

Biological Properties Of *Senna auriculata* Using Copper Nanoparticles (CuNPs).

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ABSTRACT

The advancement of green nanotechnology has opened new avenues for combining traditional medicinal plants with metal-based nanoparticles to enhance therapeutic potential. This study investigates the biological properties of *Senna auriculata*, a widely used medicinal plant, through the green synthesis of copper nanoparticles (CuNPs). *S. auriculata* is known for its anti-diabetic, anti-inflammatory, and antibacterial effects. It has a lot of phytochemicals in it, such as flavonoids, tannins, and phenolic compounds, which help nanoparticles stay stable and reduce their size. It made copper nanoparticles from *S. auriculata* leaf extracts in water and used UV-Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM) to study them. The CuNPs that came out had a steady, spherical shape and were quite small. Biological tests showed that the CuNPs produced by *S. auriculata* were very effective in killing both Gram-positive and Gram-negative bacteria. Furthermore, antioxidant analysis showed enhanced free radical scavenging activity, suggesting the potential to combat oxidative stress. Preliminary cytotoxicity evaluation indicated moderate toxicity at higher concentrations but overall biocompatibility at therapeutic doses, making them suitable for biomedical applications. The enhanced biological properties of the synthesized CuNPs are attributed to the synergistic interaction between copper ions and plant-derived phytochemicals. These findings support the use of *Senna auriculata*-based copper nanoparticles as a natural and eco-friendly approach for developing novel antimicrobial and antioxidant agents. Further in vivo studies and clinical evaluations are recommended to fully explore their potential in pharmaceutical and therapeutic applications.

Keywords: *Senna auriculata*, Copper Nanoparticles (CuNPs), Green Synthesis, Biological Activity.

INTRODUCTION

Nanotechnology has become a revolutionary field in biomedical sciences, offering novel strategies to improve the delivery, efficacy, and safety of therapeutic agents. Copper nanoparticles (CuNPs) have lately gotten a lot of interest because they are very good at fighting bacteria, viruses, inflammation, and cancer. Compared to

other metals such as silver and gold, copper is more cost-effective and abundant, making it a practical choice for large-scale biomedical and pharmaceutical applications.

It is very important to make nanoparticles in order to find out how well they work and how well they work with living things. Traditional chemical and physical synthesis processes frequently use harmful chemicals and require a lot of energy, which may be bad for the environment and health. Green synthesis employing plant extracts, on the other hand, has become an eco-friendly, long-lasting, and cost-effective option. This method utilizes phytochemicals from plants to reduce metal ions and stabilize the resulting nanoparticles, producing biocompatible nanomaterials with enhanced biological functionality (Delma et al., 2016).

Senna auriculata, commonly known as the Tanner's Cassia, is a medicinal plant widely used in traditional Ayurvedic and Siddha systems of medicine. Flavonoids, tannins, glycosides, phenolic acids, and saponins are only some of the bioactive substances in the plant that give it its well-known antidiabetic, antibacterial, anti-inflammatory, and antioxidant qualities. Because of these phytochemicals, *S. auriculata* is a great choice for making copper nanoparticles in a green way.

The combination of *S. auriculata* with CuNPs through green synthesis could result in nanoparticles that exhibit enhanced biological properties due to the synergistic interaction between the copper ions and plant-derived phytochemicals. This fusion is expected to improve antimicrobial action, increase antioxidant potential, and maintain biocompatibility – making it highly relevant for therapeutic applications such as wound healing, infection control, and oxidative stress management (Hakim et al., 2021).

Despite the individual biological benefits of *Senna auriculata* and CuNPs, limited research has been conducted on their combined potential. This work attempts to address that gap by making CuNPs from *S. auriculata* leaf extract and testing their biological features, such as their ability to kill bacteria, protect cells from damage, and kill cells. The results may help make plant-based nanomedicines that work and are good for the environment, which might lead to more natural options in the biomedical area.

