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GREEN SYNTHESIS, CHARACTERIZATION, AND BIOACTIVITIES OF COPPER OXIDE NANOPARTICLES FROM CAUSONIS TRIFOLIA: ANTIMICROBIAL, ANTIOXIDANT, AND ANTIPROLIFERATIVE PROPERTIES

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ABSTRACT

The environmentally friendly, simple, and cost-efficient nature of green nanoparticle synthesis has attracted considerable interest. Recent research has significantly expanded into plant-based nanoparticle synthesis and their biomedical applications. Among various metals, copper (Cu) is preferred for nanoparticle synthesis due to its established antimicrobial properties, medical applications, and high lethality against pathogens. In the present study, copper oxide nanoparticles (CuO NPs) were derived from the stem, leaf, and fruit extracts of *Causonis trifolia* and characterized using UV-visible spectroscopy, X-ray diffraction, scanning electron microscopy, Fourier transform infrared spectroscopy, and energy dispersive X-ray spectroscopy. The formation of CuO NPs was confirmed by spectral analysis, with UV-Vis absorbance at 280–350 nm and FTIR peaks at 500–600 cm^{-1} indicating copper oxide vibrations. SEM analysis revealed particle morphology with agglomerates, while XRD confirmed a monoclinic structure with crystallite sizes of 35.0 nm (leaf extract), 27.0 nm (stem extract), and 7.0 nm (fruit extract). The biomedical potential of CuO NPs was tested against Gram +ve and Gram -ve bacteria, fungal strains, and antioxidant & antiproliferative activities. Fruit extract-derived CuO NPs showed the maximum antibacterial activity against *Clostridium perfringens* (38 mm @ 500 µg/mL), *Bacillus licheniformis* (36 mm @ 500 µg/mL), *Vibrio cholerae* (35 mm @ 500 µg/mL), and *Salmonella e*nterica (35 mm @ 500 µg/mL). Additionally, significant antifungal activity was observed against *Saccharomyces cerevisiae* (28 mm @ 500 µg/mL) and *Candida albicans* (23 mm @ 500 µg/mL), followed by stem and leaf-derived nanoparticles. The antioxidant and antiproliferative activities were concentration-dependent, with fruit-derived CuO NPs demonstrating the highest cytotoxicity (93.47% at 1000 µg/mL), followed by stem and leaf-derived CuO NPs (71.22%). These research findings demonstrate the potential of eco-friendly synthesized CuO NPs using novel *Causonis trifolia* as effective antimicrobial, antioxidant, and antiproliferative agents, supporting their application in biotechnology, biomedical, and pharmaceutical fields.

Keywords: Green Synthesis, *Causonis Trifolia*, Cuo Nps, Antimicrobial, Antioxidant, and Antiproliferative Properties.

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INTRODUCTION

Nanotechnology has revolutionized various scientific fields, particularly through nanoparticle (NP) synthesis, due to their unique properties and broad applications.[1] NPs (1–100 nm) exhibit superior optical, magnetic, catalytic, and electrical properties,[2] making them valuable for industrial and biomedical use.[3] Conventional NP synthesis involves high temperatures, toxic chemicals, and energy-intensive methods, raising environmental concerns. Green synthesis offers an eco-friendly alternative,[4] utilizing plant extracts, biomolecules, and microorganisms, reducing toxicity and enhancing scalability.[5] Plant-based synthesis is widely explored for metal NPs due to its economic and environmental benefits[6], with fungi, yeast, algae, and bacteria also serving as bio-factories.[7] Among metal NPs, CuO NPs stand out for their thermal stability, large surface area, and exceptional catalytic properties.[8] Noble metal NPs play a vital role in antimicrobial

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medicine, biotechnology, optics, catalysis, and energy storage. Metals like silver, copper, zinc, and gold stand out for their functional properties and low toxicity.[9] Cu NPs, widely used in cosmetics, textiles, and biotechnology, exhibit strong antimicrobial and biocompatibility properties. Green synthesis using plant extracts as reducing and capping agents provides a sustainable alternative.[10] The synergy between medicinal plant bioactive compounds and Cu NPs enhances their antimicrobial and anticancer potential.[11] CuO NPs, offering cost-effectiveness and superior biocidal properties, are gaining attention for biomedical applications such as wound healing, drug delivery, and disease treatment.[12] Their high stability, catalytic efficiency, and antimicrobial activity make them suitable for pharmaceuticals, gas sensors, solar cells, and superconductors.[13] Their unique crystal structure and high surface area contribute to prolonged antimicrobial efficacy.[14] In healthcare, Cu NPs significantly reduce nosocomial infections on medical instruments and surfaces.[15] Their high surface area enhances drug attachment, optimizing therapeutic applications.[16] The integration of plant extracts in NP synthesis exemplifies a sustainable methodology[17], ensuring stability and long-term effectiveness.[18] Bioengineered CuO NPs, synthesized using bacteria, fungi, or plant extracts, offer sustainable solutions for healthcare, materials science, and environmental applications.[19] Despite extensive research on Cu NP synthesis, the green synthesis from *Causonis trifolia* remains underexplored. This ethnomedicinal plant, from the Vitaceae family, holds therapeutic value. This study investigates Cu NP synthesis using its leaf, stem, and fruit extracts. The synthesized NPs were analyzed for antibacterial, antioxidant, and cytotoxic properties, aiming to advance green nanotechnology and sustainable nanomaterials.

