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GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING THE OUTER LAYER OF *Allium cepa* L. AND THE EVALUATION OF ITS ANTIOXIDANT CAPACITY

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ABSTRACT

The green synthesis approach of synthesizing nanoparticle is one that is rapid, less toxic to the environment, uses materials that are easily accessible and is more cost effective than the chemical synthesis and physical synthesis approaches. In this study, Zinc oxide nanoparticles were synthesized from the outer layer of *Allium cepa* L. using the green synthesis method. The synthesized nanoparticles were characterized through the use of UV-Vis spectrophotometry, Fourier Transform Infrared spectroscopy (FT-IR), Dynamic light scattering (DLS), Transmission Electron Microscope (TEM), Scanning Electron Microscopy (SEM) and X-ray diffraction (XRD) analyses. The antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity assay with Ascorbic acid as the reference standard for the assay. The size range of the synthesized nanoparticles was 28- 38.0 nm and they were found to be non-smooth but spherical in shape. There was a dose dependent increase in the antioxidant activity of the biosynthesized ZnONP and they were able to inhibit 50% of the DPPH free radical at a 377.242 µg/ml concentration showing good *in vitro* antioxidant activity. This is the first report on Zinc oxide nanoparticles was being synthesised using the outer layer of *Allium cepa* L. for use as an antioxidant agent.

KEYWORDS: Nanoparticles, Antioxidant, Onions, Spectroscopy, Zinc Oxide.

INTRODUCTION

Nanotechnology is a newly emerging aspect of science that operates at the nanoscale. Nanotechnology refers to the study, control, manipulation and creation of matter at the nanometer scale (sized from 1 to 100 nanometers). The size of one nanometer is one billionth of a meter (1nm = 10^{-9}m). The prefix "*Nanos*" stems from the ancient Greek for "dwarf". In science it means one billionth (10 to the minus 9) of something, this small size is particularly useful in drug delivery. Scientists have discovered that the chemical, physical and biological properties of materials are fundamentally different when observed at the nanolevel.

This has drawn increasing interest in the synthesis of metal and metal oxide nanoparticles especially Zinc oxide nanoparticles. ZnONP have a wide bandgap semiconductor which has an energy gap of 3.37 eV at room temperature[1,2] which is why it is able to absorb ultraviolet light and also remain transparent to visible light this attribute is very useful in its application in light emitting devices (LED), solar cells as conducting film. This nanoparticle has also been used in the field of electronics, medicine and as a catalyst. In recent times, the interest in ZnONPs has increased due to its unique antimicrobial, antifungal, antioxidant, photochemical and catalytic property.[3] It has been described by the U.S. Food and Drug Administration (F.D.A.) as one of the Generally Recognized As Safe (GRAS) metal oxide nanoparticle[4] and its application can be seen in several fields . Zinc oxide nanoparticles can be synthesized through the physical, chemical or biological method, although the first two methods are associated with higher costs, production of toxic chemicals, larger spaces required for the machines used and higher energy demand, while the biological method is an alternative technique that uses natural sources such as plants or microorganisms. Many studies have reported on the synthesis of ZnONP using various plants, the phytochemicals present in the plant act as reducing agents and encapsulate the nanoparticles[5] which confer different biological activity.[6]

Oxidative free radicals are atoms that contain one or more unpaired electrons; they are produced endogenously and exogenously ie; in the body through normal biological processes or from exposure to pollutants, radiation or cigarette smoke respectively.[7] They become particularly harmful during oxidative stress which is when there are elevated levels of reactive oxygen species and reactive nitrogen species (ROS/ RNS, which are types of free radicals) more than the biological systems ability to eliminate them and oxidative stress can cause damage to cells, tissues and organs.[8] Oxidative stress has been implicated in the development of several diseases such as metabolic or chronic disorders or cancers[9,10] such as Parkinson's disease, sickle cell disease, Alzheimer's disease, vertigo, autism, myocardial infarction, atherosclerosis, depression, infection, heart failure, chronic fatigue syndrome, cancer, and fragile X syndrome[11,12] hence, the need for antioxidants. Antioxidants are substances that are capable of preventing or delaying oxidation at low concentration, oxidative stress can be treated and prevented through the use of antioxidants.

