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Biological Properties of *Hydrastis Canadensis* Using Copper Nanoparticles

Druva Chowdary Nagandla, Sreemoy Kanti Das, K. Suresh Babu
Faculty of Pharmacy, Lincoln University College, Malaysia

Abstract: The perennial plant Hydrastis canadensis, more often known as goldenseal, has a long history of usage in herbal medicine due to its many beneficial characteristics, such as its ability to enhance the immune system, reduce inflammation, and fight against microbes. Berberine, hydrastine, and canadine are three of the bioactive components of Hydrastis canadensis that have shown promising results in treating a range of medical issues, including inflammation, bacterial infections, and gastrointestinal problems. Natural plant extracts have the potential to be medicinal, but they often encounter obstacles in terms of bioavailability, stability, and site delivery. Despite these obstacles, a new strategy for improving medicinal plants' biological activity—the use of nanotechnology, and more especially nanoparticles derived from metals—has arisen.

Copper nanoparticles mediated by Hydrastis canadensis were extensively studied for their biological characteristics. Use of disc diffusion and minimum inhibitory concentration (MIC) tests allowed for the evaluation of antimicrobial efficacy against a wide range of bacterial and fungal pathogens, including Gram-positive and Gram-negative strains. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging tests were used to assess the antioxidant potential of the produced nanoparticles. The results showed that the nanoparticles had better free radical scavenging activity than either the plant extract or the copper nanoparticles alone. Biofunctionalized CuNPs also showed encouraging anticancer activity by inhibiting cancer cell growth and inducing apoptosis when evaluated on different cancer cell lines using MTT tests.

This research found that adding copper nanoparticles to Hydrastis canadensis significantly increased its biological activity. The enhanced bioavailability, surface reactivity, and stability of the nanoparticles are responsible for the observed synergistic impact. This unique nanocomposite shows promise as a therapeutic agent candidate due to its enhanced antibacterial, antioxidant, and cytotoxic capabilities relative to its constituent parts, according to the results.

Finally, this study lays the groundwork for a unique strategy in nanomedicine and pharmaceutical sciences: the investigation of copper nanoparticles mediated by Hydrastis canadensis. The green synthesis process improves medicinal plants' pharmacological potential while also providing an economical and environmentally friendly alternative to nanoparticle manufacturing. For a complete understanding of how these biofunctionalized nanoparticles work and to confirm their therapeutic potential in treating cancer, oxidative stress diseases, and drug-resistant infections, more in vivo investigations and clinical trials are needed.

Keywords: Hydrastis canadensis,Copper Nanoparticles, Green Synthesis, Antimicrobial Activity, Antioxidant Properties.

I. INTRODUCTION

The perennial plant *Hydrastis canadensis*, most often known as goldenseal, grows wild in North American forests. Due to its diverse variety of bioactive chemicals, goldenseal has become well recognized in contemporary herbal medicine, building on its long history of usage by Indigenous communities. Berberine, hydrastine, and canadine are the main components of the plant, and they have powerful antioxidant, anti-inflammatory, and antibacterial properties. *Hydrastis canadensis* has a long history of medicinal usage, including indications for gastrointestinal problems, respiratory infections, and skin diseases. Although the plant has a long history of usage in traditional medicine, the full extent of its medicinal potential has only just been explored in current scientific studies. Additional research into the mechanisms of action and possible pharmaceutical uses of these bioactive chemicals is warranted due to recent studies suggesting they may provide far-reaching health advantages (Biswas et al., 2022).

Emerging at the same time as breakthroughs in botany, nanotechnology is a game-changing area with far-reaching consequences for environmental protection, healthcare, and materials science. It has been shown that metal nanoparticles in particular display distinct chemical, biological, and physical characteristics compared to their bulk equivalents. Copper nanoparticles (CuNPs) stand out from the crowd thanks to their unique set of characteristics, which include a large surface area, high conductivity, catalytic efficiency, and relative affordability when compared to other metal nanoparticles like gold or silver. Plus, CuNPs have a ton of biological activity, including antibacterial, antioxidant, anticancer, and anti-inflammatory properties, so they might be used in biomedicine in a big way.

A new way to improve the effectiveness of treatments is to combine nanotechnology with traditional medicinal herbs. Here, a synergistic effect that enhances the biological capabilities of both materials might be achieved by mixing *Hydrastis canadensis* with copper nanoparticles. With the addition of CuNPs, the antimicrobial and antioxidant activities of the plant's natural compounds could be even stronger. This would make the treatment more effective against oxidative stress, cancer cells, and a host of other harmful microorganisms.

The purpose of this research is to investigate the possible synergistic effects of combining *Hydrastis canadensis* with copper nanoparticles and to learn more about its biological characteristics in this novel fusion. The major goals of this study are to synthesis CuNPs using *Hydrastis canadensis* extracts, to describe the nanocomposites that are formed, and to assess their biological activity by means of a battery of in vitro experiments. The research will look at the antioxidant potential using free radical scavenging tests, the cytotoxic effects on certain cancer cell lines, and the antimicrobial efficacy against common bacterial and fungal infections. The goal of these studies is to fill in the gaps in our knowledge about the synergistic effects of CuNPs and bioactive chemicals obtained from plants in the context of therapeutic applications.

The use of *Hydrastis canadensis* in nanoparticle production is supported by its extensive phytochemical profile, which not only enhances its biological activities but also stabilizes and reduces the amount of solvent needed for the process. Due to their low cost and lack of environmental impact, green synthesis techniques are becoming more and more popular. These methods employ plant extracts instead of harmful chemicals. This work presents a sustainable method for producing nanoparticles using *Hydrastis canadensis*, which is in line with green chemistry principles. It also improves the bioavailability and effectiveness of the plant's natural components.

