GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER-DOPED ZINC HYDROXIDE NANOPARTICLES USING THE LEAF EXTRACT OF *TRIDAX PROCUMBENS*

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Abstract. Ag-doped zinc hydroxide nanoparticles were synthesized using the leaf extract of *Tridax procumbens* through a chemical co-precipitation method. The synthesized sample was characterized using powder X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDAX), ultraviolet spectroscopy (UV), and Fourier transform infrared spectroscopy (FTIR). XRD analysis confirmed the preferential growth of Ag-doped zinc hydroxide nanoparticles with an average width of 42 nm. SEM imaging revealed that the nanoparticles exhibited a spherical morphology. EDAX spectra confirmed the elemental composition of the sample, while FTIR spectroscopy was used to analyze the stretching and bending frequencies of molecular functional groups. The UV spectrum determined the band gap energy of the nanoparticles to be 4.5 eV. Antibacterial studies demonstrated that the antimicrobial activity of the synthesized nanoparticles increased proportionally with concentration. Additionally, MTT assay results indicated significant cytotoxic activity against MDA-MB-231 cells, with Ag-doped nanoparticles exhibiting maximum effects on cell viability at a low concentration of 20 µg/mL, surpassing the effects observed with *Tridax procumbens* extract alone.

Key words: Ag-doped, *Tridax procumbens*, nanoparticle, structural characterization, biomedical applications.

INTRODUCTION

Zinc hydroxide ($Zn(OH)_2$) is an inorganic compound naturally found in three rare mineral forms: wülfingite, ashoverite, and sweetite [2]. It is classified as an

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amphoteric hydroxide, which can react with acids and bases [4]. While generally insoluble in water, it dissolves in strong acids to form soluble zinc salts [3]. Zinc hydroxide finds applications in medicine as an absorbent agent in surgical dressings, aiding in wound fluid absorption [34]. It is also used as an intermediate in the industrial production of pesticides and pigments [8]. Silver (Ag), with atomic number 47, is a lustrous, soft, white metal known for its exceptional ductility and malleability. It possesses the highest electrical conductivity among metals, though its high cost limits its widespread use in electrical applications. Chemically, silver is relatively inert but reacts with nitric acid and concentrated sulfuric acid. It occurs in its native form and in minerals like chlorargyrite (horn silver) and argentite. While silver does not oxidize in air, it tarnishes upon exposure to hydrogen sulfide, forming black silver sulfide. Silver has diverse applications, including photography, medicine, electronics, and catalysis. Its antimicrobial properties make it particularly valuable in medical treatments, antibacterial coatings, and wound care. Recent industrial demand, particularly in solar panel manufacturing and electronics, has driven significant price increases, with silver outperforming gold in market trends.

Tridax procumbens, a medicinal plant, produces secondary metabolites with promising anticancer properties against human lung (A549) and liver (Hep G2) cancer cell lines. Leaf extracts of *T. procumbens* have demonstrated significant cytotoxic effects, highlighting their potential in developing novel anticancer agents. Traditionally, *T. procumbens* has been used in Indian medicine for wound healing, anticoagulant, antifungal, and insect-repellent applications. Local healers have employed it to treat skin conditions such as boils, blisters, and cuts [32]. Doping nanomaterials presents a versatile approach to enhancing their properties while maintaining high surface areas. By incorporating different elements, researchers can fine-tune electronic, optical, photochemical, photoelectrochemical, and photocatalytic characteristics to meet specific needs. In this study, silver-doped zinc hydroxide nanoparticles (Ag-doped $Zn(OH)_2$ NPs) were synthesized using *T. procumbens* leaf extract as a green synthesis method. The synthesized nanoparticles were characterized using X-ray diffraction (XRD), scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), energy-dispersive X-ray spectroscopy (EDS), and UV-visible spectroscopy. Additionally, their antibacterial activity was evaluated. Nanotechnology has revolutionized biomedical, environmental, and electronic applications, with metal-doped nanoparticles playing a key role in enhancing material properties. Among these, Ag-doped $Zn(OH)_2$ NPs have garnered significant attention due to their potent antimicrobial, optical, and cytotoxic properties [9, 12, 25]. Zinc hydroxide is well-known for its biocompatibility and catalytic activity, while silver incorporation further amplifies its antibacterial and anticancer efficacy [18, 29]. In this research, *T. procumbens* leaf extract was used for the green synthesis of Ag-doped $Zn(OH)_2$ NPs, offering an eco-friendly and cost-effective alternative to conventional chemical synthesis. The structural characterization was conducted using XRD and

EDS, while UV-visible spectroscopy determined optical properties. The antibacterial efficacy of the nanoparticles was assessed against *Escherichia coli* and *Klebsiella* species, and their cytotoxic effects were examined using the MTT assay on MDA-MB-231 breast cancer cells [5, 15]. This study aims to investigate the structural, optical, and biological properties of Ag-doped $Zn(OH)_2$ NPs to evaluate their potential applications in nanomedicine, antimicrobial coatings, and cancer therapy. The findings contribute to the growing interest in green nanotechnology for developing biofunctional materials with enhanced therapeutic and environmental benefits [22, 37].

MATERIALS AND METHODS

Silver-doped zinc hydroxide nanoparticles were synthesized using the leaf extract of *Tridax procumbens* via the chemical co-precipitation method. Zinc sulfide and sodium hydroxide were accurately weighed, considering their purity, and separately dissolved in *T. procumbens* leaf extract to achieve a 0.1 M concentration. Once a homogeneous solution was obtained, the reagents were combined under continuous magnetic stirring. A 0.1 M silver nitrate solution was then added gradually to the mixture while maintaining constant stirring. The resulting precipitate was separated from the reaction mixture and thoroughly washed multiple times with distilled water and ethanol. Finally, the wet precipitate was dried and finely ground using an agate mortar to obtain a uniform powder sample.