Allium cepa L. (onion) is an herbaceous biennial plant grown for its edible bulb. Its common names in Nigeria include Yabasi (Igbo), Alubosa (Yoruba), Albasa (Hausa). It is the most cultivated and consumed vegetable in the world, widely used in cooking to add flavour to foods. The outer layer which is regarded as waste contains more flavonoids and polyphenols[13] especially quercetin which is the most abundant polyphenol. Onions vary in colour from white, yellow, red, pink or brown. For this research the red onion cultivar that has a purplish-red skin is used. Research show that coloured varieties such as yellow or red onions have greater antioxidant activity than white onion varieties which can be attributed to its higher flavonoid content[14] with quercetin and kaempferol being the most common flavonoids present in onion extracts.[15,16] *Allium cepa* L. contains many secondary metabolites that contribute to its biological activity.[17,18,19] for example it is an exceptionally antioxidant[20] and possesses antiviral, antifungal, antibacterial[21], antithrombotic, anti-inflammatory.[22,23,24,25,26], antidiabetic[27], antihyperlipidemic[28,29], anticancer properties [etc.

MATERIALS AND METHODS

Chemicals

Analytical grade ascorbic acid, Ethanol, zinc nitrate and sodium hydroxide were obtained from Onitsha, Nigeria; DPPH was obtained from Merck, Germany. All reagents and chemicals were used as received with no further purification.

Plant materials

The *Allium cepa* L. (Onion) bulb was collected from Elele, Rivers state, in the southern part of Nigeria, in the month of November 2022. The onion bulbs were authenticated and identified at the Department of pharmacognosy, Madonna University, Elele, Nigeria.

Allium cepa L. extract preparation

The outer layer of the onion bulb was peeled off, washed with tap water and boiled in the distilled water for 45 min at 100 ºC. The plant extract was filtered into a beaker to remove insoluble portion.

Green synthesis of ZnONPs

A solution of zinc nitrate was prepared by weighing a 20 g quantity of zinc nitrate and dissolving it with a 150 ml volume of distilled water in a 500 ml beaker. A 200 ml of the plant extract was mixed with 150 ml of the zinc nitrate solution and subsequently treated with 1.0 M sodium hydroxide (10 ml) and this produced a light brown mixture. The reaction was incubated at a temperature of 27^{o}C, in the dark for 6 hours in order to avoid photochemical activation of the zinc nitrate. A precipitate of yellowish zinc oxide nanoparticles (ZnONP) could be seen to have formed at the bottom of the beaker, the solution of zinc nitrate in water provided the ions that initiated the reaction. The mixture was left in an oven to dry overnight until a yellow paste was obtained; this paste was the further dried completely till a pale yellow powder was obtained which was then packed in to an airtight container for use.

Characterization

UV-vis spectroscopy (Perkin Elmer Lambda 35 UV-Vis spectrophotometer, Germany.) at a wavelength ranging from 200-800 nm was used to detect characteristic peaks and confirmed the synthesis of the prepared zinc oxide nanoparticles. The exact phytochemical responsible for reducing and encapsulating the ZnONPs were confirmed using Fourier Transform Infra-Red spectroscopy (Shimadzu 8400S, Japan) that identifies the functional groups present. The surface morphology of the prepared ZnONPs was studied using Scanning Electron Microscopy (SEM) on a TESCAN VEGA LMU, Germany as well as Transmission Electron Microscope (TEM) on a ZEISS LIBRA 120 KV- UK, at different magnifications. The X-ray diffraction (XRD) pattern of the biosynthesized sample of Zinc Oxide nanoparticles was recorded by a Bruker d8 Advanced X-ray diffractometer, using Cu Kα radiation (1.5406 Ä) at a voltage of 40 Kv, 2 ϴ/ϴ scanning mode. Dynamic light scattering (DLS) and Zeta Potential of synthesized nanoparticles were analysed to know the average size and stability of particles (DLS-Nano2S model, UK).

