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Antimicrobial and Antioxidant Potential of Optimized Copper Oxide Nanoparticles of *Cassia angustifolia* Leaf Extract against Resistant Isolates

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ABSTRACT

Objectives: The current study was aimed at synthesizing copper oxide nanoparticles (CuO-NPs) using the leaf extract of *Cassia angustifolia* using a green, eco-friendly procedure and establishing the antimicrobial and antioxidant capacity of these nanoparticles against drug-resistant microbial isolates. It was also the purpose of the study to compare the biological activity of CuO-NPs and plain ethanol extract of *Cassia angustifolia*. **Methods:** The green synthesis technique was used to prepare CuO-NPs in which the reducing and stabilizing agent was *Cassia angustifolia* leaf extract. The prepared nanoparticles were optimized and characterized (this fact is skipped to make the abstract short). Their activities were also measured in terms of their antioxidant level by DPPH radical scavenging activity and total antioxidant capacity. The antibacterial activity was established by the procedure of well diffusion agar against Gram-positive (*Bacillus subtilis, Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*) bacteria strains. **Results:** The optimized CuO-NPs manifested the drastic improvement in antioxidant activity with the inhibition of DPPH-free radical formation (85.28%) compared to plain ethanol extract (62.12%). It was also discovered that dose-dependent reduction in total antioxidant activity occurred. The CuO-NPs had great antibacterial activities, and zones of inhibition were between 18.24 and 25.78 mm against a variety of strains assayed. The modified CuO-NPs were very sensitive, with an average of 60-80 percent of the isolates tested. **Conclusion:** The green method used to synthesize CuO-NPs using an extract of *Cassia angustifolia* leaves showed a high level of antibacterial and antioxidant activity. These outcomes are suggestive of the fact that they can be used in medical fields as alternative treatment methods, especially to overcome drug-resistant infections. This enhanced activity is explainable by both the presence of compounds of bioactive secondary metabolites inside it, the extract itself, and the influence of the polarity of solvents used in synthesis.

Keywords: Cassia angustifolia, nanoparticles, Copper Oxide, Antimicrobial, Green synthesis

INTRODUCTION

Global funding on nanotechnology research has increased dramatically during the past ten years. It is a cutting-edge field of scientific and technological study with a great deal of promise for producing a number of novel goods with potential medical applications[1-3.] Metal precursors are used to create almost all metal nanoparticles, either constructively or destructively[4.] Copper, nickel, zinc, silver, and gold are a few types of metal nanoparticles. The science of green chemistry employs a non-regulatory, economically driven approach to achieve environmental preservation and sustainable development. The end of the twentieth century saw a major breakthrough in science and technology[5-6]. The effects are now beginning to manifest. Green initiatives have gained a lot of attention due to the increasing need to develop safe, nontoxic chemicals, environmentally friendly solvents, and renewable resources[7-9].

The development of efficient green chemistry methods that employ natural reducing, capping, and stabilizing agents to produce metal nanoparticles with the right size and shape has received a lot of attention lately[10-12]. Without the use of harsh, expensive, and dangerous chemicals, copper nanoparticles can be produced organically. Biological techniques make use of environmentally benign resources such as enzymes, seaweed (macroalgae), bacteria, fungi, diatoms, plant extracts, and microalgae like cyanobacteria[8, 13]. Combinations of biomolecules found in some organism extracts; including vitamins, polysaccharides, amino acids, and enzymes/proteins, can bio reduce metal ions in a method that is safe for the environment and complex chemically. Because of their relative affordability, non-toxicity, and environmental friendliness, NPs are commonly selected from plant extracts and microorganisms in biogenic syntheses[14-17]. In recent years, the use of plant extracts in the synthesis of nanoparticles has grown in importance.

Antibiotic-resistant organisms are currently impeding the treatment of bacterial illnesses. Alternative therapeutic strategies, such as the use of metal nanoparticles—which include Cu, Zn, and Ag and are mostly known for their antibacterial qualities—become more necessary as a result of these situations[11,18]. Oxidation results from CuO NPs and other metal ions adhering to the lipid membrane. In the fight against the rise of resistant diseases in recent years, the creation of novel antibacterial drugs has become essential. Offering safe and effective complementary and alternative medicine treatments could prove to be a key tactic in enhancing access to healthcare, as over one-third of people in developing countries lack access to essential pharmaceuticals[19-21].The information gathered from our study may be utilized to develop new medications made from *cassia angustifolia* that closely resemble its active ingredients.

