RJC

RASĀYAN *J. Chem.*
Vol. 18 | No. 3 |1671-1681| July-September | 2025
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

ECO FRIENDLY SYNTHESIS OF ZINC OXIDE NANO PARTICLES USING *Causonis trifolia* EXTRACT: CHARACTERIZATION AND EVALUATION OF ANTIMICROBIAL and ANTIOXIDANT ACTIVITY

Santosi Anusha Buravilli[1], Srinivasa Rao Golagani[1,✉], Venkata Ravi Sankar Ummidi[2], Sai Kishore Marothu[3], M. Vidya Kalpana[4], A. Hymavathi[4] and Ravada Kishore[1]

[1]Department of Chemistry, School of Science, GITAM (Deemed to be University, Visakhapatnam-530045 (Andhra Pradesh), India
[2]MicGene Lab, Visakhapatnam-530045 (Andhra Pradesh), India
[3]Sri Chaitanya Junior College, Visakhapatnam-530045 (Andhra Pradesh), India
[4]Visakha Govt.Degree College for Women(A), Visakhapatnam 530 020, India
✉Corresponding author: sgolagan@gitam.edu

ABSTRACT

The synthesis of nanoparticles using plants provides an environmentally friendly and sustainable alternative for developing multifunctional materials with applications in biomedicine, agriculture, and environmental remediation. Antibiotic resistance remains a global challenge, affecting healthcare in both developed and developing nations. The rise of multidrug-resistant infections has significantly impacted current antibacterial therapies, necessitating alternative strategies such as plant-mediated nanomaterials, which possess bioactive compounds with therapeutic potential. Zinc oxide nanoparticles (ZnO NPs), known for their semiconducting and antimicrobial activities, are particularly promising due to their Generally Recognized as Safe (GRAS) status and potent ability to disrupt microbial membranes via reactive oxygen species (ROS) generation. The present study was intended to synthesize ZnO NPs derived from leaf, stem, and fruit extracts of *Causonis trifolia*, an ethnomedicinal plant, and evaluate their structural, antimicrobial, and antioxidant activities. ZnO NPs were synthesized through a green synthesis route using aqueous plant extracts as stabilizing and reducing agents. Characterization was carried out by employing UV-Visible, XRD, SEM, EDX, and FTIR spectroscopic techniques. Antibacterial and antifungal efficacy was assessed via agar well diffusion against a range of pathogenic Gram-positive and Gram-negative bacteria and fungal strains. Antioxidant potential was assessed by employing DPPH radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays. Spectral and morphological analysis confirmed the successful formation of crystalline ZnO NPs, with the largest and most crystalline particles obtained from leaf extract (60.91 nm), followed by fruit (45.73 nm) and stem extracts (11.45 nm). Antibacterial studies revealed that stem-extract ZnO NPs exhibited the strongest inhibition, particularly against gram-positive*Clostridium perfringens* (30.0 mm), *Staphylococcus aureus* (28.0 mm), and gram-negative *Vibrio cholerae*, Escherichia coli (28.0 mm each),followed by *Pseudomonas aeruginosa* (25.0 mm). Antifungal assays similarly showed maximum activity from stem-derived NPs against *Candida albicans* (24.0 mm) and *Saccharomyces cerevisiae* (14.0 mm). Antioxidant evaluation revealed that stem-derived NPs had the highest DPPH scavenging activity (IC_{50} = 195.28 µg/mL), while fruit-derived NPs showed superior FRAP activity (IC_{50} = 198.96 µg/mL), highlighting phytochemical influence on bioactivity. In conclusion, *Causonis trifolia* extracts effectively mediate the green synthesis of ZnO NPs with promising antimicrobial and antioxidant potential. These findings support their relevance as alternative therapeutic agents in combating antibiotic-resistant infections and advancing biomedical nanotechnology.

Keywords:*Causonis trifolia*, ZnO Nanoparticles, Green Synthesis, Antimicrobial Ability, Antioxidant Ability, Antifungal Activity.

RASĀYAN*J. Chem.*, Vol. 18, No.3, 2025

INTRODUCTION

Plant-mediated synthesis of nanoparticles (NPs) has emerged as a sustainable option to conventional techniques, contributing eco-friendly and non-toxic routes with applications in medicine, agriculture, food

http://doi.org/10.31788/RJC.2025.1839383

processing, and water treatment[1]. Unlike chemically synthesized NPs, which may exhibit toxicity and limited efficacy[2], biosynthesized NPs benefit from enhanced stability and biocompatibility, facilitated by phytochemicals like flavonoids, proteins, and carbohydrates that aid in metal ion reduction through functional groups such as hydroxyl (-OH).[3]Since Feynman's proposal in 1959, nanotechnology has enabled the fabrication of materials with unique properties, supporting diverse biomedical applications like drug delivery, imaging, and antimicrobial therapies.[4,5]Among metal oxides, zinc oxide nanoparticles (ZnO-NPs) are especially valuable because of their wide band gap (~3.37 eV), excellent stability, and notable antibacterial activity.[6,7,8]Green synthesis using plant extracts rich in polyphenols and amino acids allows ZnO-NPs to function as both reducing and stabilizing agents[9], producing biocompatible materials recognized as GRAS with the ability to disrupt microbial membranes and generate ROS.[10]Amid rising antimicrobial resistance[11,12,13], green synthesis offers a promising route to novel antimicrobials.

Metal-based NPs, especially ZnO-NPs, show potent bactericidal potential due to their nanoscale dimension and high surface area.[14,15] Resistance to antibiotics now affects over 70% of pathogens[16,17], prompting the urgent need for alternative agents. ZnO-NPs also demonstrate synergy with antifungal agents like nystatin and fluconazole, enhancing efficacy while minimizing toxicity.[18,19]In the present study, ZnO-NPs were derived using leaf, stem, and fruit extracts of *Causonis trifolia*—a medicinal vine rich in bioactive substances like flavonoids, alkaloids, tannins, squalene, myricetin, and nimbidin.[20,21]HPLC and GC-MS analysis confirmed the presence of key phenolics and 19 phytochemicals contributing to its antioxidant, anti-inflammatory, antidiabetic, and antimicrobial effects.

