



The biosynthesis of ZnO nanoparticles using *Synadenium grantii* latex: characterisation and evaluation of their antimicrobial activities

Daniel Tirop¹ | Lemeitaron Njenga¹ | Ayabei Kiplagat¹ | Grace Lagat¹ | Martin Onani²

1. Department of Chemistry and Biochemistry, University of Eldoret, Eldoret, Kenya.

2. Department of Chemistry, DST/Mintek Nanotechnology Innovation Centre, University of the Western Cape, Bellville, South Africa.

* Corresponding Author Email: lemeitaronnjenga@gmail.com

Abstract:

The steadily rising incidences of microbial and infectious diseases pose a direct threat to both human and animal sustainability. Over the previous 20 years, the expectations for eco-friendly nanoparticles application as contemporary medicinal agents have grown. This is why, as opposed to using hazardous chemicals, researchers have recently concentrated on simple, green, sustainable, and affordable ways to create nanoparticles. This research aimed to use the latex of *Synadenium grantii* to synthesize ZnO NPs (zinc oxide nanoparticles) using a simple and environmentally friendly technique. The observation of the maximum wavelength at 365 nm using Ultraviolet-visible (UV-Vis) spectrometry signified the formation of zinc oxide nanoparticles. The FT-IR (Fourier transform-infrared) spectrometry indicated the bands of various biomolecules (mainly polyphenols at 1541 cm⁻¹ wavenumber) involved in reducing and capping zinc oxide nanoparticles. The XRD spectrum demonstrated the wurtzite phases of ZnO nanoparticles of 24 nm in size. The synthesized ZnO NPs demonstrated great anti-microbial potency compared to the positive control (ampicillin) as tested on *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). The study confirmed the presence of various chemical compounds in latex of *Synadenium grantii* responsible for the synthesis of ZnO NPs with enhanced anti-bacterial effects in comparison to the latex extract.

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1. Introduction

Nanoscale technology is gradually expanding across different scientific domains such as medicine, agriculture, and physics. The rapid increase in the synthesis of nanoparticles (NPs) for creating innovative materials with unique characteristics at the nanoscale has been noticeable in recent years (Njenga *et al.*, 2023; Gupta *et al.*, 2018). The biomedical field is interested in the biogenic formulation of ZnO nanostructures with antimicrobial potential. Metallic oxide nanomaterials have been fabricated by several chemical and physical methods. Nevertheless, chemical synthesis carries several potential risks, such as cytotoxicity, genotoxicity, and carcinogenicity. Furthermore, the end products of these processes cause harmful, poisonous compounds that stick to the produced nanomaterials' surface, reducing their usefulness for medical purposes (Akhtar *et al.*, 2013).

The biogenic methods offer a safer approach and are gaining momentum. The procedures for synthesis of nanoparticles are environmentally friendly and the particles synthesized possess exceptional chemical (Hajizadeh *et al.*, 2023), optical (Omeh *et al.*, 2024), biomedical (Somu & Paul, 2019) and electronic properties (Turki *et al.*, 2023) which can be easily tuned just like those from chemical means; these biological procedures involve the use of organisms ranging from plants, bacteria, and fungi (Ikhiya *et al.*, 2023; Ali *et al.*, 2022; Said *et al.*, 2022). The phytochemicals in plants (Hameed *et al.*, 2019) and both unicellular and multicellular organisms can synthesize and/or regulate the sizes of the biosynthesized nanomaterials (Li, 2023). The key advantages of this strategy for the environment are its low toxicity levels (Dibaba, 2023). The relationship building for the pharmaceutical and biomedical applications is amongst other advantages of the biogenic synthesis. The conventional methods for formulating nanoparticles entail applying chemical and physical processes (Khan *et al.*, 2022).

The ZnO NPs are amongst several biogenically synthesized nanoparticles. It belongs to a significant family of commercially feasible products that have special antibacterial, anticancer, and magnetic qualities (Jeyabharathi *et al.*, 2022; Selim *et al.*, 2020). It also can be both photo-catalytic and photo-oxidizing (Dulta *et al.*, 2022). The Food and Drug Administration (FDA) in the USA had classified ZnO as a “GRAS” (often thought to be secure) substance, which is another appealing quality of the material. It is noted that a significant percentage of gram-negative and gram-positive pathogens are inhibited in their growth by zinc oxide nanoparticles (Muthuvel *et al.*, 2020). Their capacity to produce higher concentrations of hydroxyl radicals, singlet oxygen i.e. Reactive Oxygen Species (ROS) and H₂O₂, which effectively destroy microbial cells, is primarily responsible for their antibacterial action (Chandrasekaran *et al.*, 2020). Inorganic oxide nanoparticles are better in contrast to antimicrobial agents based on organic chemicals due to their extended shelf life, general stability, and an effective method of treating microbial ailments (Abdelbaky *et al.*, 2022). Herbal remedies, or the custom of using plant extracts as medication for a variety of ailments, have been around for a while. In Asian nations, natural compounds like *Synadenium grantii* are commonly utilized for combating bacterial illnesses. But their efforts haven't yielded many results (Wang *et al.*, 2015).