Antibiotic-resistant organisms are currently impeding the treatment of bacterial illnesses. Alternative therapeutic strategies, such as the use of metal nanoparticles—which include Cu, Zn, and Ag and are mostly known for their antibacterial qualities—become more necessary as a result of these situations[22,23]. Oxidation results from CuO-NPs and other metal ions adhering to the lipid membrane. This work employs a range of analytical approaches to discover bioactive compounds and evaluates the antibacterial activities of generated NPs against MDR strains of bacteria both in vitro and in vivo. The anti-pathogenic evaluation of the Cuo-NPs derived from this plant is examined in this work. MIC is also examined in connection with these microorganisms.[24]

MATERIAL AND METHODS

Chemicals

We bought 98% of the copper sulphate ($CuSO_4{\bullet}5H_2O$) from Sigma-Aldrich. Merck provided the sodium hydroxide (98%) and hydrochloric acid (HCl) (35%), which were used to measure the pH. *Cassia angustifola* leaves have been gathered from various locations around Rajasthan. The agar medium was used to cultivate bacterial colonies. Every chemical and solvent used was of analytical quality.

Green Synthesis of CuO nanoparticles using *Cassia angustiolia* extract

CuO nanoparticles were physiologically produced using an alcoholic extract of Cassia leaves. 90 mL of copper sulphate solutions and 10 mL of plant extract were thoroughly mixed to produce CuO nanoparticles, which were then allowed to rest at room temperature[25]. The mixture was cultured for four days, and at regular intervals, the formation of NPs was monitored using both visual inspection and UV-vis spectroscopic measurements. After incubation, the mixture was centrifuged for 15 minutes at 5000 rpm. The resulting pellet was resuspended in a little amount of distilled water and centrifuged again. This process was repeated two or three times to eliminate the pollutants. To get rid of any last traces of moisture, the finished pellet was dried in a hot air oven. The particles were collected and described once they had dried[26-27].

Optimization of Drug Loaded Nanoparticles

To systematically analyse and optimize formulation parameters, the Box-Behnken Design (BBD-RSM) response surface methodology was utilized. The primary variables that determine the particle size and Zeta potential of the CuO-NPs loaded with *C. angustifolia* extract production include formulation variables including homogenization speed and reaction temperature.

In vitro antioxidant Activity: Assay of DPPH (1,1-diphenyl-2-picrylhydrazyl) Radical Scavenging Activity)

By interacting with the stable DPPH (1,1-diphenyl-2 picryhydrazyl) free radical, the plant extract's antioxidant capability was assessed using its ability to scavenge free radicals and a standard ascorbic acid solution in methanol. In a 2 ml propylene vial, 1 mL of each of the various dosages of plant extract and ascorbic acid (25–250µg/ml) were used to create three duplicates of the plant sample in methanol. After adding 1 milliliter of freshly made DPPH solution (0.2 mM), it was left in the dark for 30 minutes. A visible spectrophotometer was used to measure the abs of each tube's solution at 517 nm, with methanol serving as the blank[28].

% of inhibition = (Control−Test)/Control) × 100

Determination of antimicrobial activity by agar diffusion method

In compliance with CLSI guidelines, the antimicrobial susceptibility was assessed using the Kirby-Bauer disk diffusion method with commercially available antimicrobial discs (Hi-Media, Mumbai). The agar diffusion method was used to investigate the produced metal oxide nanoparticles' antimicrobial activity, including their antibacterial properties. The samples' antibacterial activity was assessed against Gram-negative bacterial strains like P. *aeruginosa* (P.A.) (ATCC 10145), E. *coli* (ATCC 25922), and *Klebsiella pneumonae* (K.P.) (ATCC 13883) as well as Gram-positive bacterial strains like *Bacillus subtillis* (B.S.) (ATCC 6051) and *Staphylococcus aureus* (S.A.) (ATCC 25923). CuO-NPs and leaf alcoholic (ethanolic) plant extracts were diluted separately. Dilution in sterile test tubes starts at 10% to 100% (µg/ml)[29]. By evaluating the zone of inhibition against the test pathogens, the produced metal oxide nanoparticles' antibacterial activity was assessed.