The importance of this study goes beyond just finding a new medicine. Additionally, it sheds light on how plant-based nanotechnology might help combat antibiotic resistance and the increasing prevalence of oxidative stress-related chronic illnesses, two of the most critical issues in world health today. In addition, further research into the molecular level interaction between nanoparticles and substances originating from plants might lead to the development of treatment alternatives that are highly targeted, efficient, and biocompatible.

Finally, by studying the biological effects of *Hydrastis canadensis* mixed with copper nanoparticles, this research hopes to close the gap between conventional herbal therapy and modern nanotechnology. Nanomedicine, phytopharmacology, and green chemistry are expanding areas of study, and this research aims to add to that body of knowledge via extensive analysis and testing. The results of this research have the ability to pave the way for new, creative, economical, and eco-friendly ways to treat a range of human health issues (Sharmin et al., 2021).

II. BACKGROUND OF THE STUDY

Because of their abundance of bioactive chemicals with possible medicinal uses, medicinal plants have been an integral part of conventional healthcare systems across the globe for a very long time. The perennial herb *Hydrastis canadensis*, more often known as goldenseal, is native to the eastern United States and certain portions of Canada and is one of these plants. For its many therapeutic uses, including the treatment of infections, gastrointestinal issues, and inflammatory problems, *Hydrastis canadensis* was highly esteemed by Indigenous peoples and early immigrants. This plant's medicinal value is mainly due to the abundance of isoquinoline alkaloids found in it, including berberine, hydrastine, and canadine. The scientific community has acknowledged the antibacterial, anti-inflammatory, antioxidant, and anticancer characteristics of these bioactive substances.

Modern scientific study has only just started to reveal the full scope of *Hydrastiscanadensis*'s pharmacological potential, despite the herb's lengthy usage in traditional medicine. The bioavailability, stability, and cellular absorption of berberine have hindered its usefulness in therapeutic applications, despite its encouraging outcomes in antibacterial and anticancer research. The transport, stability, and biological activity of chemicals generated from plants have been challenged in the past, but new developments in nanotechnology provide hope for a possible solution.

Medicine, biology, and materials science are just a few of the scientific disciplines that have been profoundly impacted by nanotechnology, a multidisciplinary discipline that manipulates materials at atomic and molecular dimensions (1-100 nanometers). Nanoparticles made of metals, and in particular CuNPs, have shown promise as a material with useful physicochemical characteristics in this area. As a trace element, copper is vital to living things. At the nanoscale, it has powerful antibacterial, antifungal, antioxidant, and anticancer effects. This is mainly because of its capacity to cause cellular metabolic processes to malfunction, break microbial membranes, and produce reactive oxygen species (ROS).

Copper nanoparticles are ideal for use in biomedicine due to their low cost and high catalytic activity, which sets them apart from other metal nanoparticles.

Their antimicrobial properties, wound healing capabilities, and drug delivery capabilities have recently been brought to light in scientific investigations. To guarantee their safe and successful usage in biological systems, meticulous formulation and stabilization procedures are required due to the intrinsic cytotoxicity of copper nanoparticles, particularly at higher concentrations.

An innovative method for increasing the therapeutic potential of both components is to combine *Hydrastis canadensis* extracts with copper nanoparticles. An environmentally benign, sustainable, and non-toxic alternative to traditional chemical synthesis techniques is biogenic synthesis, often known as green nanotechnology. It entails reducing metal ions into nanoparticles using plant extracts. In addition to reducing negative impacts on the environment, this approach has the ability to increase biological activity by combining plant phytochemicals with metal nanoparticles.

Evidence from many research points to the possibility of greater cytotoxic effects on cancer cells, higher antioxidant activity, and better antibacterial efficiency when metal nanoparticles are combined with bioactive chemicals derived from plants. However, there is a lack of study that particularly addresses the biological features of *Hydrastis canadensis* in conjunction with copper nanoparticles. The development of more effective and safer advanced therapeutic medicines may benefit greatly from a better understanding of the molecular interactions between these two components.

This work aims to address the lack of research on the biological characteristics of copper nanoparticles mediated by *Hydrastis canadensis*. Their antioxidant capacity, cytotoxic effects on some cancer cell lines, and antibacterial activity against different bacterial and fungal strains are the main goals. Critical elements determining the biological activity of manufactured nanoparticles are their physicochemical qualities; this research will also investigate these aspects, including particle size, shape, and surface charge.

Nanomedicine and green nanotechnology are rapidly expanding fields, and this study might make important contributions to both. This work has the potential to open the door to the development of new, effective, and ecologically sustainable therapeutic medicines by merging the well-established medicinal effects of *Hydrastis canadensis* with the distinctive characteristics of copper nanoparticles. In addition, this study's findings might pave the way for other studies using plant-metal nanoparticle systems, which could lead to the improvement of both conventional and alternative healthcare.

Finally, a promising new area of study in natural product and nanotechnology is the combination of the biological characteristics of *Hydrastis canadensis* with copper nanoparticles. With the hope of shedding light on how state-of-the-art nanotechnological advancements might improve traditional herbal therapy, this research seeks to increase our knowledge of these interactions. The significance of this study in tackling modern healthcare concerns is highlighted by the possible implications for better treatment results, less antibiotic resistance, and the development of environmentally friendly medicinal solutions.