Antioxidant activity

The antioxidant activity of Zinc oxide nanoparticles synthesized from the outer layer of *Allium cepa* L. bulb was determined based on the radical-scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical using the method described by Nithiya and Udayakumar (2016) with some modifications.[30] A 100µg/mL concentration stock solution of the ethanol extract of the ZnONPs synthesized and the standard (ascorbic acid) was prepared by dissolving 10mg of each sample in 100mL of ethanol, from which 6 concentrations of 3.125, 6.25, 12.5,25, 50 and 100µg/ml were obtained by serial dilution in different test tubes. A 0.04 mg/mL concentration of DPPH was freshly prepared in ethanol. 1ml of ethanol and 1 ml of the freshly prepared DPPH solution was added to 0.5ml of each concentration of ZnONP and the same was done for ascorbic acid. 1ml of DPPH and ethanol was used as the blank and kept in the dark for 30 minutes. After which the absorbance of each concentration at 517nm was measured using a UV spectrophotometer. Then the percentage inhibition was calculated using the equation below:

DPPH scavenging activity (%) = [Ab – *As*/*A*b] × 100, (1)

Where; *A*b = the absorbance of blank and *As* = absorbance of scavenging sample. A graph of percentage inhibition(%) against concentration (µm/ml) was plotted and the IC 50 values that denote the concentration of sample required to scavenge/ inhibit 50% of DPPH free radical was obtained from the calibration curve.

Statistical analysis

Image processors such as Image J software, generally used to analyses of sub-micron images and other tools including the Crystallographic toolbox (CrysTBox) were used to analyse the biosynthesized zinc oxide nanoparticles.

The antioxidant activity assay was carried out in triplicates; linear regression analysis was used to calculate the IC50 value using Microsoft Office Excel.

RESULT AND DISCUSSION

UV-vis spectroscopy

The formation of yellowish precipitate at the bottom of the beaker after the addition of the plant extract to the solution of zinc nitrate is the preliminary indicator of the successful synthesis of ZnONP. The reaction between colourless zinc nitrate solution and aqueous extract of the outer layer of *Allium cepa* L. produced a colour change from to light brown. Pale yellow ZnONPs was obtained after drying; it was then kept in an airtight container. The phytochemicals such as alkaloids, phenols, flavonoids present in onions[31,32] contain anionic radicals that can reduce zinc salts to ZnONPs, especially quercetin (a polyphenol) which further aggregates to ZnONPs, thereby providing dispersion capacity in aqueous medium. Sodium hydroxide (NaOH) was used to increase the alkalinity of the mixture, thus increasing the OH-. Larger amount of OH- causes a significant attraction between the OH- and the Zn + which lead to increased crystallization and production of ZnONPs.[33]

Uv spectroscopy can be used to predict the shape and the size of the bio synthesized nanoparticles. The UV-vis spectra obtained showed absorption peak maxima at 359 nm and this is a characteristic signature demonstrating the presence of ZnONPs (Figure 1) and the shape was predicted to be spherical According to the with Mei's theory which states: The shape of the synthesized nanoparticle is spherical if a single sharp absorbance peak was observed in the UV-vis spectrum.[34] Similar results were obtained from ZnONP synthesized from *Plectranthus amboinicus* leaf extract and ZnONP synthesized from *Mangifera indica.*[35]

