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Green synthesis, characterization, application and study of antimicrobial properties of zinc oxide nano particles using *Cyathocline purpurea* phytoextract

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Abstract
Use of phytoextract in synthesis of nanoparticles has many advantages over conventional physico-chemical methods and has various applications in Agriculture, Agrochemical and plant protection products, medicine and biology. In present study, zinc oxide (ZnO) nanoparticles (NPs) were synthesized using medicinally important herb, *Cyathocline purpurea* leaf extract and 0.1M zinc acetate dihydrate was used as a precursor in leaf extract. The structural and optical properties of NPs were characterised by ultraviolet-visible spectroscopy, Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and scanning electron microscope (SEM). The antifungal activity and potential of ZnO NPs was evaluated disc diffusion method against *Alterneria solani* as test pathogen. The minimum inhibitory concentration of ZnO NPs evaluated was 200 µL/ mL. During synthesis the colour change of reaction mixture from brown to white indicated the formation of ZnO NPs. UV maxima at 371 nm and absorption band at 530 nm, XRD pattern matching of the NPs matching with that of JCPDS card for ZnO confirmed the presence of pure ZnO NPs. FTIR spectrum revealed the presence of bioactive functional groups involved in the reduction of bulk zinc acetate to ZnO NPs. SEM analysis revealed the shape of NPs to be spherical whereas XRD pattern confirmed the particle size ranges from 80 to 120 nm. As the NPs showed strong antifungal activity against selected pathogen, suggesting that plant-based synthesis of NPs can be an excellent strategy to develop simple and eco-friendly biomedical products for environmental sustainability.

Keywords: Phytonanosynthesis, phytoextract, XRD, SEM, *Cyathocline purpurea*, nanosynthesis

1. Introduction
Nanoparticles synthesis for applications in areas like medicine, agriculture, chemistry, physics, microbiology, catalysis, electronic, information technology and environmental biotechnology; as a result, it became the century's upcoming industrial revolution and area of interest [1, 2]. Nanoparticles being prepared by various processes like physical, chemical, biological, biochemical, electrochemical, and sonochemical routes. Out of these, the biological route is most preferred, which involves microbial nanosynthesis (use of microrganisms) and phytonanosynthesis (use of plants extracts). Microbial nanosynthesis requires a long period of time to obtain adequate synthetic biomass and the same biomass cannot be reused for another synthesis. The use of plant sources in nanoparticle synthesis is a more preferred route due to a shorter period of synthesis, also due to the most plant sources satisfy the roles of stabilising, reducing, and capping effects, hence limited number of raw materials, promoting green technologies, safer nature of synthesis, biocompatibility, non-toxic nature, cost-effectiveness, sustainability, and environmental friendliness [1]. As, biosynthesis involving the use of plant extracts in aqueous medium, process becomes cheap and offers no limitations in terms of applications.

The use of plant extracts in nanoparticle synthesis also responsible for changing the oxidation state of metal ions through metabolic processes and the energy is conserved when the oxidized metal ions act as terminal electron acceptors and the capping effect provided by active components present in phytoextracts for surface modification of metal nano particle synthesised by this technique. However, all biological entities cannot be used for synthesizing nanoparticles due to their enzyme activities and adverse metabolic processes, hence, selection of the biological medium for synthesis of metal nano particle is one of the crucial steps.

Nanomaterials having great potential in various fields of science due to their excellent physico-chemical, biological characteristics and stability [8].

As, the nanoparticles (NPs) having high surface-to-volume ratio, they become appropriate and valuable entity for application-oriented performance (e.g., photocatalysis, cosmetics, plant protection products, energy reservoirs, packaging, and environmental remediation) and encourages their incorporation into a wide range of commercial products. Utilization of harmful compounds for reduction and as a capping agent in the nanoparticle synthesis causes a variety of adverse effects on the environment and the living system. As a result, plant extracts (PEs) are therefore considered as promising tool for the easy synthesis of metal oxide NPs through the green route, because of its eco-friendly approach, non-toxic, low cost, environmentally compatible and easy to apply. Additionally, the resultant particles are biocompatible and free of toxic stabilizers compared to classical chemicals. Basically, Phytoextracts contain a variety of active biomolecules that helps to reduce and stabilize NPs [1] [2]. The green synthesis technique for synthesising the metal oxide nanoparticles has evolved over the years which benefits use of plant materials along with water as a solvent, thereby making the process moderately pollutant-free and eco-friendly. Other advantages of the method would be low energy consumption, mild reaction conditions (e.g., low temperature and pressure), absence of catalyst and importantly the cost-effective process of the process. In contrast, chemical and physical processes are expensive and comparatively hazardous to the environment as a result of the toxic and unsafe reagents used for synthesis and toxic by-product released into the environment during the synthesis [3] [4].