III. LITERATURE REVIEW

Goldenseal, or *Hydrastis canadensis*, is a perennial plant with antibacterial, anti-inflammatory, and immunostimulatory characteristics that have long been utilized in North American herbal medicine. Tribes of Native Americans have relied on this plant's roots and rhizomes for generations to cure a wide range of medical conditions, including gastrointestinal issues, skin infections, and inflammations of the mucous membranes. The herb's medicinal value is due in large part to the isoquinoline alkaloids found in it, particularly berberine, hydrastine, and canadine.

There are now more opportunities than ever before to increase the bioactivity of chemicals obtained from plants by means of nanoparticle production, thanks to recent developments in nanotechnology. The remarkable antibacterial, antioxidant, and catalytic capabilities of copper nanoparticles (CuNPs) have made them stand out among the rest. Because of their very tiny size and high surface area-to-volume ratio, nanoparticles have unusual physicochemical properties that enable them to engage in molecular-level interactions with biological systems. With an eye on their complementary medicinal uses, the authors of this literature review investigate the physiological effects of combining *Hydrastis canadensis* with copper nanoparticles.

Several bioactive chemicals found in *Hydrastis canadensis* are responsible for its pharmacological actions. Berberine has shown remarkable antibacterial, anti-inflammatory, antidiabetic, and anticancer properties; it is the alkaloid from this plant that has been investigated the most. Reduces microbial pathogenicity by interfering with quorum sensing mechanisms, preventing nucleic acid production, and disrupting bacterial cell division. The traditional medicinal use of the plant is enhanced by its hydrastine and canadine, both of which possess antibacterial characteristics. Furthermore, research indicates that these alkaloids may influence the immune system by regulating the synthesis of cytokines and blocking the activity of pro-inflammatory enzymes such cyclooxygenase-2 (COX-2).

The remarkable biological activities of copper nanoparticles, such as their antibacterial, antioxidant, and anticancer capabilities, have attracted a lot of interest. They are able to breach bacterial cell walls and release intracellular contents because of their diminutive size, which allows them to cross bacterial cell membranes. Additionally, CuNPs produce reactive oxygen species (ROS), which cause oxidative stress in cancer cells and microbes, ultimately resulting in cell death by necrosis or apoptosis. In addition, these nanoparticles show promise as potential medicinal uses due to their ability to enhance wound healing via cell migration, proliferation, and angiogenesis.

Because of the complementary nature of the plant's bioactive chemicals and the nanoparticles, combining *Hydrastis canadensis* extracts with CuNPs may increase biological activity. Metal nanoparticles have the potential to enhance the bioavailability, stability, and targeted delivery of chemicals obtained from plants, according to research. Such mixtures seem to have more antibacterial, antioxidant, and anticancer benefits than the separate components, according to preliminary research. Antibiotic resistance is an increasing problem in global health, but this collaborative strategy shows promise in combating it.

The extracts of *Hydrastis canadensis* have shown promise in combating several microorganisms, including fungus, protozoa, and both Gram-positive and Gram-negative bacteria. The main way berberine works is by preventing bacteria from making DNA and by preventing efflux pumps from doing their jobs. Because CuNPs increase membrane permeability, decrease biofilm formation, and speed up bacterial cell death, their inclusion amplifies these actions. When it comes to fighting off multidrug-resistant bacterial strains, which are a major problem in hospitals, this combination strategy could work wonders.

Hydrastis canadensis and copper nanoparticles' antioxidant capabilities have been extensively studied. While CuNPs help lower ROS levels in injured tissues, berberine scavenges free radicals, lowers oxidative stress, and protects DNA. By boosting their free radical scavenging capacity, these two medicines work together to defend against illnesses caused by oxidative stress, such as cardiovascular disease and neurological disorders. In addition, the combination formulation has an intensified anti-inflammatory action, which makes it a good choice for treating inflammatory bowel disease and arthritis, which are chronic inflammatory illnesses.

Because berberine may induce apoptosis in a variety of cancer cell lines, including breast, colon, and lung cancer cells, *Hydrastis canadensis* also has cytotoxic and anticancer potential. CuNPs boost ROS production and make it easier to transfer bioactive chemicals intracellularly, both of which increase this harmful impact. Better cancer cell apoptosis, slower tumor growth, and less metastasis are the outcomes of this two-pronged attack. To further reduce the negative effects of traditional chemotherapy, CuNPs may be modified for targeted medication delivery.

One interesting approach to improving the medicinal efficiency of plant extracts is the combination of *Hydrastis canadensis* with copper nanoparticles. The safety, bioavailability, and effectiveness of these bio-nanocomposites must be confirmed by further in vivo investigations and clinical trials, even if existing research emphasizes the possible synergistic effects. Finding the sweet spot between therapeutic efficacy and safety in terms of synthesis process, nanoparticle size, and dose is an area that needs more investigation. Research into the processes by which bioactive plant chemicals interact with nanoparticles will also provide light on how to create new medicinal medicines.

Ultimately, there is great promise in enhancing the effectiveness of plant-based therapeutics across a range of biological domains via the integration of *Hydrastis canadensis* with copper nanoparticles. Antibiotic resistance, cancer therapy, and disorders connected to oxidative stress are just a few of the global health concerns that this novel technique shows promise for tackling. These bio-nanocomposites have the potential to provide the foundation for next-generation medicines that are safer, more effective, and more precisely delivered if they undergo more study(Guerrini et al., 2022).

IV. COPPER NPS

As a necessary trace mineral, copper is present in several meals. The wide variety of metabolic activities in which it functions as a cofactor for enzymes includes energy production, the production of connective tissue, neurotransmitters, collagen, and red blood cells. Brain growth, immunological system function, skin regeneration, angiogenesis, and healing all rely on copper. Additionally, it aids in the process of superoxide radical dismutation. Though it's possible to become ill from either too much or too little copper. Evidence of copper's antibacterial properties has also been around for a long time, with documentation going all the way back to 3000 BC. A number of pathogenic bacteria have therapeutic implications, and there is strong evidence that NPs based on copper may restrict their proliferation.