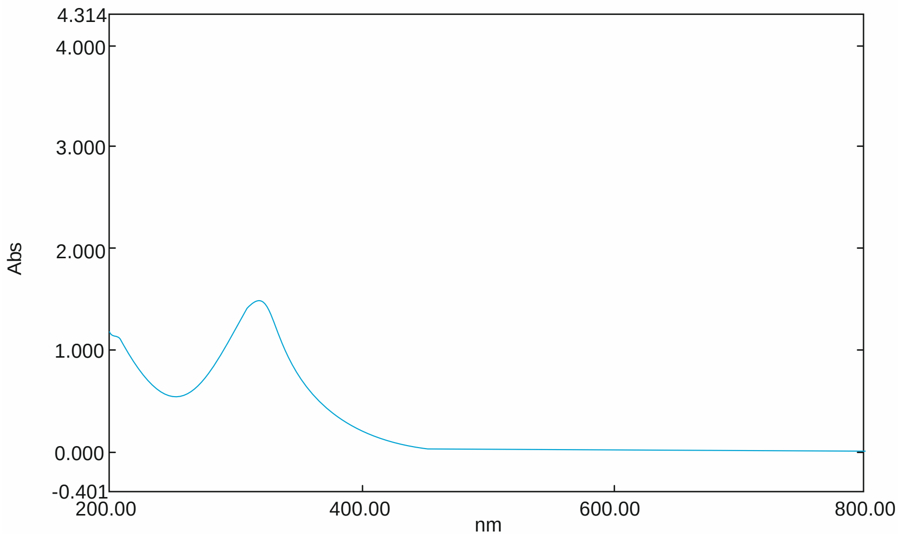


Fig. 1: UV-vis spectroscopic analysis of synthesized Zinc Oxide NPs.

FT-IR analysis

The FT-IR analysis was performed to examine the surface adsorption of the functional groups present at a spectral range of 4000-500 cm^{-1}.an observation of the spectra data (figure 2) reveals that the main absorption peaks were detected in the range of lower wavenumbers, the biosynthesized nanoparticles exhibit broad absorption peaks at 3313 cm^{-1} this corresponds to O-H bond, the peaks at 1620 – 1652 cm-1 represent the C=O stretching vibrations for amides, the peaks observed 1389 cm-1 and 1068 cm^{-1} represent the stretching vibrations of amide I and II and a characteristic peak of Zn-o at 620

cm^{-1}.[36] The FT-IR analysis depict the presence of polyphenols and primary amines which are functional groups identified in the phytochemicals present in onions that play a role in capping of phytochemicals on the surface of nanoparticles.

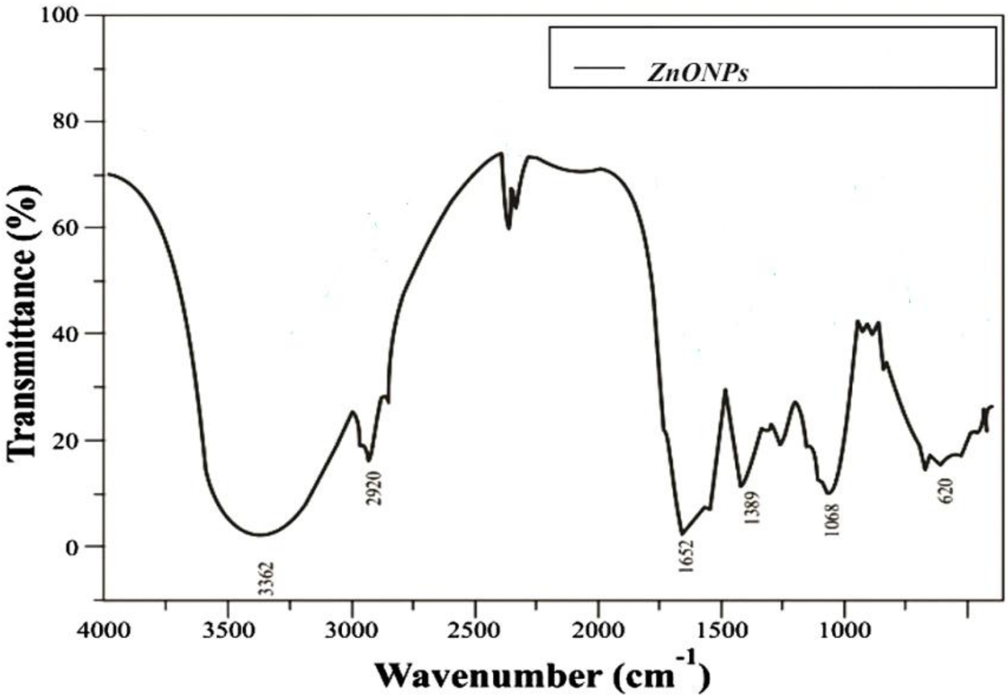


Figure 2: FT-IR of the synthesized ZnONPs.

