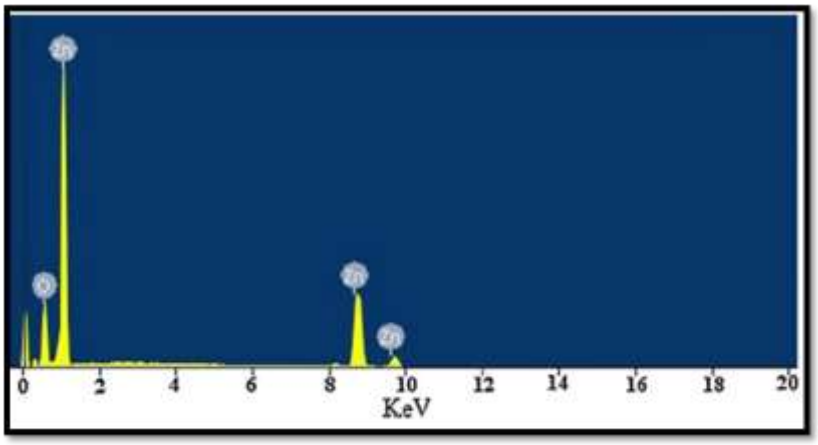

Figure 3: EDX analysis of ZnO nanoparticles

Optical properties (UV)

A Shimadzu device used a UV-2550 spectrophotometer at room temperature where UV absorption spectra were recorded. The "UV spectrum" of the manufactured "Zinc oxide nanoparticles" is shown In Figure 4. The shrill peak at "376 nm" The shrill peak indicates the regular size of the manufactured molecules due to the "Surface Plasmon" and absorption of "Zinc oxide nanoparticles". Surface plasmon absorption in metal oxide nanoparticles is due to the collective oscillation of electrons in free conduction rays excited by the event of Electromagnetic radiation. When the wavelength of the incident light exceeds a large diameter of the particles, this type of resonance is observed. [29].

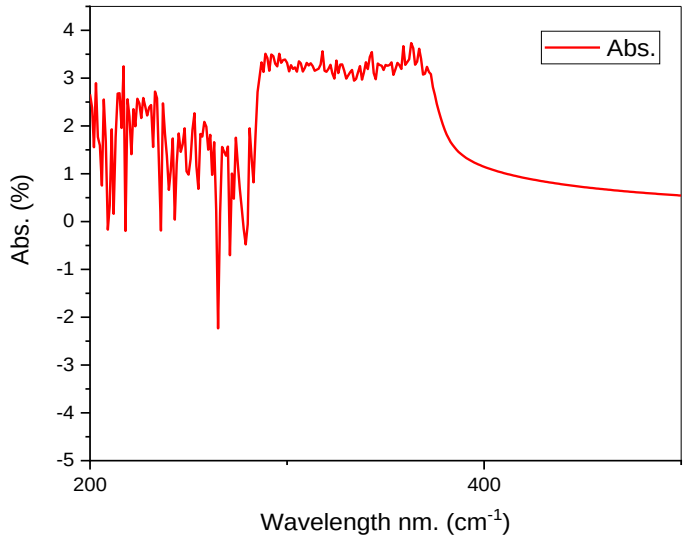

Figure 4: UV spectra of ZnO nanoparticles

Fourier transform infrared spectroscopy analysis (FTIR)

The infrared spectrum was studied in the range (400-4000 cm^{-1}) at room temperature, and the absorption spectra give information about the vibration phase of the network and the structural structure of the spinel, as well as how the ions are distributed between the octahedral and tetrahedral sites. The results of FTIR, or Fourier transform infrared, spectroscopy the high purity of ZnO-NPs obtained from the chemical-mechanical reaction approach is demonstrated in Figure 5, where it was detected in the 4000-280 cm−1 range. Possessing a wide absorption band at roughly 375 cm −1. The band at 375 cm-1 is associated with the hexagonal zinc oxide Raman active E2 mode." Many tiny absorption bands were found at 930, 1050, and 3400 cm−1. These absorption bands can be disregarded since they are probably connected to carbon dioxide ("CO") and hydrogen ions (H2O (OH) that are taken up from the atmosphere (air). [30].

