GREEN ROUTE SYNTHESIS AND CHARACTERIZATION OF ZnO NANOPARTICLES USING *SPATHODEA CAMPANULATA*

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ABSTRACT

Nanoparticles have most of their constituting atoms on their surfaces and are similar to biomolecules making them have unique and noble properties compared to their bulk counterparts. They have thus received tremendous research focus and immense applications in sensors, medicine, cosmetics, optoelectronics, environmental protection, information storage and in catalysis. Biological methods of synthesizing nanoparticles specifically using plants are currently under exploitation due to the fact that they are cost effective and environmentally friendly way of synthesizing nanoparticles. In the present study, zinc oxide nanoparticles (ZnO NPs) were successfully synthesized using zinc nitrate and aqueous leave extract of *Spathodea campanulata* as the reducing as well as stabilizing agent. Formation of nanoparticles was monitored using UV-Visible spectroscopy and a characteristic absorption peak at 328nm was observed. From the transmission electron microscope (TEM) images, the synthesized ZnO nano-crystallites were of uniform morphology and size range of 20-50nm. XRD characterization revealed the face-centered cubic of highly crystalline nanoparticles indexed to (JCPDS card No. 36-1451). SEM images revealed spherically shaped and smooth surfaced nanoparticles arranged on top of one another. The EDX gave strong signals for zinc and oxygen at energies 137.6888 units and 118.0190 units, respectively, indicating the occurrence of the nanoparticles in their oxide form rather than the pure zinc form. From the FTIR spectra of the zinc oxide nanoparticles, the characteristic absorption peak of Zn-O bond was observed at 848cm^{-1}. The spectra of the *Spathodea campanulata* extract gave an O-H stretch of polyphenols at 3222cm^{-1}, nitrile group from proteins at 2339cm^{-1} and double substituted aromatic bending at 682cm^{-1}.

KEYWORDS: *Green, synthesis, ZnO, nanoparticles, Spathodea.*

INTRODUCTION

The various routes that have been used for the synthesis of ZnO nanoparticles include sol–gel synthesis [1, 2], hydrothermal, solvothermal methods [3, 4], microemulsion methods and precipitation method [5]. These methods are associated with environmental contamination, high temperatures, high pressures and expensive equipment. Biological methods specifically the use of plants are becoming the most preferred methods as they are clean, cost effective, considerable rapid and often single step protocols [6,7]. In addition, nanoparticles prepared using plant extract as the reducing as well as the capping agents have been found to be nontoxic and can be employed in medicine [8]. According to [9], biosynthetic routes provide nanoparticles of better defined sizes and morphology as compared to other physicochemical methods of producing nanoparticles. Plant biomolecules are known to mediate the reduction of ions to nanoparticles and in the stabilization of the same nanoparticles [10]. Vidya *et al.* [11]) reported synthesis of ZnO nanoparticles using leaves extract of *Calotropis Gigantea*. The biosynthesized nanoparticles had size range of 30-35nm and were spherical in nature. "Green chemistry" synthesis of ZnO

nanopowders using gelatin was also reported by Darroudi *et al.,* [12]. The reduction of the ions was ascribed to the polysaccharides, terpenoids and phenolic compounds that were present in the extract. Synthesis using biomolecules present in plant extracts is in congruent with green chemistry principles and the enormous plant diversity presents a wide research area that requires exploitation.

Spathodea campanulata is a medicinal plant belonging to the family Bignoniaceae and monotypic genus Spathodea [13]. It mainly grows as a tree with height ranges between 7-25 meters, but occurs as a shrub in the open savanna. Commonly known as Nandi flame, African tulip or Fountain tree, *Spathodea campanulata* is indigenous to most parts of tropical Africa and southern Asia. It is a traditional source of drug in Africa and has been widely used to treat inflammations, stomach aches and skin diseases due to bacterial infections [13, 14, 15]. An extensive phytochemical analysis of the water extracts of the leaves of the *Spathodea Campanulata* has revealed presence of polyphenols, caffeic acid, alkaloids, proteins and carbohydrates in varying proportions [16, 17].

