Acacia Concinna extracts mediated synthesis of Copper oxide nanoparticles against pathogenic bacteria

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ABSTRACT:
Background: Nanotechnology has emerged as a powerful tool for developing innovative solutions to global health challenges, particularly in combating pathogenic microorganisms. This study explores using *Acacia Concinna* extract to synthesize copper oxide (CuO) nanoparticles (NPs) and evaluates their anti-inflammatory/ and anti-microbial activity against pathogenic bacteria. The eco-friendly synthesis approach not only minimizes environmental impact but also enhances the biocompatibility of the nanoparticles. This study aims to evaluate the anti-inflammatory and anti-microbial activity of *Acacia Concinna*.

Materials and Methods: In the present study, *Acacia Concinna* is used to synthesize copper nanoparticles which were characterized using SEM and EDX analysis. The anti-microbial activity was assessed by using the agar well diffusion method and the anti-inflammatory activity was by protein denaturation assay.

Results and discussion: The synthesized nanoparticles were 20 nm in size, round, hexagonal, and crystalline in form. The anti-microbial activity of *Acacia Concinna* and Cu Nps extract was evaluated against Gram-positive and Gram-negative bacteria. At 100 µg/ml, the highest zone of inhibition was seen in S.aureus (16mm). The anti-inflammatory activity of synthesized Cu Nps considerably decreased protein denaturation in a dose-dependent manner at different doses.

Conclusion: Copper nanoparticles synthesized using *Acacia Concinna* extract through an eco-friendly green synthesis process exhibit significant anti-inflammatory and antibacterial properties. Their antibacterial activity effectively targets both Gram-positive and Gram-negative bacteria by disrupting cell membranes and generating reactive oxygen species (ROS). Additionally, the phytochemicals in *Acacia Concinna* enhance their therapeutic potential, making them suitable for biomedical applications.

KEYWORDS: copper nanoparticles; *Acacia Concinna;* anti-inflammatory, anti-bacterial, eco-friendly.

INTRODUCTION:

The emergence of microorganisms resistant to multiple antimicrobial agents has sparked interest in developing new bactericides based on inorganic materials. This shift is driven by the limitations of traditional organic agents, including low heat resistance, high decomposability, and short lifespan (1). The most rapid adopters of nanotechnology are the areas of information and communication such as electrical and optoelectronic sectors, food technology, energy technology, and medical products including several pharmaceuticals and drug delivery systems, diagnostics, and medical technology (2,3). NPs synthesized through this green method increased in stability, prevented the agglomeration and deformation of the nanoparticles (NPs), and allowed the adsorption of phytochemicals on the surface of the NPs, which enhanced the reaction rate of NPs (4,5). These days, there is increased interest in metal oxide NPs, such as copper oxide (CuO), iron oxide (Fe_3O_4), and zinc oxide (ZnO), because of their unique visual, physical, and biological properties (6).

CuO NPs, one of these metal oxide NPs, are gaining attention due to their importance in the biopharmaceutical and environmental remediation sectors (7). CuO NPs produced by environmental methods have been widely utilized as important base materials due to their non-toxic nature, cost-effectiveness, and widespread plenitude, with good electrical and optical properties (8). The primary drawback of employing CuO nanoparticles (CuONPs) in medicine is their potential toxicity to mammalian cells as well as those of vertebrates and invertebrates (9). Despite its usage in biomedical applications, CuO NPs can increase the generation of reactive oxygen species in living cells (10). The prevalence of diseases that are not responding to the existing traditional medicines is one of the biggest problems facing the healthcare industry today. CuONps have the aptitude to have a physiological influence because of their anti-inflammatory, anti-bacterial, anti-biofilm, anti-cancer, and anti-free radical properties (11).