For nanoparticles synthesis using phytoextracts, the extracts are mixed with an appropriate metal precursor to form a solution. The rate of formation, morphology, and stability of the nanoparticles are determined by parameters such as types and concentration of phytochemicals in the plant extract, metal–salt concentration, pH and temperature of the reaction media. The reduction of metal oxide precursors is carried out by the phenolic compounds and compounds containing hydroxyl group in the plant extracts. Stabilization of nanoparticle growth is promoted by amino acids, protein, and lipids present in the plant extracts which can act as biological surfactants [3].

In general, three steps are involved in the synthesis of metal/metal oxide nanoparticles from plant extracts, (i) the activation phase: bio reduction of metal ions/salts and nucleation process of the reduced metal ions); (ii) the growth phase: spontaneous combination of tiny particles with greater ones, called Ostwald ripening; (iii) the termination phase: determines the final shape of the nanoparticles [3].

The main focus of green chemistry is to design chemical products by safer way and reduce or eliminate the use or generation of hazardous substances that affects human health, environment and, in that context, one should develop nanoparticles that not only take care of controlling the vector breeding environments, but also exhibit the antimicrobial potentials. Several methods have been employed for prevention of the proliferation of mosquito-borne diseases. Most common of them is the application of synthetic insecticides, such as organochlorine and organophosphate compounds on mosquito-infested areas [1]. However, the introduction of chemically-based insecticides in the environment possesses some health challenges due to its toxic and non-degradable nature and increases the mosquito's resistivity to these common insecticides [1]. Hence, identification, isolation, and synthesis of bioactive compounds which can work against mosquitoes will be simple and eco-friendly way for managing the mosquito-borne diseases. Similarly, implementation of these bioactive plant components on nanoparticles has been investigated as potential alternatives for the chemical insecticides.

Zinc oxide (ZnO) is one of the very promising nanomaterials and has recently attracted the attention of many scientists for the biosynthesis of NPs due to its unique properties and multiple applications such as drug delivery, solar cells, photocatalytic degradation and personal care products like sunscreens and cosmetics, agrochemical and micronutrient in agriculture and horticulture [5] [6] [7]. ZnO NPs have been biosynthesized from several plant extracts such as Cassia auriculata, Aloe vera, Duranta erecta, Cinnamomum verum, Bauhinia tomentosa, Vitex trifolia, Moringa oleifera, Azadirachta indica, Artocarpus gomezianus and Olea europaea, etc [1, 2, 3, 9].

Considering literature review and valuable antimicrobial, medicinal and antioxidant properties [10, 11] of *Cyathocline purpurea* L, plant from Asteraceae family, to date, there have been no data found on its use for green synthesis of NPs, author decided to use the phytoextract of the same for synthesising the ZnO NPs.

Cyathocline purpurea L. is annual and occasionally perennial weed in paddy field. Flowers is usually purple in colour and occurs in corymbs at the end of branches. This weed plant is also shows great importance in medicinal field mostly as anti-inflammatory agents. The plant roots are used in medicine for treating stomach pain.

The aim of this study was to explore the application of *Cyathocline purpurea* L. as a capping and reducing agent for the biosynthesis of ZnO NPs. The biosynthesized ZnO NPs were characterized and confirmed by various spectroscopic and microscopic techniques, i.e., UV-Vis spectroscopy, FTIR, XRD and SEM, in addition, the antifungal activity and effect on seed germination and plant growth was also evaluated against known pathogens strains.

2. Materials and Methods

2.1 Collection and preparation of plant material

Fresh leaves of *Cyathocline purpurea* L. were collected from the Garden of Indofil Industries Limited, Thane, India in October 2021. The leaves were completely air dried in the shade before ground into a fine powder in mixer and sieved using a 24-mesh sieve. The powdered leaves were maintained in an air-tight container at room temperature (30 ± 2 °C) and kept away from light until use.