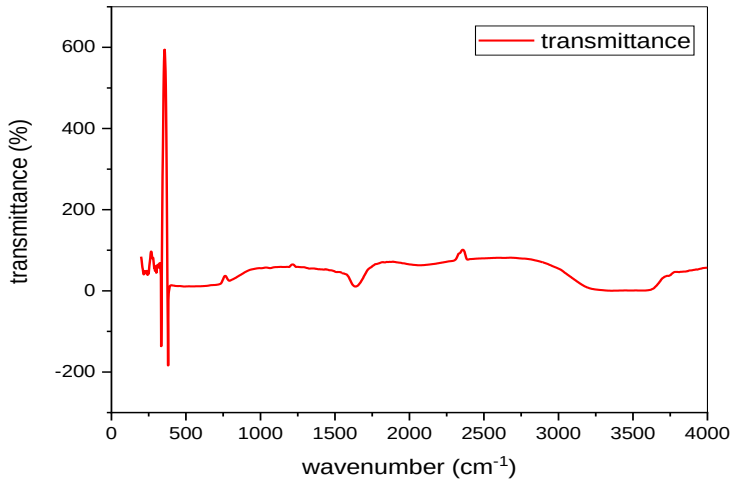

Figure 5: FTIR of ZnO nanoparticles

Antioxidant activity measurements

Anti-oxidant activity of zinc acetate and zinc oxide nanoparticles was determined using three methods. Preparation of Standard Ascorbic Acid (A.A) in Ethanol To prepare a standard solution of ascorbic acid, take 2 mg of ascorbic acid and dissolve it completely in 20 ml of ethanol (100 µg / ml) Preparation of working solution of Ascorbic Acid (A.A) in ethanol To prepare a working solution by diluting a standard solution of ascorbic acid. Preparation of zinc acetate solution and NPs. To prepare a solution of zinc acetate, 2 mg of the zinc acetate was dissolved in a small amount of DW (1000 ppm), and 2mg of NPs were dissolved in 20 mL of

ethanol. Preparation of working solution of zinc acetate and NPs To prepare a working solution (10-100)ppm by diluting the standard solution of zinc acetate(ZA) and NPs.

DPPH assay

1. According to Niraimathi et al., the antioxidant activity of the zinc acetate ZA and NPs was assessed using the DPPH assay.

2. Mix 1 ml of the DDPH solution with 1 ml of each concentration of the produced NPs and aqueous zinc acetate (10–100 mg in ethanol).

3. After that, these solutions were incubated for an hour at room temperature in the dark.

4. Next, the absorbance of the combination was measured at 517 nm.

5. A positive control was ascorbic acid.

6. The following formula demonstrated the sample's capacity to scavenge DPPH radicals:

$$\%\textbf{DPPH scavenging} = (\textbf{A of control} - \textbf{A of sample})/\textbf{A of control} \times \textbf{100}$$

A sample is the absorbance of the sample "DPPH solution and samples, and a control is the absorbance of the control samples DPPH solution without samples. [31].

Assay for total antioxidants

We assessed NPs and zinc acetate's overall antioxidant activity using the methodology outlined by Prieto et al.

1. Three milliliters of reagents and 0.3 milliliters of various concentrations of ZA and NPs, produced as previously mentioned, were combined in test tubes.
2. Reagent solution: 28 mM sodium phosphate, 0.6 M sulfuric acid, and 4 mM ammonium molybdate.
3. For ninety minutes, these solutions were incubated at 95 °C.
4. Following the tubes' cooling, the solution's absorbance at 695 nm was measured, and the antioxidant activity was computed using the formula below:

$$\%\textbf{Total antioxidant} = \textbf{A of control} - \textbf{A of}\frac{\textbf{sample}}{\textbf{A}}\textbf{of control} \times \textbf{100}$$

5. As a positive control, ascorbic acid was used [32].

Power assay

According to the methods described by Singh et al. The iron limit strength of “ZA and NPs” was evaluated

