Synthesis of Zinc Oxide Nanoparticles using Aqueous Extract of Sarcostemma Acidum and their Antibacterial Activities

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ABSTRACT:
Nanotechnology involves the production and use of nanoscale materials. Zinc oxide nanoparticles (ZnONPs) are considered one of many inorganic nanoparticles with applications in antibacterial, antifungal, antiviral, anti-inflammatory, wound healing, antiiflamatory disease, and eye treatment. Nanoparticles were produced using sarcostemma acidum stem extract and characterized by uv- visible spectroscopy, x-ray diffractometer (XRD), Fourier transform infrared spectroscopy, scanning electron microscopy (SEM).

Keywords: **Nanoparticals, Green Synthesis, X-ray diffraction.**
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1. Introduction

Natural products had been indispensably used by many cultures and traditions from thousands of years. They are intensively explored for their bioactive pharmacophores in modern pharmaceutical products.

Sarcostemma acidum sp. commonly known as Somlata is a member of family Asclepiadaceae and had a ritual importance in Indian mythology. Susrutha Samhita, mentioned twenty four varieties of Soma plants based on their habitat, name and medicinal properties. The plant is used in ritual practices by Indian sages from ages Somayaga. The plant is used by various tribal communities in Kerala and Tamil Nadu and different other parts of India. The soma is generally used for restoration and is neither hallucinogenic nor intoxicating.

Somlata (Sarcostemmaacidum Wight. & Arn.) is a perennial jointed trailing shrub with green, cylindrical, fleshy glabrous branches containing milk white latex, The shrub is distributed wildly in India, Sri Lanka, Pakistan and European countries. In India it is distributed in dry rocky places in Coast of Coromandel , Konkan, Deccan, Karnataka, Andhra Pradesh, Tamil Nadu , Western Ghats (Sahya mountain ranges)Maharashtra, Madhya Pradesh, Kerala, Bihar and Bengal[1-2].

The chemical constituent of the plant shows variation according to occurence and distribution. The plant occurring in warm places in India mainly contains sucrose Malic acid, Succinic acid, Alkaloids, Phytosterols, traces of tannin, Alpha and beta amyrins, Beta sito sterol Lupeol and lupeol acetate[3-4].

Nanotechnology involves the use of materials with nanoscale dimensions of 1-100 nm. Working with nanomaterials allows scientists to better understand biology. Green synthesis of nanoparticles greatly reduces the use of physical and chemical methods. Various chemical reactions have been proposed such as [5-6].

Recent advances in the development of nanotechnology, especially the ability to prepare large nanoparticles of all sizes and shapes, have led to the creation of new biocides. Nanomaterials are called “the miracle of modern medicine.” It is claimed that the antibacterial device can kill six different types of bacteria, while the nano device can kill approximately 650 cells. Metal nanoparticles have many functions that cannot be found in different phases [7] and are widely used for medicals and protective purposes due to their unique catalytic, opticles, electrical, magnetic and antibiotic properties [8-9] in this study [10]. The synthesis of nanoparticles in the size of 10-100 nm has been the focus of research and interest because it can be used in many scientific and technological fields. In the last few years, iron oxide nanoparticles have become widely used in medicine.

Metal oxide nanoparticles have environmental applications as they can help or eliminate toxic and harmful chemicals in the environment by acting as a catalyst [11]. Among metal oxide nanoparticles, zinc oxide is of interest due to its much application in many fields such as optics, piezoelectric, magnetic and gas sensing. In addition to these properties, ZnO nanostructures also have high catalytic activity, strong adsorption capacity and are increasingly used in sunscreen production [12], ceramic and rubber processing, wastewater treatment, and become bactericidal [13-14]. Various physical and chemical methods have been used to produce large metal nano particles in a short time. Chemical treatment causes some chemicals to adsorb to the surface, which may cause adverse effects in medical use.

They can also be used in medical applications such as drug delivery, antioxidants and other activities and disease detection. ZnO NPs have been reported to have antibacterials activity against various bacteria such as Salmonella, Staphylococcus aureus and Escherichia coli using the disk diffusion method [15-16]. ZnO NPs have been reported to have high antibacterial activity against infectious disease [17]. Additionally, the antibacterial activity of synthesized ZnO NPs against different bacteria was also evaluated. The aim our research is to produce ZnO NPs from the Sarcostemma acidum plant and anti inflammatory properties.

2. MATERIAL AND METHODS

2.1. Plant collection

The stem of Sarcostemma Acidum was collected from campus of Govt. girls college Rewa (M.P). It is a medicinally important plant. Fresh stem were harvested all over the year.