TESTS CONDUCTED

X-ray diffraction (XRD) is a highly effective technique for determining the crystallite size of powder samples. This method relies on accurately measuring the broadening of diffraction peaks. In this study, the XRD line broadening method was employed to estimate the crystallite size of the synthesized powder. The surface morphology of the samples was analyzed using scanning electron microscopy (SEM), while the elemental composition was examined through energy-dispersive X-ray (EDAX) analysis. Fourier-transform infrared (FTIR) spectroscopy was conducted using a SHIMADZU FTIR 8400S spectrometer with the KBr pellet method. Ultraviolet-visible (UV-Vis) spectroscopy was performed in absorbance mode within the wavelength range of 200–800 nm. Additionally, antibacterial studies were carried out to evaluate the antibacterial properties of the sample.

CELL LINE PROCUREMENT AND MAINTENANCE

The MDA-MB-231 metastatic breast cancer cell line was obtained from the National Centre for Cell Sciences (NCCS), Pune, and used in the current study. The

cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10 % fetal bovine serum (FBS), and an antibiotic solution containing streptomycin (100 mg/mL) and penicillin (100 U/mL). Cells were maintained as a monolayer in culture flasks at 37 °C and pH 7.4, in a humidified atmosphere with 5 % CO_2. Once the cells reached confluence, they were trypsinized using a trypsin-ethylenediaminetetraacetic acid (EDTA) solution and used for subculturing and experimental analysis.

CELL VIABILITY ASSAY

To assess the cytotoxic potential of Ag-doped nanoparticles and *T. procumbens* (tridase) on MDA-MB-231 cells, a 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide (MTT) assay was performed. Briefly, the cells were seeded at a density of 1×10^4 in 96-well microtiter plates and incubated at 37 °C for 24 hours. The following day, the cells were subcultured and treated with various concentrations of Ag-doped nanoparticles and tridase (20, 40, 80, 100, 150, and 200 µg/mL), with control cells maintained in a normal medium. After 24 hours of incubation, 100 µl of MTT solution was added to each well and incubated for 4 hours. The resulting dark purple formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and the absorbance was measured at 570 nm using an enzyme-linked immunosorbent assay (ELISA) reader. The percentage of cell viability or growth inhibition was calculated using the formula:

$$\% \, viability = \frac{(A_T - A_B)}{(A_C - A_B)} \cdot 100 \quad (1)$$

A_T serves as absorbance of treated cells; A_B serves as absorbance of blank and A_C serves as absorbance of control.

RESULTS AND DISCUSSION

XRD – PARTICLE SIZE CALCULATION

The X-ray diffraction (XRD) patterns of the synthesized silver-doped zinc hydroxide nanoparticles using *T. procumbens* leaf extract are presented in Table 1 and Fig. 1. XRD analysis confirms that the nanoparticles are crystalline and in the nanoscale range. The broadening of X-ray diffraction peaks indicates the fine particle nature of the sample. The crystallite size of the synthesized silver-doped zinc hydroxide nanoparticles was determined using the Scherrer equation.

$$D = \frac{0.9\lambda}{\beta \cos\theta} \tag{2}$$

where λ represents the wavelength of X-rays, β denotes the half-width at half maximum, and θ indicates the diffraction angle.

The average grain size of the particles is determined to be 42 nm [1].

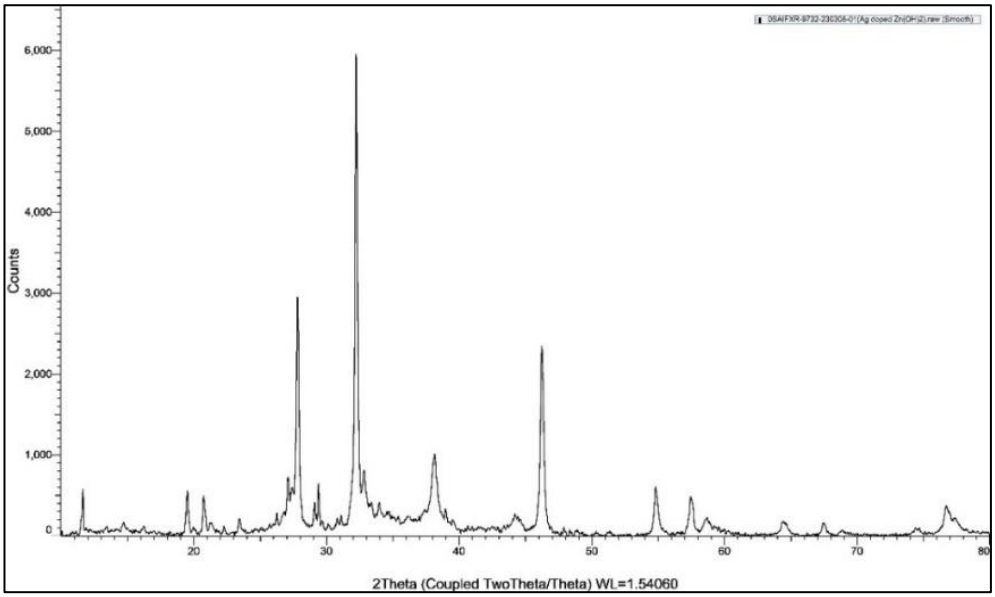

Fig. 1. XRD pattern of Ag-doped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens*.

The X-ray Diffraction (XRD) analysis of silver-doped zinc hydroxide nanoparticles synthesized using *T. procumbens* leaf extract confirms the formation of a crystalline structure. The obtained diffraction peaks closely match the JCPDS standard 26-0952, indicating that the synthesized material retains the characteristic phase of zinc hydroxide. The diffraction angles (2θ) observed at 19.506°, 20.740°, 27.822°, 32.237°, 38.159°, and 46.230° correspond to the expected reflections, with slight shifts compared to the standard values. These minor variations in D-spacing suggest possible lattice distortions due to silver doping, which may introduce strain or induce slight structural modifications. The presence of sharp and intense peaks indicates a highly crystalline nature, while any observed peak broadening could be attributed to the nanoscale dimensions of the particles. The XRD analysis confirms that silver incorporation does not significantly alter the primary phase of zinc hydroxide, ensuring the retention of its structural integrity.