Concerns about the potential risks of NPs to both human and environmental health persist despite their widespread use. Even though copper is not very hazardous to people, there is no information available on the toxicity of CuNPs.

Copper nanoparticles are toxic to the reproductive system, kidneys, liver, and spleen, according to new studies. Nanoparticles of copper likewise halted the proliferation of human extravillous trophoblast cells and induced cell death in SW480 cells. The content of the biological agent, the precursor salt used, the conditions of synthesis, and the final particle size are some of the factors that influence the characteristics and potential toxicity of CuNPs.

Improving the synthesis process to get NPs with low toxicity and strong antibacterial activity is a challenging and laborious task. The characterisation of CuNPs may provide further information about their physical properties and potential detrimental mechanisms in living organisms (Miu&Dinischiotu, 2022).

V. GREEN SYNTHESIS OF NANOPARTICLES

When discussing conventional approaches to NP synthesis, two major camps have emerged: the top-down and the bottom-up schools of thinking. What differentiates these categories from one another is the raw materials used to make NPs, which vary significantly. In top-down techniques, the bulk material is broken down into NPs using a variety of physical, chemical, and mechanical processes, such as sputtering, mechanical milling, and laser ablation. Many bottom-up methods, including chemical reduction, biological/green synthesis, spray pyrolysis, and vapor deposition, begin with atoms or molecules as their building blocks to create NPs. It is possible to create and stabilize metal nanoparticles (NPs) utilizing a variety of physical and chemical methods in both top-down and bottom-up methods. However, a lot of the current methods need very expensive and energy-intensive specialized machinery. The employment of harmful chemicals as reagents and the subsequent generation of harmful waste make traditional NP synthesis a much more detrimental environmental impact.

One of the most well-liked bottom-up approaches is green synthesis of NPs, which aims to solve many of the issues plaguing conventional methods, including their high cost, toxicity, lack of safety, and detrimental impact on the environment. Through the integration of nanotechnology and green chemistry, novel biosynthetic routes have been established for the synthesis of NPs from microorganisms and plants. You may walk safely along these trails without worrying about harming the ecosystem.

Nanoparticle biogenesis, sometimes called green synthesis, makes use of a wide variety of plant components, including roots, stems, leaves, fruits, peels, and seeds. The end result would not have been possible without the contributions of each of these parts. Beneficial uses are found for metabolites produced by microbes (such as bacteria, actinomycetes, yeast, and algae), virus particles, and biomass waste. The bioactive reduction capacities of plant metabolites—including terpenoids, alkaloids, and proteins—make them essential to the green synthesis concept. In addition, the poly-hydroxyl groups of secondary metabolites are essential for NP synthesis by metal ion reduction.

Naturally, NPs may be synthesized by both plants and bacteria. Both intracellular (endogenous) and extracellular (exogenous) methods exist for living things to perform biogenic synthesis. Essential for endogenous biosynthesis is the ability of some bacteria and plant cells to hyper-accumulate metals from their surroundings. The cytosol of a cell is where metals are reduced and kept as nanoparticles. Plants have the ability to hyper-accumulate and excrete heavy metals, which are very toxic even at low concentrations, according to research.

Secondary metabolites are required for exogenous biosynthesis and are secreted by plant roots in response to metal stress. These waste products chelate metal ions, reducing their size to a more manageable form. Even though scientists are continually trying to figure out how to make nanoparticles, the specifics of biogenic synthesis are still a mystery. These discoveries have led to the discovery of new and interesting uses, as well as novel methods for synthesizing and improving control over the size and shape of NPs. That depends on the interactions between certain metal or metal oxide crystal faces and biomolecules or secondary metabolites, which serve as capping agents. In the synthesis of NPs, a broad range of naturally existing biological resources, such as bacteria and plants, may act as capping agents.

The synthesis method is fine-tuned to take advantage of NP production and optimum efficiency based on the natural source employed. For instance, bacteria may be cultured in a liquid medium with relative ease, and the filtrates, both extracellular and intracellular, can be used as a reducing agent to create nanoparticles. Gathering the plant materials, cleaning and drying them, mechanically grinding them, mixing them with a solvent at high heat, and finally filtering the solvent extract are the six primary steps in a green synthesis process that employs plant extracts (Wang et al., 2021).

The primary components of a metal nanoparticle production process are a solvent, reducing and stabilizing agents, and NPs, or nanoparticles of metal. In the context of green synthesis, a number of phytocomponents act as stabilizers and reductors when mixed with an aqueous solution of the precursor metal salt. What follows is a detailed explanation of how to extract NPs from plants. To summarize, under carefully regulated reaction conditions, plant extracts are mixed with solutions of metallic copper precursor salts. Many synthesis factors influence the NPs' properties, including the precursor salt, pH, temperature, and precursor-to-extract ratio.

So far, the following procedures have been proposed for the environmentally friendly production of NPs: The ionic state of copper is first decreased. Some plant compounds may be able to absorb copper ions due to their reducing potential. After the ionic form is neutralized by reduction, aggregation and growth follow, leading to oxidation and coating. The coating is made by reacting with compounds found in plants. In order to make the final NPs more stable, these interactions are necessary. Various phytocomponents obtained from plant extracts play crucial roles in the green production of CuNPs, as shown in Figure 1. Additional information on this synthesis method is provided in Section 7.