2.2 Preparation of the plant extract.

5 g of stem were washed with running tap water followed by distill water and then cut, and soaked in a 250 ml Erlenmeyer flask containing 100 ml distill. The solution was boiled at 70 °C for 8 min. The stem extract was allowed to cool to room temperature, filtered through Whatmann number-1 filter paper, and the filtrate was stored for further experimental use.

2.3. Synthesis of ZnO NPs

1 mM Zinc acetate $[Zn(O_2CCH_3)_2(H_2O)_2]$ was dissolved in 100 ml distilled water and kept on stirring for 20 minutes [18]. Then 20 mL of NaOH solution was added dropwise followed by stirring the solution for 1 hour. Add 25 mL of plant extract into the solution and then left for incubation for 1 hour. After incubation a yellow coloured solution appeared. After incubation the solution was stirred for 2 hour and then centrifuged. The precipitate was separated from the reaction solution by centrifugation at 8000 rpm at 60 °C for 15 min and pellet was collected. Pellet was dried using a hot air oven operating at 80 °C for 2 h and preserved in air-tight bottles for further studies.

2.4. Characterization of biosynthesized ZnO NPs

Synthesizing ZnO NPs by various analytical technique were Optical properties of ZnO NPs were characterized based on UV absorption spectra with the wavelength range of 200–800 nm. X- ray diffraction (XRD) analysis was performed on X-ray diffractometer (miniflex 600) operating at 30 kV and 40 mA. A X- ray crystallography is used for the determination of crystal density, purity and size of the nanoparticles 2 mg of ZnO NPs was mixed with 200 mg of potassium bromide (FTIR grade) and pressed into a pellet for FTIR characterization. The sample pellet was placed into the sample holder and FTIR spectra were recorded in FTIR spectroscopy at a resolution of 4 cm^{-1}[19] . Shape and size were analyzed by using SEM.

2.5. Antibacterial activity of synthesized ZnO NPs

The antibacterial activity of synthesized ZnO NPs was performed by agar disc diffusion method against infection pathogens. Fresh overnight culture of each strain was swabbed uniformly onto the individual plates. The 25 µl, 50 µl and 75 µl of ZnO NPs solution impregnated disc were placed onto the plates and incubated for 24 h at 37 °C. Commercial antibiotic discs were placed as control. After incubation period got over, different levels of zonation formed around the disc was measured.

3. RESULTS AND DISCUSSIONS

3.1. Visual observation

ZnO NPs have attracted great attention because of their superior optical properties. Visual color change is the preliminary test for nanoparticle synthesis represents the synthesis of ZnO.NPs synthesized using freshly prepared *sarcostemma acidum* stem extract. Color change from half white to pale yellow represents the synthesis of ZnO NPs.

Fig.:1 Before and After formation of Nps

3.2 UV Analysis

UV-visible spectroscopy is usually conducted to confirm the synthesis of ZnO NPs. Conducting electrons start oscillating at a certain wavelength range due to surface Plasmon resonance (SPR) effect. (Fig. 2) represents the UV-visible spectra of freshly prepared ZnO NPs. Peaks obtained at 371 nm clearly demonstrated that presence of ZnO Nps in the reaction mixture.

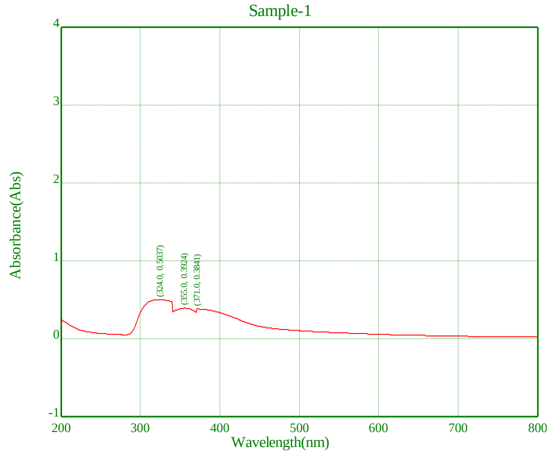


Fig.2: UV Analysis of ZnO Nanoparticle prepared by Sarcostemma Acidum

3.3. SEM

SEM analysis is done to visualize shape and size of nanoparticle. FEI Quanta 250 Scanning electron microscope was used to determine the shape ZnO NPs . SEM images were seen in different magnification ranges like 2 µm–200 nm which clearly demonstrated the presence of spherical shaped nanoparticle with mean average diameter of 70 nm [20]. The presence of carbon in trace amount indicates the involvement of plant phytochemical groups in reduction and capping of the synthesized ZnO NPs [21] (Fig. 3)