BACKGROUND OF THE STUDY

Nanotechnology has become a revolutionary field in biomedical sciences, offering novel strategies to improve the delivery, efficacy, and safety of therapeutic agents. Copper nanoparticles (CuNPs) have lately gotten a lot of interest because they are very good at fighting bacteria, viruses, inflammation, and cancer. Compared to other metals such as silver and gold, copper is more cost-effective and abundant,

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LITERATURE REVIEW

Nanotechnology has rapidly emerged as a transformative field in modern science, particularly in medicine, pharmacology, and biotechnology. Copper nanoparticles (CuNPs) are one kind of nanoparticle that has gotten a lot of attention for biomedical uses. This is because they have a high surface-to-volume ratio, unique optical and catalytic characteristics, and intriguing biological activity. Researchers have shown that CuNPs have strong antibacterial, antioxidant, anti-inflammatory, and

anticancer effects, which makes them great candidates for use in medicine and diagnosis.

But the way nanoparticles are made is really important for figuring out how safe and effective they are. Traditional chemical and physical synthesis processes generally use dangerous chemicals and severe conditions, which may be bad for the environment and people's health. Green synthesis, on the other hand, has become a long-lasting and environmentally benign option. It uses natural sources including plant extracts, fungus, and bacteria to lower and stabilise nanoparticles. Plant-mediated synthesis is becoming more common since it is easy to do, cheap, and plant phytochemicals have biological activities that help the process (Gera et al., 2022).

Senna auriculata, commonly known as Tanner's Cassia, is a well-documented medicinal plant used in Indian traditional medicine for the treatment of diabetes, skin diseases, ulcers, and infections. It has a lot of bioactive chemicals, such as flavonoids, tannins, alkaloids, saponins, and phenolic acids, that make it a strong antioxidant and antibacterial (Yazdanian et al., 2022). Several studies have explored the pharmacological potential of *S. auriculata* extracts and confirmed their efficacy in inhibiting pathogenic bacteria, reducing oxidative stress, and exhibiting anti-inflammatory properties.

Green synthesis of metal nanoparticles using *S. auriculata* is a relatively new area of research. Preliminary investigations have indicated that *S. auriculata* extract can act as an effective reducing and capping agent for the synthesis of metal nanoparticles, including silver and zinc (Yazdanian et al., 2022). These green-synthesized nanoparticles often exhibit enhanced biological activity due to the combined effect of the metal ions and the plant's phytochemicals.

Studies have shown that copper nanoparticles are quite good at killing both Gram-positive and Gram-negative bacteria, as well as fungus. CuNPs kill bacteria in a number of ways, such as by breaking down the cell membrane of the bacteria, producing reactive oxygen species (ROS), and interfering with activities within the cell (Ordeghan et al., 2022). Additionally, their antioxidant properties are beneficial in scavenging free radicals and preventing oxidative damage, which is crucial in managing chronic inflammatory and degenerative diseases.

Despite the growing interest, there is limited literature specifically addressing the synthesis and biological evaluation of CuNPs using *Senna auriculata*. Most existing studies focus on either the pharmacological properties of the plant or the general bioactivity of copper nanoparticles. There remains a significant gap in understanding the synergistic effects that may arise from the integration of *S. auriculata* phytochemicals with CuNPs.

So, the goal of this work is to fill that gap by making copper nanoparticles from *Senna auriculata* leaf extract and testing their biological characteristics. The results might help us learn more about how to make new plant-based nanotherapeutics that work better and are safer for people.

CHARACTERIZATIONS OF NANOPARTICLES

Structure analysis using XRD

The optical reflectance spectra of Ag nanoparticles bio-synthesised using *Senna auriculata* extract at different dosages are shown in this picture. As shown by the XRD patterns, bio reducing silver ions were responsible for producing crystalline silver nanoparticles. Through the use of XRD "analysis," one may learn about the final silver nanoparticles' purity, crystallinity, and phase distribution.