2.2 Preparation of *Cyathocline purpurea* L leaf extract

The air-dried powder 40 of *Cyathocline purpurea* L leaves was taken and immersed in 400 mL of deionized water. The extraction process was performed via the ultrasonic assisted solvent extraction by placing the conical flask in a Sonicator at room temperature (32 ± 2 °C) for 30 min. The aqueous solution was filtered using muslin cloth first and then Whatman filter paper No. 42. The filtrate solution of *Cyathocline purpurea* L. leaf extract was kept in a refrigerator to be utilized for further use.

2.3 Green synthesis of ZnO nanoparticles (NPs)

After heating 200 millilitres of *Cyathocline purpurea* L. leaf extract at 60 ± 5 °C for 10 min, 100 millilitres of 0.1 M zinc

acetate dihydrate (Zn (CH3COO)2·2H2O) (2.0453 g of zinc acetate dihydrate was dissolved in 100 mL of deionised water) was added drop-by-drop to it under stirring at 1200 rpm, that resulted in cream-colored zinc oxide precipitate formation. For the complete reduction in zinc oxide, the reaction mixture was left for 2 hours. Change of colour from yellow to light brown and red to of-white indicates completion reaction. Then the precipitate was centrifuged at 16,000 rpm for 10 min at room temperature (32 ± 2 °C) and the residue was washed with deionised water followed by ethanol repeatedly in order to remove the impurities. The precipitate was dried overnight in an oven at 105 ± 2 °C. The obtained dried powder was calcined in a muffle furnace at 600 ± 10 °C for 2 h and the white powder of ZnO NPs was obtained after calcination as shown in Figure 1. The resulted powder was used for further characterization.

2.3 Characterization of ZnO nanoparticles (NPs)

2.4.1 UV-Visible spectroscopy

In order to study the optical characteristics of green synthesized ZnO NPs, a known amount of ZnO NPs (0.1 g) was dispersed in 10 mL of ethanol (96%). The absorption spectrum was recorded by using a UV-Vis (1900 series) UV-Visible spectrophotometer (Shimadzu, Japan) in between a wavelength scan of 200–800 nm [2-5].

2.4.2 Fourier Transform Infra-Red (FTIR) spectroscopy

FTIR analysis of ZnO NPs was employed to identify the functional groups (FGs) involved in biosynthesized ZnO NPs by using ATR (Bruker, Berlin, Germany). At a wavelength of 4000–400 cm−1, the FTIR spectra was scanned with a resolution of 4.0 cm−1 [8-12].

2.4.2 Scanning Electron Microscopy (SEM)

Scanning electron microscope (SEM) analysis was employed for determination of Surface morphology and the particle size using a (ZEISS) instrument. Thin film of the sample was prepared on a carbon coated copper grid by just dropping and spreading a very small amount of the sample on the grid [11-12].

2.4.3 X-Ray Diffraction (XRD)

The crystalline structure of ZnO NPs was analysed by an X-ray diffractometer (Bruker D8 DISCOVER, Bruker, Germany) with Cu-Kα radiation (λ = 1.54060 Angstrom). The relative intensity data were collected over a 2θ range of 25º–75º, 2θ values and relative intensities (I/Io) were determined from the chart, and the minerals of core materials were identified with JCPDS carts [10-12].