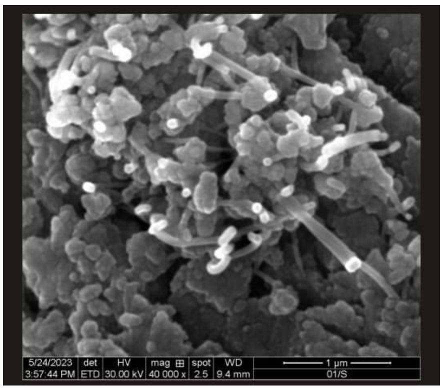


Fig.3: SEM analysis of ZnO Nps

3.4. X-ray diffraction (XRD)

XRD Spectra provides an insight about the crystallinity of nanoparticle. (Fig.4) represents XRD Spectra of ZnO NPs synthesized using stem extract. Size of the nanoparticle was calculated using Debye–Scherrer equation. Joint Committee on Powder Diffraction Standards (JCPDS) was used as a reference to assign the lattice planes according to the peaks obtained. Average size of the synthesized nanoparticle was found to be 37.67 nm.

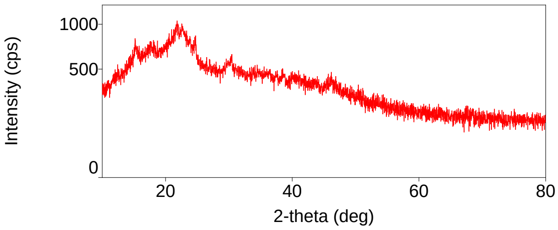


Fig.4: Graph of X- ray Diffraction

Table 1:- Parameter calculation for average size calculation for nanoparticles

2 ϴ	h k l	FWHM	D (nm)
15.590	1 1 0	0.390	5.674
17.070	0 1 2	0.540	5.071
20.331	1 0 3	0.177	4.388
21.050	2 0 2	0.250	4.108
22.984	0 0 4	0.272	3.917
23.590	3 0 1	0.650	3.685
26.540	2 0 4	0.600	3.512

$D = K\lambda\beta cos\theta$where, D- particle size in nm, λ- X-ray wavelength, β- FWHM, θ- Bragg's angle of reflection.

3.5. FT-IR analysis

Substance-specific vibrations of the molecules lead to the specific signals obtained by IR spectroscopy. FT-IR spectra and functional group involved in ZnO NP synthesis illustrated peak in the range of 500–4000 cm $^{-1}$ (Fig. 5 (a) and (b) and Table 2-3). Broad peak obtained at 3545.28 corresponded to O-H stretching vibrations, peak in the range of 3479.70 to 3315.74 corresponded to N-H stretching, peaks from 3122.86 to 2742.87 corresponds to C-H stretching, weak peaks from 2191.21 to 2650.28 corresponds of C ≡ C alkyne stretching. Broad peaks 1523.82 corresponds to N-O stretching and 1408.08 corresponds to S=O stretching of sulfonyl chloride.

Table 2:- FTIR Spectral peaks of Zinc Nanoparticles

S .No	Absorption peak	Functional group
1	3545.28	O-H Streching
2	3479.70	N-H Streching
3	3122.86	C-H Streching
4	2191.21	C ≡≡ C Streching
5	1523.82	N-O Streching
6	1408.88	S=O Streching

Plant extract itself had shown a broad range at 3581.93 corresponds to O-H streching ,sharp peaks obtained at 3045.70 corresponds to C-H stretching 3387.11 corresponds to N-H stretch of amide groups. Weak peaks from 1703.20 to 1747.57 corresponds to ester (RCOOR) and (RCOOH)

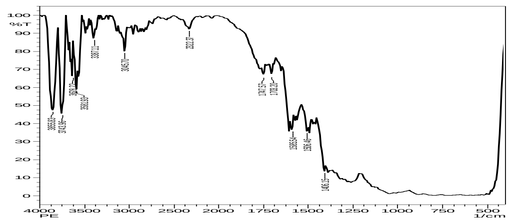
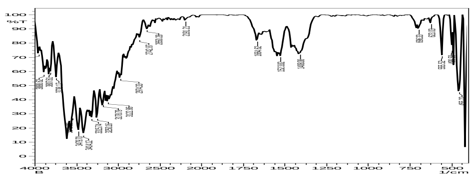


Fig. 5 (a) Functional groups involved in ZnO Nps



(b) Plant extract analyzed by FTIR

Table 3:- FTIR Spectral peaks of plant extract

S.No	Absorption Peaks	Functional Groups
1	3581.93	O-H
2	3387.11	N-H
3	3045.70	C-H
4	1703.20	RCOOR & RCOOH

3.6. TEM Analysis

Transmission electron microscope (TEM) study was performed to estimate the shape and size of the nanoparticles. ZnNPs synthesized were mostly seen in spherical shape and size varied from 40-90 nm when analyzed.