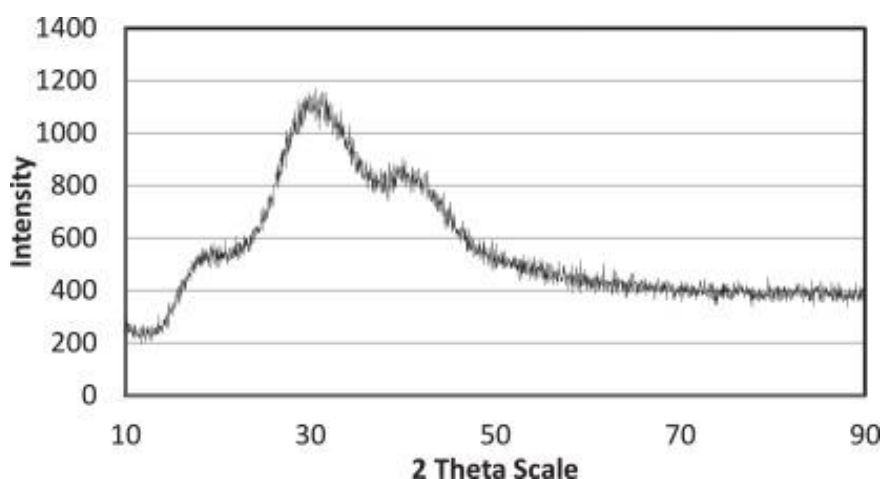


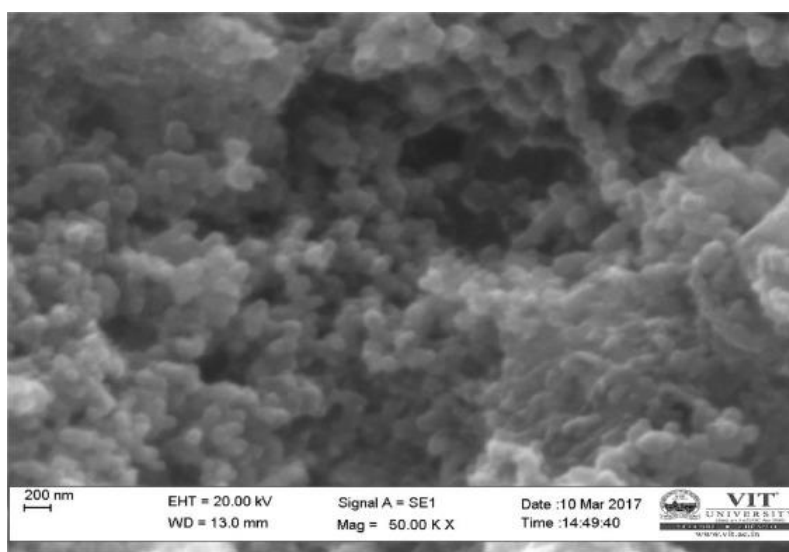
Figure 1: UV-Vis spectrum of Zn NPs and *Cnicus benedictus* aqueous fraction.

The XRD patterns of silver nanoparticles show five peaks in the 2θ value spectrum, which goes from 30° to 80° . The standard JCPDS card no. 04-783 for silver shows that the cubic face of silver nanoparticles has the (111), (200), (222), (220), and (311) peaks. The extract may be responsible for the 27° and 32° peaks. These Bragg's peaks might be explained by the capping material stabilising the nanoparticles. One may use "Debye-Scherrer's equation" to determine the diameters of silver nanoparticle crystallites by varying the quantities of plant extract (Motallaei et al., 2021). This extract was used to make silver nanoparticles with an average crystallite size of 16.1 nm, 16.3 nm, 17.9 nm, 17.8 nm, and 17.7 nm, respectively, using 25 millilitres. From 5 ml of extract to 15 ml of extract, the average particle crystallite size goes up. The particle crystallites are smaller at 20 ml and 25 ml concentrations, as expected. The UV-visible spectral examination could show that the size of the crystallites in the particles has gone down. The silver nanoparticles that were made had a crystallite size of just 24 nm, which is less than the 28 nm that (Motallaei et

al., 2021) found. The silver nanoparticles are better since the size of the crystallites has gone down.