Minimal inhibitory concentration (MIC)

The lowest concentration that prevents discernible development is known as the MIC. The extract and NPs were synthesized in two-fold serial dilutions using the dilution procedure, with concentrations ranging from 250 to 4000 µg/ml. Nine milliliters of Muller Hinton Agar were combined with one milliliter of each extract dilution. On the plates with different extract concentrations, 10 microliters of each standardized broth culture (1.5× 108 CFU/ml) were grown. After that, the plates were incubated in accordance with each organism's growth requirements, and any observable bacterial growth was monitored[30]. The lowest extract and CuO-NP concentration that prevented any discernible growth on the agar surface was known as the minimum inhibitory concentration

Statistical analysis

Values were expressed as Mean ± SD for each group and statistically significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Duncan's test for multiple comparisons.[31]

RESULTS

In vitro antioxidant activity of *C.* angustifolia leaves and optimized CuO Nanoparticles DPPH radical scavenging activity

Stronger scavenging ability was shown by lower IC_{50} findings, which measure the concentration of substance that causes 50% inhibition. The amounts of ethanol extract, ascorbic acid, and CuO-NPs varied from 20 to 100 µg/mL. Because the extract contains vitamin C, CAEE and CANPs had high antioxidant activity. The CAEE and CANPs leaves exhibited positive IC_{50} values of 46.25 ± 0.3 and 34.42 ± 0.4, respectively, when compared to the reference standard of ascorbic acid, which had an IC_{50} of 28.26 ± 0.15 Table 1. The data showed that for each tested concentration, the percentage of free radical scavenging potency rose in a dose-dependent way.

Table 1. Leaf extract's potential for antioxidants using the DPPH method

Conc µg/mL	DPPH (%) Inhibition		
	Ethanol Extract (CAEE)	CuO-NPs (CANPs)	Ascorbic Acid
20	13.25±0.08	15.5±0.18	17.52±0.52
40	23.25±0.42	29.12±0.18	35.7±0.4
60	40.27±0.1	50.14±1.2	55.12±1.5
80	49.25±1.2	64.25±0.7	72.08±1.9
100	62.12±1.15	85.28±1.4	91.64±2.1
IC_{50}	**46.25±0.3**	**34.42±0.4**	**28.26±0.15**

Total antioxidant activity (TAA)

The combined antioxidant capacity of the C. *angustifolia* leaf extract, CuO-NPs, and ordinary ascorbic acid is displayed in Table 2 and Figure 2. Leaf extract, ascorbic acid, and CuO-NPs all shown dose-dependent antioxidant activity. The half inhibition concentrations (IC_{50}) for ascorbic acid, CuO-NPs, and leaf extract were 50.3, 42.12, and 38.5 µg/ml, respectively. The CuO-NPs dramatically reduced TAA activity in a dose-dependent manner. Compared to C. *angustifolia* extract, CuO-NPs are close to reference and might have higher antioxidant activity.

Table 2. Total antioxidant activity of C. *angustifolia* extract and CuO-NPs

Conc µg/mL	(%) Inhibition		
	Ethanol Extract (CAEE)	CuO-NPs (CANPs)	Ascorbic Acid
20	10.85±0.04	13.24±0.09	16.23±0.42
40	19.42±0.12	24.75±0.25	29.42±0.3
60	35.1±0.25	43.2±1.5	48.6±1.2
80	42.4±0.8	56.46±1.3	65.23±1.1
100	60.36±1.3	78.5±1.2	84.25±1.8
IC_{50}	**50.3±0.3**	**42.12±0.4**	**38.5±0.24**

Anti-microbial activity of Alcoholic extracts of leaf and their optimized CuO-NPs against resistant isolates

Green CuO-NPs from C. *angustifolia* leaf extract had a substantial inhibitory impact after 24 hours of incubation when compared to both conventional amikacin and C. *angustifolia* leaf extract. The bactericidal activity of these samples was compared using the zone of inhibition (ZoI) as a metric. The extracts' CuO-NPs showed a zone of inhibition (ZOI) comparable to that of common antibiotics. The standard treatment for all bacterial infections was amikacin. The study found that specific parts of the leaves of C. *angustifolia* showed the capacity to fight off gram-positive and gram-negative bacteria that are resistant to a variety of drugs.