1. In the first phase, equal volumes of solutions containing "sodium phosphate (0.2 M, pH = 6.6) and "potassium hexacyanoferate (1% weight/volume) were combined with 1 mL of various concentrations (10–100 ppm) of ZA and NPs.
2. These eventual combinations were incubated at 50 °C for 20 minutes. 1 ml of Trichloroacetic acid TCA was added to stop the reaction.
3. Then, the mixtures were placed in a centrifuge at 10,000 rpm for 10 minutes.
4. For ten minutes, a final supernatant solution of 1.5 mL was combined with 1.5 mL of distilled water and 0.1 mL of a solution of 0.1% weight/volume ferric chloride (FeCl3).
5. Then, at 700 nm, the absorbance of the resultant materials was measured in comparison to an empty solution.
6. Each sample was tested in three repetitions, and the reaction mixture's higher absorbance values indicated a larger capacity for reduction. The mean was noted. [33]

$$\% \text{ Inhibition} = (A \text{ of control} - A \text{ of sample}) / A \text{ of control} \times 100$$

Antioxidant activit

The biological process known as oxidation is important because it helps living things produce energy. It can also lead to the production of free radicals, which are created when oxygen reacts with certain molecules, including carbohydrates, proteins, nucleic acids, and fats [34]. Numerous illnesses result from this, including "liver cancer, heart disease, vascular sclerosis, and kidney failure. Antioxidants are considered important factors, as they limit the harmful effects of these oxidative reactions. By doing this, free radicals may be prevented or eliminated permanently, boosting immunity and lowering illness. [35]. In this research, the effect of using nano-zinc oxide prepared mechanically as an antioxidant for antioxidant activity was studied Screening of zinc nanoparticles has been tested using three different methods. The antioxidant capacity was evaluated with high accuracy. The activity of eliminating free radicals is considered an important test in the measurement of antioxidant activity due to stability ease of use, Stability, and speed of testing. Then DPPH [36] was measured as shown in the results in Figure: 6. the results showed that the NPs had a higher DPPH activity of 86% compared to the NPs [37] at a concentration of 400 µg/ml. The results of this study are in line with earlier

research, and NPs exhibit an increase in activity with increasing concentrations, making them promising therapeutic agents for a variety of oxidative stress-related disorders. [38-39].

Table 1: Result of DPPH assay

		%Inhibition	
Conc. of solutions	A.A	NPs	ZA
10	35.7	12.8	6.6
25	40.8	18.4	8.2
50	55.2	24.1	10.5
75	65.6	33.8	14.6
100	80.9	52.9	18.6

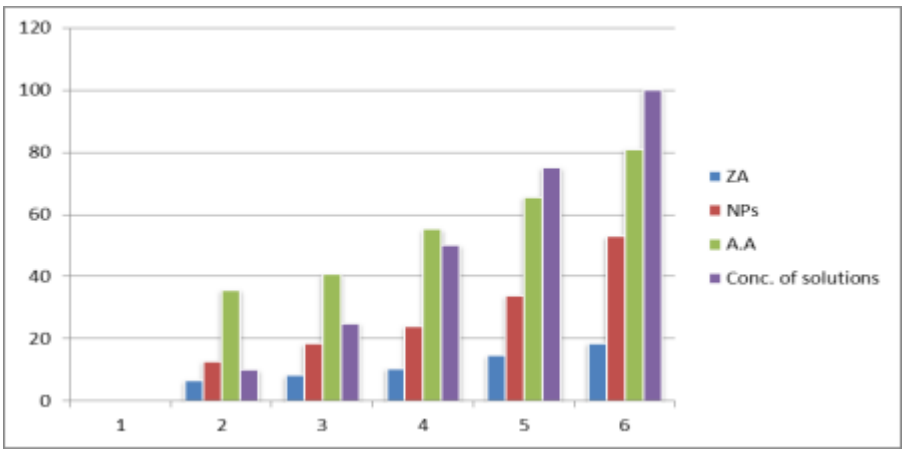

Figure 6: The figure shows the Result of DPPH assay

The reduction of Mo(VI) to Mo(V) by the antioxidant molecule and the creation of the green phosphate, Mo(V), constitute the basis of the total antioxidant phosphomolybdate technique. According to the results displayed in the figure, ZnNPs have higher antioxidant activity than AA or ZA.