EXPERIMENTAL

Collection of Plant Material and Chemicals

Leaves, stems, and fruits of *Causonis trifolia* were collected from Machavaram, Andhra Pradesh (16°51'11.0"N, 81°59'01.2"E). Copper sulfate pentahydrate ($CuSO_4.5H_2O$) (98%), hydrochloric acid (HCl) (35%), and sodium hydroxide (NaOH) (98%) were procured from Merck. A 0.01 M stock solution was prepared by dissolving 2.5 g of $CuSO_4.5H_2O$ in 100 mL of distilled water. Analytical-grade chemicals were used, and microbial cultures were grown in specific media.

Preparation of Plant Extracts

Fresh plant material was washed, air-dried, and ground into a fine powder. Aqueous extracts were prepared by mixing 5 g of powdered material with 100 mL of double-distilled water, heating for 5 min, and filtering through Whatman No.1 filter paper. Extracts were stored at 4°C for later use.[20]

Green Synthesis of CuO NPs

CuO nanoparticles were synthesized by dissolving 2.5 g of $CuSO_4.5H_2O$ in 100 mL of deionized water. Then, 50 mL of *Causonis trifolia* extract was added, and pH was adjusted to 7 using NaOH. The solution was refluxed at 70°C for 5 hours, turning from pale green to deep brown. After centrifugation, the precipitate was washed with deionized water and ethanol, dried at 60°C for 4 hours, and stored at 4°C.[20]

Characterization of CuO Nanoparticles

CuO nanoparticles were characterized using UV-Vis spectroscopy (200–800 nm)[21] for confirmation, FTIR (4000–400 cm^{-1})[22] for functional group analysis, XRD (CuKα, λ = 1.5406 Å, 2θ: 30–70°)[23] for crystalline structure, EDX for elemental composition[24], and SEM for morphology, shape, and size.[25]

Biological Assays

Antimicrobial Activity

Stock CuO nanoparticle solutions (1 mg/mL in Dimethyl Sulfoxide (DMSO) were diluted to 100, 200, and 500 µg/mL. Bacterial (*Bacillus subtilis, B. licheniformis, Clostridium sp., Staphylococcus epidermidis, S. aureus, Aeromonas sp., Vibrio cholerae, Escherichia coli, Pseudomonas aeruginosa*, and *Salmonella paratyphi*) and fungal (*Aspergillus niger, A. flavus, Candida albicans,* and *Saccharomyces cerevisiae*) strains were obtained from Microbial Type Collection Centre (MTCC) and sub-cultured on nutrient agar and Potato Dextrose Agar (PDA). The agar well diffusion method (6 mm wells, 50 µL sample) was used to evaluate antimicrobial activity. Controls included DMSO (negative), Streptomycin (bacteria), and Fluconazole (fungi). Plates were incubated at 32°C for 24 hours, and inhibition zones were measured.[26]

Antioxidant Activity

DPPH Assay

A 0.1 mM DPPH solution was mixed with test samples (200–1000 µg/mL) and incubated in the dark for 30 min. Absorbance was recorded at 517 nm, with ascorbic acid (20–100 µg/mL) as the standard. Higher inhibition indicated stronger antioxidant potential.[27]

FRAP Assay

Test samples were incubated with FRAP reagent (acetate buffer, TPTZ, $FeCl_3$) at 37°C for 30 min, and absorbance was measured at 593 nm. Lower absorbance indicated higher antioxidant activity.[28]

Antiproliferative Activity

Saccharomyces cerevisiae (MTCC 4742) was cultured in PDB and incubated for 24 hours at 37°C. Viability was assessed using a methylene blue staining method. Yeast cells were treated with plant extracts (5 and 10 mg/mL), quercetin (positive control), or distilled water (control) and incubated at 32°C for 24 hours. Viable cells remained transparent, while dead cells stained blue. Cell count was determined using a hemocytometer.

RESULTS AND DISCUSSION

UV-Visible Spectroscopy Analysis

UV-Visible spectroscopy confirms CuO nanoparticle (CuO NPs) synthesis *using Causonis trifolia* leaf, stem, and fruit extracts (Fig.-1). The absorption spectrum exhibits a peak at 280–350 nm, characteristic of CuO NPs due to electronic transitions and charge transfer. These findings align with prior studies, including Karuppannan *et al.*[29] (264, 333 nm) and Kothari[30] (252.5 nm). Broader SPR bands have also been reported: Asghar & Asghar[31] (563–582 nm), and Hasanin *et al.*[32] (561 nm), Ginting *et al.*[33] (480–620 nm). The observed absorption band confirms nanoscale CuO formation, with a steep absorbance rise indicating narrow size distribution and well-defined optical properties. The absence of additional peaks supports high purity, minimizing interference from organic residues.