The derived ZnO-NPs were evaluated for antibacterial and antifungal efficacy in opposition to Gram +ve, Gram -ve, and fungal pathogens. Although ZnO-NPs have shown potential toxicity via ROS and inflammasome activation in cancer cells[22,23,24], green synthesis significantly enhances their safety profile.[25,26,27]This study underscores the potential of *Causonis trifolia*-mediated ZnO-NPs as effective antimicrobial agents, promoting their application in biomedicine and supporting plant-based nanoparticle synthesis as a viable, eco-friendly alternative.

EXPERIMENTAL

Collection of Plant Specimens and Reagents

Leaves, stems, and fruits of *Causonis trifolia* were collected from Machavaram, Andhra Pradesh (16°51'11.0"N, 81°59'01.2"E). Analytical-grade zinc sulfate pentahydrate ($ZnSO_4$), hydrochloric acid (35% HCl), and sodium hydroxide (98% NaOH) were obtained from Merck. A 1 × 10-2 M $ZnSO_4$ stock solution was prepared by suspending 2.5 g in 100 mL of deionized water. Microbial cultures were maintained in appropriate media for further studies.

Aqueous Plant Extract Preparation

Plant specimens were first washed thoroughly with distilled water, then shade-dried at ambient temperature and finely ground. To prepare the extract, 5 grams of the powdered sample were heated up toa boil in 100 mL of distilled water using microwave heating for 5 minutes. The resulting solution was filtered two times by employing Whatman No. 1 filter paper and kept at 4°C for further use.

Green Synthesis of ZnO NPs

ZnO NPs were prepared by combining 50 mL of each plant extract (leaf, stem, and fruit) with 100 mL of 1×10^{-2} M $ZnSO_4$ solution. The pH was makeup to 7.0 using NaOH, and the mixture was refluxed at 70°C for 5 hours under continuous stirring. The appearance of a milky white color indicated nanoparticle formation. After allowing the reaction to stand for 24 hours, the mixture was subjected to centrifugation, and the precipitate was cleaned three times with deionized water and once with absolute ethanol.The purified ZnO nanoparticles were dried for 4 hours at 60°C and kept at 4°C for future use.

Characterization of ZnO NPs Derived via the Green Method

ZnO NPs were synthesized and characterized using different analytical methods to verify and validate their structure, morphology, and composition as detailed in Table-1.

Preparation of Nanoparticle Stock Solutions and Microbial Cultures

Stock solutions of ZnO nanoparticles were initially prepared in Dimethyl Sulfoxide (DMSO) at a concentration of 1 mg/mL, then diluted to working concentrations of 100, 200, and 500 µg/mL for use in

biological assays. The bacterial and fungal strains employed in the study were sourced from the 'Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.' Bacterial strains were cultured on nutrient agar, while fungal strains were maintained on potato dextrose agar (PDA). All microbial species were identified using standard microbiological techniques.

Table-1: Characterization Techniques Used for Green-Synthesized ZnO Nanoparticles

Technique	Purpose	Instrument/Method Details	Reference(s)
UV–Visible Spectroscopy	Confirmation of nanoparticle formation	SHIMADZU UV-2600i; wavelength range: 200–800 nm	Akintelu and Yao[28]
FTIR (Fourier Transform Infrared Spectroscopy)	Functional groups identification	KBr pellet method; range: 4000–400 cm^{-1}; resolution: 4 cm^{-1}	Akintelu *et al.*[29]
XRD (X-ray Diffraction Analysis)	Crystalline phase and structure determination	Bruker D8 Advance; CuKα radiation (λ = 1.5406 Å); 2θ range: 30–70°	Sharma *et al.*[30]; Jamdade *et al.*[31]
EDX (Energy Dispersive X-ray Spectroscopy)	Analysis of elemental composition	Standard EDX detection of element-specific X-ray peaks	Noruzi[32]
SEM (Scanning Electron Microscopy)	Analysis of morphology, shape, and size	JEOL (Japan) SEM	Prabhu *et al.*[33]

Antimicrobial Assay

Test Organisms

Bacterial strains, including '*Bacillus subtilis* (MTCC 441), *Bacillus licheniformis* (MTCC 7445), *Clostridium* sp. (MTCC 11101), *Staphylococcus epidermidis* (MTCC 3615), *Staphylococcus aureus* (MTCC 2940), *Aeromonas* sp. (MTCC 4033), *Vibrio cholerae* (MTCC 3906), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa (*MTCC 424), and *Salmonella paratyphi* (MTCC 735), along with fungal strains *Aspergillus niger* (MTCC 282), *Aspergillus flavus* (MTCC 277), *Candida albicans* (MTCC 227), *Saccharomyces cerevisiae* (MTCC 4742) and *Phytophthora* were collected from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India.' Standard bacterial and fungal cultures were sub-cultured into freshly prepared nutrient agar and potato dextrose agar (PDA), respectively, and incubated at 32°C for 24 hours to obtain fresh cultures for antibacterial and antifungal activity.