Table 2: Result of total anti-oxidant assay

		% Inhibition	
Conc.	NPs	A.A	ZA
10	9.9	7.7	6.1
25	14.4	12.2	9.5
50	22.7	19.1	14.4
75	28.5	21.4	18.6
100	48.3	40.4	25.1

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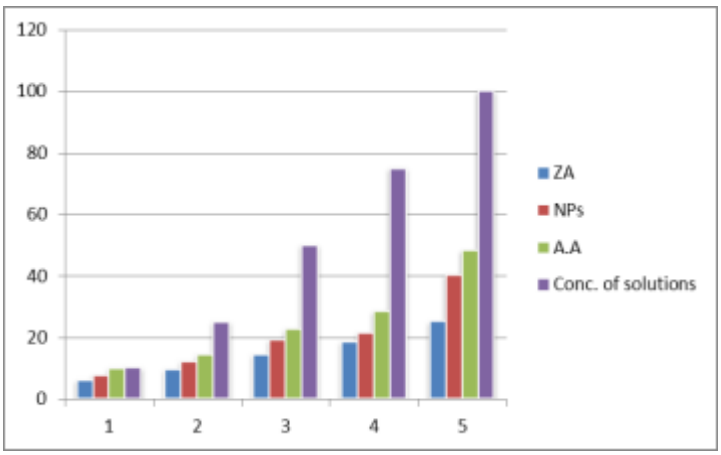

Figure 7: The figure shows the total anti-oxidant assay

When a reaction happens between samples and potassium ferricyanide (Fe3+) to generate potassium ferrocyanide (Fe2+), followed by a reaction with ferric chloride (FeCl3) to make a ferric-iron complex (44), the ability of a reductase to donate an electron is tested to determine the antioxidant capacity. NPs have higher antioxidant activity than A.A. and Z.A., as the figure illustrates. At 100 µg/mL, the highest and lowest optical densities of NPs about A.A and Z.A were found to be 1.22, 0.96, and 0.49, respectively.

Table 3: Result of reducing power assay

		% Inhibition	
Conc.	NPs	A.A	ZA
10	0.28	0.101	0.03
25	0.49	0.22	0.09
50	0.72	0.47	0.17
75	0.99	0.65	0.31
100	1.22	0.96	0.49

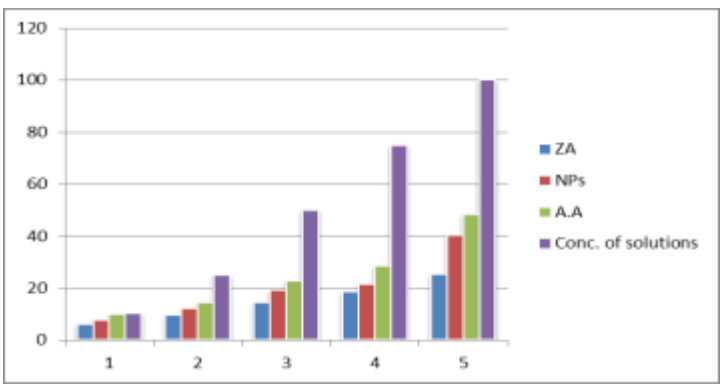

Figure 8: The figure shows the reducing power assay

The results of ZnO NPs using the DPPH evaluation method showed the presence of RSA significantly increased with an increase in the concentration of NPs. Where is the half-maximal inhibitory concentration (IC50) of NPs As in Figure 7. The activity at the concentrations mentioned above in Table (3) Ascorbic acid showed a positive control of above 75%. Suppression at 50 µg mL. Antioxidant potential. The suppression ability of the prepared nano-sized NPs can also be attributed to the Synthesis process [39]. The photosynthesis of ZnO NPs showed better Antioxidant properties when evaluated by DPPH [40].

Conclusion

In conclusion, we created ZnO nanoparticles through direct thermal breakdown. ZnO nanoparticles are quite simple to manufacture. The creation of pure wurtzite-structured ZnO nanoparticles is shown by the XRD pattern, and the average particle size is determined to be 46.49 nm. Studies using FESEM further back ZnO nanoparticle" production. The effectiveness of ZnO nanoparticles as antioxidants rises with concentration. The particles' ability to scavenge free radicals by up to 91% in 90 minutes provides strong evidence for antioxidant activity.

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