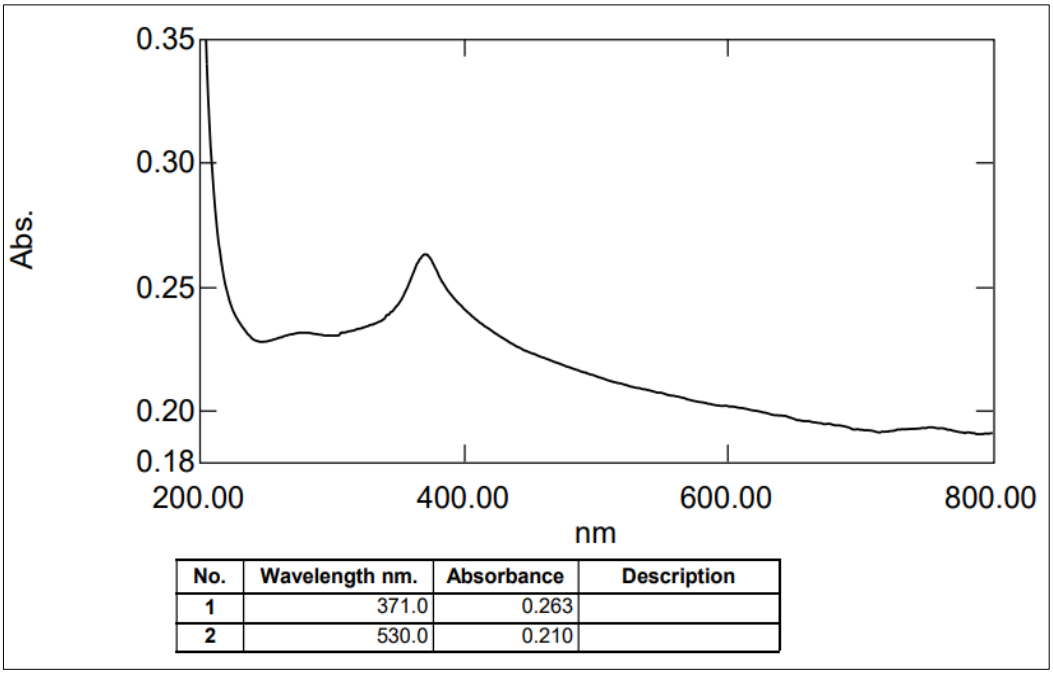
2.5 Antifungal activity of ZnO nanoparticles (NPs)

The antifungal activity of synthesised ZnO NPs was tested against the pathogen, *Alterneria solani* by dispersing the ZnO NPs in sterilised deionised water at two different concentrations (100 µL/mL and 200 µL) against the deionised water as a control [7, 11-12].

2.6 Effect of ZnO nanoparticles (NPs) on Seed germination and plant growth

The effect of synthesised ZnO NPs on seed germination and plant growth was determined by treating the seeds of *Zea maize* with ZnO NPs before sowing and the effect was concluded after seed germination and vital growth [7].

Fig 1: ZnO nanoparticles synthesised using *Cyathocline purpurea* L. leaf extract



No.	Wavelength nm.	Absorbance	Description
1	371.0	0.263	
2	530.0	0.210	

Fig 2A: UV- Visible spectra of ZnO nanoparticles in Zinc acetate and *Cyathocline purpurea* L. leaf extract solution after 2 hours

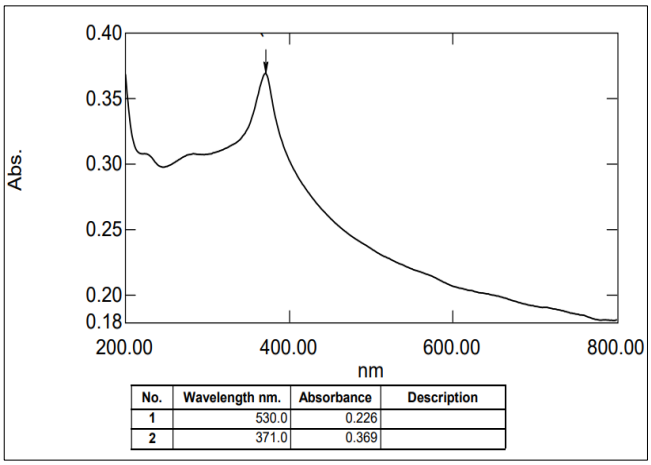


Fig 2B: UV- Visible spectra of green synthesised ZnO nanoparticles using *Cyathocline purpurea* L. leaf extract after drying at 105 °C for 2 hours

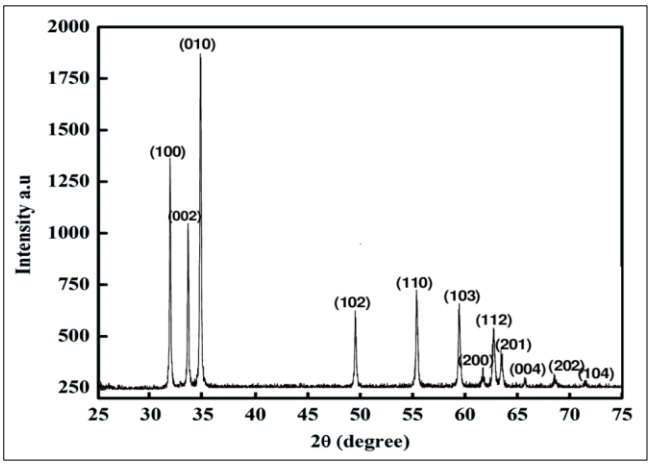


Fig 3: XRD pattern indicating formation of ZnO nanoparticles using *Cyathocline purpurea* L. leaf extract after drying at 105 °C for 2 hours