Procedure

Fresh microbial (both bacteria and fungi) cultures standardized to 0.5 McFarland were swabbed onto respective agar plates. Wells (6 mm) were loaded with 50 µL of ZnO NPs, plant extracts (leaf, stem, fruit), DMSO (-ve control), and standard antibiotics Streptomycin and Fluconazole for bacteria and fungi, respectively. Inoculated plates were kept for 24 hours at 32°C, and inhibition zones were noted in mm. The assay was performed in triplicate at three concentrations @ 100, 200, and 500 µg/mL.[34,35]

Antioxidant Activity

DPPH Radical Scavenging Method

Antioxidant potential was measured by employing the DPPH technique. A 0.1 mM DPPH ethanol solution was combined with sample extracts at concentrations of 200–1000 µg/mL and kept in the dark for 30 minutes. Absorbance was recorded at 517 nm. Ascorbic acid (20–100 µg/mL) served as the positive control. The percent inhibition was calculated as:

$$DPPH\ scavenging\ effect\ (\%)\ or\ Percent\ Inhibition\ = \frac{Control\ - Test/Standard}{Control} \times 100$$

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP solution (acetate buffer, TPTZ, $FeCl_3$ in a 10:1:1 ratio) was mixed with sample extracts at a 1:30 ratio and kept for 30 min at 37°C. Absorbance was measured at 593 nm. Lower absorbance indicated higher antioxidant activity. As positive control, Ascorbic acid was employed (20–100 µg/mL). All assays were conducted three times.

RESULTS AND DISCUSSION

UV-Visible Spectroscopic Analysis

UV-Vis analysis confirmed the successful synthesis of ZnO nanoparticles from *Causonis trifolia* extracts. Absorption peaks between 300–400 nm indicated band gap excitation characteristic of ZnO. Fruit, stem,

and leaf-derived nanoparticles showed maximum absorbance around 350 nm, with declining trends at higher wavelengths, reflecting stable dispersion. The presence of phytochemicals like flavonoids and tannins facilitated reduction and capping, affirming nanoparticle stability (Fig.-1).

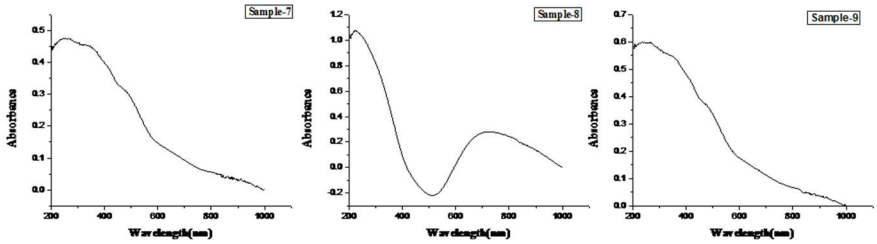

Fig.-1: UV–Vis Absorption Spectra of ZnO NPs Derived from Fruit, Stem, and Leaf Extracts of *Causonis trifolia*

Energy Dispersive X-ray Spectroscopy (EDS) Assay

EDS analysis of fruit-derived ZnO nanoparticles revealed strong Zn peaks at ~1.0, 8.6, and 9.6 keV, and an O peak at ~0.5 keV, confirming ZnO formation. Minor peaks for C, K, P, and S suggest the involvement of phytochemicals in nanoparticle stabilization. The dominance of Zn and O signals affirms the purity and green synthesis pathway.

XRD Analysis of ZnO NPs Synthesized Using *Causonis trifolia* Extracts

XRD established the hexagonal Wurtzite construction of ZnO in all samples. Fruit-derived nanoparticles showed intense peaks, particularly at 26.61°, with a mean crystallite size of 45.73 nm. Stem-derived particles exhibited broader peaks and a smaller size (11.45 nm), indicating strain and defects. Leaf-derived particles showed well-defined peaks and the largest crystallite size (60.91 nm), suggesting high crystallinity. No secondary phases were detected (Fig.-4).

FTIR Spectrum

FTIR spectra illustrate O–H, N–H, C–H, C=O, and C–O functional groups, confirming the presence of phytochemicals involved in nanoparticle synthesis. Zn–O stretching vibrations appeared below 700 cm^{-1}, validating ZnO formation. Bioactive substances in the extracts performed both reducing and capping agents (Fig.-3).

Scanning Electron Microscopy Analysis

SEM images revealed mixed spherical and irregular morphologies with varying degrees of agglomeration across all samples. Leaf-derived particles showed moderate agglomeration; stem-derived ones exhibited pronounced aggregation; fruit-derived particles presented mixed shapes with partial aggregation. These differences reflect the influence of phytochemical composition on nanoparticle morphology (Fig.-2).

Antibacterial Potential of ZnO NPs in Opposition to Gram-positive and Gram –ve bacteria

ZnO NPs derived from various parts of *Causonis trifolia* (stem, leaf, and fruit) exhibited variable antibacterial ability against both Gram-positive and Gram –ve bacteria. The antibacterial efficacy was assessed via the well diffusion assay at concentrations of 100, 250, and 500 µg/well. Against Gram-positive bacteria, stem-derived ZnO NPs showed the highest efficacy, notably inhibiting *Clostridium perfringens* (30.0 mm) and *Staphylococcus aureus* (28.0 mm), followed by moderate activity against *Bacillus licheniformis* (23.0 mm) and *Staphylococcus epidermidis* (11.0 mm). *Bacillus subtilis* remained resistant. Leaf-extract ZnO NPs showed moderate activity, while fruit-derived NPs were least effective, with minimal inhibition zones (7.0–8.0 mm) (Table-2 and Fig.-6). Against Gram-negative bacteria, strong inhibition was observed for stem-derived NPs against Vibrio cholerae and Escherichia coli (28.0 mm), with P. aeruginosa (25.0 mm), Salmonella enterica (18.0 mm), and Aeromonas hydrophila (16.0 mm) showing slightly lower inhibition. Leaf-based NPs exhibited moderate inhibition, while fruit-based NPs were largely ineffective (Table-3 and Fig.-7). The enhanced antibacterial ability of stem and leaf ZnO NPs is likely due to their higher phytochemical content, aiding nanoparticle stabilization and bioactivity. The findings are consistent with previous studies. Al-darwesh *et al.*[36] reported inhibition zones of 10.3

mm (K. aerogenes) and 3.3 mm (P. aeruginosa). Imade *et al.*[37] demonstrated activity against S. enterica, K. pneumoniae, and S. aureus using plantain peel ZnO NPs. Kalaba *et al.*[38] showed nanofluids from Streptomyces baarnensis were highly effective (36 mm for MRSA).