The present work investigated the biosynthesis of ZnO nanoparticles by incubating zinc acetate solution with *Synadenium grantii* latex. The study assessed the antibacterial studies of the synthesized nanomaterials in comparison to the latex adopting the disc diffusion methodologies

against microbial isolates of *Escherichia coli* (gram-negative) *Staphylococcus aureus* (gram-positive) and zones of inhibition recorded.

2. Materials and methods

2.1. Chemicals and reagents

Sodium Hydroxide (NaOH) and Zinc Acetate Dihydrate ($\text{Zn}(\text{CH}_3\text{CO})_2 \cdot 2\text{H}_2\text{O}$) were purchased at Himedia Lab, Nairobi, and used directly without further purifications. The Distilled Water (DW) and laboratory strains were collected in the University of Eldoret Chemistry and Microbiology Labs, respectively.

2.2. Plant extracts collection and preparation

Latex extracts from *Synadenium grantii* to be used in this research work were collected from Ziwa in Eldoret, Uasin-Gishu County, Kenya. The latex was collected by slitting the trunk of *Synadenium grantii* longitudinally, pure latex was collected in dark bottles and protected from light. For further application, the latex extract is refrigerated at 4 °C. 10 mL of the extract is applied in the synthesis of ZnO NPs.

2.3. Biosynthesis of ZnO nanoparticles

The synthesis method was borrowed from (Gilavand *et al.*, 2021) with few modifications. Briefly, 50 mL of DW and 1 mM of $\text{Zn}(\text{CH}_3\text{CO})_2 \cdot 2\text{H}_2\text{O}$ were mixed for 20 minutes on a hot plate. Then, 25 mL of plant extract was added to the mixture. The pH of the mixture was adjusted to 7 by adding 1.8 M NaOH dropwise. For one hour, the resulting reaction mixture was swirled. The solution's hue changed to yellow, indicating that ZnO NPs had formed. A centrifuge was used to separate the precipitate from the reaction liquid for 35 min at 6000 rpm at room temperature. The collected mass for additional research, is oven dried for 4 hours at 60 °C and stored in airtight vials.

2.4. Characterization of ZnO nanoparticles

- Optical analysis: The surface plasmon resonance of ZnO nanostructures was examined using an UltraViolet-visible spectrophotometer (Shimadzu, UV-1800 series). A synthetic sample was scanned between wavelengths of 300-500 nm while suspended in sterile distilled water (Rahman *et al.*, 2022).
- The X-Ray Diffraction (XRD) analysis: Pattern of biosynthesized ZnO NPs measurements were done on Bruker D8 Advance X-ray diffractometer system. The intensity of the diffracted Cu-K α radiation ($\lambda = 0.154$ nm, 40 kV, and 40 mA) was measured in a 2θ range between 10° and 90° with an increment of 0.0194° (Dibaba, 2023).
- Fourier Transform Infrared (FT-IR) spectroscopy analysis: To confirm which bioactive phytoconstituents were present and responsible for the stabilization, decrease, or control of the size of the ZnO nanoparticles, an FTIR spectrophotometer (Perkin-Elmer

Spectrum 1000) was utilized. At ambient temperature, KBr pellets were used to acquire ZnO nanoparticle FTIR spectra at 4000–400 cm⁻¹ scan range.

- **Anti-microbial activity:** The procedure was adopted from (Radhakrishnan *et al.*, 2021) with several modifications. The ZnO nanomaterials were assessed for their antimicrobial potential by growing both *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) laboratory bacterial strains sourced from Kenya Medical Research Institute (KEMRI). The agar diffusion technique methodology was adopted to ascertain the antimicrobial activity of oven-dried zinc oxide nanomaterials. In a nutshell, the microbes were inoculated for eight hours in Luria Bertani broth. Sterilized cotton swabs were used to seed the isolates onto Luria Bertani agar plates. A sterile borer was used to create wells on the surface of agar (7 mm diameter). The varying amounts of zinc oxide nanoparticles (25–75 ug/mL), latex extracts of *Synedenum grantii*, and positive control (ampicillin) were added to separate wells. For 24 hours at 37 °C, the plates were incubated.