EXPERIMENTAL METHODS

All chemicals such as zinc nitrate and ethanol were of analytical grade and were used as received without further purification. Ethanol and zinc nitrate were purchased from Sigma-Aldrich chemicals. Ultra-purified water used throughout the research work to prepare solutions was obtained from a Milli-Q Water purification System (Millipore Corp., Bedford, MA, USA).

Preparation of plant extract

Fresh leaves of *Spathodea campanulata* were collected next to the Department of chemistry, Chiromo campus, University of Nairobi. They were then taken for characterization at the herbarium, School of Biological Sciences (SBS), University of Nairobi. After characterization they were washed thoroughly under running deionized water and then rinsed thoroughly with ultra-purified water and left to air dry. The plant leaves were then sliced into pieces and boiled at different temperatures (37^{O}C, 50 ^{O}C, 75 ^{O}C, 100 ^{O}C and 125 ^{O}C) for 15 minutes. The resulting solutions were filtered using Whatman filter paper No. 41 and then stored in a refrigerator at 16^{O}C for further use.

Synthesis of ZnO nanoparticles

20ml of *Spathodea campanulata* extracted at 37^{O}C and 5ml of 10Mm Zn $(NO_3)_2$ solutions were mixed together and the mixture left to stand for 2 hours at room temperature. The mixture having turned deep yellow was then centrifuged at 14000 rpm for 30 minutes and the supernatant poured out. The light yellow paste obtained was redispersed in ultra-purified water to remove excess biological molecules. The process of centrifugation and redispersion in ultra-purified water was repeated three times to completely purify the nanoparticles. The light yellow paste collected was then dried in an oven at 60^{o}C overnight, packed and stored for characterization. The synthesis procedure was repeated for each plant extract prepared at the other temperatures (50 ^{O}C, 75 ^{O}C, 100 ^{O}C and 125 ^{O}C). Different plant extract volumes (5ml, 10ml and15ml) were also mixed with 5ml of 10Mm Zn $(NO_3)_2$ solutions in different containers and the procedure discussed above for the synthesis of ZnO nanoparticles repeated for all.

Characterization of the synthesized nanoparticles

Ultraviolet-Visible (UV-VIS) Spectrophotometer

The resulting effects of varying plant extraction temperatures and concentrations on the properties of synthesized Zinc oxide nanoparticle were investigated using A Nicolet Evolution 100 (Thermo Electron Cooperation, UK) UV-Vis instrument.

X-ray Diffractometer

The crystallization and crystal structure of the biosynthesized ZnO nanoparticles were checked through a PAN analytical Xpert Pro θ-2θ powder X-ray diffractometer (Model-D8 Advance, BRUKER Germany) using Cu Kα radiation at 45 kV and 40 mA.

Fourier Transform-Infra Red (FT-IR)

The spectra of *Spathodea campanulata* extract and the synthesized ZnO nanoparticles were recorded in the range of 500 to 4000cm^{-1} using ATR-FTIR (a Nicolet

Magna-IR system 560 FTIR spectrometer of resolution 4 cm $^{-1)}$.

Scanning Electron Microscope (SEM)

The biosynthesized Zinc oxide nanoparticles images at various magnifications were taken using a Hitachi X-650 SEM analyzer (acceleration voltage +25 k, resolution 20µm).

Transmission electron microscopy (TEM)

Morphology of the biosynthesized Zinc oxide nanoparticles were investigated using Philips CM20T-LaB6 TEM microscope operating at acceleration voltage of 120 kV.

RESULTS AND DISCUSSION

Ultraviolet-Visible spectroscopy

Confirmation and monitoring of the reduction of zinc ions to zinc oxide nanoparticles was UV- Vis spectrum in the range of 200–1000 nm. The spectrum (figure 1) showed a distinct peak, centered at 328nm, specific for ZnO NPs [18].