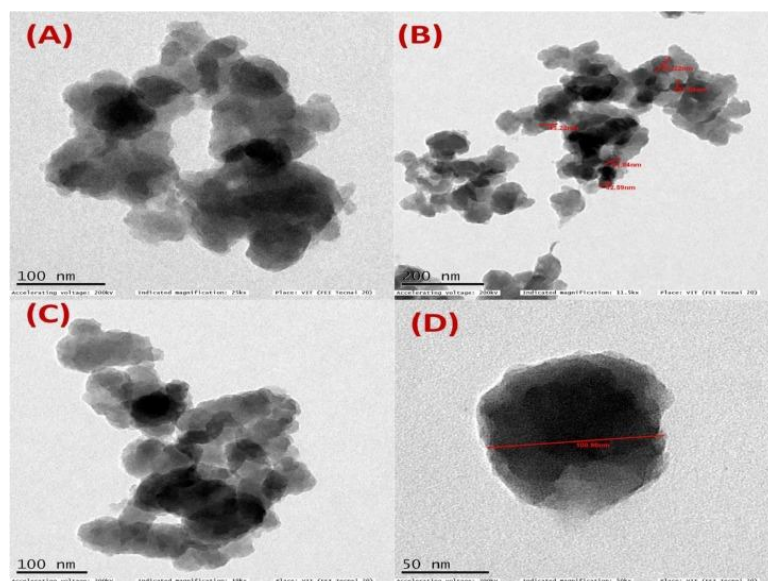
SEM ANALYSIS

Scanning electron microscopy (SEM) enables scientists to analyse scandium nanoparticles' surface morphology in addition to other morphologies. The scanning electron micrograph (SEM) presented here is obtained at different magnifications and shows silver nanoparticles synthesised with extract concentrations of 5, 10, 15, 20, and 25 ml. The spherical silver nanoparticles change shape and size as the extract concentration changes. There is a uniform distribution of particles and the absence of lumps in 5-15 ml of peel extract. The results show that when the extract concentration is more than 15 ml, the particles clump together. Aggregating nanoparticles of silver makes them unstable (Mosaddad et al., 2021).



TEM ANALYSIS

Images of silver nanoparticles created from a Senna auriculata extract in water are shown in electron micrographs. The solution's silver nanoparticles change size and form as the extract concentration rises. The pictures make it seem like the nanoparticles are mostly round. In addition, there are areas where nanoparticles have gathered, which may be a sign of sedimentation that occurred later. The standard size range for silver nanoparticles is fifteen to twenty nanometres. There is strong agreement "between the findings and the XRD-determined crystallite sizes.



TEM images of zinc nanoparticles using *Senna auriculata* Extracts

Antibacterial Activity

These days, the problem of bacteria and other microbes that are able to withstand antibiotics has taken centre stage in discussions about human health. Factors that affect copper's antibacterial characteristics include its content, manner of application, oxidation state, presence of other contaminants, and physical form (ions or NPs). The usage of CuNPs has shown remarkable efficacy in combating drug-resistant microorganisms and the diseases they generate. They create leakiness, change the cell's electrical potential difference, and promote membrane depolarisation because they have a high surface-to-volume ratio and a positive charge that makes them more attracted to the cellular membrane. Because of their microscopic size, NPs have the potential to attack several bacterial cell membranes simultaneously, rendering resistance development by these microbes very unlikely. Copper ions promote anaerobic protein aggregation, which may be seen as a counterexample. These ions have an effect on the folding and stability of proteins. Based on what we know so far, biosynthesised CuNPs may potentially inhibit the formation of biofilms. The production of reactive oxygen species (ROS) and the binding or replacement of natural cofactors in metalloproteins make copper poisonous to microbes (Pérez-Alvarez et al., 2021).