Table 1

Experimental and standard diffraction angles of Ag-doped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens*

Experimental		Standard – JCPDS 26-0952	
Diffraction angle (2θ in degrees)	D-spacing (Å)	Diffraction angle (2θ in degrees)	D-spacing (Å)
19.506	4.54712	19.028	4.66
20.740	4.27943	20.687	4.29
27.822	3.28783	27.856	3.20
32.237	2.77463	32.386	2.7620
38.159	2.35654	38.505	2.3360
46.230	1.96215	46.991	1.9320

XRD – DISLOCATION DENSITY

Dislocation density is defined as the length of dislocation lines per unit volume of the crystal. In materials science, dislocations are crystallographic defects or irregularities in the crystal structure. The presence of dislocations can significantly affect the material properties, as their movement is hindered by other dislocations within the sample. Therefore, a higher dislocation density typically correlates with increased hardness of the material.

X-ray line profile analysis is commonly used to determine dislocation density. The dislocation density (ρ\rhoρ) can be calculated using the following equation:

$$\delta = 1/D^2 \tag{3}$$

where δ is the dislocation density, and *D* is the crystallite size.

Results of the dislocation density calculated from the formula are given in Table 2. This equation helps in estimating the dislocation density by analyzing the broadening of X-ray diffraction peaks, which occurs due to the presence of dislocations in the crystal lattice.

The structural parameters of silver-doped zinc hydroxide nanoparticles were analyzed using X-ray Diffraction (XRD), and the calculated crystallite size, dislocation density, and number of unit cells indicate significant variations due to silver doping. The crystallite size, determined using Scherrer's equation, ranges from 20.81 nm to 49.46 nm, with the largest size observed at 19.506° and the smallest at 38.159°. This variation suggests that silver incorporation affects nanoparticle growth, possibly due to lattice strain and synthesis conditions.

The dislocation density, which represents crystal defects per unit area, follows an inverse trend with crystallite size, where the highest dislocation density (2.309×10^{15} m^{-2}) corresponds to the smallest crystallite size at 38.159°, indicating increased structural defects. Conversely, the lowest dislocation density (4.088×10^{14} m^{-2}) at 19.506° suggests better crystalline quality. Similarly, the number of unit cells is highest (0.310×10^5) at 19.506°, where larger crystallites contain more unit cells, and lowest (0.023×10^5) at 38.159°, corresponding to the smallest crystallites with the highest defect density. These findings align with previous studies [10, 20] which report that metal doping often induces lattice distortions, reducing crystallite size and increasing defect concentration. The increasing dislocation density with decreasing crystallite size is also consistent with the Williamson-Hall analysis [30]. Overall, the results confirm that silver doping influences the structural properties of zinc hydroxide nanoparticles by introducing lattice strain, modifying crystallite size, and increasing defect density. These structural variations are crucial in determining the potential applications of the synthesized nanoparticles in catalysis, biomedical fields, and electronic devices.

Table 2

Dislocation density and number of unit cells from XRD of Ag-doped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens*.

2θ (deg)	Particle size D (nm)10^{-9}	Dislocation density (m^2) $\times 10^{14}$ $\delta = 1/D^2$	Number of unit cells $\times 10^5$
19.506	49.45778001	4.08819E+14	0.310702064
20.740	43.19262452	5.36020E+14	0.206951732
27.822	42.19095543	5.61774E+14	0.192884960
32.237	41.97965781	5.67443E+14	0.190001472
38.159	20.80833181	2.30954E+15	0.023139399
46.230	30.96056942	1.04323E+15	0.076219677

From the tabulated data and the observations in Figs. 2, 3, and 4, it is evident that dislocation density is inversely proportional to particle size and the number of unit cells. As particle size and the number of unit cells decrease, the dislocation density increases. The number of unit cells can be calculated using the following equation:

$$n = \pi \left(\frac{4}{3}\right) \cdot \left(\frac{D}{2}\right)^3 \cdot \left(\frac{1}{V}\right) \quad (4)$$

where D is the crystallite size, and V is the cell volume of the sample.

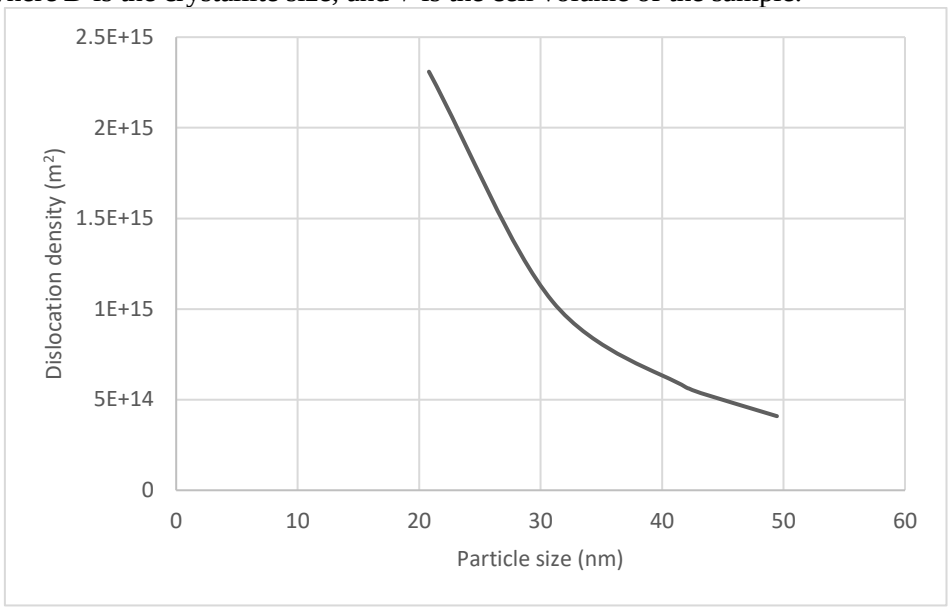

Fig. 2. Particle size vs dislocation density curve of Ag-doped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens*.