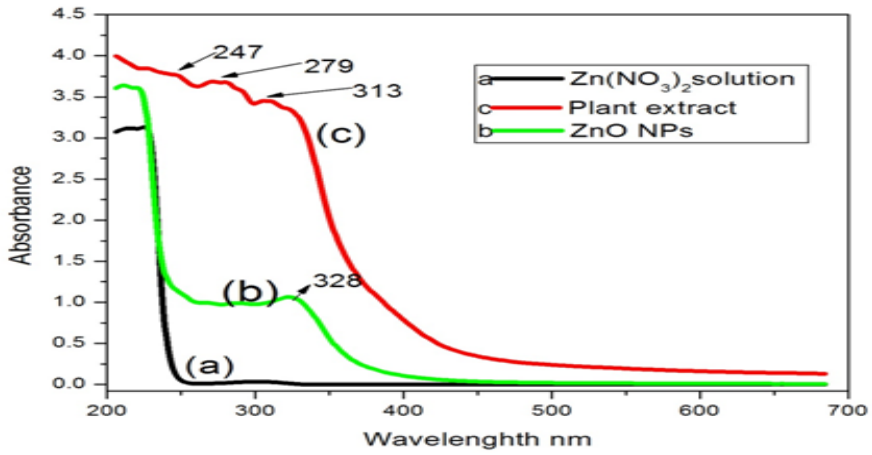

Figure 1: Comparison UV-Vis spectra for Zinc nitrate solution (a), ZnO NPs (b) and plant extract (c).

The absorption band at 328nm was attributed to the excitation of valence electrons of ZnO arranged in the nanoparticles. This implies that the nanoparticles absorb light in the ultra-violet region [19]. The characteristic absorption peak at 328 nm indicates the successful biosynthesis of ZnO NPs and is in good agreement with previous work reported by [20]. The zinc nitrate solution showed no absorption band. Nagarajan and Arumugan (2013) also reported no absorption band for Zinc nitrate solution [21].The plant extract had absorbance bands at 247nm, 279nm and 313nm.These were attributed to the polyphenol compounds and proteins present in the plant extract.

X-ray diffractometer

Figure 2 shows the x-ray diffractometer (XRD) spectrum of the biosynthesised zinc oxide nanoparticles.

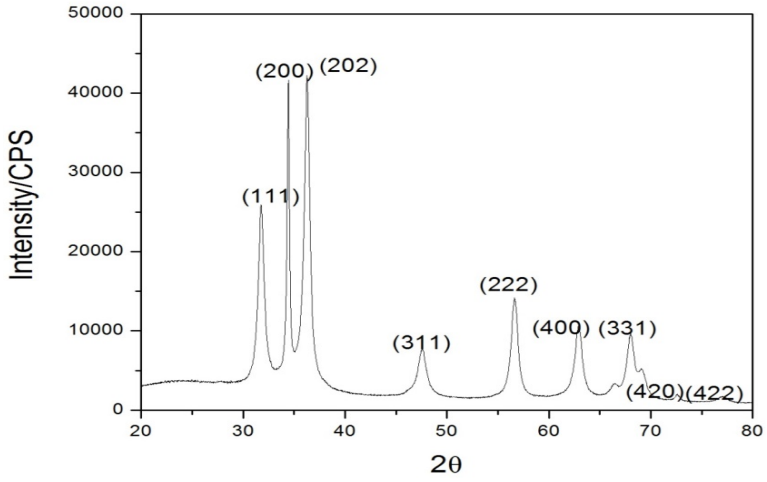

Figure 2: XRD pattern for the biosynthesized Zinc Oxide nanoparticles.

The major peaks correspond to Bragg reflections with 2θ values of 31.41°, 34.31° 36.12°, 47.79°, 56.54°, 62.81°, 67.97°, 72.91° and 77.17°. These location of the characteristic Bragg reflections were indexed to (1 1 1), (2 0 2), (3 1 1), (2 2 2), (4 0 0), (3 3 1), (4 2 0) and (4 2 2) planes of ZnO wurtzite structure (standard JCPDS card 36-1451) and this confirmed the presence of zinc oxide nanoparticles. The appearance of sharp diffraction patterns confirms the small size as well as high crystallinity of the synthesized nanoparticles. The XRD patterns obtained in this study were also similar to XRD patterns obtained for biologically synthesized ZnO nanoparticles reported in literature [22]; [8]].

Transmission Electron Microscope (TEM)

From the TEM images (**figure 3**), the biosynthesized ZnO nanoparticles had large grain size, uniform morphology and nearly spherical in shape.