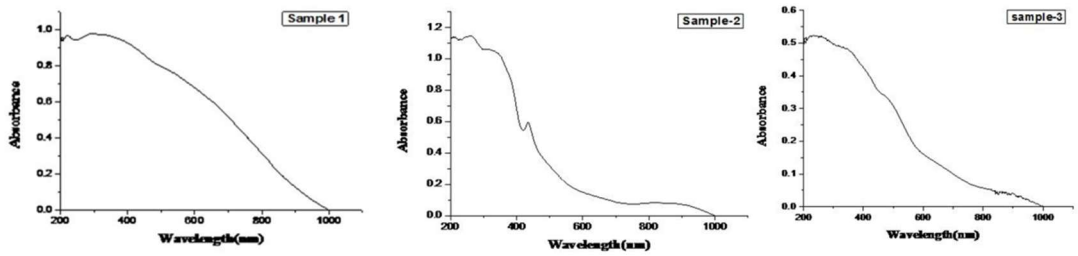

Fig.-1: UV-Visible Spectrum Analysis of Ecofriendly Synthesized CuO NP's from (Sample-1) Fruit (Sample-2) Stem (sample-3) Leaf Extracts of *Causonis trifolia*

Energy Dispersive X-ray Spectroscopy (EDS) Analysis

EDS analysis (Fig.-2) confirms CuO NPs synthesis, with distinct Cu peaks (~8 keV, Cu-Kα; ~1 keV, Cu-Lα) and O at ~0.5 keV (O-Kα). These results are consistent with ELhabal *et al.*[34] (Cu peaks at 1, 2, 3, 8 keV), Velsankar *et al.*[35] (*Allium sativum*-derived CuO NPs: 44.40% Cu, 55.60% O), and Asghar & Asghar[31] (Cu-Lα at 0.93 keV, Cu-Kα at 8.04 keV). Liu *et al.*[36] (*Cinnamomum zeylanicum*-derived CuO NPs) and Qamar et al.[37] (*Momordica charantia*) reported similar peaks, while Amaliyah *et al.*[38] (*Piper retrofractum*) detected Cu (70.3%), O (15.0%), and trace elements. Trace elements (Ca, Si, S, K, C, Na) suggest phytochemical-mediated stabilization. Sulfur (~2.3 keV) likely originates from stabilizing compounds, while carbon (~0.3 keV) may stem from plant residues. The minimal presence of foreign elements confirms high purity. *C. trifolia* extracts facilitate eco-friendly CuO NPs synthesis without chemical stabilizers, ensuring sustainable nanoparticle production.

X-Ray Diffraction (XRD) Analysis

XRD analysis confirms the successful synthesis of crystalline CuO nanoparticles using *Causonis trifolia* leaf, stem, and fruit extracts (Fig.-3). Diffraction peaks at ~32°, 35°, 38°, 48°, and 61° correspond to the

(110), (002), (111), (202), and (113) planes, matching the monoclinic CuO phase (JCPDS Card No. 05-0661). The absence of impurity peaks indicates high phase purity, while peak broadening confirms nanoscale crystallinity. The crystallite sizes calculated via the Debye-Scherrer equation are 35.0 nm (leaf extract), 27.0 nm (stem extract), and 7.0 nm (fruit extract).

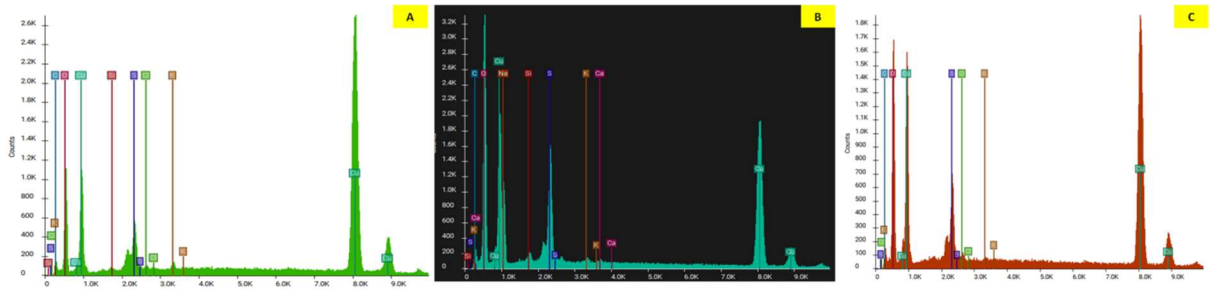

Fig.-2: Energy Dispersive X-ray Spectrum Analysis of Eco-Friendly Synthesized CuO NP's from (A) Stem, (B) Leaf (C) Fruit Extracts of *Causonis trifolia*

These results align with previous studies: Rajeshkumar *et al.*[39] (68 nm), Rajesh Kumar & Rinitha[40] (42–90 nm), Kothari[30] (13 nm), and Velsankar[35] (25–30 nm). Typical CuO NPs diffraction peaks at ~35°, 38°, 48°, and 61° confirm the monoclinic phase. Qamar *et al.*[37] reported peaks at 32.64°, 35.1°, 38.9°, and 48.9° (6 nm crystallites), while Liu *et al.*[36] identified peaks at 35.77°, 38.97°, and 48.88°, indexing them to (-111), (111), and (-202) planes. Rajesh Kumar & Rinitha[40] observed peaks at 24°, 30°, and 42°, while Amaliyah *et al.*[38] reported 37.079°, 42.693°, and 61.922° (56.8 nm).