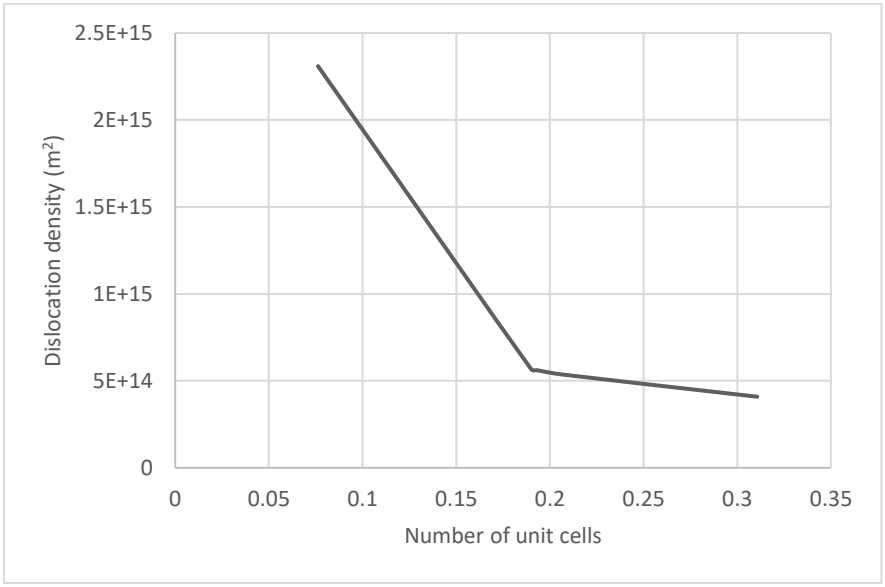

Fig. 3. Number of unit cells vs dislocation density curve of Ag-doped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens*.

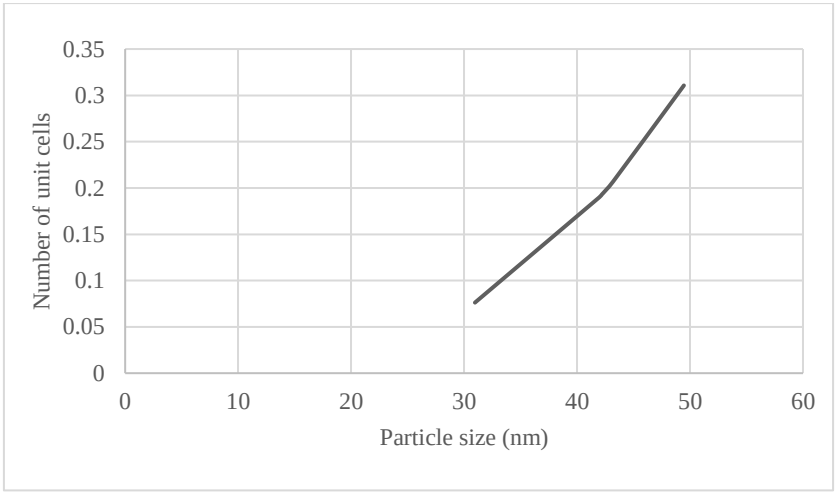

Fig. 4. Particle size vs number of units cell curve of Ag-doped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens*.

XRD – MORPHOLOGY INDEX

A XRD morphology index (*MI*) is calculated from *FWHM* of XRD data using the relation

$$MI = \frac{FWHM_{\mathrm{h}}}{FWHM_{\mathrm{h}} + FWHM_{\mathrm{p}}} \tag{5}$$

where *MI* is the morphology index, $FWHM_{\mathrm{h}}$ is the highest *FWHM* value obtained from peaks and $FWHM_{\mathrm{p}}$ is the value of a particular peak's *FWHM* for which *MI* is to be calculated.

The relation between morphology index and particle size is shown in Table 3.

The analysis of silver-doped zinc hydroxide nanoparticles based on full width at half maximum (*FWHM*), particle size, and morphology index provides valuable insights into their structural and morphological characteristics. The crystallite size, calculated using Scherrer's equation, ranges from 20.81 nm to 49.46 nm, with the largest particle size observed at 49.46 nm for an *FWHM* value of 0.002843 radians, and the smallest at 20.81 nm for an *FWHM* of 0.007048 radians. The inverse relationship between *FWHM* and particle size suggests that broader peaks correspond to smaller crystallites due to increased lattice strain and structural defects. The morphology index, which indicates the shape anisotropy of the nanoparticles, varies from 0.420 to 0.642, with higher values signifying more uniform and well-defined morphologies. The highest morphology index (0.642) is observed for the largest crystallite size at 49.46 nm, suggesting a more regular shape,

whereas the lowest index (0.420) for the smallest crystallite size at 20.81 nm indicates increased structural distortions and shape irregularities.

These findings are consistent with previous research on metal-doped zinc hydroxide nanoparticles, where doping has been shown to influence crystallite size and morphology due to strain effects and atomic substitutions [9, 26]. Additionally, the decreasing morphology index with decreasing particle size aligns with studies [17], which suggest that smaller crystallites tend to exhibit increased surface roughness and irregular shapes. Silver incorporation changes the size and shape of zinc hydroxide nanoparticles, impacting their use in optoelectronics, catalysis, and biomedicine.

Table 3

Morphology index of Ag-doped zinc hydroxide nanoparticles
using the leaf extract of *Tridax procumbens*

FWHM (β) radians	Particle size *D* (nm) $\times 10^{-9}$	Morphology index (unitless)
0.002843	49.45778001	0.642533873
0.003262	43.19262452	0.610406327
0.003384	42.19095543	0.60163229
0.003437	41.97965781	0.597948731
0.007048	20.80833181	0.420362433
0.004867	30.96056942	0.512226899

It is observed that *MI* has a direct relationship with particle size (Fig. 5).