100 µl of each of the 10%, 25%, 50%, 75%, and 100% diluted extracts and CuO-NPs were impregnated into sterile paper discs (6 mm in diameter). The discs were then dried in a hot air oven at 100 °C for two hours before being dispensed over the top of the inoculated agar plate. Following that, the plates were incubated in accordance with each organism's growth requirements. The zones of inhibition were measured and recorded in millimeters to assess the antibacterial activity of each sample, which was tested in triplicate.

Effect of Cassia angustifolia Ethanol Extract (CAEE) against resistant isolates

The CAEE leaf of the plant under study showed test organism suppression. The ethanol extract of the leaves (CAEE) showed significant efficacy against both *Bacillus* species and P. *aeruginosa* in all dilutions from 10-100%, with a zone of inhibition (ZOI) ranging from 4.25-14.25 mm for *Bacillus* species and 7.75-16.6 mm for P. *aeruginosa* Table 3. S. *aureus*, E. *coli*, and K. *pneumoniae* exhibited resistance at lower concentrations, and only at higher concentrations was a zone of inhibition discernible. All *microorganisms* were well inhibited by the leaf ethanol extract, although P. *aeruginosa* had the largest zone of inhibition (16.6 mm at 100% dilution). P. *aeruginosa*, K. *pneumoniae*, and *Bacillus* species all showed a zone of inhibition that was dependent on concentration. E. *Coli* showed sensitivity at higher concentrations but resistance at lower dosages.

The corresponding zones of inhibition for the gram-positive bacteria B. subtilis and S. *aureus* were 4.25–14.25 mm and 10.5–15.6 mm, respectively. At full intensity, the inhibitory zones for gram-negative bacteria were 8.25 mm to 12.65 mm for E. *Coli* at 40–100% dilution and 7.75 mm to 16.6 mm for P. *aeruginosa.* At a concentration of 80%, only P. *aeruginosa* and E. *coli* revealed an inhibition zone that was marginally less intense than maximal intensity; no inhibition was visible in the other microorganisms.

Effect of *Cassia angustifolia* optimized CuO-NPs against resistant isolates

The study's findings show that microorganisms with very low ZOI are very little affected by CuO-NPs at concentrations of 10–20%. Group analysis conducted during the study shows that, in comparison to baseline, microbial growth is dramatically reduced by 60% and 80% after treatment with CuO-NPs. On several microbiological strains, the maximum ZOI (18.24 to 25.78) was achieved in 60–80% of CuO-NPs.

CuO-NPs of the leaf extract from the plant investigated exhibited suppression of the test organisms. The ZOI for gram-positive organisms ranged from 5.75 to 23.47 mm for B. subtilis and 3.12 mm for S. *aureus* at the lowest concentration of 10% and 24.3 mm at the highest concentration of 80% Table 4. When it came to gram-negative bacteria, E. *coli* showed 26.8 ZOI in conc 100% and P. *aeruginosa* displayed 24.2 mm at maximum intensity. According to the aforementioned research, the extract's effectiveness may be attributed to the broad-spectrum antibiotic components or secondary metabolites it contains, or it may be that any potential antibacterial activity depends mostly on the solvent's polarity.

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Comparative Antimicrobial Effect of Extract & CuO-NPs with Amikacin

Generally speaking, tiny CuO-NPs with a high surface area interact with bacteria's cell surfaces more successfully than large NPs, ultimately exhibiting superior antimicrobial qualities. The produced CuO-NPs' antibacterial activity against pathogenic bacteria, such as

Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa (Gram-Ve bacteria), *Streptococcus aureus*, and B. *subtilis* (Gram-Ve bacteria), was tested using the agar well diffusion method.

All *microorganisms* varying sensitivity to CuO-NPs nanoparticles is dependent on the size of the particles, the synthesis temperature, the structure of the bacterial cell wall, and the extent of contact between the organisms and the nanoparticles. In contrast to S. *aureus*, which lacks such layers in its membrane, B. *subtilis* has several layers made up of lipids, proteins, and lipopolysaccharides that protect it from CuO. S. *aureus* has a single, thick layer of peptidoglycan that is made up of a combination of amino acids and sugars. As a result, S. *aureus* and CuO interact more strongly than B. *subtilis* does.