Acacia Concinna is a medicinal plant that grew in Southern Asia's tropical rain forests whose fruits are used to cleanse hair and encourage hair growth (12). In traditional medicine, shikakai is also used to treat dandruff, gum disease, leprosy, psoriasis, jaundice, constipation, skin issues, itching, pimples, and hyperpigmentation (13). As a herbal medicine, the leaves, bark, and pods have been utilized for expectorant, purgative, and emetic purposes (14). Leaves are used to prevent dermatitis and diabetes in Thailand, India, and Myanmar ((14,15). This study explores the synthesis of CuO NPs mediated by *Acacia Concinna* extract, focusing on its effectiveness against various pathogenic bacteria. The methodology leverages the plant's natural reducing and stabilizing agents, producing CuO NPs with desirable properties. Characterization of the nanoparticles, along with antibacterial efficacy testing, provides insights into their potential as an alternative to conventional antibiotics and a valuable addition to antimicrobial agents in medical and environmental applications. The aim of this is to evaluate the anti-inflammatory and anti-microbial activity of *Acacia Concinna*.

MATERIALS AND METHODS:

Preparation of the extract: *Acacia Concinna* was purchased from the herbal care center. 2 grams of *Acacia Concinna* were measured and added to a conical flask and dissolved in 100ml of distilled water. Then the extract was heated at 60°C for 7-8 minutes in a heating mantle. Using a blotting paper the extract was then filtered into another conical flask using a Whatmann No.1 filter paper and the filtrates were re-filtered through a 0.45 µm nylon membrane filter. The collected filters were dried in different processes. The extract was concentrated using a rotary evaporator with a water bath at (80˚-90℃) and dried by freeze drier at (-62˚C).

Synthesis of nanoparticles:

20 mill moles of copper sulfate are mixed with 40 mL of plant extract and 60 mL of distilled water in a conical flask. The flask was then placed in the orbital shaker at 65 rpm and then in a magnetic stirrer at 450 rpm for uniform distribution. The color change was noted regularly with an interval of one hour for two days. With the help of UV spectroscopy, the prepared copper containing *Acacia Concinna* extract was recorded to check for the synthesis of nanoparticles. It was then subjected to centrifugation at 10,000 rpm for 10 mins in a centrifuge. The copper nanoparticle pellets were then collected to perform antimicrobial and anti-inflammatory activity tests.

CHARACTERISATION:

SEM analysis:

After synthesis, the size and shape of the nanoparticles were examined using a scanning electron microscope. The synthesized nanoparticles were placed on a sample holder and veiled in a thin layer of conductive material before being inspected under a high-resolution microscope.

EDX analysis:

After being removed from the *Acacia Concinna* extract, the NPs' surface was scanned with an electron beam, allowing the electrons to impact and activate the substance. As each element returned to its previous energy state, it immediately released X-rays with different energies and wavelengths. The corresponding elements were labeled, and the X-ray wavelength and intensity were shown on the X and Y axes, respectively. The X-axis peak values were compared with the known wavelengths of each element to determine the elemental composition of the sample.

Antimicrobial activity of *Acacia Concinna :*

The antimicrobial efficiency of copper nanoparticles was assessed using the agar well diffusion method. The antibacterial activity of copper nanoparticles was tested against four different bacterial isolates *E. Coli* and *S. aureus*. Fresh bacterial cultures were prepared on the surface of Muller-Hinton agar plates in a broth medium. Different concentrations of copper nanoparticles (25, 50, and 100 µL) were incorporated into the wells and the plates were incubated at 37°C for 24 hours. After incubation, the zone of incubation formed around the discs was measured and noted down.

Anti-inflammatory of *Acacia Concinna :*

0.05 mL of *Acacia Concinna* of various fixation (200µg/ml,300µg/ml,400µg/ml) was added to 0.45 ml bovine serum albumin (1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. Care should be taken while micro-pipetting to avoid bias, standard values which were obtained prior are used for comparison. These samples were incubated at room temperature for 20 min and then heated at 55 °C in a water bath for 30 min. The samples were cooled and the absorbance was estimated spectrophotometrically at 660 nm.