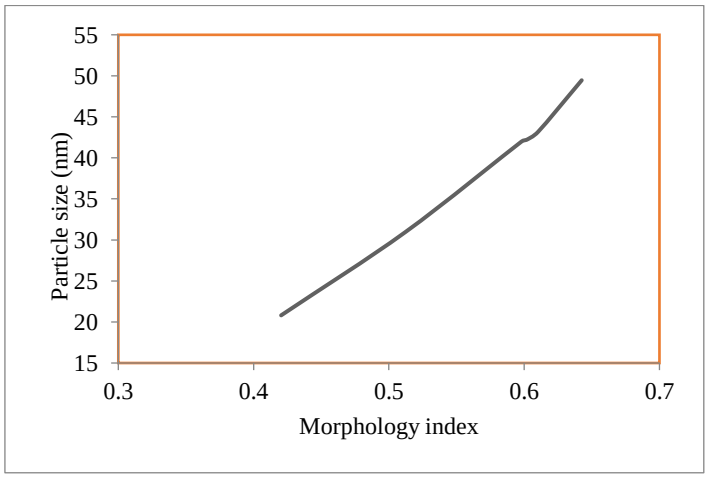

Fig. 5. Morphology index vs particle size curve of Ag-doped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens*.

SEM STUDIES

Scanning electron microscopy (SEM) was employed to analyze the morphology and size of the silver-doped zinc hydroxide nanoparticles synthesized using the leaf extract of *T. procumbens* [19]. Figure 6 presents the SEM images of these nanoparticles at various magnifications. The SEM images reveal that the nanoparticles have a spherical shape [24]. It is observed that the particle sizes are slightly increased, and there is also a noticeable agglomeration of particles, which can be attributed to the presence of silver [35].

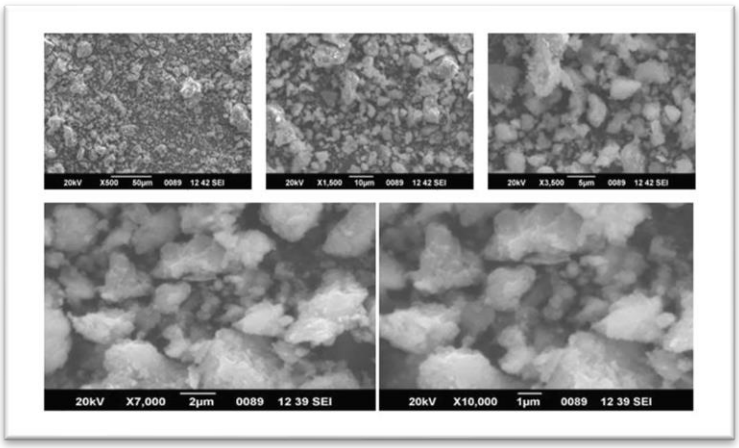

Fig. 6. SEM images of Ag-doped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens* at various magnifications.

EDAX STUDIES

The EDAX elemental analysis of silver-doped zinc hydroxide nanoparticles, synthesized using the leaf extract of *T. procumbens*, confirms the presence of key elements – O, Zn, and Ag – indicating that these elements are major components of the sample [16, 27]. The presence of the Na element may be attributed to the use of sodium hydroxide during the synthesis process. The EDAX spectrum of these nanoparticles (Fig. 7.) The weight percentage of the elements present in the sample is provided in Table 4.

The elemental composition of silver-doped zinc hydroxide nanoparticles was analyzed using energy dispersive X-ray spectroscopy (EDS), revealing the presence of oxygen (O), sodium (Na), magnesium (Mg), sulfur (S), chlorine (Cl), potassium (K), calcium (Ca), zinc (Zn), and silver (Ag). Oxygen exhibits the highest atomic percentage (62 %), confirming the hydroxide nature of the synthesized nanoparticles. The presence of zinc (Zn) at 6.19 atomic % and silver (Ag) at 7.41 atomic % indicates the successful incorporation of silver into the zinc hydroxide matrix. The relatively high weight

percentage of silver (27.4 %) suggests significant doping, which could alter the electronic and structural properties of the nanoparticles. The detection of elements such as Na, S, Cl, K, and Ca may be attributed to residual precursors from the synthesis process or impurities from the plant extract used in the green synthesis method.

The presence of silver in the nanoparticles is particularly important, as silver-doped nanomaterials are widely recognized for their enhanced antimicrobial, catalytic, and optical properties [12, 26]. The observed elemental composition aligns with previous studies on metal-doped zinc hydroxide nanoparticles, where silver incorporation has been reported to modify lattice parameters and enhance functional properties [17, 28]. Recent research [6, 14] has demonstrated that silver doping significantly improves the antibacterial and antifungal activities of metal hydroxides, making them promising candidates for biomedical applications. Furthermore, studies [22] indicate that Ag-doped Zn-based nanoparticles exhibit enhanced photocatalytic efficiency for environmental remediation. The presence of elements such as sodium and sulfur also suggests potential modifications in surface chemistry and charge transport properties, which are crucial for their use in biosensors and optoelectronic devices [13, 3]. The elemental analysis confirms the successful synthesis of Ag-doped zinc hydroxide nanoparticles, indicating their potential applications in healthcare, catalysis, and environmental purification.

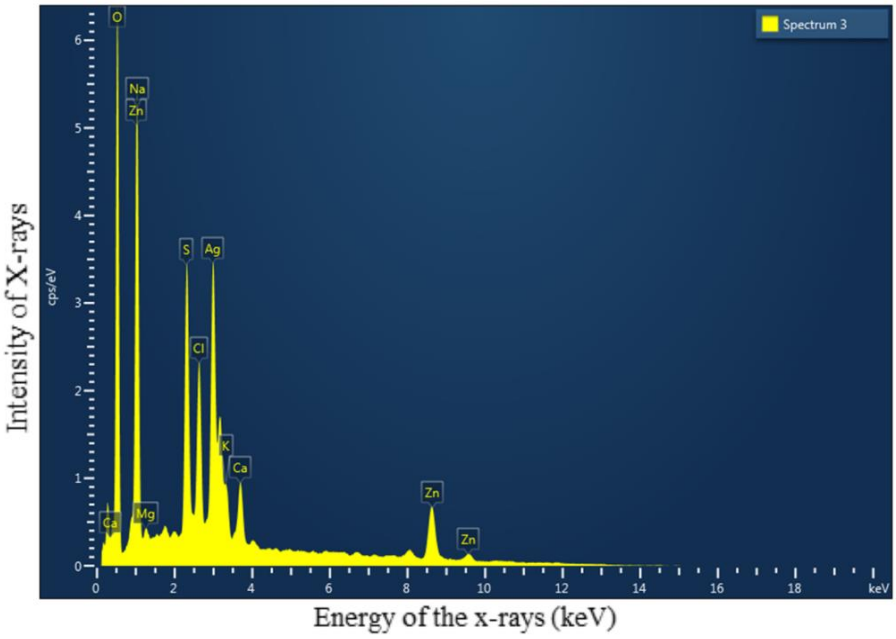

Fig. 7. EDAX spectrum of Ag-doped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens*.