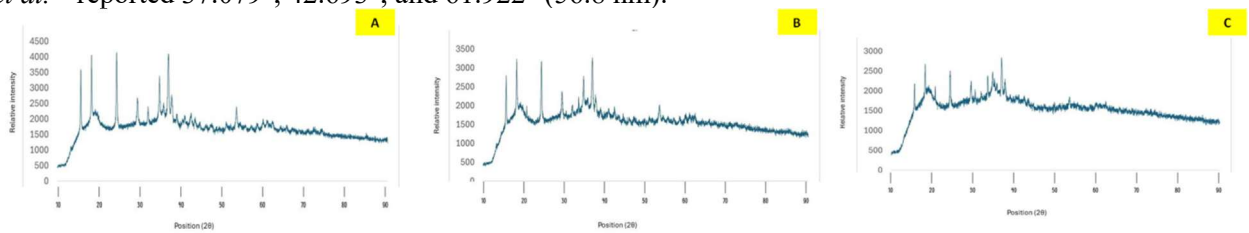

Fig.-3: X-Ray Diffraction Analysis of Ecofriendly Synthesized CuO NP's from (A) Stem, (B) Leaf, (C) Fruit Extracts of *Causonis trifolia*

Fourier Transform Infrared (FTIR) Analysis

FTIR analysis confirms the role of *Causonis trifolia* extracts in CuO nanoparticle synthesis and stabilization (Fig.-4). The broad O-H stretching peak (3200–3500 cm^{-1}) indicates hydroxyl groups from plant bioactives acting as capping agents, while the strong C=O stretching peak (1600–1700 cm^{-1}) suggests phytochemical-mediated Cu^{2+} reduction. Peaks at 1300–1500 cm^{-1} correspond to C-H bending, and characteristic Cu-O stretching vibrations (500–600 cm^{-1}) confirm CuO formation. These results align with previous studies (Reddy *et al.*[41]; Ghidan *et al.*[42]; Amin *et al.*[43]; Liu *et al.*[36]), corroborating plant-based CuO nanoparticle synthesis.

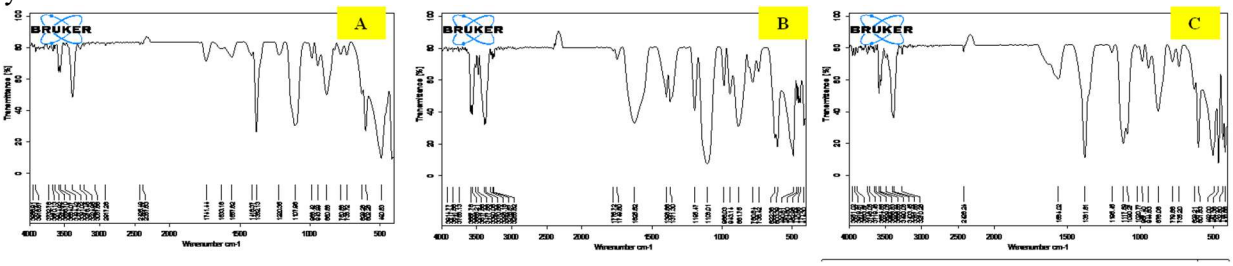

Fig.-4: Fourier Transform Infrared Spectroscopy (FTIR) Spectrum of Green Synthesized CuO NP's from *Causonis trifolia* using: (A) Leaf, (B) Stem, and (C) Fruit

Scanning Electron Microscopy (SEM) Analysis

SEM images (50 and 100 nm magnification) reveal CuO nanoparticles with irregular morphology and partial aggregation (Fig.-5). The rough texture and heterogeneous distribution indicate phytochemical

involvement in nanoparticle formation. These findings align with previous studies (Ghidan *et al.*[42]; Mahdavi *et al.*[44]; Baghayeri *et al.*[45]), confirming the successful green synthesis of CuO NPs.

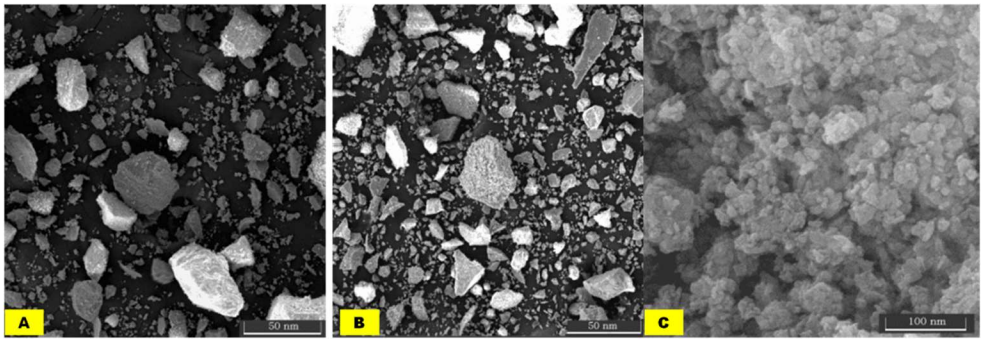

Fig.-5: Scanning Electron Microscope images at Different Magnifications of Green Synthesized CuO NP's from (A) Stem, (B) Leaf, (C) Fruit Extracts of *Causonis trifolia*

Antibacterial Activity of CuO Nanoparticles

CuO NPs synthesized from *Causonis trifolia* stem, leaf, and fruit extracts exhibited dose-dependent antibacterial activity against five Gram-positive (*Bacillus subtilis, B. licheniformis, Clostridium perfringens, Staphylococcus epidermidis, Staphylococcus aureus*) and five Gram-negative (*Aeromonas hydrophila, Vibrio cholerae, Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi*) bacterial strains (Tables-1 & 2). Stem-derived CuO NPs showed strong inhibition (12–38 mm), with *C. perfringens* (38 mm) and *Salmonella enterica* (38 mm) being most sensitive. Leaf-derived CuO NPs displayed moderate activity (10–35 mm), with *C. perfringens* (35 mm) and *V. cholerae* (31 mm) showing the highest susceptibility. Fruit-derived CuO NPs exhibited the strongest antibacterial effects (13–38 mm), particularly against *C. perfringens* (38 mm) and *V. cholerae* (35 mm) as shown in Fig.-6 & 7. The superior efficacy of fruit-derived CuO NPs suggests enhanced bioactivity due to higher phytochemical content. These findings align with previous studies (Velsankar *et al.*[35]; Asghar & Asghar[31]), confirming plant-based CuO NPs' broad-spectrum antibacterial potential. The mechanism involves ROS generation, membrane disruption, and biomolecular damage, making CuO NPs promising antimicrobial agents.