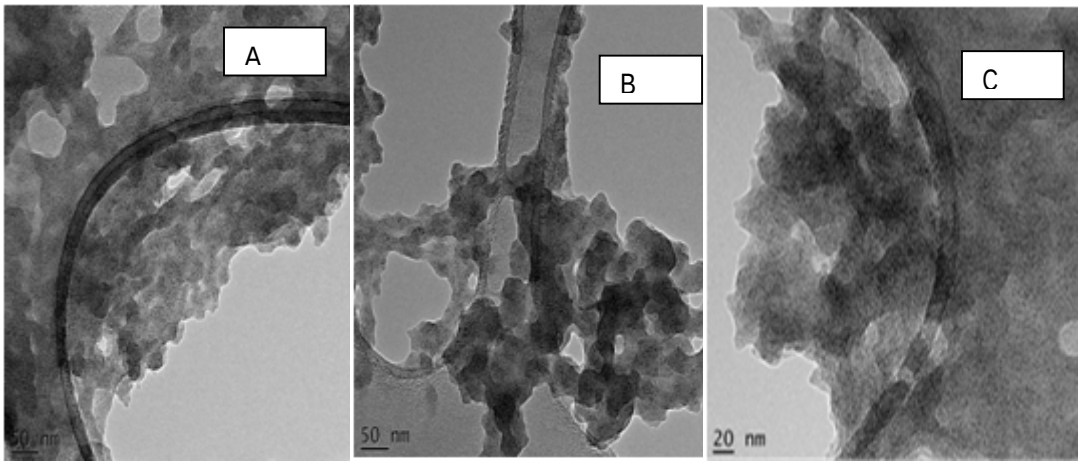

Figure 3: TEM images of biosynthesized ZnO nanoparticles (A-C).

The ZnO nanoparticles were prepared using *Spathodea campanulata* extracted at 90°C (figure 3A), 50°C (figure 3B) and at 37°C (figure 3C).

Metal oxide nanoparticles prepared using *Spathodea campanulata* extracted at 100°C revealed fine particle morphology with significantly low agglomeration and particle size of 50nm (figure 3A), while nanoparticles plant leaves extracted at 37°C (figure 3C) revealed a highly aggregated nano-powder with particle size of 20nm. This was attributed to the fact that extraction at 100°C was efficient and most phytochemicals were obtained and used for reduction as well as stabilization of the nanoparticles. The above results also suggest possible nanoparticle size control by use of temperature variations.

Energy Dispersive Spectroscopy (EDX)

EDX analyses were carried out to confirm the presence of ZnO nanoparticles and to determine the elemental composition of the ZnO nanoparticles. The EDX spectra (figure 4) revealed a strong signal for zinc at energy 137.6888 units and a prominent oxygen peak at energy 118.0190 units.

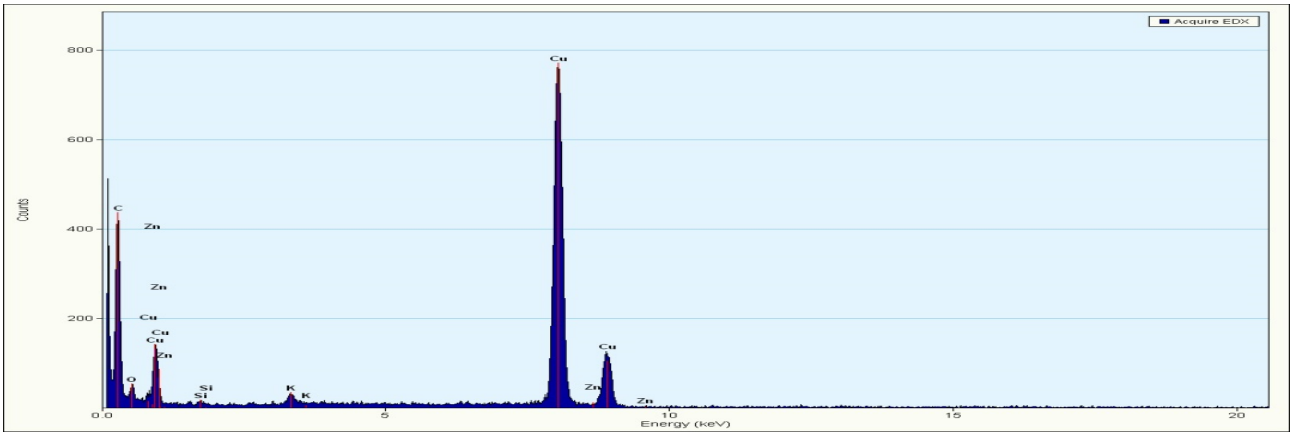

Figure 4: EDX elemental analysis of ZnO nanoparticles.