Dynamic light scattering (DLS) Particle size distribution

Figure 3 depicts the DLS image of the biosynthesized nanoparticles, the nanoparticles were uniform in size ranging from 15-45 nm, and the mean particle size distribution of this nanoparticle is 28.21±6.369 which is similar to the XRD report (28.24nm). the zeta potential which is an estimate of the surface load was found to be 2.61 mV and The synthesized nanoparticles are moderately dispersed in the medium and are positively charged.

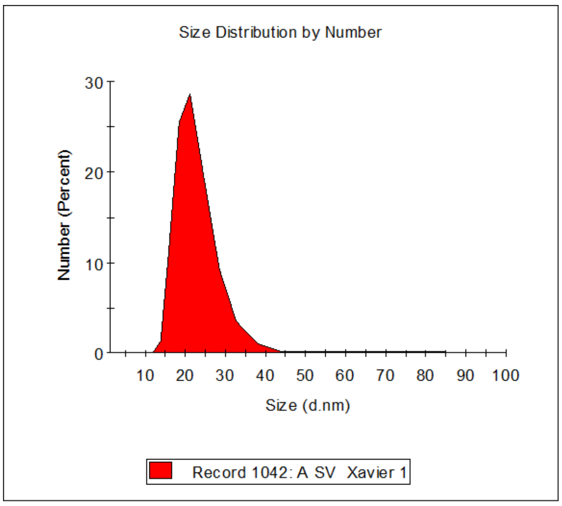


Figure 3.4: Dynamic light scattering (DLS) Particle size distribution of synthesized ZnONP.

XRD analysis

Data was obtained for the 2 ϴ (10° – 100°) in a step proceeding of 0.0204 degree. At 2 ϴ values of 6 peaks (18.203°, 20.937°, 23.671°, 30.108°, 34.608°, and 40.204°) corresponding to (020), (022), (022), (111), (120), and (200) integer on the 'hkl' planes respectively. This result correlates with data from International Centre for Diffraction Data (ICDD). Using Debby-Scherrer equation:

$$D = \frac{K\lambda}{_\beta \, Cos \, \theta}$$

(where D is the thickness of the nanocrystal, K is a constant; λ is the Bragg`s angle 2ϴ).The average particle size of the ZnONPs was calculated to be 8.24 nm at an operational Optimum Bragg reflection obtained 2 ϴ of 18.402°. This result is similar to that of the DLS – Zeta sizer which gave 28.21±6.369 average particle size. Unassigned crystalline peaks (42.8, 48.2 and 51°) are also recorded though the presence of crystallized phytochemicals on the surface of the Zinc oxide can be responsible for this.