Percentage of protein denaturation was
determined utilizing the following equation,
% of inhibition = absorbance of sample x 100/ absorbance of control

Results :

SEM and EDX analysis:
SEM was done to analyze the surface morphology of the nanoparticles synthesized. Fig 1a shows the SEM analysis of CuO Nps synthesized from *Acacia Concinna*. The average particle size of the nano copper was observed around 20 nm. Morphologically, nanoparticles appear spherical and hexagonal. It showed the presence of heterogeneous sizes of particles formed. The presence of Cu, while some traces of O, C, and Al were also found which were due to the presence of secondary metabolites in the leaf extract. EDX spectra of copper nanoparticles using *Acacia Concinna* shown in Fig. 1 indicated the presence of copper nanoparticles. Binding energy peaks at 0.83 keV also show the presence of copper. Other peaks at 0.32 keV, 0.61 keV, and 1.33 keV represented the presence of C, O, and Al respectively, which shows the presence of carbon stabilizers and certain impurities. This aggregation may be due to the presence of secondary metabolites in the leaf extract.

Anti-inflammatory activity:
Copper nanoparticles synthesized from *Acacia Concinna* effectively inhibited protein denaturation. All of the measured concentrations (200 µg/ml, 300 µg/ml, 400 µg/ml, and 500 µg/ml) significantly reduced the protein denaturation in a dose-dependent pattern. At the highest concentration (500 µg/ml), the maximum percentage of inhibition was 82.2 % and 15.9% inhibition at the lowest concentration of extract (Fig 2).

Antimicrobial activity :

The biosynthesized nanoparticles exhibited significant antimicrobial activity against *E. coli* and *S. aureus*. The highest zone of inhibition was 16 mm for *S.aureus* and 14 mm for *E. coli* at 100 µg/ml whereas the lowest zone of inhibition was 10 mm for *S.aureus* and 9 mm for *E. Coli* (Fig-3&4)These synthesized CuO Nps were effective against *S.aureus* compared to *E. coli*