3. Results and discussion

3.1. Biosynthesis of ZnO nanoparticles

The change in the constituent mixture's hue was very prominent. This was confirmed as the first indication that zinc oxide nanoparticles were forming. In the present work, a change of color from white to red then to yellow indicated the synthesis of zinc oxide nanoparticles by latex extracts of *Synedenum grantii* (Gilavand *et al.*, 2021).

3.2. Optical properties

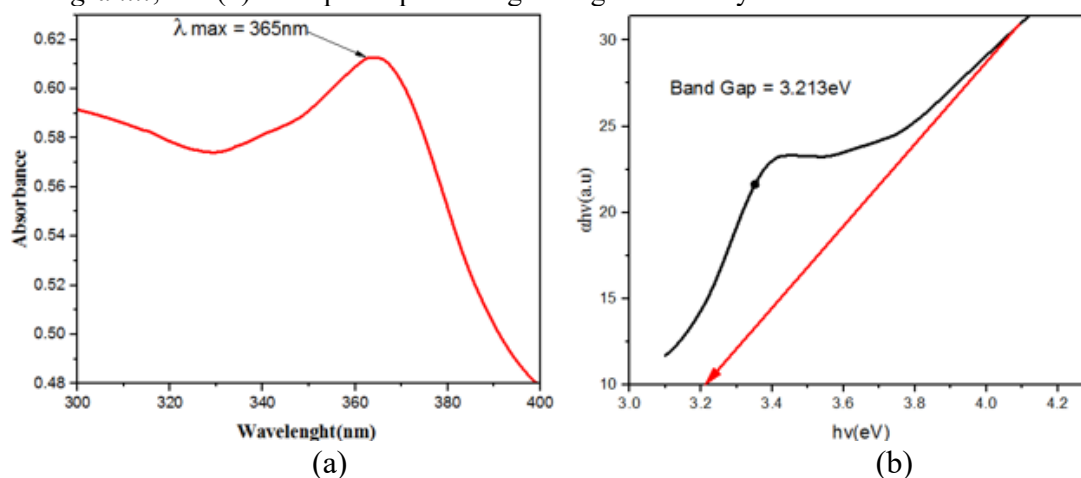
UV-vis spectroscopy is a common and widely used technique to characterize the surface plasmon resonance of synthesized NPs. Figure 1(a) represents the UV-visible maximum absorption peaks of the green biosynthesized zinc oxide nanoparticles. Zinc oxide nanoparticles' highest absorption peak was located at 365 nm (with a band gap of 3.213eV). The observation of a single peak at approximately 365 nm signified the formulation of ZnO NPs. This finding is in tandem with previous findings of green synthesized ZnO NPs that were found to be located in the 324–390 nm range (Alharthi *et al.*, 2021). This highly red-shifted absorption maximum band (from the bulk usually around 373 nm) is an indication of nano sized ZnO NPs formation.

Tauc's relation was applied to fit the absorption data to obtain the E_g (optical band gap energy) of the nanomaterial (Muhammad *et al.*, 2019).

$$\alpha h\nu = E(h\nu - E_g)E(h\nu - E_g)^{\frac{1}{2}} \quad (1)$$

Where α is the optical absorption coefficient and is derived from the absorption data, and $h\nu$ is the photon energy, E_g is the straight band gap, and E is a constant (Abbas *et al.*, 2019). As shown in Figure 1(b), a plot of $(\alpha h\nu)^2$ as photon energy ($h\nu$) function and the curve linear portion extrapolated to zero absorption gives the value of the direct band gap (E_g). It was observed that the band-gap energy was 3.213eV which is closer to that of typical ZnO (3.33 eV).

Figure 1: (a) UV-vis absorption spectra of synthesized ZnO nanostructures by latex of *Synadenium grantii*, and (b) Tauc plot representing the E_g of the biosynthesized ZnO nanostructures



3.3. XRD analysis

The primary peaks of the green-synthesized zinc oxide nanoparticles' observed XRD patterns (Figure 2(a): According to JCPDS Card No. 00-065-3411) appear at 2θ values of 31.7, 34.5, 36.3, 47.5, 56.5, 62.8, 66.2, 67.9, 69.0, 72.6, and 76.9 $^\circ$ indexed to (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3), (2 0 0), (1 1 2), (2 0 1), (0 0 4) and (2 0 2) planes that verified wurtzite phases of the formulated nanomaterials (Lopez-miranda *et al.*, 2023). The ZnO NPs' good crystalline nature is indicated by the sharp, narrow peaks. Scherrer's equation estimates the size of zinc oxide nanostructures (Aklilu, 2022). The biogenic ZnO NPs possessed an sizes of 24 nm (Table 1). Sharp diffraction peaks in the ZnO NPs' X-ray diffraction spectra were indicative of their structural crystallinity.