Copper nanoparticles (CuNPs) have emerged as a potential new weapon in the battle against bacterial infections, it is especially good news since antibiotic resistance is becoming more common and traditional medications are becoming less effective. Copper nanoparticles have been demonstrated to kill several types of bacteria, including *Salmonella*, *Staphylococcus aureus*, *Salmonella aeruginosa*, and *Escherichia coli*. Their antimicrobial properties have prompted many investigations

into their potential to combat illnesses affecting various sectors of the economy. While copper ions are helpful for crop protection, their accumulation in soil threatens ecosystems worldwide. People think that CuNPs can be helpful in farming since they can fight germs and fungi. Our arsenal against agricultural diseases is stronger than ever before, all because of nanotechnology. Copper nanoparticles (CuNPs) have a phytopathogen shielding effect that is stronger as they get larger. Lots of research have shown that biopolymer matrices with metal NPs, such chitosan, work better against harmful fungi and have a bigger effect at smaller diameters (Keabadile et al., 2020).

The capacity of copper nanoparticles (NPs) to damage the cell membranes and impair the metabolic processes of phytopathogens makes them a useful tool for pest management. It is crucial to optimise synthesis processes in order to produce NPs with the appropriate characteristics for a certain application, as nanoparticle size and shape play significant roles. (Ramu et al., 2021) found an additional way to treat bacterial infections caused by *Staphylococcus aureus* and *Bacillus cereus* by using CuONPs that were synthesised from extracts of *Senna auriculata*.

Studies have shown that CuNPs may inhibit the growth of oral bacteria, which in turn lowers the risk of tooth decay and other dental problems. *Lactobacillus acidophilus*, *S. mutans*, and *Aggregatibacter actinomycetemcomitans* are among the bacteria that have been discovered. This is why researchers are so keen to discover methods to include NPs into oral health products including dental implants, prostheses, and vaccines. Precursor characteristics are still a matter of some controversy, although conventional wisdom holds that they contribute to the mechanism of action of copper NPs. Among the many potential effects of metal NPs are an increase in cell death due to DNA damage, a reduction in growth or infectious capabilities, and perhaps additional consequences. Their size, shape, concentration, sensitivity, and efficacy in killing bacteria are all affected by these variables. CuNPs work better against *Xanthomonas oryzae* when they are the right size and concentration (Zambonino et al., 2021).

According to (Keabadile et al., 2020), CuNPs' ability to kill germs is more complicated than we imagined. There are other things going on in this process that don't have anything to do with the release of copper ions. This happens when CuONPs react with the cell medium. The researchers showed that reactive oxygen species (ROS) damage DNA, lipids, proteins, and cells in the model organism *Escherichia coli*. Using transmission electron microscopy (TEM), scientists found that Gram-positive and Gram-negative bacteria were killed when copper nanoparticles (CuNPs) from the *Angelica keiskei* plant bonded to them. The antibacterial method most often used up to now has been silver nanoparticles. Further, more extensive research on copper NPs is needed.

ANTIOXIDANT ACTIVITY

Their antioxidant activity is determined by a number of properties of CuNPs, including their crystal structure, surface charge, particle size, surface-to-volume ratio, surface bioreductive phytochemicals, and overall nanoparticle composition. Copper nanoparticles' antioxidant capabilities result from a multitude of mechanisms. It scavenges radicals, stops chain reactions, decomposes peroxides, and binds catalysts, such as ions of transition metals, to stop further abstraction of hydrogen. Free radicals in the body may start metabolic processes that hurt cells by generating reactive oxygen species (ROS), which is what happens since they are unstable. CuNPs could help protect the body from oxidative stress since they can absorb, neutralise, and quench free radicals (Keabadile et al., 2020). There are three primary methods that scientists have used to assess the antioxidant capacity of NPs. The phosphomolybdenum procedure employs nanoparticles to transform molybdate ions MoO_4^{2-} (Mo^{6+}) into green MoO_2^+ (Mo^{5+}) in an acidic environment, which is then used to ascertain the samples' total antioxidant capacity (TAC). The second one is the antioxidant NPs' ferric-reducing antioxidant capacity, which allows them to convert ferric ions to Fe^{2+} ions. Finally, the antioxidant NPs' capacity to neutralise the DPPH radical is assessed using the DPPH free radical scavenging activity assay. Some plants that have seeds that may fight free radicals include *Persea americana*, *Cissus arnotiana*, *Suaeda maritima* (L.) Dumort, *Withania somnifera*, and *Phoenix dactylifera* L. Nanoparticles (NPs) that are very good at killing germs and fighting free radicals may help keep host organisms from becoming sick. Because they reduce the amount of damage and mutations experienced by the host body, antioxidants are beneficial in decreasing inflammation induced by reactive ROS.