Table-1: Antibacterial Activity of CuO NP Synthesized from Different Parts of *Causonis trifolia* against Gram-Positive Bacteria

Plant parts	Concentration of CuO 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)				
Cuo NPs from Stem extract	100	12.0	19.0	29.0	22.0	19.0
	250	16.0	21.0	35.0	25.0	25.0
	500	21.0	29.0	38.0	26.0	31.0
Cuo NPs from Leaf extract	100	10.0	12.0	27.0	12.0	15.0
	250	13.0	14.0	32.0	15.0	17.0
	500	16.0	26.0	35.0	16.0	25.0
Cuo NPs from Fruit extract	100	15.0	32.0	29.0	13.0	23.0
	250	17.0	35.0	33.0	15.0	26.0
	500	19.0	36.0	38.0	19.0	31.0

Antifungal Activity of CuO Nanoparticles

CuO NPs synthesized from *Causonis trifolia* stem, leaf, and fruit extracts exhibited dose-dependent antifungal activity against four fungal strains (Table-3). Stem-derived CuO NPs showed moderate inhibition (0–24 mm), with *Saccharomyces cerevisiae* (24 mm) and *Candida albicans* (23 mm) being the most susceptible. *Aspergillus niger* exhibited weak inhibition (8 mm) only at 500 µg/mL.

Leaf-derived CuO NPs displayed varied activity (0–28 mm), with *S. cerevisiae* (28 mm) showing the highest inhibition, followed by *C. albicans* (19 mm). *Aspergillus flavus* exhibited moderate susceptibility (12 mm). Fruit-derived CuO NPs exhibited the highest antifungal efficacy (0–28 mm), particularly against

S. cerevisiae (28 mm) and *C. albicans* (23 mm), with moderate inhibition of *A. flavus* (13 mm). Fruit-derived CuO NPs were the most effective, likely due to enhanced bioactive compound content. Previous studies (Velsankar *et al.*[35]; Qamar *et al.*[37]; Rajesh Kumar & Rinitha[40]; Rai & Lall[46]; Tahvilian *et al.*[47]) confirm plant-based CuO NPs' antifungal potential. Their mechanism involves ROS-mediated membrane disruption and biomolecular damage, highlighting their potential as antifungal agents.

Table-2: Antibacterial Activity of CuO NP Synthesized from Different Parts of *Causonis trifolia* against GramAZ Negative Bacteria

Plant parts	Concentration of CuO NP (µg/well)	*Aeromonas hydrophila* MTCC 4033	*Vibrio cholera* MTCC 3906	*Escherichia coli* MTCC 443	*Pseudomonas aeruginosa* MTCC 424	*Salmonella enterica* MTCC 735
		Diameter of Inhibition zone (mm)				
Cuo NPs from Stem extract	100	16.0	18.0	25.0	22.0	26.0
	250	19.0	27.0	28.0	26.0	34.0
	500	25.0	33.0	32.0	32.0	38.0
Cuo NPs from Leaf extract	100	15.0	15.0	19.0	19.0	23.0
	250	17.0	23.0	23.0	23.0	26.0
	500	23.0	31.0	26.0	28.0	29.0
Cuo NPs from Fruit extract	100	19.0	28.0	24.0	27.0	28.0
	250	22.0	32.0	29.0	29.0	31.0
	500	29.0	35.0	32.0	31.0	35.0

Fig.-6: Inhibition Zone at Various Concentrations of Eco-Friendly Synthesized CuO NP's from *Causonis trifolia* Fruit Extract Against Gram-Positive Bacteria: (A) *Bacillus subtilis,* (B) *Bacillus licheniformis,* (C) *Clostridium perfringens,* (D) *Staphylococcus epidermidis*, and (E) *Staphylococcus aureus*