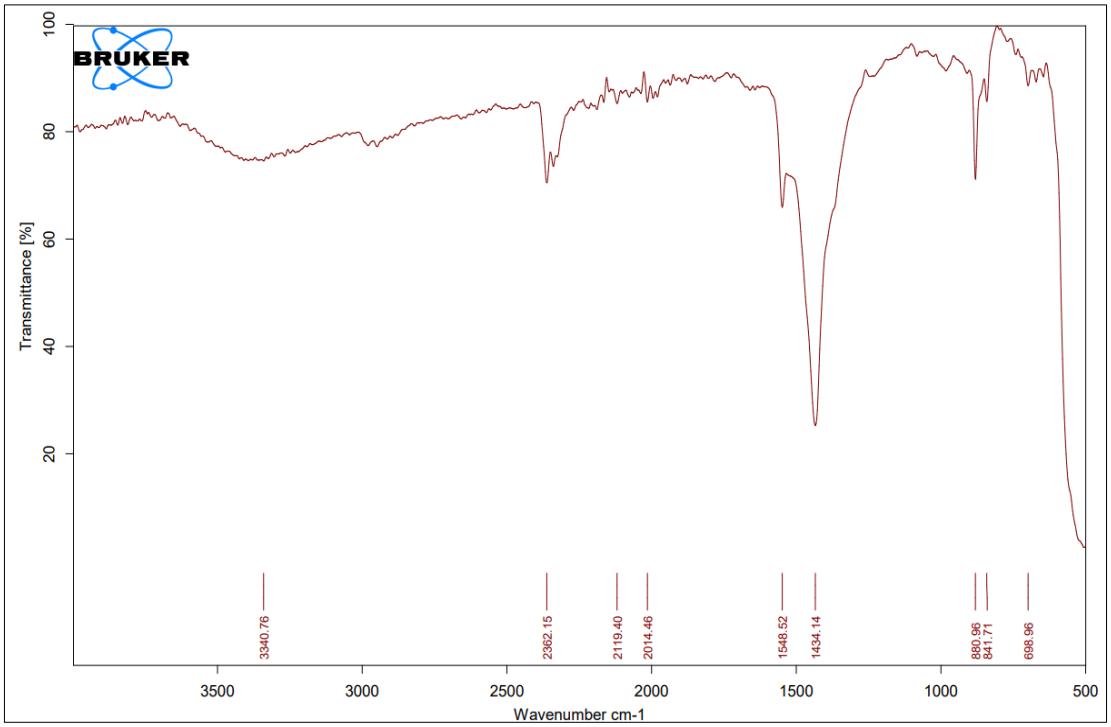
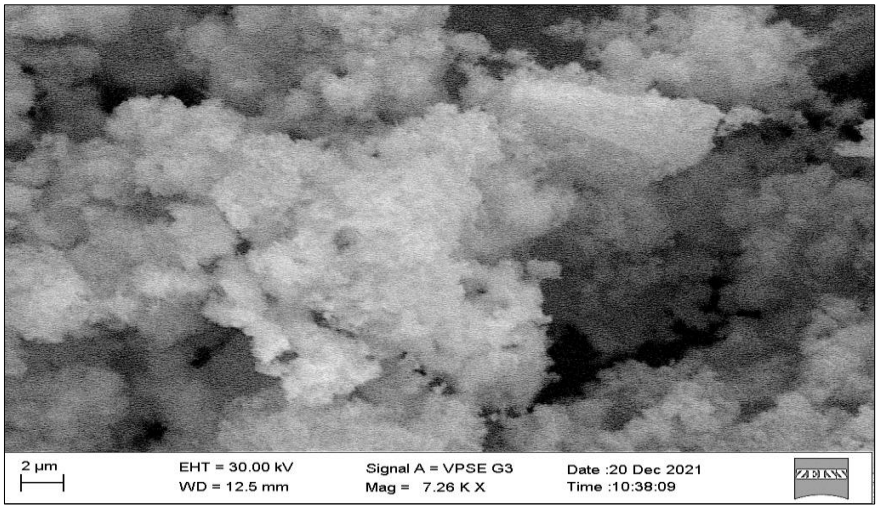
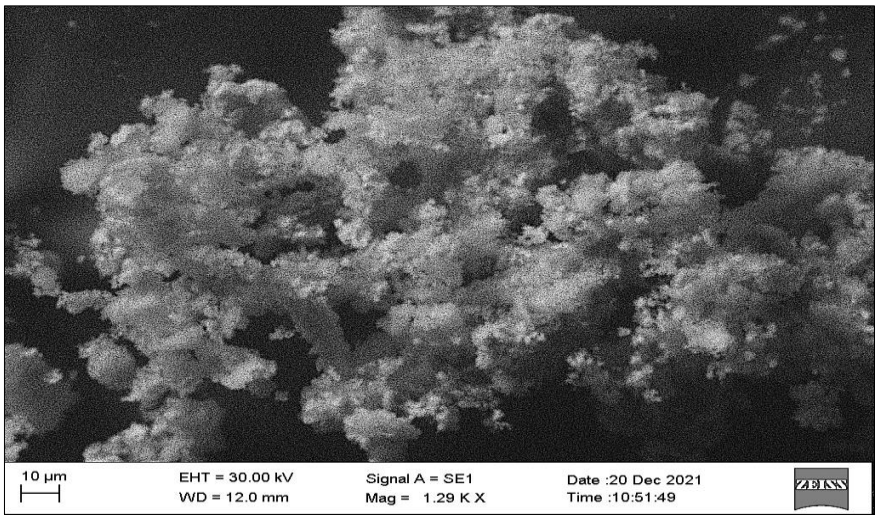