Antibacterial activity assay by minimum inhibitory concentrations (MIC)

The minimal inhibitory concentration (MIC) of the extract and CuO-NPs against the five bacteria ranged from 250 to 4000 µg/mI. S. *aureus* reveals MIC value of 2000 and 1000 µg/mI for extract and their CuO-NPs respectively. The MIC value for B. *Subtilis* at examination of CAEE and CuO-NPs is 2000 and 500 µglmI. Similar patterns were shown by all micro-organisms. Lower MIC of CuO-NPs indicate their effectiveness against all resistant strains compared to plain ethanolic extract.

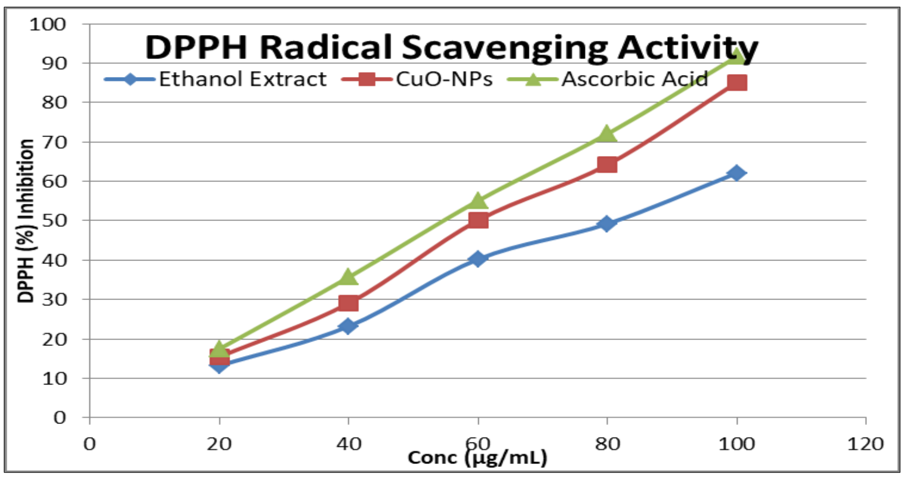

Figure. 1. Leaf extract's potential for antioxidants using the DPPH method

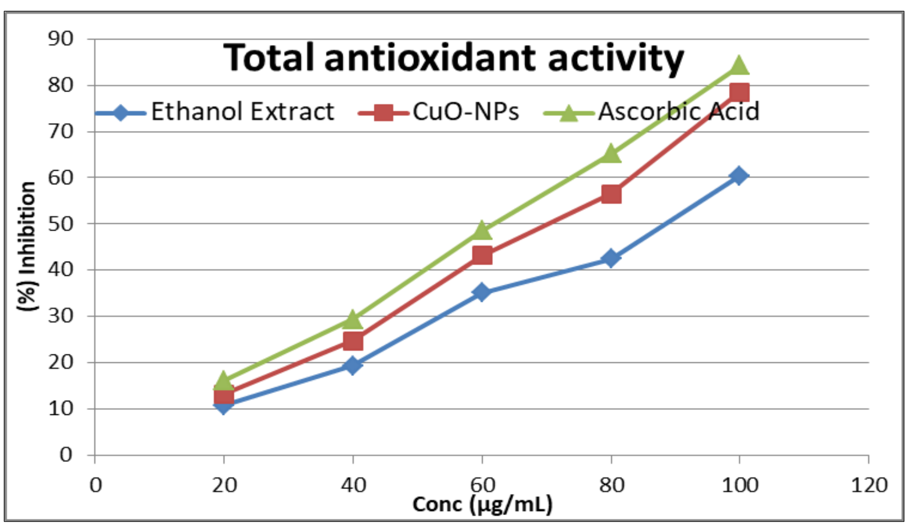

Figure. 2. Total antioxidant activity of C. *angustifolia* extract & CuO-NPs

Table 3. The *Cassia angustifolia* ethanol extract (CAEE) leaves for antibacterial properties against a range of microorganisms