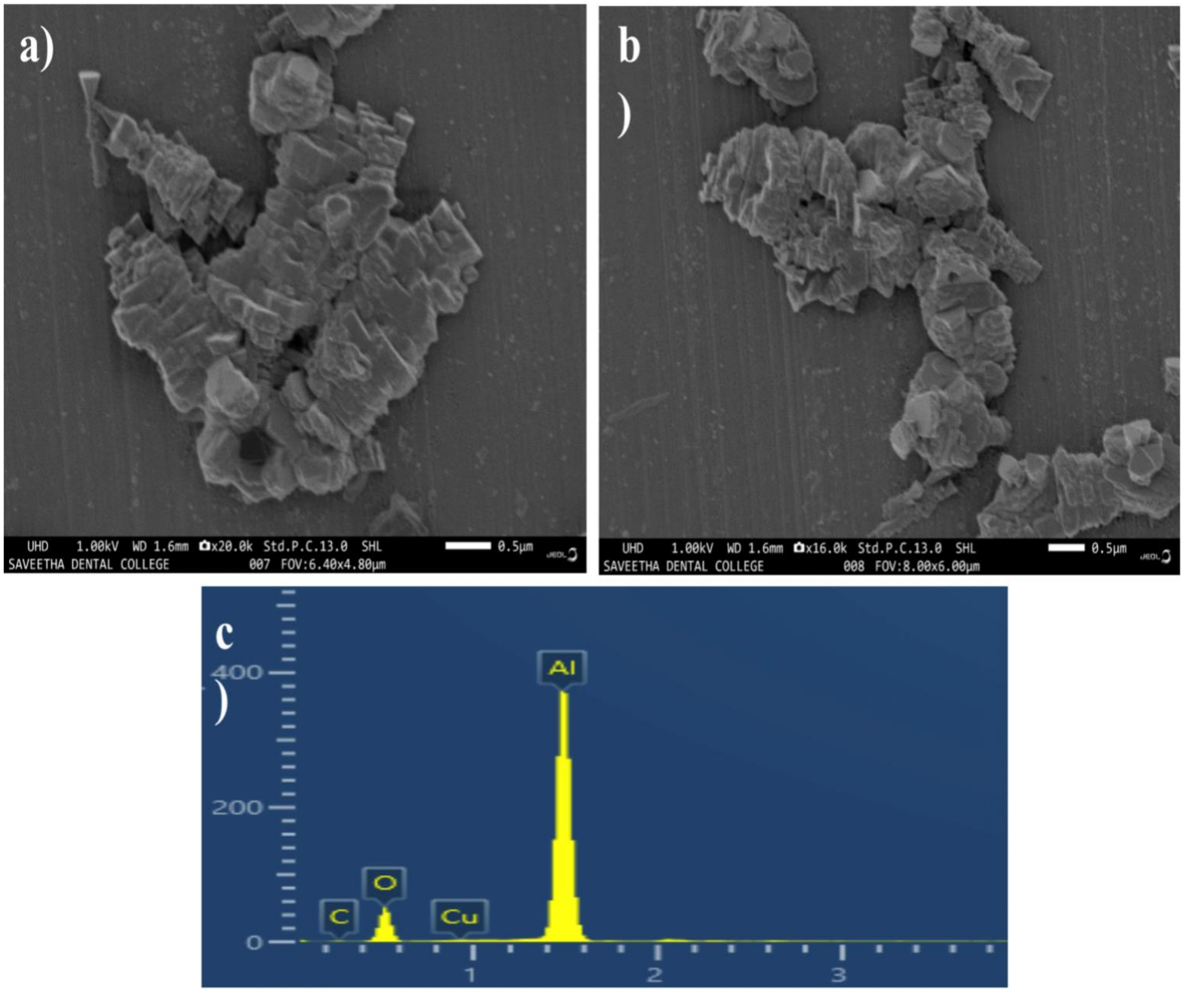

Fig 1 - SEM and EDX images of synthesized CuO NPs using *Acacia Concinna*.

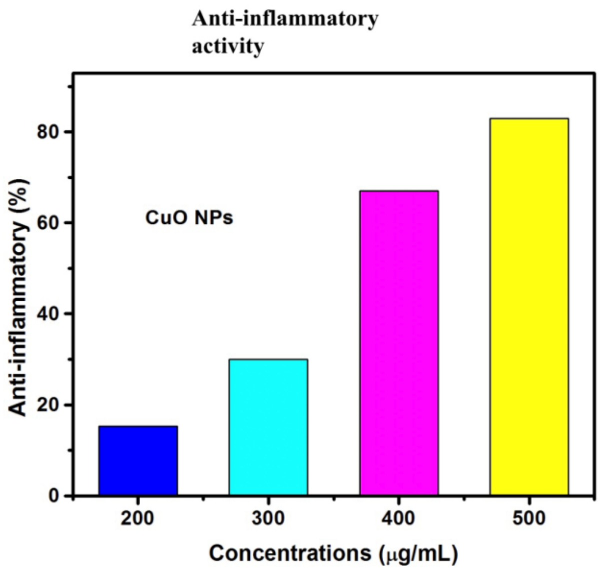

Fig-2: Inhibition of protein denaturation activity of synthesized copper nanoparticles