This EDX results further confirms the biosynthesis of the ZnO nanoparticles and the occurrence of the zinc nanoparticles in its oxide form rather than in pure zinc form.

The spectra also indicated that ZnO nanoparticles were composed of impurities like silicon, chlorine and potassium. The impurities (Cl, K and Si) could be of biological origin present in the plant extract. The presence of copper and carbon elements were due to carbon-coated copper adhesive used as sample holder.

Scanning Electron Microscope (SEM)

The SEM images (figure 5) show individual zinc oxide nanoparticles and a number of aggregates of the nanoparticles.

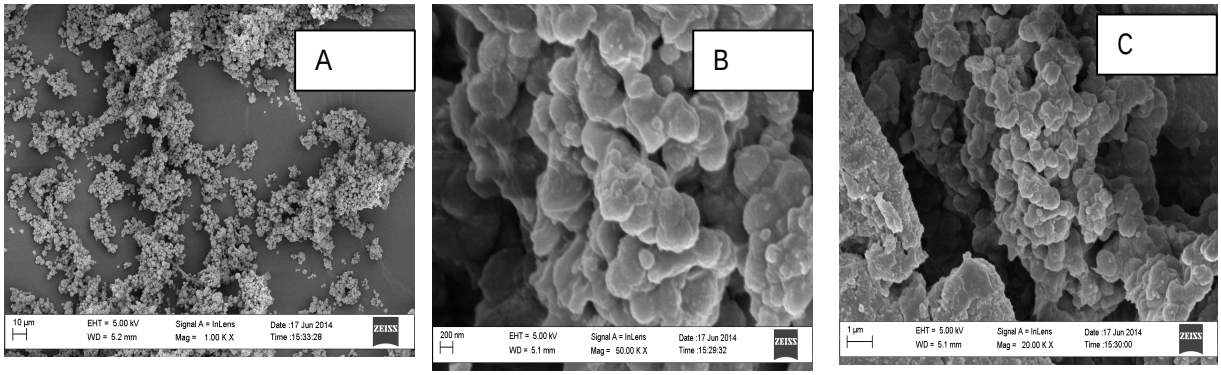

Figure 5: SEM images of the biosynthesized ZnO nanoparticles.

The nanoparticles are present in the bright regions of the image while the dark regions were covered with platinum which acted as the substrate. The shapes of the nanoparticles were nearly spherical and at high magnifications of 1 and 10µm showed formation of aggregates of nanocrystallites (**figure 5B and 5C**). The SEM analyses also indicated that the ZnO nanoparticles were arranged on top of one another and were nearly spherical in shape.

Fourier-Transform Infra-Red (FTIR)

The Attenuated Fourier-Transform Infra-Red (ATR-FTIR) analysis was carried out to investigate the biomolecules involved in the synthesis as well as stabilization of the ZnO nanoparticles

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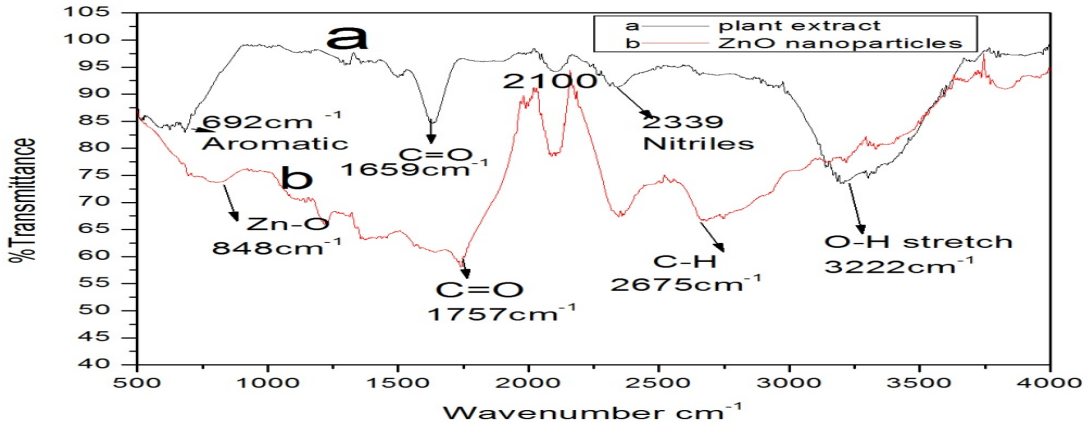