It has been shown that CuNPs may be formed by a broad range of medicinal plants. Because medicinal plants contain such a wide diversity of components, the resulting nanoparticles exhibit a tremendous variation of shapes, sizes, and properties. Depending on their form, NPs may be categorized in many ways: rod-shaped, spherical, amorphous, triangular, cylindrical, and prismatic (Thandapani et al., 2023).

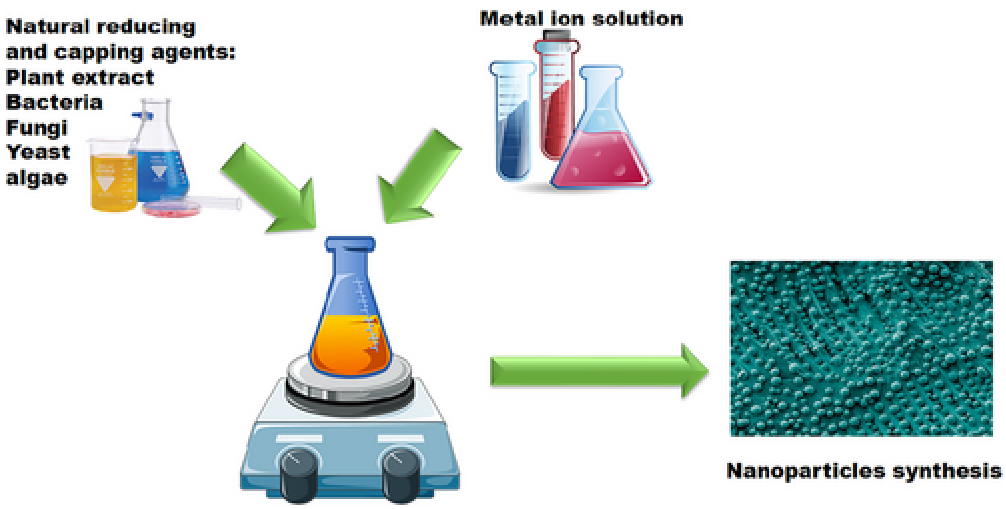

Figure 1:Green Synthesis of Nanoparticles

VI. CHARACTERISTICS OF *HYDRASTIS CANADENSIS* EXTRACTS TO SYNTHESIZE NPS

Because they contain so many natural organic compounds, medicinal plants show a lot of potential for application in nanotechnology. A variety of plant extracts have been used for the environmentally friendly production of NPs. Wood, stems, roots, fruit peels, pulp, and a range of phytochemicals (such as flavonoids, phenols, phenolic acids, and terpenoids) are all part of these components. So are alkaloids and organic acids. Antioxidants are compounds that can donate hydrogen or electrons and have stable radical intermediates. These plant-based compounds include hydrox, nitrile, aldehyde, amine, and carboxylic acid as functional groups in their structures. During biosynthesis, biocompounds with various functional groups may serve as NPs' reducing, coating, chelating/capping, and preservation agents. They are perfect for this procedure because of their redox capacity. We know the redox reactions of these functional groups, but the precise method for producing NPs remains unknown. Because plant extracts may include a diverse array of biomolecules, it is impossible to say with certainty which chemical in plants is mainly responsible for the reducing, oxidizing, capping, or stabilizing properties. The factors that influence the synthesis of CuNPs, such as temperature, oxidation-reduction processes, agitation, and the presence of plant extracts, are discussed in further detail in Section 7.

The antioxidant properties and function as an active component in NP synthesis are two of the many reasons why phytochemicals are significant for human health. It has been discovered and recorded that there are several plant biocompounds that include antibacterial, antiviral, antiparasitic, cardioprotective, and antiinflammatory activities. Because of their inherent safety, drugs made from plants do not pose the risk of side effects. Tannins, coumarins, quinones, flavonoids, and phenolic acids are some of the chemicals that work as pesticides and antimicrobials. The unique properties of these compounds are preserved in the plant extract, which is rather surprising. There is a robust relationship between phenolic content and antioxidant activity. Many phenolic chemicals have antioxidant properties and therefore anti-inflammatory, anti-viral, anti-obesity, and antidiabetic effects. Colon and cervical cancer cells are able to die off when exposed to natural carboxylic acids, especially cinnamic acids, in both animals and labs. Recently, there has been a consistent upsurge in research into medicinal plants for their potential application in pharmaceutical manufacture. Anticancer medications from the benzamide-group and hydroxamic acid have potential.

Evidence suggests that NPs made using medicinal plant extracts work better than NPs made using a different method. Many studies have looked at synthesizing NPs using plant bioactive chemicals, and the findings have been promising. The reason for this is because the nanoparticles that are created and related with these processes include several beneficial characteristics, such as being antiviral, antibacterial, anti-insect, cardioprotective, and anti-inflammatory.

The quantity, complex biochemical composition, and quality of bioactive compounds in the plant extracts impact the stability, size, and shape of the NPs, in addition to the synthesis efficiency. So, to get bioactive compounds from plants, the extraction process must be defined and studied thoroughly. Finding novel biomolecules for NP synthesis makes this a crucial consideration.