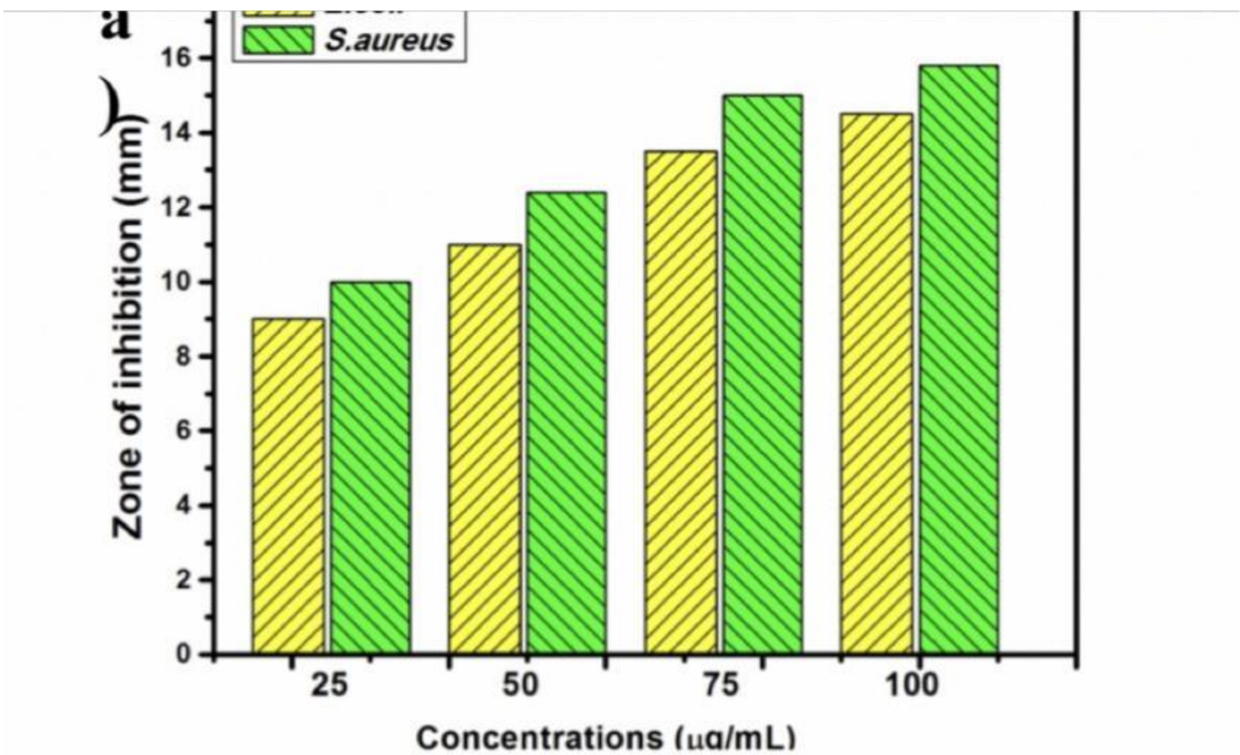

Figure-3: a) Bar graph showing antimicrobial activity of synthesized CuO NPs using *Acacia Concinna* against gram-positive and gram-negative bacteria. The yellow color depicts *E.coli*. *The* Green bar depicts *S. aureus*.

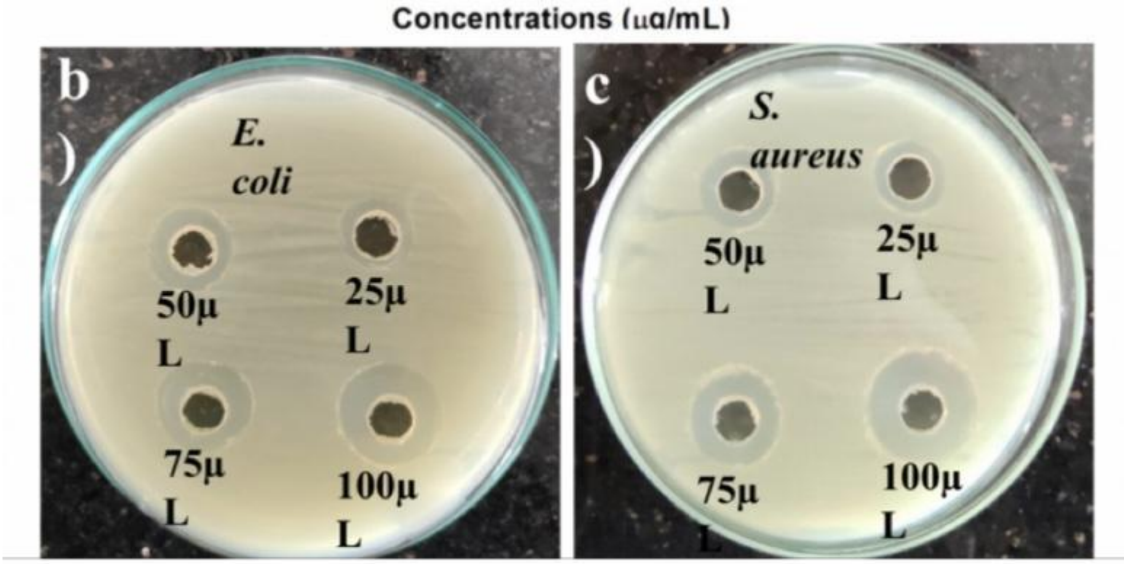

Figure-4: Antimicrobial activity of synthesized CuO NPs using *Acacia Concinna*. B) Zone of inhibition of synthesized extract against *E.coli*. C) Zone of inhibition of synthesized extract against *S. aureus.*