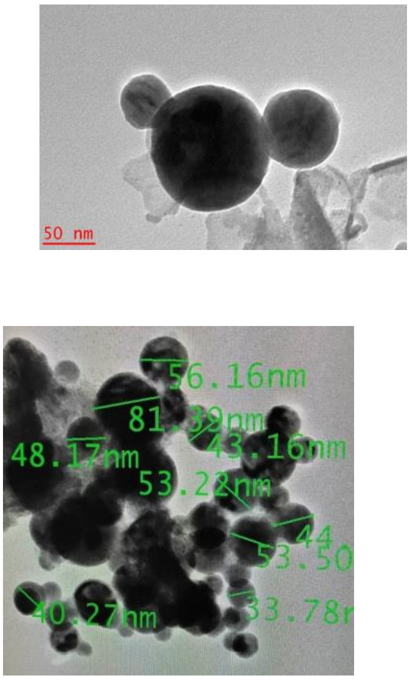


Fig 6: The Image of Zno nanoparticle synthesized using an aqueous extract of S.Acidium .

3.7. Antibacterial activity

Agar disc diffusion technique was adopted to perform the assay. Anti-bacterial effect of ZnO NPs was visualized against infection pathogen like *E.coli,*. Ampicillin disc was used as a control. Results clearly demonstrate that the nanoparticle showed antibacterial effect in a dose-dependent manner (Fig. 7 and Table 4).

Maximum zone of inhibition was observed against a gram- bacterium (*E. coli*). Minimum zone of inhibition was observed against a gram + bacteria (*Enterococcus sp.*).

Different mechanism of action of nanoparticle against gram + and gram –bacteria has been already reported in previous literature because of difference in structural composition.

Zone of inhibition obtained using nanoparticle was much lower than the standard disc used which depicts the need of further engineering of nanoparticle to obtain desirable effects. The entire tests were done in triplicate. The zinc oxide nanoparticles are inhibit the microbial growth in *in-vitro* antimicrobial activities [22-24].

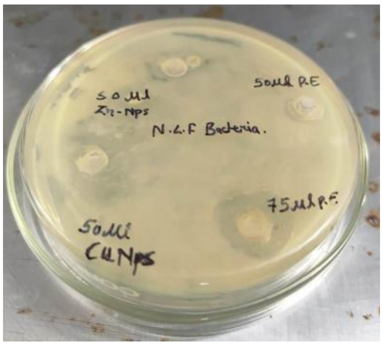


Fig: 7 – Antibacterial Activities of Zn oxide nanoparticles

Table 4:-Antibacterial activity of ZnO Nps

Bacteria	50µl	75µl
L.F Bacteria	20 mm	No results
N.L.F Bacteria	No results	15 mm

Table 5:-Antibacterial activity of S.Acidum Plant Extract

Bacteria	Ampicillin	Tetracyclin	Gentamycin
L.F Bacteria	No results	20 mm	No results
N.L.F Bacteria	No results	25 mm	No results

Table 6:-Antibiotic activity against pathogenic bacteria.

Bacteria	25µl	50 µl	75µl
L.F Bacteria	No results	No results	25 mm
N.L.F Bacteria	No results	No results	No results

4. Conclusion

Synthesis of nanoparticles used in this experiment is found to be eco-friendly, non-toxic and less usage of chemicals compared to physical and chemical method. The presence of phytochemicals in the leaf extract itself helps in the synthesis of metal oxide nanoparticle by inducing oxidation and reduction reaction. The functional groups of phytochemicals induce the nanoparticle synthesis were amines and alkanes that are widely seen in secondary metabolites such as terpenoids, flavonoids, alkaloids, etc.

A preliminary confirmation, the rapid synthesis of ZnO NP was measured using the UV-Visible spectroscopy at a maximum absorbance of 371 nm. Further the XRD analysis proved the crystalline nature of the ZnO NP, while the The TEM analysis of ZnO NP demonstrated the size approximately in the range of 40–90 nm. Also the anti-bacterial activity of ZnO NPs has proved that these can be used as potent anti-bacterial agent against infection.

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