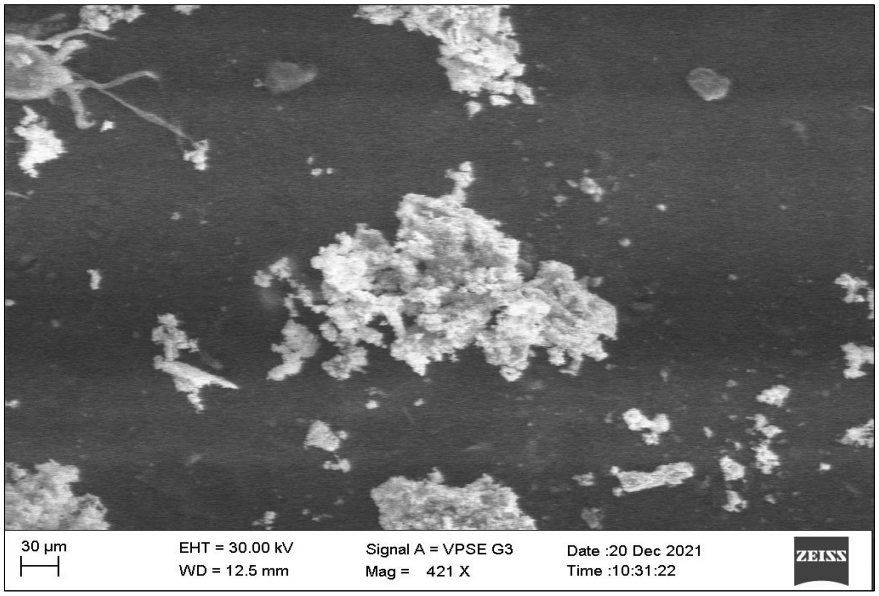
Fig 4: FTIR Spectrum of ZnO nanoparticles synthesised by *Cyathocline purpurea* L. leaf extract