DISCUSSION:

The biological synthesis of nanoparticles using plants as bio-reductants offers significant advantages over other biological processes, particularly in terms of scalability for large-scale production and the elimination of the need for cell culture maintenance (16). The method used for nanoparticle synthesis plays a crucial role in determining their stability. Green synthesis approaches, which utilize plant extracts or microorganisms, often result in nanoparticles with enhanced stability due to the presence of natural capping agents (17,18). In this study, A. Concinna extract was utilized as a reactant to facilitate nanoparticle nucleation in solution. It contains biomolecules that can reduce metal ions to metal NPs, including flavonoids, saponins, alkaloids, and polyphenols. This study is unique in that it is the first to report the biosynthesis of CuO NPs using *Acacia Concinna* extract against pathogenic bacteria. The CuO NPs synthesized using *Acacia Concinna* extract exhibit strong antimicrobial properties against a range of pathogenic bacteria, including Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli). The antibacterial mechanism includes disruption of the bacterial cell membrane, DNA, and protein Damage. CuO NPs penetrate bacterial cells, leading to oxidative stress and eventual cell death. Similar results reported by Bernard et al., 2021, Cu Nps exhibited significant antimicrobial activity against *S. aureus* compared to *E. coli* with a zone of inhibition diameters of 26.00 ± 0.58 mm and

30.00 ± 0.58 mm (19). Sathiya Vimal. et.al. reported Cu Nps prepared from *Sida Acuta to* extract highly inhibited *E.coli* compared to *S. aureus* with Zone of inhibition diameters of 15 mm (20). Similar to this study, Cu NPs showed a significant level of antibacterial activity against *Escherichia coli* (43.33 ± 1.15 mm) and *Proteus vulgaris* (42.66± 0.57 mm). Whereas, moderate action is seen for Cu NPs against *Staphylococcus aureus* (38.66 ± 1.15 mm) and *Pseudomonas aeruginosa* (21.66 ± 0.57 mm) (21). The antibacterial activity was found to increase with the increase of concentration of nanoparticles which is in agreement with our study. A study done by Jasmin. et.al.2023 copper nanoparticles synthesized using *Catharanthus roseus* showed maximum inhibition of protein denaturation at 54.15% at a concentration of 400 µg/ml compared to the FeNps (22). Tejitha.et.al,2023, copper nanoparticles showed similar anti-inflammatory activity compared to the gold standard drug at the highest concentration (50 µg/ml) (23). Similar results were shown by another study, A. K. Al-Jubouri et.Al., 2022 CuNPs demonstrated a significant anti-inflammatory activity when compared to a standard drug (24). This biosynthesis approach using *Acacia Concinna* extract aligns with eco-friendly nanotechnology, reducing the need for toxic chemicals in nanoparticle synthesis. In the future, Comprehensive in vivo and in vitro studies should be conducted to evaluate the cytotoxicity and biocompatibility of CuO NPs for medical applications.

CONCLUSION:

This present study concluded that one of the herbs *Acacia Concinna* extract was used in this study to successfully produce copper oxide nanoparticles, offering a cost-effective, simple, and bio-compatible. The CuO NPs were characterized by SEM and EDX. The synthesized nanoparticles were spherical and hexagonal in shape and crystalline in nature, with size of 20 nm. The green synthesized Cu NPs exhibited excellent antibacterial and anti-inflammatory activity against gram-positive and gram-negative bacteria. Cu Nps was found to be effective at various concentrations. Further, the application of green synthesized Cu NPs can be extended to anticancer, antioxidant, and anti-diabetic activities.

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