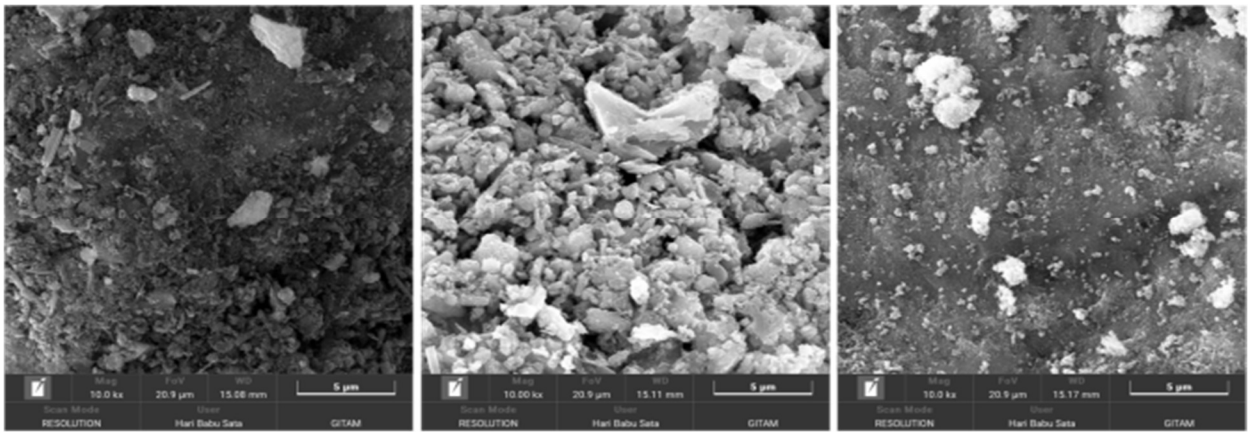

Fig.-2: SEM Images of ZnO NPs Derived from Leaf, Fruit, and Stem Extracts of *Causonis trifolia*

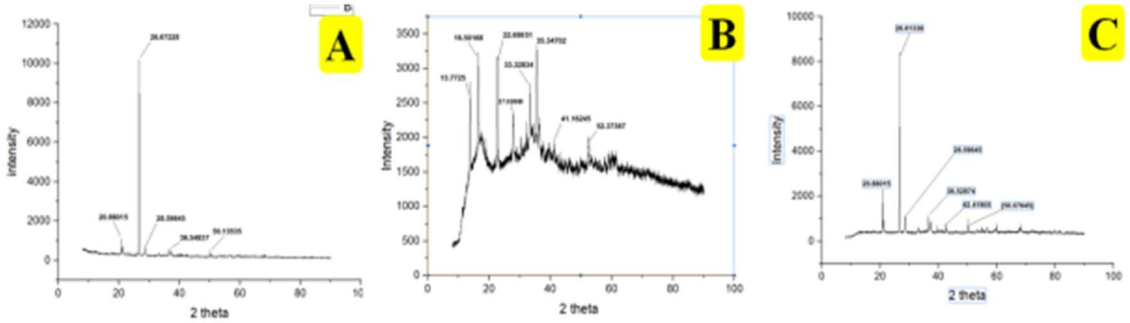

Fig.-3: FTIR Spectra of ZnO NPs Derived from Leaf (A), Fruit (B), and Stem (C) Extracts of *Causonis trifolia*

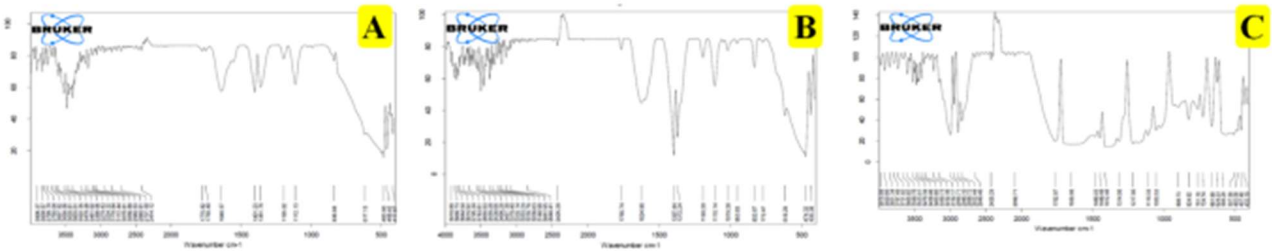

Fig.-4: XRD Spectra of ZnO NPs Derived from Leaf (A), Stem (B), and Fruit (C) Extracts of *Causonis trifolia*

Table-2: Antibacterial Potential of ZnO NP Synthesized from DifferentParts of *Causonis trifolia* against Gram-Positive Bacteria

Plant parts	Concentration of ZnO NP (µg/well)	*Bacillus subtilis* MTCC 441	*Bacillus licheniformis* MTCC 7445	*Clostridium perfringens* MTCC 11101	*Staphylococcus epidermidis* MTCC 3615	*Staphylococcus aureus* MTCC 2940
		Diameter of Inhibition zone (mm)				
Zn NPs from Stem extract	100	--	15.0	25.0	7.0	20.0
	250	--	20.0	22.0	8.0	21.0
	500	--	23.0	30.0	11.0	28.0
	Positive control	15.0	12.0	29.0	10.0	17.0
	Negative control	--	--	--	--	--
Zn	100	--	8.0	16.0	--	12.0

NPs from Leaf extract	250	--	14.0	18.0	8.0	15.0
	500	--	20.0	24.0	9.0	24.0
	Positive control	14.0	12.0	29.0	11.0	17.0
	Negative control	--	--	--	--	--
Zn NPs from Fruit Extract	100	--	--	--	--	--
	250	--	--	--	--	--
	500	7.0	--	--	8.0	--
	Positive control	19.0	12.0	28.0	12.0	18.0
	Negative control	--	--	--	--	--

'--' indicates Resistance

ZnO NPs typically show greater activity against Gram-negative bacteria due to their thinner peptidoglycan layer and negatively charged lipopolysaccharides, which promote nanoparticle adhesion and membrane disruption (Fig.-5). In conclusion, stem- and leaf-derived ZnO NPs from C. trifolia possess strong antibacterial ability, especially opposed to S. aureus, C. perfringens, E. coli, and V. cholerae. The lower efficacy of fruit-based NPs highlights the role of phytochemical composition in nanoparticle bioactivity. Literature comparisons affirm the suitability of eco-friendly synthesized ZnO NPs for antimicrobial applications.