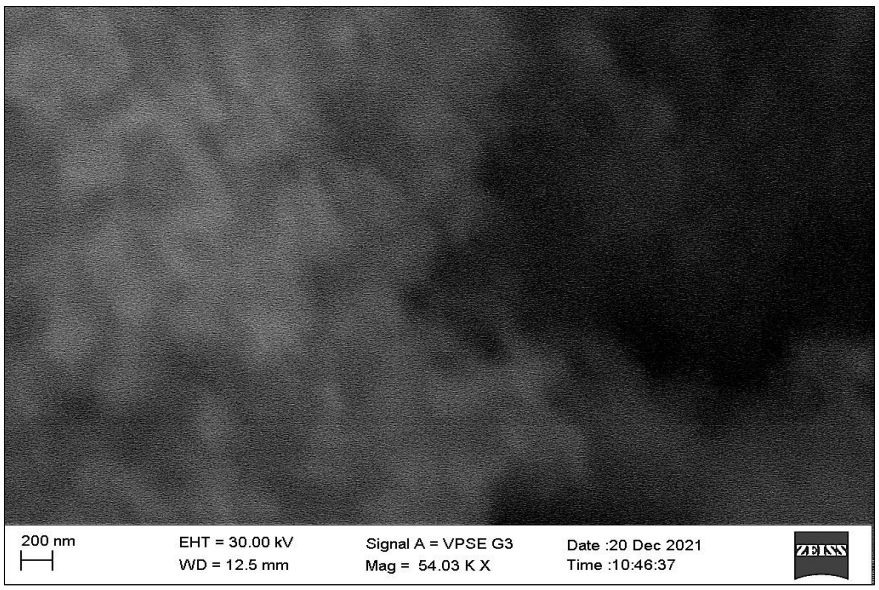
5A.



5B



5C



5D

Fig 5: SEM images of ZnO nanoparticles showing their morphology at different resolutions.

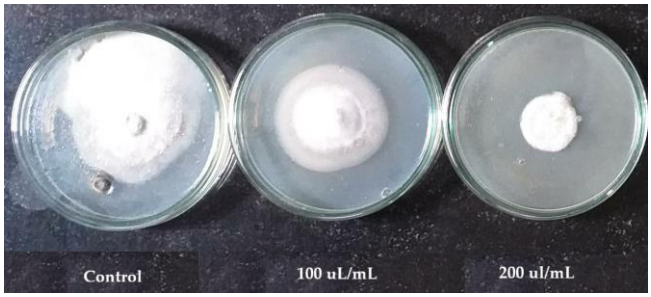


Fig 6: Antifungal activity of ZnO nanoparticles against *Alerneria solani*

7A

7B

Fig 7: Effect of ZnO nanoparticles on seed germination and plant growth

3. Result and Discussion

3.1 Uv Visible Spectroscopy

The reduction of zinc ions to ZnO nano particles was monitored using the UV- Visible spectrophotometer with respect to time. The size, shape, interaction between the particles, and the absorbed molecules present on the surface of the nanoparticles have a strong influence on the optical properties of metal nanoparticles. A sharp absorption band at 371 nm in UV- Visible spectrum of reaction mixture of Zinc acetate and *Cyathocline purpurea* L. leaf extract solution after 2 hours indicates formation of ZnO nanoparticles (Figure-2 A.). The synthesised were isolated by centrifugation, washed with deionised water, dried in thermostatically controlled oven at 105 °C for 2 hours and the UV spectrum was recorded to reconfirm the formation of ZnO NPs. The UV Spectrum shows sharp at 371 nm confirms formation of ZnO nanoparticles. As more than 20 phytochemicals identified in *Cyathocline purpurea* L. leaf extract, plays vital role in reduction of zinc ions in formation of ZnO nano particles [8-12].

3.2 Fourier Transform Infra-Red (FTIR) spectroscopy

The Figure-4. Shows FTIR Spectrum of ZnO nanoparticles synthesised by *Cyathocline purpurea* L. leaf extract after drying at 105 °C for 2 hours. From figure it was observed, that the bands are at 3340 cm^{-1}, 2362 cm^{-1}, 2119 cm^{-1}, 1548 cm^{-1}, 1434 cm^{-1}, 880 cm^{-1}, 841 cm^{-1} and 608 cm^{-1}. The FTIR spectrum of ZnO nanoparticles was recorded in the range of 500–4000 cm^{-1}. The peak in the region between 400 and 600 cm^{-1} is allotted to Zn–O stretching.

Absence of peaks in the region of 3500 and 2500 cm^{-1} indicated no characteristic OH and N-H stretching of aldehydes. The bands at 1434–1548 cm^{-1} correspond to amide I and amide II regions arising due to carbonyl stretching in proteins and that of 1400 to 1000 cm−1 correspond to methylene from the proteins in the solution and C-N stretching vibrations of amine. The peak located at 2362 cm^{-1} is considered for C≡C bond stretching vibrations in alkyne [8-12].