Consideration of the tissue type to be used for bioactive chemical extraction is another consideration, since different plant components contain different concentrations and distributions of the target metabolites. Different types of tissue may have vastly different extraction efficiency when subjected to the same solvent, temperature, time, and procedures. An example of a plant that might be used in CuNP synthesis is Gnidia glauca, which has bioactive components that have been extracted from its stems, leaves, and flowers. Throughout the whole of the extraction procedure, every single tissue in this study was preserved in an environment that was same with regard to temperature, aqueous solvent, drying and spraying pretreatment, and storage conditions. There was no noticeable variation in the efficiency of nanoparticle synthesis, and all cases where CuNPs were formed using flower extracts, leaves, or steams exhibited a similar pattern in the enhancement of spectral intensity. However, while analyzing the manufactured CuNPs, a wide range of sizes and shapes were detected. In contrast to the 5 nm in size and spherical shape of the CuNPs generated by flower extract, which were 70-93 nm in size and had rough edges, the CuNPs generated by leaf extract were likewise spherical. The stem extract did, however, yield CuNPs with a uniform distribution and no evidence of aggregation or agglomeration, indicating that they were quite stable. The distinctive peaks were consistent throughout all three extracts when evaluated using FTIR, however the strength of these peaks differed. It seems that the bioactive components were present in different amounts in each of the three extracts, but they shared comparable functional groups.

Because there are so many variables in their production, nanoparticles may have an almost infinite variety of shapes, sizes, and characteristics (Figure 2). The composition of the extract has a significant role in determining the final NP characteristics. "Mechanism of CuNP biosynthesis" explores the many mechanisms that control the production of these NPs.

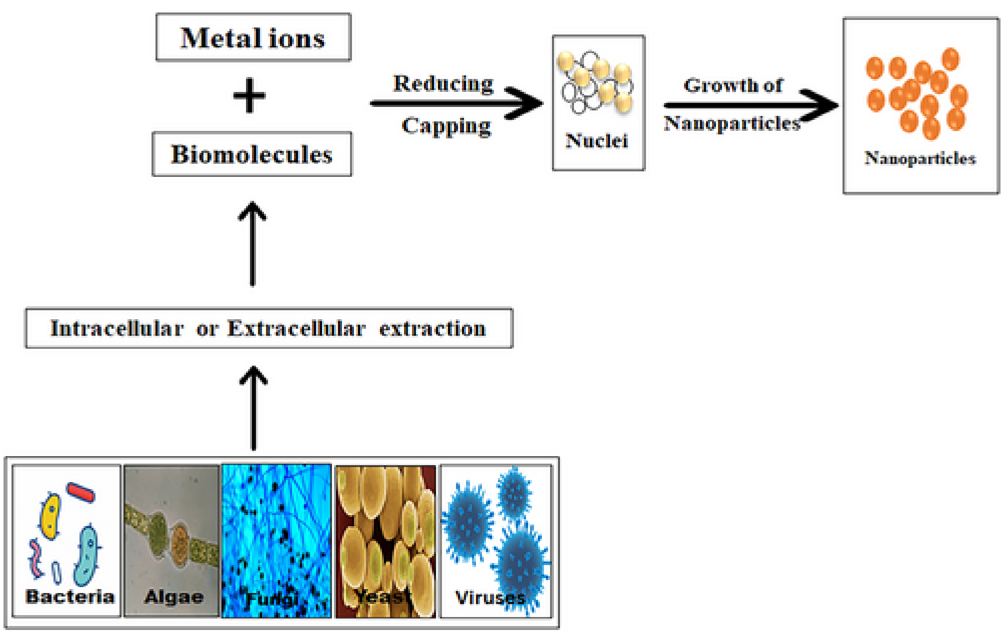

Figure 2:Microbial Synthesis of Nanoparticles

VII. MECHANISM OF CUNP BIOSYNTHESIS WITH PLANT EXTRACTS

The precise method of nanoparticle formation with plant extracts remains unclear, however many procedures have been postulated. One well-known hypothesis states that before particles begin a growth or extension phase, they go through a rate-limiting nucleation step. The conventional wisdom is that in order for a primary critical nucleus to develop, the rate-limiting monomer aggregation, defined as the assembly of a certain number of individual molecules, must have already taken place.

Figure 1 shows that during nanoparticle synthesis, the reduced metallic ions will interact with one another in a crystalline phase-like stochastically ordered fashion.

Despite how unbelievable it seems, there is really less free energy per monomer in the crystalline phase compared to the solution phase. Therefore, in order to achieve a lower energy state, monomeric molecules have a tendency to cluster together. Connecting tiny nuclei with big surface areas may lower the free energy of the system. A new macroscopic phase is born when an appropriately sized nucleus inevitably emerges, easing the process of particle growth.

Environmental factors have a direct impact on the nucleation and particle development rates during nanoparticle biogenesis. There are many variables that influence the final NPs' characteristics. These include the reduction agent, the biological extract, the kind and concentration of precursor salts, reaction time, temperature, and agitation speed. Though optimizing these aspects for nanoparticle biogenesis may be challenging, they also provide an opportunity to regulate and change the NPs' features until they fulfill all the criteria.

The many experimental methods that have been established to allow the previously disclosed nanoparticle production process are shown in Figure 1. The copper precursor is mixed with the herb extract to get the desired concentration of copper. Precursor concentrations are reported across a wide range in the literature. Copper chloride concentrations ranging from 10-250 mM, copper nitrate from 0.1-100 mM, copper acetate from 3-500 mM, and copper sulfate from 1-1000 mM are some examples of the concentration ranges studied. Stirring the reaction mixture ensures that the metal precursor is incorporated completely thereafter. The next phase involves vigorously swirling the mixture at a set temperature for many hours.