Table 4

The weight percentage of the elements of Ag-doped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens*

Element	Line type	Weight %	Atomic %
O	K series	34.43	62.8
Na	K series	7.26	9.22
Mg	K series	0.31	0.37
S	K series	7.26	6.61
Cl	K series	5.12	4.21
K	K series	1.34	1
Ca	K series	3.01	2.19
Zn	L series	13.86	6.19
Ag	L series	27.4	7.41
Total:		100	100

FTIR STUDIES

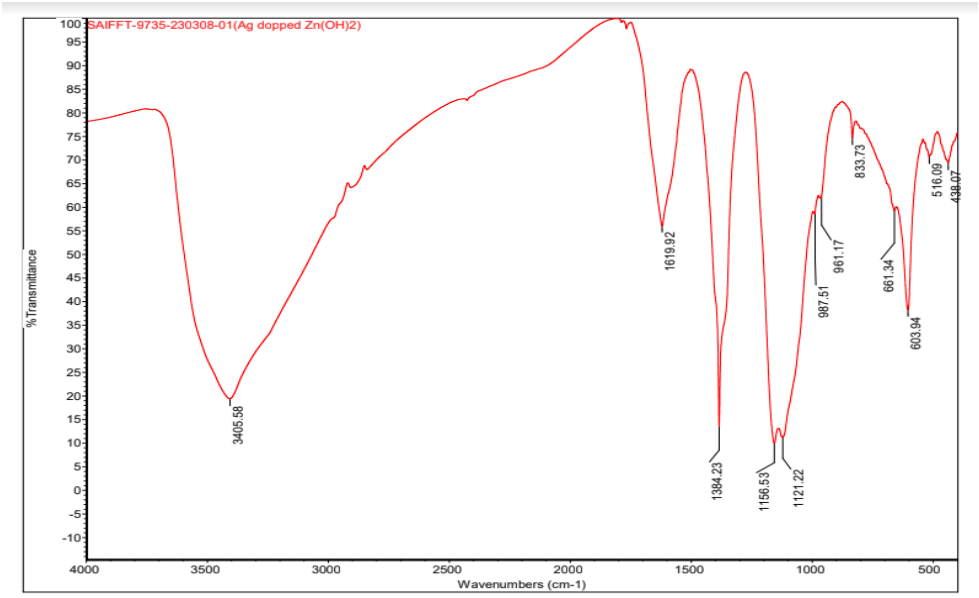

Fig. 8. FTIR spectra of Ag-dopped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens*.

The FTIR spectrum of silver-doped zinc hydroxide nanoparticles, synthesized using the leaf extract of *T. procumbens*, is shown in Fig. 8. The FTIR analysis reveals several key peaks that provide insight into the functional groups present in the sample. The peak at 3405.58 cm^{-1} corresponds to the hydroxyl (OH) group, indicative of the Ag-Zn-O system [21, 31]. The 1619.92 cm^{-1} peak is attributed to

the H-O-H bending vibration, representing water molecules [3]. A peak at 1384.23 cm^{-1} is associated with the presence of nitrate ions (NO_2), likely originating from the silver nitrate solution used during synthesis [33]. The peak at 661 cm^{-1} is attributed to emodin, a bioactive compound derived from the *T. procumbens* leaf extract [7]. The peaks at 603.94 cm^{-1} and 516.09 cm^{-1} are due to the stretching vibrations of the Ag-Zn-O bond, while the peak at 438.07 cm^{-1} corresponds to the stretching vibration of the Zn-O bond [11, 21].

UV STUDIES

The band gap of the silver-doped zinc hydroxide nanoparticles, synthesized using the leaf extract of *T. procumbens*, was determined through UV-vis spectroscopy. Figures 9 and 10, present the UV-visible absorption spectra of these nanoparticles, with the maximum absorption occurring at a wavelength of 554 nm. To determine the band gap, Fig. 11., displays the Tauc plot derived from the UV-vis data. X axis is energy (V), Y axis is $(\alpha h\nu)^2$ (eV cm^{-1}), in that α – absorption coefficient, h = Planck's constant (6.626×10^{-34} J·s), ν = frequency of the photon. From this graph, the optical band gap of the silver-doped zinc hydroxide nanoparticles is calculated to be 4.5 eV, which is consistent with previous studies on metal-doped hydroxide materials [12, 26]. Additionally, the optical transmittance of these nanoparticles, as shown in Fig. 8, is observed to be 97 %, indicating their high transparency and potential applications in optoelectronics and photovoltaics [17, 22].

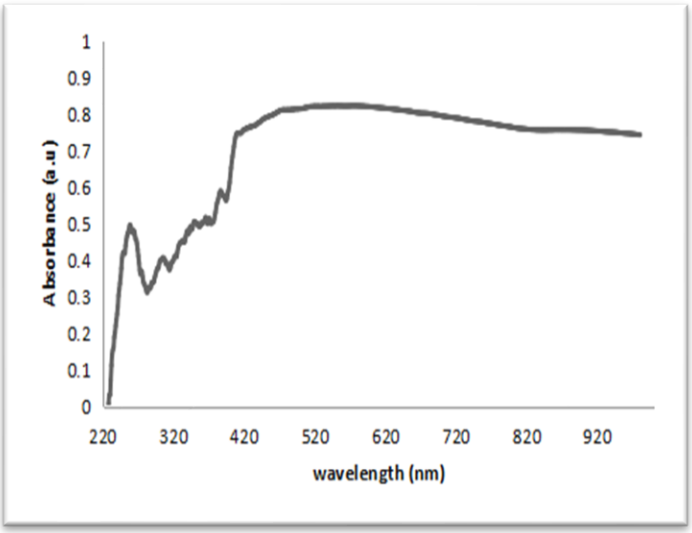

Fig. 9. UV-Visible absorption spectra of Ag-doped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens*.