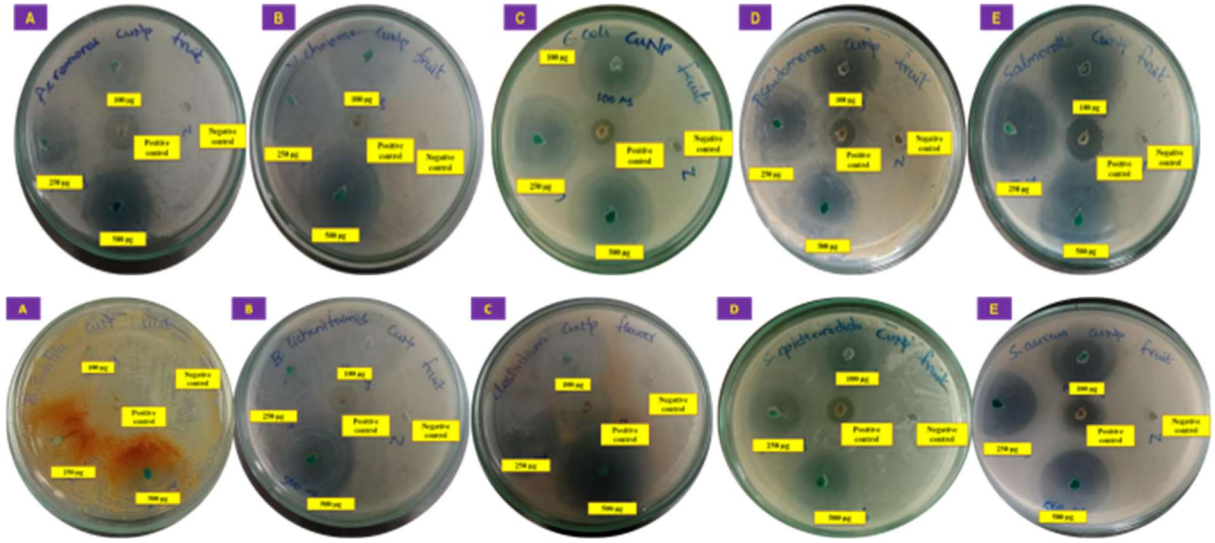

Fig.-7: Inhibition Zone at Various Concentrations of Ecofriendly Synthesized CuO NP's from *Causonis trifolia* Fruit Extract Against Gram-Negative Bacteria: (A) *Aeromonas hydrophila,* (B) *Vibrio chlolera,* (C) *E. coli,* (D) *P. aeruginosa*, and (E) *S. enteric*

Antioxidant Activity of CuO Nanoparticles

The antioxidant potential of CuO nanoparticles (CuO NPs) synthesized from *Causonis trifolia* stem, leaf, and fruit extracts was evaluated using DPPH and FRAP assays, demonstrating a concentration-dependent increase in activity. Fruit-derived CuO NPs exhibited the highest antioxidant activity, with DPPH scavenging ranging from 47.46% to 92.39% (IC_{50}: 93.72 µg/mL) and FRAP reduction from 40.27% to 93.82% (IC_{50}: 88.94 µg/mL).

Stem-derived CuO NPs showed strong activity, with DPPH scavenging of 33.33%–87.32% (IC_{50}: 88.66 µg/mL) and FRAP reduction of 38.72%–91.25% (IC_{50}: 121.5 µg/mL). Leaf-derived CuO NPs demonstrated moderate efficacy, with DPPH scavenging of 27.17%–80.43 % (IC_{50}: 289.60 µg/mL) and FRAP reduction of 29.45%–82.49 % (IC_{50}: 321.99 µg/mL) as presented in Table-4.

Table-3: Antifungal Activity of CuO NP Synthesized from Different Parts of *Causonis trifolia* against Various Fungal Strains

Plant parts	Concentration of CuO NP (µg/well)	*Aspergillus niger* MTCC 282	*Aspergillus flavus* MTCC 277	*Candida albicans* MTCC 227	*Saccharomyces cerevisiae* MTCC 4742
		Diameter of Inhibition zone (mm)			
Cuo NPs from Stem extract	100	R	R	10.0	15.0
	250	R	R	18.0	18.0
	500	8.0	12.0	23.0	24.0
Cuo NPs from Leaf extract	100	R	8.0	10.0	16.0
	250	R	8.0	12.0	18.0
	500	9.0	12.0	19.0	28.0
Cuo NPs from Fruit extract	100	R	8.0	14.0	23.0
	250	R	9.0	16.0	26.0
	500	R	13.0	23.0	28.0

Note: 'R' indicates Resistance

The superior antioxidant potential of fruit-derived CuO NPs is attributed to their enriched bioactive compounds, enhancing redox properties. Similar trends were observed in CuO NPs synthesized from *Blumea balsamifera* (Ginting *et al.*[33]), *Cissus arnotiana* (Rajeshkumar *et al.*[39]), and *Parthenium hysterophorus* (Rai & Lall[46]), reinforcing their potential as effective antioxidants.

Table-4: DPPH Scavenging & FRAP Activity of CuO Nanoparticles from different Plant Extracts at different Concentrations

Concentration (µg)	CuO NP from Stem extract		CuO NP from leaf extract		CuO NP from fruit extract	
	Per cent Inhibition (%)					
Method	DPPH	FRAP	DPPH	FRAP	DPPH	FRAP
62.5	33.33	38.72	27.17	29.45	47.46	40.27
125	52.17	55.20	35.51	38.72	58.33	58.29
250	68.84	76.83	63.41	50.05	71.74	77.86
500	82.25	85.07	68.84	67.04	86.59	85.07
1000	87.32	91.25	80.43	82.49	92.39	93.82
IC_{50}	88.66	121.50	289.60	321.99	93.72	88.94

Antiproliferative Activity of CuO Nanoparticles

The antiproliferative activity of CuO nanoparticles (CuO NPs) synthesized from *Causonis trifolia* leaf, stem, and fruit extracts was assessed based on their cytotoxicity across different concentrations, revealing a dose-dependent increase. Fruit-derived CuO NPs exhibited the highest cytotoxicity, ranging from 21.32% (62.5 µg/mL) to 93.47% (1000 µg/mL), suggesting strong antiproliferative potential. Stem-derived CuO NPs followed, with cytotoxicity increasing from 15.34% to 89.32% over the same concentration range. Leaf-derived CuO NPs showed the lowest cytotoxicity, increasing from 11.54% to 71.22%, indicating moderate activity. At 1000 µg/mL, fruit-derived CuO NPs demonstrated the most potent cytotoxic effect, followed by stem and leaf extracts (Table-5). The findings highlight the influence of phytochemical composition on the antiproliferative potential of CuO NPs, supporting their application in biomedical research.