CAEE 100 µg	ZOI (mm) CAEE (LEAF)				
Test organisms	***S. aureus* (+ve)**	***P. aeruginosa* (-ve)**	***E. Coli* (-ve)**	***K. pneumonia* (-ve)**	***B.* Subtilis (+ve)**
10%	N	7.75± 0.8	N	N	4.25 ± 1.1
20%	N	8.52± 0.26	N	6.45± 0.82	6.25± 0.45
40%	10.5± 0.42	12.25± 0.75	8.25± 0.25	7.2± 0.42	8.18± 0.78
60%	11.4± 1.12	14.1± 1.12	9.27± 0.52	8.75± 0.5	9.56± 0.95
80%	13.2± 0.65*	16.5± 1.23*	11.78± 1.05*	10.48± 0.75	12.95± 1.21*
100%	15.6± 1.2**	16.6± 0.95	12.65± 1.24	11.85± 0.6*	14.25±1.08**

**Each value represents mean + SEM;(n=3) N-No zone of Inhibition *compared to the 10% Base control, **Compared with the negative control*

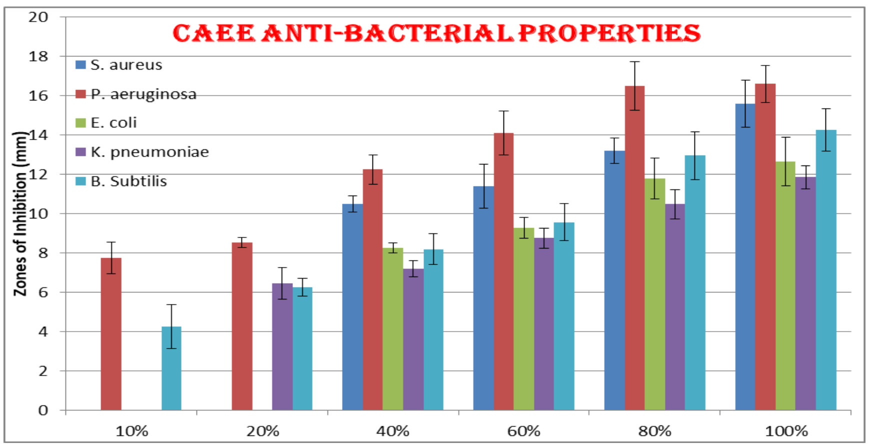

Figure. 3. *Cassia angustifolia* leaf ethanol extract exhibits antibacterial activity against a variety of microbes

Table 4. The *Cassia angustifolia* CuO-NPs for antibacterial properties against a range of microorganisms

CuO-NPs 100 µg	ZOI (mm) CuO-NPs (LEAF EXTRACT)				
Test organisms	***S. aureus* (+ve)**	***P. aeruginosa* (-ve)**	***E. Coli* (-ve)**	***K. pneumonia* (-ve)**	***B.* Subtilis (+ve)**
10%	3.12 ± 0.5	6.9± 0.8	5.12 ± 0.35	5.45 ± 1.12	5.75 ± 0.85
20%	7.75 ± 0.7	10.45± 0.78	8.64 ± 1.05	9.1± 0.75	8.90± 0.92
40%	14.8± 1.15*	16.46± 0.92*	14.72± 0.65	16.4± 1.1	14.54± 0.25
60%	18.6±1.42**	19.54± 1.24	19.78± 1.2*	20.75± 0.85*	18.24± 1.45*
80%	24.1±1.63**	23.9± 1.45**	25.78±1.55**	25.35±1.75**	22.78±1.10**
100%	24.3± 1.4	24.2± 2.2	26.8± 1.75	25.65± 2.2	23.47± 1.45

**Each value represents mean + SEM; *compared to the 10% Base control, **Compared with the negative control*

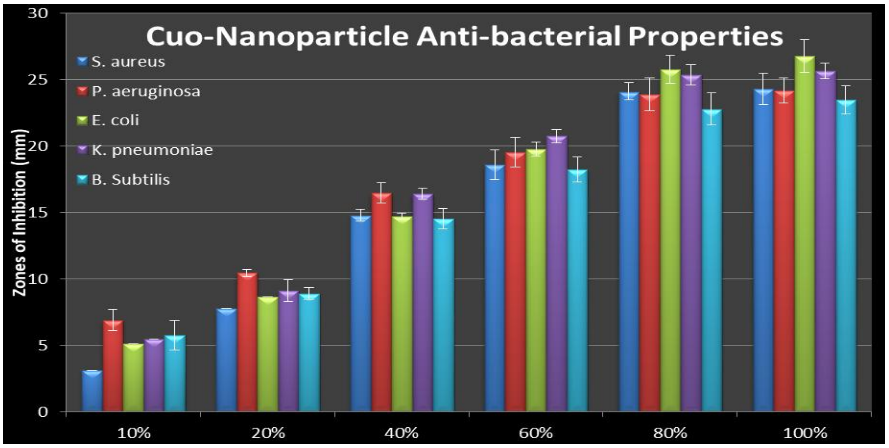

Figure. 4. Antibacterial activity against a range of bacterial species is demonstrated by the CuO-NPs of extract of the leaves of *Cassia angustifolia*