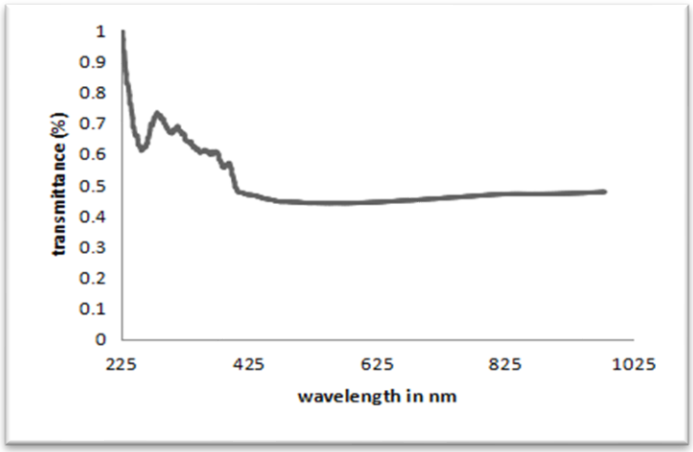

Fig. 10. Optical transmittance of Ag-doped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens*.

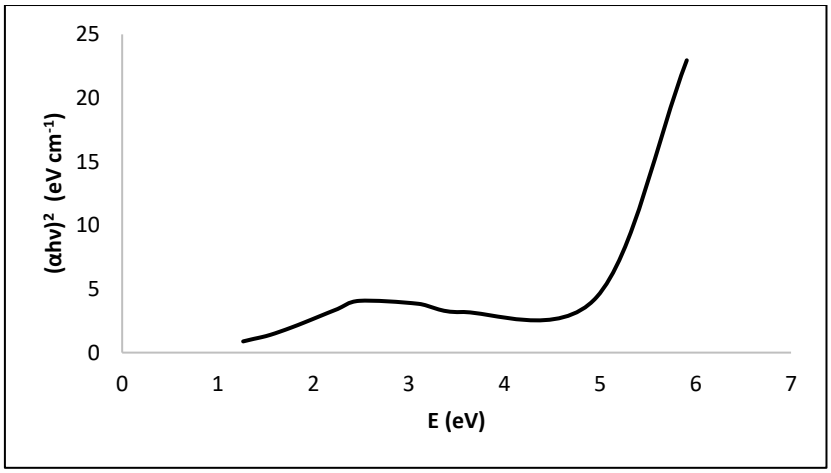

Fig. 11. Graph to find the band gap of Ag-doped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens*.

ANTIBACTERIAL STUDIES

The antibacterial activity of silver-doped zinc hydroxide nanoparticles, synthesized using the leaf extract of *T. procumbens*, was evaluated at different concentrations (50 and 100 µg/mL) against two Gram-negative bacteria, *Escherichia coli* and *Klebsiella* sp. The antibacterial potential of these nanoparticles was assessed based on the zone of inhibition of bacterial growth. The results of this antibacterial activity are presented in Table 5.

Table 5

Results of antibacterial activity of Ag-doped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens*

Zone of inhibition (mm)			
Bacterial culture		*E. coli*	*Klebsiella*
Antibiotics	Gentamycin	18	14
	Ampicillin	12	10
Sample concentration (µg/mL)	50	8	12
	100	12	16

The antibacterial activity of silver-doped zinc hydroxide nanoparticles was evaluated against *E. coli* and *Klebsiella* using the zone of inhibition (ZOI) test (Fig. 12). The results were compared with standard antibiotics gentamycin and ampicillin. Gentamycin exhibited the highest inhibition (18 mm for *E. coli* and 14 mm for *Klebsiella*), followed by ampicillin (12 mm and 10 mm, respectively). The nanoparticle sample demonstrated concentration-dependent antibacterial activity, with higher inhibition at 100 µg/mL (12 mm for *E. coli* and 16 mm for *Klebsiella*), indicating its effectiveness against gram-negative bacteria.

The observed antibacterial properties can be attributed to silver ions disrupting bacterial cell membranes and interfering with DNA replication [12, 26]. Similar studies report enhanced antibacterial activity in silver-doped nanomaterials, suggesting their potential as alternative antimicrobial agents [5, 18]. Moreover, recent research indicates that Zn-based nanoparticles exhibit synergistic effects with plant extracts, boosting their efficacy [15, 23]. These findings highlight the potential of green-synthesized Ag-doped $Zn(OH)_2$ nanoparticles in biomedical applications and antimicrobial coatings.

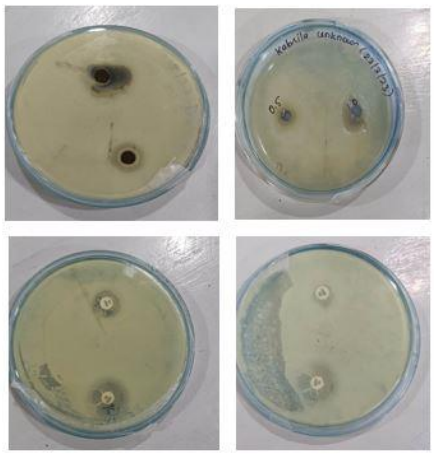

Fig. 12. Antibacterial activity of Ag-doped zinc hydroxide nanoparticles using the leaf extract of *Tridax procumbens*.

ANTICANCEROUS ACTIVITY

The cytotoxic effects of Ag-doped nanoparticles and *T. procumbens* extract (tridase) at various concentrations were evaluated using the MTT assay. The results indicated that Ag-doped nanoparticles exhibited significant cytotoxic activity against MDA-MB-231 cells (Table 6), as shown in Figs. 13 and 14., while tridase (Table 6), did not show any cytotoxic effect across the tested concentration range. At the lowest concentration of 20 µg/mL, Ag-doped nanoparticles maintained 97 % cell viability. However, as the concentration increased (40, 60, 80, 100, 150, and 200 µg/mL), cell viability was progressively reduced to 59 %, 67 %, 64 %, 65 %, 70 %, and 68 %, respectively. In contrast, treatment with tridase did not produce any significant changes in cell viability from 24 to 72 hours (Fig. 15).