Table-1: ZnO nanoparticles size determination using Scherrer equation

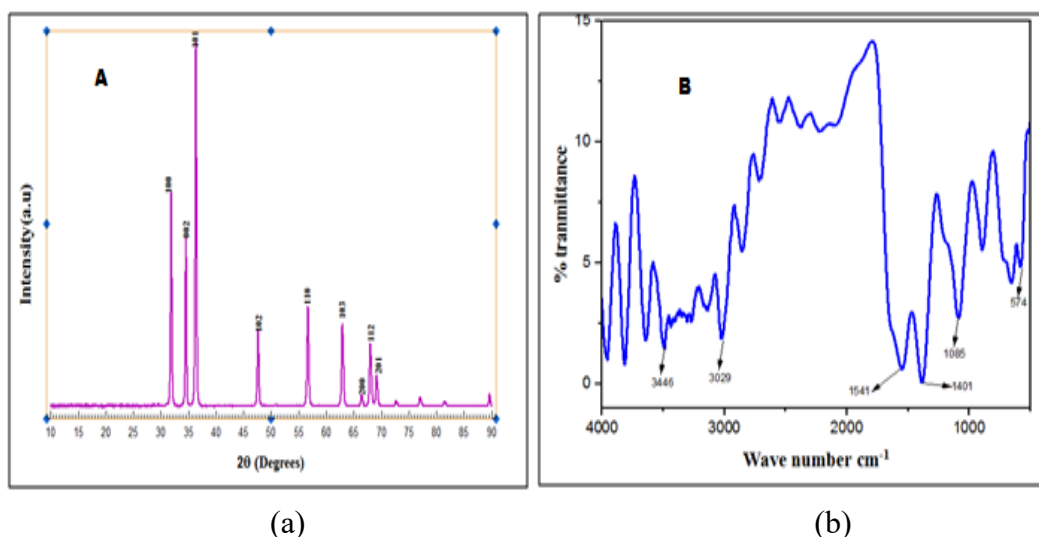
XRD spectrum peak	2θ values ($^\circ$)	FWHM	Particle size $(0.9 \times 0.154) / (\beta \sin \theta)_{(\text{nm})}$	Average particle size (nm)
1	31.7964	0.3386	24.3851	23.9611
2	34.4474	0.2899	28.6790	
3	36.2823	0.3393	24.6303	
4	47.5665	0.3810	22.7791	
5	56.6166	0.3832	23.5402	
6	62.8758	0.4054	22.9582	
7	67.9616	0.4130	23.1869	
8	69.0923	0.4478	21.5297	

3.4. FTIR studies

The bioactive agents are reported to be specified and are involved in reduction of metal oxide nanoparticles. Phenols, alkaloids, saponins, and terpenes, (Luz *et al.*, 2015) are secondary metabolites in the latex of *Synadenium grantii* and are involved in the formation of zinc oxide

nanomaterials and possess biological properties including anti-oxidant and anti-cancer activities. It can be reported that the $\text{Zn}(\text{CH}_3\text{CO})_2 \cdot 2\text{H}_2\text{O}$ (zinc acetate dihydrate) is reduced to ZnO nanoparticles by phenols because of their massive -OH groups shown in the FT-IR spectra (Figure 2(b)).

Figure 2: (a) X-ray diffraction patterns of ZnO NPs and (b) FTIR profiles of ZnO NPs



The FTIR spectra show bands in 3446 cm^{-1} due to vibration stretch of the hydroxyl group of phenol. The C=C, C=O=C and C=O vibrations of heterocyclic constituents may act as a stabilizer (The -OH groups from the phenol, in addition, perform as an encapsulating agent) (Yadav *et al.*, 2018; Naiel *et al.*, 2022). Low-intensity peak around 1541 cm^{-1} corresponded to C=C stretches of aromatized rings of polyphenols (Ebadi *et al.*, 2019). Absorption at around 1541 cm^{-1} may also exhibit the linkage with ZnO NPs which may be assigned to the carbonyl stretch in proteins. Prominent lower peaks at 574 cm^{-1} are assigned to the fingerprinting Zn-O vibrations of ZnO NPs (Hussain *et al.*, 2019). Stabilization and encapsulation of green synthesized ZnO NPs result from C=O -and OH functional groups of proteins and phenolic group molecules respectively.