Figure 6: ATR FT-IR spectra of (a) plant extract, (b) ZnO nanoparticle.

In figure 6**,**the spectra of the aqueous extract of *Spathodea campanulata* showed main peaks at 3222cm^{-1}, 2339 cm^{-1}, 1659 cm^{-1}and 692 cm^{-1} . The peak at 3222 cm^{-1} was attributed to the OH stretch in the extracted polyphenols. This was in agreement with phytochemical analysis studies carried on the aqueous extract of *Spathodea campanulata* that showed presence of polyphenols [16]. The peak at 2339 cm^{-1} was attributed to triple bond of nitrile group from proteins. The peak at 1659 cm^{-1} was attributed to C=O band of unsaturated aldehyde, while the peak at 692 cm^{-1} was assigned to aromatic bending. These peaks occurred due to phytochemicals like steroids, flavanoids, cardiac glycosides, alkaloids, tannins, caffeic acid and flavanols present in *Spathodea campanulata* aqueous extract [23].

The peak at 848 cm^{-1} was attributed to characteristic absorption peak of Zn-O bond. Reported similar investigations have shown that metal oxides give absorption bands in the fingerprint regions below 1000 cm^{-1} arising from inter-atomic vibrations [24]. The peaks at 2339 cm^{-1} and 1659 cm^{-1} in the extract and nanoparticle spectra remained considerable intact while the OH peak almost vanished. This implies that phenolic compounds containing OH groups like flavanols and caffeic acid present in the extract might have been responsible for the synthesis of ZnO nanoparticles. In addition, it was observed that the nitrile groups from proteins showing stretching band around 2341cm^{-1}, carbonyl groups at 1757 cm^{-1} and 2100 cm^{-1} were still present in the spectrum of the ZnO nanoparticles. These compounds might have been responsible for the capping of the ZnO nanoparticles by forming a layer covering the nanoparticles and thus preventing agglomeration and providing stability of the material.

CONCLUSIONS

ZnO nanoparticles were successfully biosynthesized using *Spathodea campanulata* as the reducing as well as stabilizing agent. The biosynthesized ZnO nanoparticle solution exhibited absorbance bands at 328 nm attributed to the excitation of valence electrons of ZnO arranged in the nanoparticles. Transmission electron microscope analysis demonstrated that the nanoparticles had nearly spherical shapes and having size range of 20-50 nm. The scanning electron microscope at resolutions of 1µm, 10µm and 200nm showed nearly spherically shaped nanoparticles with smooth surfaces and arranged on top of one another.

The EDX revealed a strong signal for Zinc at 137.6888 units and oxygen at 118.0190 units, thereby confirming occurrence of the zinc nanoparticles in their oxide form, rather than in their pure zinc form. The sharp peaks in the XRD pattern clearly illustrated the high crystallinity of the nanoparticles and were all indexed to standard JCPDS card no. 36-1451. FTIR spectra peak at 848 cm^{-1} indicated characteristic absorption bands of ZnO nanoparticles. The FTIR spectra of the aqueous *Spathodea campanulata* leaf extract showed presence of OH stretch due to polyphenols at 3222cm^{-1}, nitrile group from proteins at 2339 cm^{-1} and double substituted aromatic bending at 682cm^{-1}. The phytochemicals involved in the synthesis and stabilization of the ZnO nanoparticles can be presumed to be polyphenols and proteins. Further studies is contemplated to isolate all the chemical constituents of the aqueous *Spathodea campanulata* so as to confirm the exact chemicals that lead to bio-functionalization of Zn ions to ZnO NPs. The use of plant extracts is thus a potentially exciting and green method for the synthesis of zinc oxide nanoparticles and presents adverse area for further studies.

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