Table-3: Antibacterial Activity of ZnO NP Synthesized from DifferentParts of *Causonis trifolia*Against Gram-Negative Bacteria

Plant parts	Concentration of ZnO NP (µg/well)	*Aeromonas hydrophila* MTCC 4033	*Vibrio cholera* MTCC 3906	*E. Coli* MTCC 443	*P. aeruginosa* MTCC 424	*Salmonella enteric* MTCC 735
		Diameter of Inhibition zone (mm)				
Zn NPs from Stem extract	100	10.0	20.0	20.0	10.0	14.0
	250	15.0	21.0	22.0	18.0	16.0
	500	16.0	28.0	28.0	25.0	18.0
	Positive control	20.0	20.0	15.0	19.0	13.0
	Negative control	--	--	--	--	--
Zn NPs from Leaf extract	100	--	--	9.0	10.0	7.0
	250	--	17.0	18.0	14.0	13.0
	500	16.0	19.0	21.0	18.0	14.0
	Positive control	18.0	21.0	15.0	20.0	15.0
	Negative control	--	--	--	--	--
Zn NPs from Fruit Extract	100	--	--	--	--	--
	250	--	--	--	--	--
	500	--	--	12.0	--	--
	Positive control	19.0	19.0	15.0	20.0	14.0
	Negative control	--	--	--	--	--

'--' indicates Resistance

Antifungal Activity of ZnO NPs Derived from Various Parts of *Causonis trifolia*

ZnO nanoparticles synthesized from the stem, leaf, and fruit extracts of *Causonis trifolia* were tested against *A. niger, A. flavus, C. albicans, Saccharomyces cerevisiae*, and *Phytophthora* spp. Using the well diffusion method at 100, 250, and 500 µg/well (Table-4 andFig.-8).Stem-derived ZnO NPs exhibited the strongest antifungal activity, particularly against *C. albicans* (24.0 mm at 500 µg/well) and *S. cerevisiae* (up to 14.0 mm), with moderate inhibition of *A. flavus* and minimal effect on *A. niger; Phytophthora*

showed complete resistance. Leaf-derived NPs showed moderate inhibition against *C. albicans* (18.0 mm) and *S. cerevisiae* (16.0 mm), while other strains were largely unaffected.

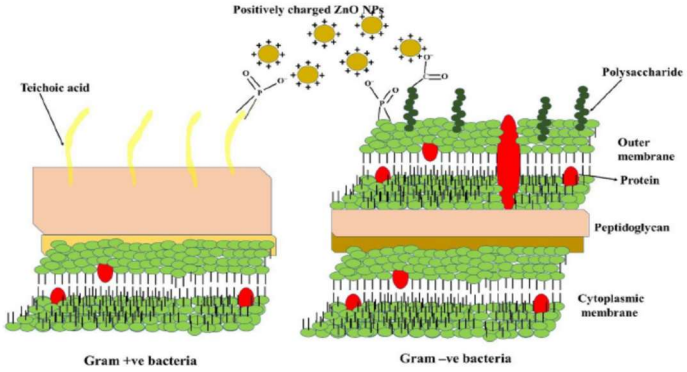

Fig.-5: Mechanism of Action of ZnO NP's on Gram +ve and Gram ve organisms (Singh *et al*.,[39])

Fruit-derived NPs were least effective, displaying weak inhibition only against *A. niger, C. albicans,* and *Phytophthora* (7.0 mm each), with no activity against *A. flavus* or *S. cerevisiae*.Positive controls showed significant inhibition across all strains (17.0–32.0 mm), confirming the antifungal efficiency of the synthesized NPs. The superior performance of stem-based NPs is attributed to higher phytochemical content, influencing nanoparticle characteristics and antifungal potency. These results are in line with prior studies. Abomuti *et al.*[40] reported that biogenic ZnO NPs significantly inhibited *C. albicans* isolates with MICs between 1.95 to 7.81 µg/mL, suggesting mechanisms involving ergosterol biosynthesis inhibition and membrane damage. Similarly, Yassin *et al.*[41] demonstrated strong antifungal activity of ZnO NPs derived from pomegranate peel, with inhibition zones of 18.68–23.12 mm against *Candida* spp. Iqbal *et al.*[42] also confirmed the antifungal effects of ZnO NPs synthesized from *Elaeagnus angustifolia* against fungi such as *A. flavus, A. niger*, and *C. albicans*, with effective inhibition observed at concentrations between 37.5 and 1200 µg/mL. Overall, *C. trifolia* stem-based ZnO NPs show considerable promise for antifungal applications, particularly in treating opportunistic yeast infections. Further mechanistic studies and formulation optimization are recommended for clinical and agricultural applications.