Table 5. Comparative antimicrobial effect of extract and their CuO-NPs for antibacterial properties using standard

Sample	Zone of Inhibition (mm)					
Test organisms	*Dose* µg	*S. aureus* (+ve)	*P. aeruginosa* (-ve)	*E. coli* (-ve)	*K. pneumonia* (-ve)	*B.* Subtilis (+ve)
CAEE	30	17.22 ± 0.8	16.4± 1.1	20.05 ± 1.25	17.53 ± 1.62	18.45 ± 1.35
CuO-NPs	30	23.65 ± 1.7	24.8± 1.54	25.22 ± 1.4	24.5± 1.32	22.82± 1.54
Amikacin	30	26.5± 2.1	25.22± 1.76	25.9± 0.95	26.6± 1.62	25.12± 1.25

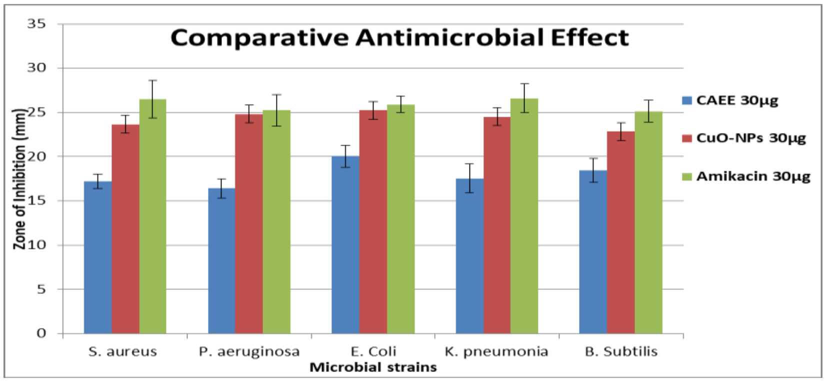

Figure. 5. Comparative antimicrobial activity pure extract of *Cassia angustifolia*, their CuO-NPs and standard Amikacin

Table 6. MIC value of plant extract and CuO-NPs against microorganisms

Microbial Strains	Gram	MIC (µg /ml)	
		Ethanol Extract	CuO-NPs
S. aureus	Gram-+VE	2000	1000
***B.* Subtilis**	Gram-+VE	2000	500
P. aeruginosa	Gram--VE	1000	500
E. coli	Gram--VE	2000	500
K. pneumoniae	Gram--VE	2000	1000