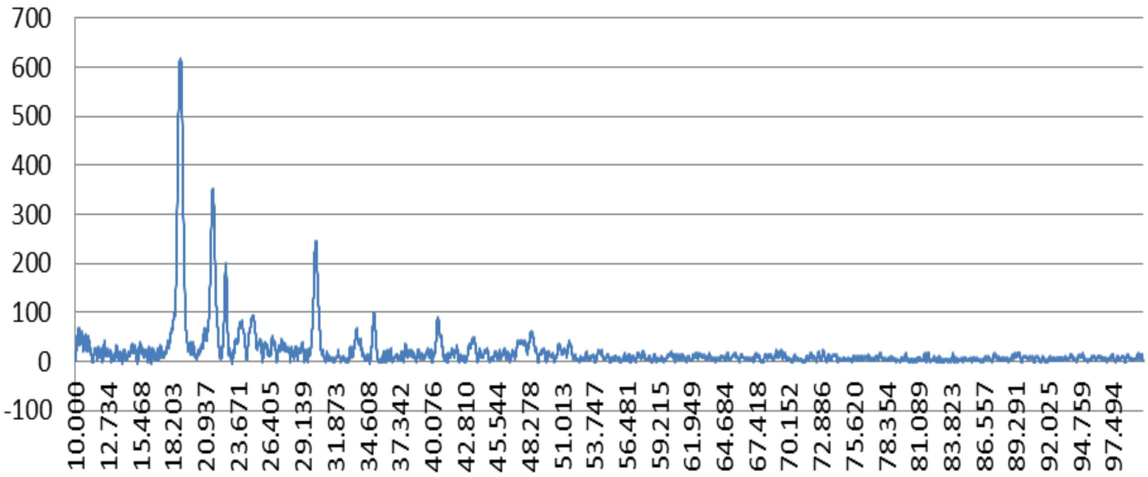
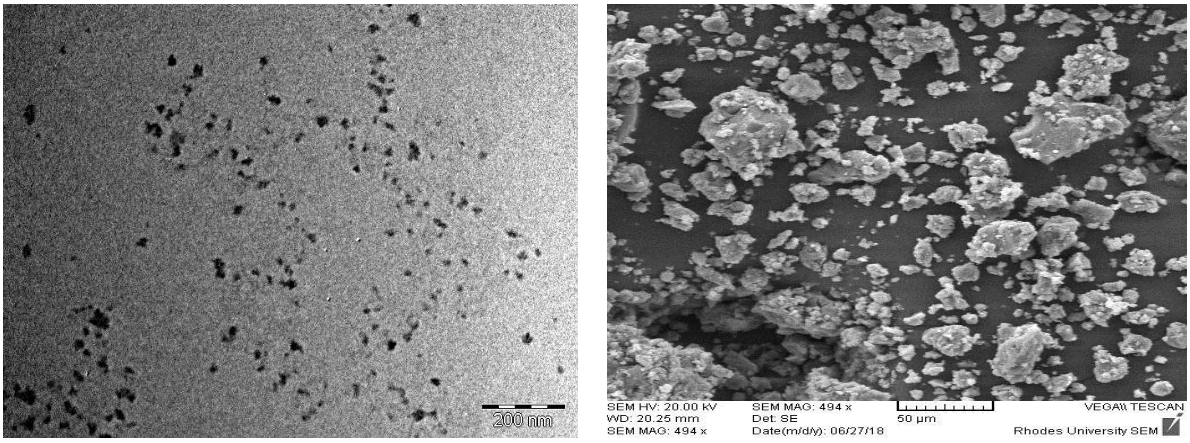


Fig. 2: XRD analysis of synthesized ZnO NPs.

TEM and SEM analysis

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) image of the biosynthesized zinc oxide nanoparticles is represented below (fig 4) and it reveals that the nanoparticles ate spherical in shape, discreet, well dispersed with size range 34 – 40 nm and average particle size of 38.0±2 nm calculated using the ImageJ SOFTWARE. Scanning electron microscopy marked the surface morphology of the synthesized nanoparticle to be spherical in shape with few non spherical crystalline structures.



(a) **(b)**

Fig. 3: *a* TEM image and *b* SEM image of biosynthesized ZnONPs.

Antioxidant Activity Assay Results

The antioxidant activity test for the biosynthesized ZnONPs was performed using the DPPH free radical scavenging assay; this is a widely used preliminary test for the radical scavenging activity of a compound. DPPH is a stable free radical that changes colour from violet to yellow when it is reduced by a electron donation of the oxygen atom in the ZnO to the nitrogen atom of DPPH that has odd electron. The test showed a comparative antioxidant activity between ascorbic acid and the biosynthesized nanoparticles and revealed that at 517nm the absorbance of DPPH decreases as the concentration of ascorbic acid and ZnONPs increases. The % inhibition of DPPH free radical by ascorbic acid ranged from 11.24% at 3.125 µg/ml to 88.868% at 100µg/ml while the % inhibition of DPPH free radical by ZnONPs ranged from 12.8655% at 3.125 µg/ml to 23.682% at 100µg/ml meaning that all the concentrations of the ZnONPs used have the ability to scavenge free radicals this is in line with previous studies supporting the antioxidant activity of ZnONPs the IC_{50} values for both the ascorbic acid and the ZnONPs were obtained using the simple linear regression technique and recorded.