Table-5: Antiproliferative Activity of CuO Nanoparticles from different Plant Extracts at different Concentrations

Plant part	Concentration (µg)	% Cytotoxicity
CuO NP from leaf extract	62.5	11.54
	125	32.86
	250	46.32
	500	58.74
	1000	71.22
CuO NP from stem extract	62.5	15.34
	125	36.87

	250	55.32
	500	76.32
	1000	89.32
CuO NP from fruit extract	62.5	21.32
	125	38.32
	250	52.35
	500	78.97
	1000	93.47

CONCLUSION

The eco-friendly synthesis of copper oxide nanoparticles (CuO NPs) utilizing *Causonis trifolia* extracts presents a sustainable and cost-efficient method for nanoparticle production. This approach minimizes reliance on toxic chemicals and expensive reagents, making it a viable option for large-scale applications. The formation and characteristics of CuO NPs were thoroughly analyzed using UV-Vis, XRD, SEM, FTIR, and EDS techniques, confirming their structural and morphological attributes. The biomedical potential of CuO NPs was evident through their strong antibacterial, antifungal, antioxidant, and antiproliferative activities. Among the synthesized nanoparticles, those derived from fruit extract demonstrated the highest bioactivity, followed by stem and leaf-derived CuO NPs. The significant antibacterial activity against Gram-positive and Gram-negative bacteria, as well as antifungal activity, suggests that these metal oxide nanoparticles could serve as effective alternatives to standard antibiotics. Additionally, the antioxidant and cytotoxic properties highlight their potential therapeutic applications in medicine and healthcare. Present research findings confirm that CuO NPs synthesized from *Causonis trifolia* can be effectively utilized in biotechnological, biomedical, and pharmaceutical fields, offering a promising green alternative for antimicrobial and anticancer treatments. Further research is warranted to explore their mechanism of action, biocompatibility, and in vivo applications.

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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:

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REFERENCES

1. T.C. Madzokere, L. T. Murombo, and H. J. M. T. P. Chiririwa, *Materials Today: Proceedings*, **45**, 3709(2021), https://doi.org/10.1016/j.matpr.2020.12.674
2. N. Baig, I. Kammakakam, and W. Falath, *Materials Advances*, **2(6),** 1821(2021), https://doi.org/10.1039/D0MA00807A

3. I. Khan, K. Saeed, and I. Khan, *Arabian Journal of Chemistry*, **12(7),** 908(2019), https://doi.org/10.1016/j.arabjc.2017.05.011
4. N. M. Noah, and P. M. Ndangili, *Sensors International*, **3,** 100166(2022), https://doi.org/10.1016/j.sintl.2022.100166
5. V. Harish, D. Tewari, M. Gaur, A. B. Yadav, S. Swaroop, M. Bechelany, and A. Barhoum, *Nanomaterials*, **12(3),** 457(2022), https://doi.org/10.3390/nano12030457
6. H. N. Chaudhari, *Materials Research Foundations*, **169** (2024), https://doi.org/10.21741/9781644903261-3
7. C. Pandit, A. Roy, S. Ghotekar, A. Khusro, M. N. Islam, T. B. Emran, ...and D. A. Bradley, *Journal of King Saud University-Science*, **34(3),** 101869(2022), https://doi.org/10.1016/j.jksus.2022.101869
8. P. Sharma, S. Pant, P. Poonia, S. Kumari, V. Dave, and S. Sharma, *Journal of Inorganic and Organometallic Polymers and Materials*, **28(6),** 2463(2018), https://doi.org/10.1007/s10904-018-0933-5
9. G. Murugappan, and K. J. Sreeram, *Colloids and Surfaces B: Biointerfaces*, **197,** 111386(2021), https://doi.org/10.1016/j.colsurfb.2020.111386
10. 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
11. H. A. Murthy, T. Desalegn, M. Kassa, B. Abebe, and T. Assefa, *Journal of Nanomaterials*, **2020(1),** 3924081(2020), https://doi.org/10.1155/2020/3924081
12. D. Vaidehi, V. Bhuvaneshwari, D. Bharathi, and B. P. Sheetal, *Materials Research Express*, **5(8),** 085403(2018), https://doi.org/10.1088/2053-1591/aad426
13. M. L. Knetsch, and L. H. Koole, *Polymers*, **3(1),** 340(2011), https://doi.org/10.3390/polym3010340
14. F. Perreault, S. P. Melegari, C. H. da Costa, A. L. D. O. F. Rossetto, R. Popovic, and W. G. Matias, *Science of The Total Environment*, **441,** 117(2012), https://doi.org/10.1016/j.scitotenv.2012.09.065
15. D. Mitra, E. T. Kang, and K. G. Neoh, *ACS Applied Materials & Interfaces*, **12(19),** 21159(2019), https://doi.org/10.1021/acsami.9b17815
16. R. N. Saha, S. Vasanthakumar, G. Bende, and M. Snehalatha, *Molecular Membrane Biology*, **27(7),** 215(2010), https://doi.org/10.3109/09687688.2010.510804
17. M. Nasrollahzadeh, F. Ghorbannezhad, Z. Issaabadi, and S. M. Sajadi, *The Chemical Record*, **19(2-3),** 601(2019), https://doi.org/10.1002/tcr.201800069
18. A. Verma, S. P. Gautam, K. K. Bansal, N. Prabhakar, and J. M. Rosenholm, *Medicines*, **6(1),** 39(2019), https://doi.org/10.3390/medicines6010039
19. M. Priya, R. Venkatesan, S. Deepa, S. S. Sana, S. Arumugam, A. M. Karami, ... and S. C. Kim, *Scientific Reports*, **13(1),** 18838(2023), https://doi.org/10.1038/s41598-023-46002-5
20. S. Rajeshkumar, N. T. Nandhini, K. Manjunath, P. Sivaperumal, G. K. Prasad, S. S. Alotaibi, and S. M. Roopan, *Journal of Molecular Structure*, **1242,** 130724(2021), https://doi.org/10.1016/j.molstruc.2021.130724
21. S. A. Akintelu, B. Yao, and A. S. Folorunso, *Plasmonics*, **16(1),** 157(2021), https://doi.org/10.1007/s11468-020-01274-9
22. 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
23. 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
24. M. Noruzi, *Bioprocess and biosystems engineering*, **38(1),** 1(2015), https://doi.org/10.1007/s00449-014-1251-0t
25. 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
26. W. PA, *CLSI Document,* **M27-A2** (2002), https://doi.org/10.11263/jsotp.28.95
27. W. Weecharangsan, P. Opanasopit, M. Sukma, T. Ngawhirunpat, U. Sotanaphun, and P. Siripong, *Medical Principles and Practice*, **15(4),** 281(2006), https://doi.org/10.1159/000092991