The dose-dependent cytotoxicity observed in Ag-doped nanoparticles suggests their potential for anticancer applications, particularly against triple-negative breast cancer (MDA-MB-231) cells [9, 26]. Studies have demonstrated that silver nanoparticles induce apoptosis via ROS generation and mitochondrial damage, contributing to reduced cell viability [1, 5, 17]. The lack of cytotoxicity in tridase indicates its biocompatibility, supporting its potential use as a stabilizing or bioenhancing agent in nanoparticle formulations [15, 23]. These findings suggest that green-synthesized Ag-doped nanoparticles may serve as promising candidates for targeted cancer therapy.

Table 6

Details of Ag-doped zinc hydroxide nanoparticles and tridase
for the cell viability test for MTT assay

Name of the cells	MDA-MB-231
Name of the drug	Ag-doped Tridase
Date of the cell seeding	08.04.2023
Date of the drug treatment	09.04.2023
Date of the MTT assay	10.04.2023 (24 hours)
Concentration of the drug	20, 40, 60, 80, 100, 150, 200 µg/mL
Preparation of the drug	1 mg of the drug in 1 mL of serum-free media

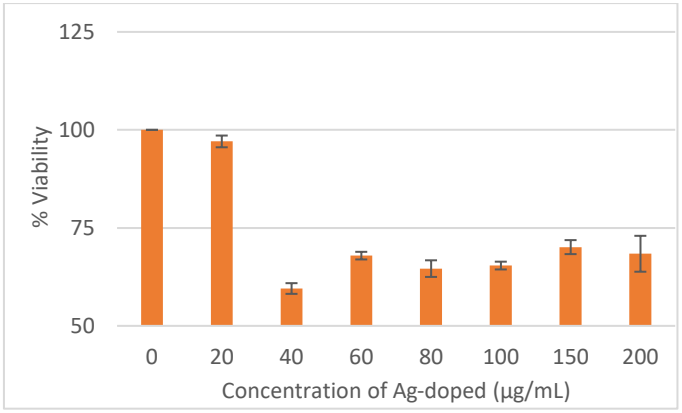

Fig. 13. Effect of various concentration of Ag-doped *Tridax procumbens* on cell viability.

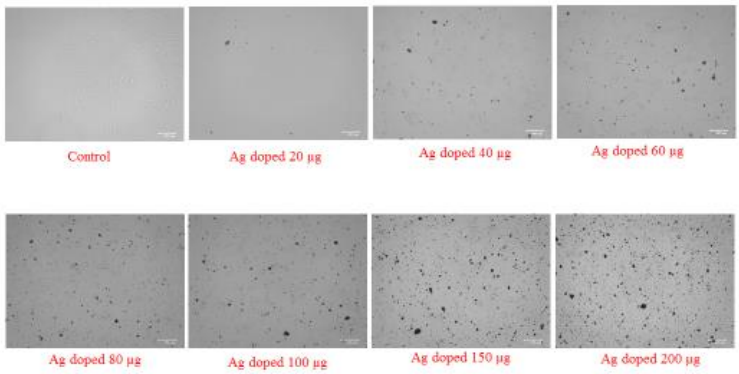

Fig. 14. Morphological assessment of Ag-doped *Tridax procumbens* treated with MDA-MB-231 cells.

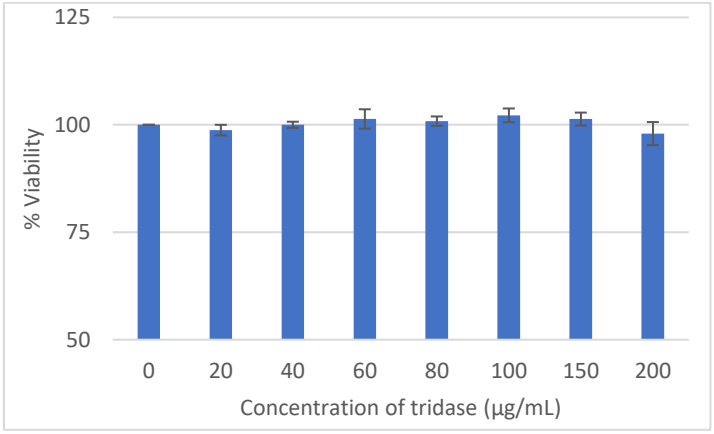

Fig. 15. Effect of various concentration of tridase on the cell viability.

CONCLUSION

The research successfully produced silver-doped zinc hydroxide nanoparticles by utilizing *Tridax procumbens* leaf extract and examined their structural, optical, antimicrobial, and cytotoxic characteristics. XRD analysis confirmed the formation of nanoparticles with slight shifts in diffraction peaks due to silver incorporation, influencing the crystal structure. EDAX analysis validated the elemental composition, revealing the presence of oxygen, silver, zinc, and trace elements, supporting successful doping. UV-vis spectroscopy determined the optical band gap (4.5 eV) and high transmittance (97 %), indicating potential optoelectronic applications. The antibacterial activity of the nanoparticles, evaluated through zone of inhibition studies, demonstrated enhanced efficacy against *E. coli* and *Klebsiella* compared to standard antibiotics. Additionally, MTT assay results indicated a dose-dependent cytotoxic effect of Ag-doped nanoparticles on MDA-MB-231 cancer cells, while *T. procumbens* extract (tridase) exhibited no cytotoxicity, suggesting its biocompatibility. Overall, the study highlights the potential biomedical applications of Ag-doped zinc hydroxide nanoparticles, including antimicrobial agents, cancer therapeutics, and environmental remediation materials. Further studies are recommended to explore their mechanism of action, biocompatibility, and real-world applications in nanomedicine and biotechnology

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