Table-4: Antifungal Activity of ZnO NP Synthesized from Different Parts of *Causonis Trifolia* Against Various Fungal Strains

Plant parts	Concentration of ZuO NP (µg)	*'A. Niger* MTCC 282'	*'Aspergillus flavus* MTCC 277'	*'C. albicans* MTCC 227'	*'Saccharomyces cerevisiae* MTCC 4742'	*Phytophthora*
		Diameter of Inhibition zone (mm)				
Zn NPs from Stem extract	100	--	--	15.0	8.0	--
	250	--	10.0	20.0	11.0	--
	500	6.0	12.0	24.0	14.0	--
	Positive control	32.0	31.0	19.0	19.0	29.0
	Negative control	--	--	--	--	--
Zn NPs from Leaf extract	100	--	--	--	--	--
	250	--	--	14.0	11.0	--
	500	6.0	--	18.0	16.0	--
	Positive control	30.0	29.0	20.0	17.0	27.0
	Negative control	--	--	--	--	--
Zn NPs from Fruit Extract	100	--	--	--	--	--
	250	--	--	--	--	--
	500	7.0	--	7.0	--	7.0
	Positive control	31.0	30.0	19.0	20.0	29.0
	Negative control	--	--	--	--	--

Antioxidant Activity of ZnO Nanoparticles Synthesized from *Causonis Trifolica* Extracts

The antioxidant potential of ZnO NPs derived from stem, leaf, and fruit extracts of *Causonis trifolia* was evaluated through two standard analyses: DPPH radical scavenging and FRAP. As shown in Table-5 and 6, both methods demonstrated a clear dose-dependent response within the tested concentration range of 62.5 to 1000 µg/mL.ZnO NPs derived from stem extract exhibited the strongest DPPH radical scavenging ability, with an IC50 of 195.28 µg/mL (Table-5). This suggests effective electron or hydrogen donation capability, likely due to the stem extract's richness in reducing and capping agents that enhance nanoparticle surface activity. Interestingly, fruit-derived ZnO NPs demonstrated the highest FRAP activity (IC50 = 198.96 µg/mL), indicating superior ferric-reducing potential (Table-6). The observed activity could be attributed to a higher concentration of electron-donating phytochemicals in the fruit extract. These results highlight the influence of phytochemical diversity among different plant parts on the antioxidant behaviour of synthesized nanoparticles. Stem-based ZnO NPs were more effective in free radical neutralization (DPPH), while fruit-based ZnO NPs were superior in ferric ion reduction (FRAP), suggesting part-specific bioactivity profiles.Zinc's known role in cellular antioxidant defence is likely enhanced by bioactive plant molecules capping the green-synthesized ZnO NPs. Literature supports these findings: Arumugam *et al.*[43] demonstrated that ZnO NPs from *Syzygium cumini* leaves effectively scavenged DPPH radicals, with an IC50 of 70.85 µg/mL. Iqbal *et al.*[42] reported 93.51% DPPH scavenging for ZnO NPs synthesized using Elaeagnus angustifolia, affirming the strong antioxidant potential of green-synthesized nanoparticles.In conclusion, ZnO NPs synthesized from *C. trifolia* stem and fruit extracts exhibit significant antioxidant properties, reinforcing their potential in developing antioxidant formulations for biomedical, pharmaceutical, and nutraceutical applications. Additional research is necessary to discover the precise phytoconstituents involved and to validate these findings through in vivo models.

Table-5: DPPH Free Radical Scavenging Activity

Concentration (µg)	Stem extract ZnO NP	Leaf extract ZnO NP	Fruit extract ZnO NP
	Per cent Inhibition (%)		
62.5	21.48	26.51	27.85
125	26.85	31.54	31.88
250	45.64	38.59	34.56
500	61.41	54.03	55.70
1000	66.78	71.14	69.80
IC_{50}	195.28 µg	364.92 µg	379.36 µg

Fig.-6: ZnO NPs Synthesized from Stem Extract Demonstrated the Highest Antibacterial Activity, Notably Against Gram +ve *Clostridium perfringens* (30.0 mm) and *Staphylococcus aureus* (28.0 mm)

Table-6: FRAP (Ferric Reducing Antioxidant Power) Activity

Concentration (µg)	Stem extract ZnO NP	Leaf extract ZnO NP	Fruit extract ZnO NP
	Per cent inhibition (%)		
62.5	21.90	16.99	25.18
125	37.19	30.09	44.84
250	48.66	50.85	53.03
500	60.68	54.12	62.32
1000	63.95	62.32	71.05
IC_{50}	249.66 µg	275.91 µg	198.96 µg

Fig.-7: Stem-extract ZnO NPs showed the Strongest Antibacterial Ability in Opposition to Gram –ve *Vibrio cholerae* and *E. coli* (28.0 mm each), next by *Pseudomonas aeruginosa* (25.0 mm)

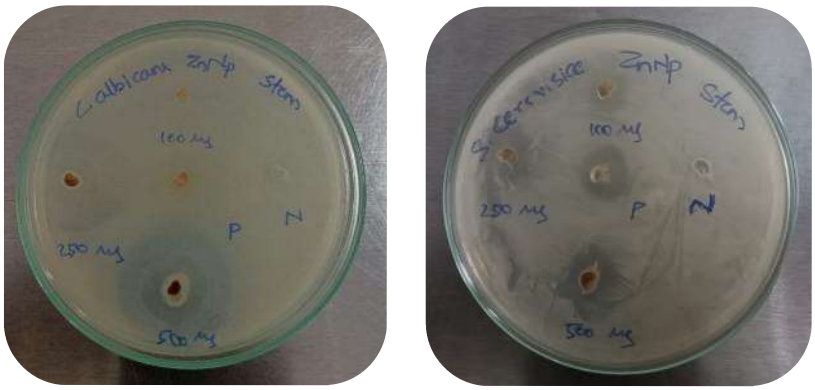

Fig.-8: Stem-derived ZnO Nanoparticles Exhibited Notable Antifungal Activity against *Candida albicans* (24.0 mm) and *S. cerevisiae* (14.0 mm)

CONCLUSION

The present study explored the eco-friendly synthesis of ZnO NPs derived from leaf, stem, and fruit extracts of *Causonis trifolia*, aiming to assess their structural, antimicrobial, and antioxidant properties. Characterization techniques confirmed the successful synthesis of ZnO NPs with a hexagonal Wurtzite model, phytochemical-assisted stabilization, and varied morphologies. Among the synthesized variants, stem-derived ZnO NPs demonstrated the most significant antibacterial and antifungal activity, particularly in opposition to *S. aureus, Clostridium perfringens, E. coli, Vibrio cholerae*, and *C. albicans*. These nanoparticles also showed the highest DPPH radical scavenging ability, indicating strong antioxidant potential. While leaf-derived NPs exhibited moderate bioactivity, fruit-derived NPs were less effective, except for their relatively high ferric-reducing capacity. The findings highlight the influence of plant part-specific phytochemical profiles on nanoparticle biofunctionality and support the application of *C. trifolia*-mediated ZnO NPs as sustainable, multifunctional agents in antimicrobial and antioxidant therapies.