28. Y. S. Lim, S. S. H. Lee, and B. C. Tan, *Fruits*, **68(6),** 483(2013), https://doi.org/10.1051/fruits/2013088
29. S. K. Karuppannan, R. Ramalingam, S. M. Khalith, M. J. H. Dowlath, G. D. Raiyaan, and K. D. Arunachalam, *Biocatalysis and Agricultural Biotechnology*, **31,** 101904(2021), https://doi.org/10.1016/j.bcab.2020.101904
30. R. Kothari, *Rasayan Journal of Chemistry*, **16(3)**, 1298(2023), http://doi.org/10.31788/RJC.2023.1638274
31. M. A. Asghar, and M. A. Asghar, *International Journal of Biological Macromolecules*, **160,** 1168(2020), https://doi.org/10.1016/j.ijbiomac.2020.05.198
32. M. Hasanin, M. A. Al Abboud, M. M. Alawlaqi, T. M. Abdelghany, and A. H. Hashem, *Biological Trace Element Research*, **200**, 2099(2022), https://doi.org/10.1007/s12011-021-02812-0
33. B. Ginting, I. Maulana, and I. Karnila, *Surfaces and Interfaces*, **21,** 100799(2020), https://doi.org/10.1016/j.surfin.2020.100799
34. 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
35. K. Velsankar, A. K. RM, R. Preethi, V. Muthulakshmi, and S. Sudhahar, *Journal of Environmental Chemical Engineering*, **8(5),** 104123(2020), https://doi.org/10.1016/j.jece.2020.104123
36. H. Liu, G. Wang, J. Liu, K. Nan, J. Zhang, L. Guo, and Y. Liu, *Journal of Experimental Nanoscience*, **16(1),** 410(2021), https://doi.org/10.1080/17458080.2021.1991577
37. H. Qamar, S. Rehman, D. K. Chauhan, A. K. Tiwari, and V. Upmanyu, *International Journal of Nanomedicine*, **2020**, 2541-2553 (2020), https://doi.org/10.2147/IJN.S240232
38. S. Amaliyah, D. P. Pangesti, M. Masruri, A. Sabarudin, and S. B. Sumitro, *Heliyon*, **6(8),** e04636(2020), https://doi.org/10.1016/j.heliyon.2020.e04636
39. S. Rajeshkumar, M. Vanaja, and A. Kalirajan, *Bioinorganic Chemistry and Applications*, **2021(1),** 1222908 (2021), https://doi.org/10.1155/2021/1222908
40. S. RajeshKumar, and G. Rinitha, *OpenNano*, **3,** 18(2018), https://doi.org/10.1016/j.onano.2018.03.001
41. K. R. Reddy, *Journal of Molecular Structure*, **1150,** 553(2017), https://doi.org/10.1016/j.molstruc.2017.09.005
42. A. Y. Ghidan, T. M. Al-Antary, and A. M. Awwad, *Environmental Nanotechnology, Monitoring and Management*, **6,** 95(2016), https://doi.org/10.1016/j.enmm.2016.08.002
43. F. Amin, B. Fozia, Khattak, A. Alotaibi, M. Qasim, I. Ahmad,... and R. Ahmad, *Evidence-Based Complementary and Alternative Medicine*, **2021(1),** 5589703(2021), https://doi.org/10.1155/2021/5589703
44. B. Mahdavi, S. Paydarfard, E. Rezaei-Seresht, M. Baghayeri, and M. Nodehi, *Applied Organometallic Chemistry*, **35(8),** e6264(2021), https://doi.org/10.1002/aoc.6264
45. M. Baghayeri, B. Mahdavi, Z. Hosseinpor-Mohsen Abadi, and S. Farhadi, S. *Applied Organometallic Chemistry*, **32(2),** e4057(2018), https://doi.org/10.1002/aoc.4057
46. A. Rai, and R. Lall, *International Journal of Multidisciplinary Research and Analysis* **4,** 101(2021), https://doi.org/10.47191/ijmra/v4-i2-01
47. R. Tahvilian, M. M. Zangeneh, H. Falahi, K. Sadrjavadi, A. R. Jalalvand, and A. Zangeneh, *Applied Organometallic Chemistry*, **33(12),** e5234(2019), https://doi.org/10.1002/aoc.5234

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