Table-2: ZOI values of zinc oxide nanoparticles, *Synadenium grantii* latex extracts, and ampicillin against two bacterial strains; *S. aureus* and *E. coli* by Agar Diffusion Technique

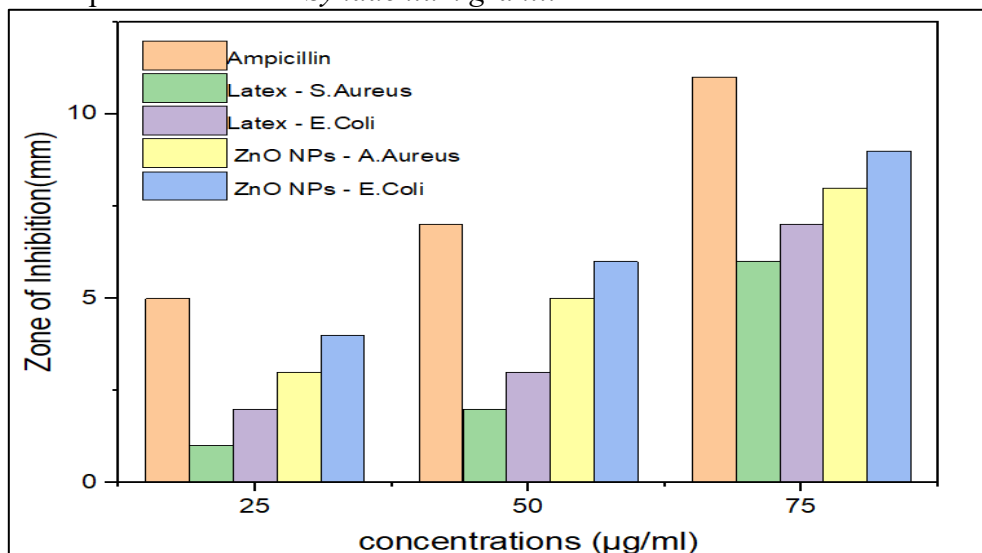
Sample Name	Zones of inhibition (mm)					
	<i>S. aureus</i>			<i>E. coli</i>		
	25 ug/ml,	50 ug/ml,	75 ug/ml	25 ug/ml,	50 ug/ml,	75 ug/ml
Zinc Oxide nanoparticles	3, 5, 8			4, 6, 9		
<i>Synadenium grantii</i> latex extracts	1, 2, 6			2, 3, 7		
Ampicillin	5, 7, 11			5, 7, 11		

3.5. The anti-microbial activity

The anti-bacterial potentials of the formulated ZnO NPs were assessed against two laboratory isolates: *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) pathogens. The standard antibiotic ampicillin (positive control) and the solvent (negative control) is used to compare

the activities. Table-2 and Figure 3 and represent the ZOI (Zones of inhibition) of the green synthesized ZnO NPs, latex extracts, and ampicillin against the select microbes. ZnO NPs showed enhanced or improved anti-microbial potentials on both bacteria compared to latex used to synthesize the ZnO NPs. Similar findings were discussed by (Kiani *et al.*, 2022) signifying the synergistic characteristics of the biosynthesized ZnO NPs.

Figure 3: Bar graphs showing the inhibition zones of ZnO NPs against bacterial strains compared to latex of *Synadenium grantii*



4. Conclusion

The study demonstrated the successful formulation of ZnO nanoparticles using latex of *Synadenium grantii*. The ZnO nanostructures were spherical and had average nano sizes of 24 nanometer as demonstrated by XRD spectra. This approach has the benefit of being fast, eco-friendly, and convenient for the green synthesis of ZnO nanoparticles. The band gap of 3.213 eV was sufficient evidence for ZnO semiconductors formulation. The investigation confirmed the stronger antibacterial potentials of the green synthesized ZnO nanoparticles compared to latex extracts. This is because ZnO nanoparticles include antibacterial ROS (reactive oxygen species) in addition to being capped by anti-bacterial substances such phenols, alkaloids, saponins, terpenes, triterpenes, ketones, sugars, fatty acids, proteins, and aromatic acids from latex extracts of *Synadenium grantii*. Thus, metal oxide-formulated nanoparticles containing latex extracts of *Synadenium grantii* are suitable for curing microbiological diseases.

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ORCID iD:

Daniel Tirop <https://orcid.org/0009-0003-1393-2069>

Lemeitaron Njenga <https://orcid.org/0009-0007-9476-4464>

Ayabei Kiplagat <https://orcid.org/0000-0002-5930-3407>

Martin Onani <https://orcid.org/0000-0002-4735-3669>

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