ACKNOWLEDGMENTS

The authors are thankful to the authorities of their institutions for providing basic facilities for this research.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

Santosi Anusha Buravilli https://orcid.org/0009-0006-9183-9697

Srinivasa Rao Golagani https://orcid.org/0000-0001-9647-7206
Venkata Ravi Sankar Ummidi https://orcid.org/0000-0002-6060-3773
Sai Kishore Marothu https://orcid.org/0009-0001-2874-0954
M.Vidya Kalpana https://orcid.org/0000-0002-8282-3291
A.Hymavathi https://orcid.org/0009-0004-9373-1239
Ravada Kishore https://orcid.org/0000-0002-7570-8447

REFERENCES

1. C. Arab, R. El Kurdi, and D. Patra, *Chemosphere*, **276**, 130158(2021), https://doi.org/10.1016/j.chemosphere.2021.130158
2. G. Yashni, A. Al-Gheethi, R.M.S.R. Mohamed, N.V. Dai-Viet, A.A. Al-Kahtani, M. Al-Sahari, and S. Alkhadher, *Chemosphere*, **281**, 130661(2021), https://doi.org/10.1016/j.chemosphere.2021.130661
3. M. Naseer, U. Aslam, B. Khalid, B.,and Chen, *Scientific Reports*, **10(1)**, 9055(2020), https://doi.org/10.1038/s41598-020-65949-3
4. A.H. Alrajhi, and N.M Ahmed, in *Handbook of Green and Sustainable Nanotechnology: Fundamentals, Developments and Applications*, pp. 1–21(2023), Springer, Cham. https://doi.org/10.1007/978-3-030-69023-6_44-1
5. V. Amendola, D. Amans, Y. Ishikawa, N. Koshizaki, S. Scirè, G. Compagnini, S. Reichenberger, and S. Barcikowski, *Chemistry – A European Journal*, **26(42)**, 9206(2020), https://doi.org/10.1002/chem.202000686
6. U. Özgür, Y.I. Alivov, C. Liu, A. Teke, M.A. Reshchikov, S. Doğan, and A.H. Morkoç, *Journal of Applied Physics*, **98(4)**, 041301(2005), https://doi.org/10.1063/1.1992666
7. D. Suresh, R.M. Shobharani, P.C. Nethravathi, M.P. Kumar, H. Nagabhushana, and S.C. Sharma, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **141**, 128(2015), https://doi.org/10.1016/j.saa.2015.01.048
8. R. Gill, S. Ghosh, A. Sharma, D. Kumar, V.H. Nguyen, D.V. Vo, T.D. Pham, and P. Kumar, *Materials Letters*, **277**, 128295(2020), https://doi.org/10.1016/j.matlet.2020.128295
9. M. Ramesh, M. Anbuvannan, and G. Viruthagiri, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **136**, 864(2015), https://doi.org/10.1016/j.saa.2014.09.105
10. M.S. Jadhav, S. Kulkarni, P. Raikar, D.A. Barretto, S.K. Vootla, and U.S. Raikar, *New Journal of Chemistry*, **42(1)**, 204(2018), https://doi.org/10.1039/C7NJ02977B
11. C.D. Romero, S.F. Chopin, G. Buck, E. Martinez, M. Garcia, and L. Bixby, *Journal of Ethnopharmacology*, **99(2)**, 253(2005), https://doi.org/10.1016/j.jep.2005.02.028
12. H.W. Boucher, G.H. Talbot, J.S. Bradley, J.E. Edwards, D. Gilbert, L.B. Rice, and J. Bartlett, *Clinical Infectious Diseases*, **48(1)**, 1(2009), https://doi.org/10.1086/595011
13. G.H. Talbot, J. Bradley, J.E. Edwards, D. Gilbert, M. Scheld, and J.G. Bartlett, *Clinical Infectious Diseases*, **42(5)**, 657(2006), https://doi.org/10.1086/499819
14. I. Kim, K. Viswanathan, G. Kasi, S. Thanakkasaranee, K. Sadeghi and J. Seo, *Food Reviews International*, **38(4)**, 537(2022), https://doi.org/10.1080/87559129.2020.1737709
15. D. Jain, A.A. Shivani, Bhojiya, H. Singh, H.K. Daima, M. Singh, and A. Singh, *Frontiers in Chemistry*, **8**, 778(2020), https://doi.org/10.3389/fchem.2020.00778
16. B. Mubeen, A.N. Ansar, R. Rasool, I. Ullah, S.S. Imam, S. Alshehri, and I. Kazmi, *Antibiotics*, **10(12)**, 1473(2021), https://doi.org/10.3390/antibiotics10121473
17. A.E. Mohamed, W.E. Elgammal, A.M. Eid, A.M. Dawaba, A.G. Ibrahim, A. Fouda, and S.M. Hassan, *International Journal of Biological Macromolecules*, **207**, 242(2022), https://doi.org/10.1016/j.ijbiomac.2022.02.173
18. P. Chand, S. Kumari, N. Mondal, S.P. Singh, and T. Prasad, *Frontiers in Nanotechnology*, **3**, 624564(2021), https://doi.org/10.3389/fnano.2021.624564