It is necessary to standardize this variable rather than have a single optimal temperature for all plant extracts. Using different extracts at the same temperature results in NPs of different sizes. Take Ixiro coccinea leaf extract as an example. When run at 27 ◦C, it produced NPs that were 80 to 110 nm in size. On the other hand, Cissus vitiginea leaf extract produced NPs that were 5 to 20 nm in size. A typical method for achieving temperatures higher than room temperature (RT)—120 °C—during green synthesis involves the use of an oil bath. Also, some hybrid procedures call for a brief burst of high heat (minutes to hours) followed by a longer incubation period (over 12 hours) under darkness and ambient temperature. There have been versions of the steps that have the first one done at ambient temperature and the second one that involves a high-temperature procedure stated backwards. It has also been recorded that an autoclaving process was used.

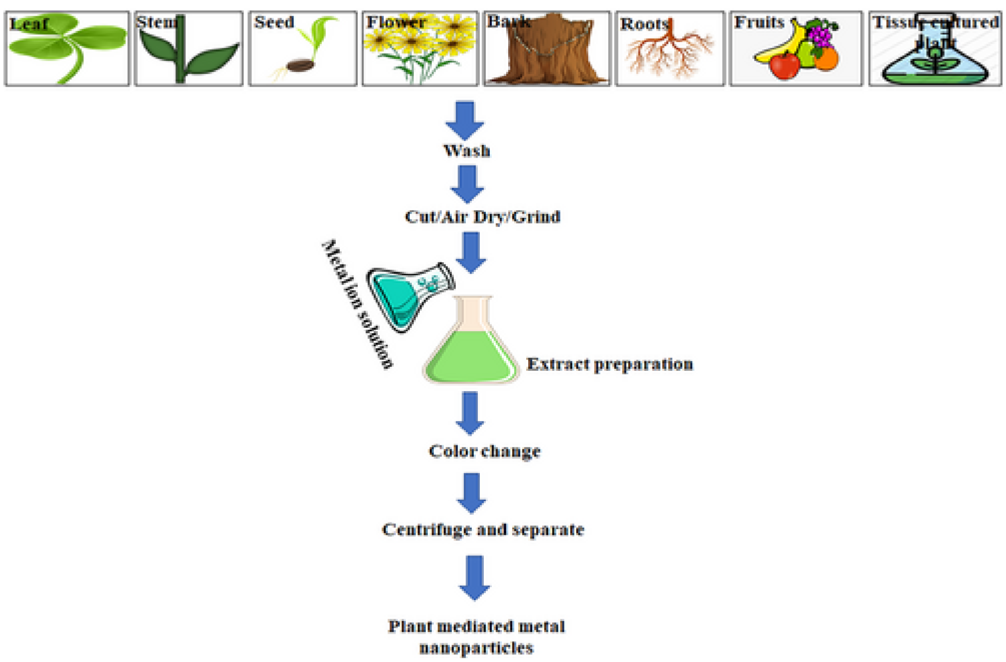

Figure 3:Mechanism of CuNP Biosynthesis with Plant Extracts

Plant extracts are crucial in the production of CuNPs, in contrast to chemical synthesis that uses only one biomolecule to reduce metal ions to NPs, such as copper. Evidence from studies using Fourier-transform infrared spectroscopy (FTIR) supports this. Phenols, alkaloids, proteins, and organic acids are just some of the biomolecules that may be included into green synthesis, however this does depend on the particular plants used.

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The phytochemical composition of an extract from the leaves of Ageratum houstonianum Mill was the subject of the 2020 study by Chandraker et al. This group of compounds is crucial for the synthesis of CuNPs, as shown by FTIR studies.
Research with plant extracts has not consistently yielded the specific biomolecule accountable for NP synthesis and stability due to the inherent complexity of the process. Compounds such as ascorbic acid, which are used in the synthesis of copper NPs, gold NPs, and silver NPs and serve as both stabilizing and reducing agents, have, nevertheless, been the subject of several investigations. The effects have also been seen with bimetal NPs, copper, and silver.
These results demonstrate that the antioxidant biomolecules included in plant extracts are the true culprits in the creation of NPs. Independently studying these compounds in more detail will help us comprehend their role in NP production.