3.3 Scanning electron microscopy (SEM)

Figure. 5 shows SEM micrographs (images) of the ZnO NPs synthesized by the reduction of Zinc acetate by *Cyathocline purpurea* L. leaf extract. The images were recorded at magnification of 2 µm, 10 µm, 30 µm and 200 nm. Topographical view indicates that nanoparticles are spherical in nature, clustered together and surface of the aggregates seems to be rough. The SEM images also indicates that the synthesised nano particles are entirely pure and the leaves extract *Cyathocline purpurea* L. has tremendous capability of synthesising the ZnO NPs. From the SEM images it was observed, that the ZnO nanoparticles were agglomerated with a particle size ranging from below 100–190 nm [8-12].

3.4 X-Ray Diffraction (XRD)

The XRD pattern of biosynthesized ZnO NPs using *Cyathocline purpurea* L. leaf extract is illustrated in Figure 3. The XRD diffraction peaks existed at 2θ angles of 31.85º, 34.55º, 36.35º, 47.69º, 56.75º, 63.09º, 66.56º, 68.17º, 69.29º, 72.87º and 77.21º corresponding to lattice planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202), respectively [11]. These peaks are in accordance with those of (JCPDS card No: 36-1451), which is indicating the confirmation of the hexagonal wurtzite structure of ZnO NPs formation [10-12]. The average crystalline size of biosynthesized ZnO NPs was calculated using Deby-Scherrer's formula [11] and the Average crystalline size of the ZnO NPs was estimated to be 75-80 nm, which is derived from the full width at half maximum of the most intense peak corresponding to (101) plane located at 35.15º. Furthermore, the XRD pattern revealed no additional peaks other than the characteristic ZnO peaks, confirming the purity of the produced ZnO NPs. Additionally, the narrow and strong diffraction peak clearly indicates that the ZnO NPs have an optimal crystalline structure [8-12]. The XRD study confirmed the presence of even smaller particles than the SEM examination. The larger nanoparticles of ZnO (about 80-120 nm) in the sample result from the agglomeration of smaller nanoparticles, whose presence is confirmed by X-ray diffraction (XRD). The XRD method allowed for the identification of smaller sizes of nanoparticles. The agglomeration of smaller nanoparticles occurs as we are dealing with biological material.

3.5 Antifungal activity of ZnO nanoparticles (NPs)

Figure-6 shows antifungal activity of ZnO nanoparticles

against the *Alerneria solani*. The activity was performed using 100 µL/mL and 200 µL /mL concentration of the ZnO nanoparticles against the control as deionised water. From the study it was observed, that the minimum inhibitory concentration for *Alerneria solani* was 200 µL /mL [7].

3.6 Effect of ZnO nanoparticles (NPs) on Seed germination and plant growth

Figure-7 shows effect of synthesised ZnO NPs on seed germination and plant growth. 100 seeds of *Zea maize* were treated with 100 mg ZnO NPs and seed germination was checked on growth medium. From the figure it was observed, that the average percentage of seed germination was 90%. After germination, the seeds were sown in pots and observed for their vegetative growth. From the study it was observed, that all the seedlings became healthy, vigorous and acquired maximum height without any mortality [7].

4. Conclusion

From the study it was concluded that the *Cyathocline purpurea* L. leaf extract. showed excellent potential as reducing agents in the formation of ZnO NPs. Characterization of synthesised NPs was done by Structural and optical techniques like UV, FTIR, XRD and SEM analysis and confirmed the formation of high purity ZnO NPs. The synthesised ZnO NPs found effective in controlling the fungal strains like *Alerneria solani* at very low concentration, 200 µL /mL. As the plants like *Cyathocline purpurea* L., containing phenolic compounds are found to be more effective than that of others synthesis techniques of NPs and biological activities due to their diverse biochemical compositions. In conclusion, NPs synthesis using phytoextracts of medicinal plants could have useful antifungal, antiviral, and antibacterial applications in plants and animals. However, further studies will be required to validate the efficacy of these NPs in various field like Agrochemical, Agriculture, medical, engineering etc.

5. Abbreviations

ZnO: Zinc Oxide
NPs: Nano Particles
FTIR: Fourier Transform Infrared Spectroscopy
XRD: X-Ray Diffraction
SEM: Scanning Electron Microscopy
mL: Millilitres

6. Acknowledgment

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