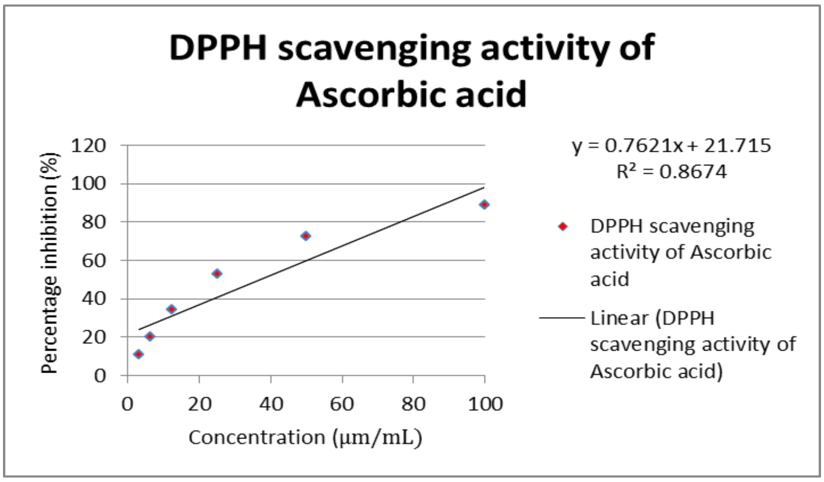


Figure 3.6: DPPH scavenging activity of Ascorbic acid.

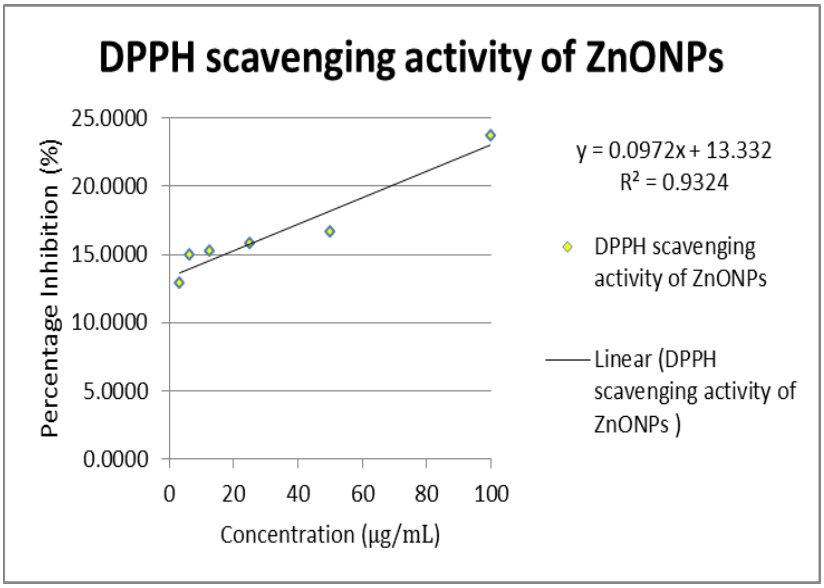


Figure 3.7: DPPH scavenging activity of zinc oxide nanoparticles (ZnONPs).

CONCLUSSION

In this present study, zinc oxide nanoparticles were successfully synthesized from the aqueous extract of the outer layer of *Allium cepa* L. The biosynthesized ZnONPs exhibited good antioxidant activity, although its IC_{50} was considerably higher than that of the standard, the results for the antioxidant activity of ZnONPs is very promising and can definitely be applied in the pharmaceutical, food and biomedical industry as a natural substitution for the synthetic antioxidants currently in use. The green synthesis method used also adds economic benefits as it is affordable and the outer layer of *Allium cepa* L. is easily accessible.

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