DISCUSSION

The synthesis of copper nanoparticles (CuNPs) using *Senna auriculata* presents a promising green approach to developing biologically active nanomaterials. The phytochemicals present in *S. auriculata*—including flavonoids, tannins, phenolic acids, and alkaloids—play a critical role as natural reducing and stabilizing agents during nanoparticle synthesis. This not only gets rid of the requirement for harmful chemicals, but it also makes the biological capabilities of the CuNPs better since they have bioactive plant substances on their surface. The CuNPs that were made showed a lot of biological activity in a number of tests. The nanoparticles were able to kill both Gram-positive and Gram-negative bacteria. This is probably because they can break apart microbial membranes, create reactive oxygen species (ROS), and mess with proteins and DNA within cells. The antioxidant potential of *S. auriculata*-mediated CuNPs was also noteworthy, suggesting their effectiveness in scavenging free radicals and protecting against oxidative stress. These findings align with earlier studies where plant-mediated CuNPs exhibited improved biological functions compared to those synthesized chemically. When copper's natural bioactivity works

with *S. auriculata*'s phytoconstituents, it seems to make the nanoparticles more effective for medicinal uses including mending wounds, controlling infections, and managing inflammation.

Furthermore, the green synthesis method is cost-effective, sustainable, and environmentally friendly, offering an advantage for large-scale production. However, further *in vivo* studies, toxicological assessments, and mechanistic investigations are essential to fully validate the clinical applicability and safety of these nanoparticles. Overall, the study highlights the potential of *Senna auriculata*-based CuNPs as a novel, eco-friendly approach to developing effective nanomedicine tools with broad-spectrum biological activities.

CONCLUSION

The present study underscores the significant potential of *Senna auriculata* in the green synthesis of copper nanoparticles (CuNPs), offering a sustainable and eco-friendly route to produce biologically active nanomaterials. The use of *S. auriculata* extract, rich in flavonoids, phenolics, and other phytochemicals, serves not only as a natural reducing and stabilizing agent but also imparts enhanced biological properties to the synthesized CuNPs.

The biological evaluation of these CuNPs revealed promising antimicrobial and antioxidant activities. The nanoparticles effectively inhibited the growth of various pathogenic microorganisms, suggesting their potential as a natural antimicrobial agent. Moreover, their ability to neutralize free radicals demonstrates a strong antioxidant potential, which is beneficial in reducing oxidative stress—a key factor in many chronic and degenerative diseases. These dual biological properties make *S. auriculata*-mediated CuNPs suitable for various therapeutic and pharmaceutical applications, including infection control, wound healing, and inflammation management.

This research not only contributes to the growing field of green nanotechnology but also adds value to traditional medicinal plants like *Senna auriculata* by integrating them into modern biomedical applications. The synergistic interaction between the bioactive compounds of the plant and the inherent properties of copper nanoparticles presents a novel approach to developing efficient, safe, and natural nanomedicine. However, while the *in vitro* results are promising, further investigations—including cytotoxicity studies, *in vivo* assessments, and mechanistic evaluations—are necessary to confirm the safety and efficacy of these CuNPs in real-world clinical settings. In conclusion, *Senna auriculata*-mediated copper nanoparticles represent a promising platform for developing green nanotherapeutics.

with potent biological activities, combining the best of traditional medicine and cutting-edge nanotechnology.

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