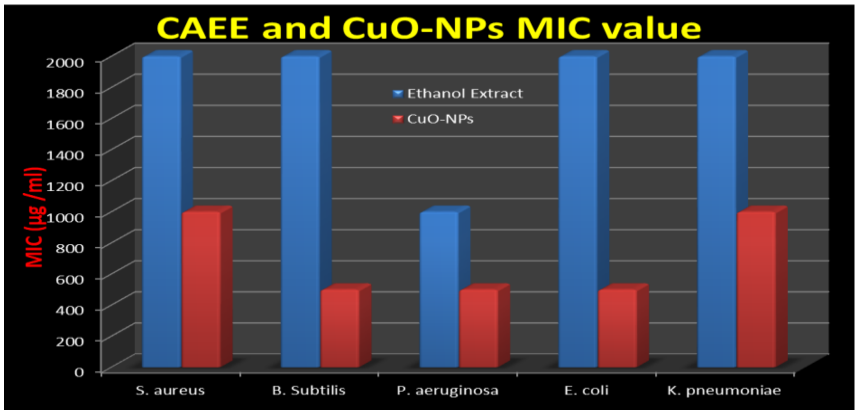

Figure. 6. MIC value of CAEE and CuO-NPs against microorganisms

DISCUSSION

The results demonstrated that the antioxidant activity of C. angustifolia ethanol extract (CAEE), *C. angustifolia*-derived nanoparticles (CANPs), and CuO-NPs was concentration-dependent, with lower IC_{50} values indicating stronger scavenging potential. The presence of vitamin C in the extract likely contributed to this enhanced activity. Interestingly, CuO-NPs exhibited strong antioxidant behavior, with an IC_{50} of 42.12 µg/mL, which was lower than that of ascorbic acid (50.3 µg/mL) and comparable to the leaf extract (38.5 µg/mL). This suggests that CuO-NPs possess intrinsic antioxidant properties, possibly due to their ability to donate electrons or scavenge free radicals effectively. The dose-dependent increase in radical scavenging activity across all tested concentrations further supports their potential as antioxidant agents.

Notably, CANPs showed better antioxidant performance than the crude extract (CAEE), likely due to the enhanced bioavailability and reactivity of nanoparticles. The proximity of CuO-NPs IC_{50} to that of ascorbic acid indicates that they may serve as a viable alternative or complementary antioxidant in biomedical and pharmaceutical applications.[31] However, further studies are needed to elucidate the exact mechanisms behind their scavenging activity and potential synergistic effects with natural antioxidants like vitamin C.

The antimicrobial evaluation of the *C. angustifolia* Cuo-NPs revealed significant inhibitory effects against both Gram-positive and Gram-negative bacteria, with varying degrees of sensitivity depending on the bacterial strain and extract concentration. The differential sensitivity between Gram-positive and Gram-negative bacteria could be attributed to variations in cell wall structure. Gram-negative bacteria possess an outer membrane that often confers resistance to antimicrobial agents, yet P. *aeruginosa* was highly susceptible, possibly due to the extract's ability to disrupt its membrane integrity or interfere with essential metabolic pathways.[32] Meanwhile, Gram-positive bacteria, which lack this protective outer layer, were generally more sensitive, particularly at higher concentrations.

These findings highlight the potential of Cu-NPs of C. angustifolia leaf extract as a natural antimicrobial agent, particularly against resistant strains like P. *aeruginosa*. However, further studies are needed to isolate and characterize the specific bioactive compounds responsible for this activity and to evaluate their mechanisms of action. Additionally, synergistic effects with conventional antibiotics could be explored to enhance therapeutic efficacy against multidrug-resistant pathogens.

CONCLUSION

In conclusion, both C. angustifolia-derived compounds and CuO-NPs exhibit promising antioxidant properties, with CuO-NPs showing particularly strong activity. These findings highlight their potential as natural or synthetic antioxidants for therapeutic or industrial use, warranting further investigation into their efficacy and safety. CAEE exhibits promising antibacterial properties, with the strongest effects observed at higher concentrations. Its broad-spectrum activity, particularly against P. aeruginosa, positions it as a potential candidate for developing plant-based antimicrobial treatments. This study confirms that CuO-NPs derived from C. angustifolia exhibit significant, concentration-dependent antibacterial activity, with optimal efficacy at 60–80% concentrations. The broad-spectrum inhibition, particularly against Gram-negative bacteria, positions these nanoparticles as a promising candidate for antimicrobial applications. Future studies should focus on mechanistic elucidation and translational potential in clinical settings as well as optimizing extraction methods and assessing in vivo efficacy to validate its therapeutic potential.

Conflict of interest

Regarding the publishing of this work, the authors state that they have no conflicts of interest.

List of abbreviations

ANOVA - Analysis of Variance
ATCC - American Type Culture Collection
BBD-RSM - Box–Behnken Design–Response Surface Methodology
CAEE - Cassia angustifolia Ethanol Extract
CANPs - Cassia angustifolia Nanoparticles
CFU - Colony Forming Units
CLSI - Clinical and Laboratory Standards Institute
CuO-NPs - Copper Oxide Nanoparticles
DPPH - 1,1-Diphenyl-2-picrylhydrazyl
HCl - Hydrochloric Acid
IC_{50} - Half Maximal Inhibitory Concentration
MIC - Minimum Inhibitory Concentration
NaOH - Sodium Hydroxide
NPs - Nanoparticles
Rpm - Revolutions per Minute
SD - Standard Deviation
SEM - Scanning Electron Microscope
TAA - Total Antioxidant Activity
UV–Vis - Ultraviolet–Visible (Spectroscopy)
ZOI - Zone of Inhibition
µg/mL - Micrograms per Millilitre

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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