19. L. Scorzoni, F. Sangalli-Leite, J. de Lacorte Singulani, C.B. Costa-Orlandi, A.M. Fusco-Almeida, and M.J.S. Mendes-Giannini, *Journal of Microbiological Methods*, **123**, 68(2016), https://doi.org/10.1016/j.mimet.2016.02.005
20. S. Hazra, A.S. Ray, S. Das, A. Das Gupta, and C.H. Rahaman, *Plants*, **12**(7), 1495(2023), https://doi.org/10.3390/plants12071495
21. L. Chabib, H. Hamzah, W. Rahmah, S.F. Sammulia, E. Setyowati, and A. Nurfitriani, *Pakistan Journal of Biological Sciences*, **26(2)**, 91(2023), https://doi.org/10.3923/pjbs.2023.91.100
22. A. Miri, N. Mahdinejad, O. Ebrahimy, M. Khatami, and M. Sarani, *Materials Science and Engineering C*, **104**, 109981(2019), https://doi.org/10.1016/j.msec.2019.109981
23. X. Liang, D. Zhang, W. Liu, Y. Yan, F. Zhou, W. Wu, and Z. Yan, *Toxicology and Industrial Health*, **33(10)**, 737(2017), https://doi.org/10.1177/0748233717712409
24. C. Bi, J. Li, L. Peng, and J. Zhang, *Biomedical Research.*, **28**, 2065(2017).
25. L.K. Ruddaraju, S.V.N. Pammi, G.S. Guntuku, V.S. Padavala, and V.R.M. Kolapalli, *Asian Journal of Pharmaceutical Sciences*, **15(1)**, 42(2020), https://doi.org/10.1016/j.ajps.2019.03.002
26. S. Saif, A. Tahir, and Y. Chen, *Nanomaterials*, **6(11)**, 209(2016), https://doi.org/10.3390/nano6110209
27. X. Zhu, K. Pathakoti, and H.M. Hwang, in *Green Synthesis, Characterization and Applications of Nanoparticles*, Elsevier, pp. 223–263(2019), https://doi.org/10.1016/B978-0-08-102579-6.00010-1
28. S. A. Akintelu, B. Yao, and A. S. Folorunso, *Plasmonics*, **16(1),** 157(2021), https://doi.org/10.1007/s11468-020-01274-9
29. S. A. Akintelu, S. C. Olugbeko, F. A. Folorunso, A. K. Oyebamiji, and A. S. Folorunso, *Journal of Chemistry*, **2020(1),** 2876019(2020), https://doi.org/10.1155/2020/2876019
30. P. Sharma, S. Pant, V. Dave, K. Tak, V. Sadhu, and K. R. Reddy, *Journal of Microbiological Methods*, **160,** 107(2019), https://doi.org/10.1016/j.mimet.2019.03.007
31. D. A. Jamdade, D. Rajpali, K. A. Joshi, R. Kitture, A. S. Kulkarni, V. S. Shinde, ... and S. Ghosh, *Advances in Pharmacological and Pharmaceutical Sciences*, **2019(1),** 9080279(2019), https://doi.org/10.1155/2019/9080279
32. M. Noruzi, *Bioprocess and Biosystems Engineering*, **38(1),** 1(2015), https://doi.org/10.1007/s00449-014-1251-0t
33. Y. T. Prabhu, K. V. Rao, V. S. Sai, and T. A Pavani, *Journal of Saudi Chemical Society*, **21(2),** 180(2017), https://doi.org/10.1016/j.jscs.2015.04.002
34. W. PA, *CLSI Document* **M27-A2** (2002), https://doi.org/10.11263/jsotp.28.95
35. S. F. ELhabal, H. M. Elwy, S. Hassanin, A. A. El-Rashedy, A. A. Hamza, and M. A, Khasawneh, *International Journal of Nanomedicine*, **17,** 6095(2022), https://doi.org/10.2147/IJN.S385543
36. M.Y. Al-Darwesh, S.S. Ibrahim, and M.A. Mohammed, *Results in Chemistry*, **7**, 101368(2024), https://doi.org/10.1016/j.rechem.2024.101368
37. E.E. Imade, T.O. Ajiboye, A.E. Fadiji, D.C. Onwudiwe, and O.O. Babalola, *Scientific African*, **16**, e01152(2022), https://doi.org/10.1016/j.sciaf.2022.e01152
38. M.H. Kalaba, G.M. El-Sherbiny, E.A. Ewais, O.M. Darwesh, and S.A. Moghannem, *BMC Microbiology*, **24(1)**, 254(2024), https://doi.org/10.1186/s12866-024-03392-4
39. N. Singh, R. Singh, K. Shah, and B.K. Pramanik, *Chemosphere*, **327**, 138497(2023), https://doi.org/10.1016/j.chemosphere.2023.138497
40. M.A. Abomuti, E.Y. Danish, A. Firoz, N. Hasan, and M. A. Malik, *Biology*, **10(11)**, 1075(2021), https://doi.org/10.3390/biology10111075
41. M.T. Yassin, A.A.F. Mostafa, A.A. Al-Askar, and F.O. Al-Otibi, *Crystals*, **12(6)**, 774(2022), https://doi.org/10.3390/cryst12060774
42. J. Iqbal, B.A. Abbasi, T. Yaseen, S.A. Zahra, A. Shahbaz, S.A. Shah,... and P. Ahmad, *Scientific Reports*, **11**, 20988(2021), https://doi.org/10.1038/s41598-021-99839-z
43. M. Arumugam, D.B. Manikandan, E. Dhandapani, A. Sridhar, K. Balakrishnan, M. Markandan, and T. Ramasamy, *Environmental Technology & Innovation*, **23**, 101653(2021), https://doi.org/10.1016/j.eti.2021.101653

[RJC-9383/2025]