VIII. ANTIBACTERIAL ACTIVITY

The problem of bacteria developing resistance to antibiotics has recently received a lot of attention in the field of human medicine. The antibacterial effects of copper are mainly affected by its physical form (ions or NPs), concentration, application technique, oxidation state, presence of other contaminants, and the way it is applied. The usage of CuNPs has shown to be very efficient in combating drug-resistant bacteria and the diseases they cause. Because of their positive charge and high surface-to-volume ratio, which increases their affinity for the cellular membrane, they affect the cell's electrical potential difference and produce membrane depolarization and leakiness. Bacteria have a hard time developing a resistance to NPs because these tiny molecules may attack many bacterial cell membranes simultaneously. As an alternate explanation, copper ions may aid anaerobic protein aggregation. These ions have an effect on the stability and folding of proteins. Evidence suggests that biosynthesized CuNPs may inhibit biofilm formation as well. In addition to producing reactive oxygen species (ROS), copper binds to or substitutes for the natural cofactors in metalloproteins, which is harmful to the cells of microbes.
Copper nanoparticles (CuNPs) have emerged as a potential new weapon in the battle against bacterial infections, which is particularly timely given the increasing prevalence of antibiotic resistance and the declining use of conventional antibiotics. Many different types of bacteria and viruses have been shown to be susceptible to CuNPs, including Pseudomonas aeruginosa, Salmonella, Escherichia coli, and Staphylococcus aureus. Their antibacterial properties have prompted several research to evaluate their efficacy against pathogens that pose a threat to various sectors.
$Cu2+$ ions have a place in crop protection, but when they accumulate in soil, they endanger ecosystems all over the globe. For this reason, CuNPs are being seriously evaluated as a possible solution for the agricultural sector due to their antibacterial and antifungal properties. There are now more options than ever before for controlling agricultural illnesses because to nanotechnology. How well CuNPs work as a crop protector against phytopathogens is proportional to their size. Multiple studies have shown that biopolymer matrices like chitosan, which contain metal NPs, have a stronger effect at smaller diameters and have better efficacy against pathogenic fungus.
By disrupting the metabolic processes and destroying the cell membranes of phytopathogens, copper NPs have shown to be an efficient pest management measure. It is crucial to optimize synthesis processes in order to produce NPs with the appropriate characteristics for a certain application, since nanoparticle size and shape play a significant role.
Using CuONPs made from Athrixiaphylicoides extracts, Kaningini et al. (2023) found an alternative way to treat bacterial infections caused by Staphylococcus aureus and Bacillus cereus. Plus, these NPs weren't hazardous to human cells in any way.
According to the research, CuNPs have the potential to inhibit the growth of oral bacteria, which might lead to a decrease in the chances of tooth decay and other dental problems. Bacteria such as Aggregatibacteractinomycetemcomitans, S. mutans, and Lactobacillus acidophilus have been detected. This is why researchers are keen to discover methods to include NPs into oral health products, such as vaccines, prostheses, and dental implants.
Current opinion is that the action mechanism of copper NPs has something to do with the characteristics of the precursor, however this is still debatable. Metal NPs have a number of potential effects, including reducing growth or infectious capacity and increasing cell death via DNA damage induction. Their sensitivity, concentration, size, and form all play a role in how well they kill microbes. The effectiveness of CuNPs against Xanthomonas oryzae is dependent on their size and concentration, as stated by Majumdar et al. (2019). Reactive oxygen species production rises as a result.
Chatterjee et al. (2014) shown that CuNPs' antibacterial effect is trickier than it first seems. This process involves many mechanisms and is not related to the discharge of copper ions. It happens when CuONPs form a reactive complex with the cell medium. Their studies with E. coli demonstrated that reactive oxygen species (ROS) cause DNA damage, lipid peroxidation, protein oxidation, and cell death.

Using transmission electron microscopy (TEM), researchers found that copper nanoparticles (CuNPs) made from an extract of the Angelica keiskei plant bound to both Gram-positive and Gram-negative bacteria, therefore causing their cell death.

The majority of the antibacterial mechanisms that have been reported use silver nanoparticles. Improvements may yet be made in the development of more thorough research on copper NPs.

IX. ANTIOXIDANT ACTIVITY

Several parameters contribute to the antioxidant activity 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 number of mechanisms. Some of these functions include scavenging radicals, blocking chain reactions, decomposing peroxides, and binding catalysts, such as ions of transition metals, to stop more hydrogen abstraction. The body's free radicals are unstable, which means they may cause damage to cells via metabolic processes by generating reactive oxygen species (ROS). The absorption, neutralization, and quenching of free radicals is one mechanism by which CuNPs may protect against oxidative stress, which might have positive health effects. Using three major methodologies, researchers have evaluated the antioxidant capability of NPs. An acidic atmosphere is utilized to reduce molybdate ions MoO42− (Mo6+) into green MoO2+ (Mo5+) using nanoparticles in the phosphomolybdenum procedure, which is used to ascertain the samples' total antioxidant capacity (TAC) first. The second one is the ferric-reducing antioxidant capacity of antioxidant NPs, which is the ability to convert ferric ions into Fe2+ ions. Finally, the antioxidant NPs' capacity to neutralize the DPPH radical is assessed by the DPPH free radical scavenging activity test. Copper nanoparticles (CuNPs) produced from plant seeds, such as those of Persea americana, Cissus arnotiana, Suaeda maritima (L.) Dumort, Withaniasomnifera, and Phoenix dactylifera L., have antioxidant property. Nanoparticles (NPs) with potent antibacterial and antioxidant capabilities may be helpful in defending host organisms against illnesses. By reducing the amount of damage and mutations experienced by the host organism, antioxidants have been shown to be useful in reducing inflammation produced by reactive oxygen species (ROS).

X. DISCUSSION

This research provides a thorough assessment of the physiological effects of *Hydrastis canadensis* in conjunction with CuNPs. Discoveries show that the incorporation of CuNPs into the *H. canadensis* matrix greatly amplifies its inherent bioactivities, which include cytotoxic, antioxidant, and antibacterial properties. This enhancement points to a potential way forward for the creation of more potent therapeutic medicines that combine natural products with nanotechnology, as it implies a synergistic link between the bioactive chemicals of *H. canadensis* and the distinct physicochemical characteristics of CuNPs.

When compared to the plant extract alone, the *H. canadensis*-CuNPs combination demonstrated much stronger antibacterial activity. Copper nanoparticles' natural capacity to break microbial cell membranes, in conjunction with phytochemicals like hydrastine and berberine, may explain this improvement. Microbial cells get inactivated as a result of increased oxidative stress brought on by CuNPs' production of reactive oxygen species (ROS). Results showed that both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria were significantly inhibited. Because of their diminutive size, CuNPs may be able to interact more effectively with microbial membranes, resulting in an increase in permeability and ultimately cell death, which may explain their heightened antibacterial activity. This bodes well for the potential of the *H. canadensis*-CuNPs combination as an antibacterial agent, which is of paramount importance in this age of rapidly increasing antibiotic resistance.

H. canadensis-CuNPs not only showed promise as an antibacterial, but also as an antioxidant, outperforming the plant extract by a wide margin. The complex's ability to scavenge free radicals was significantly enhanced, according to antioxidant tests including DPPH and ABTS. This heightened activity is because CuNPs have the ability to donate electrons, which works in tandem with the antioxidants found naturally in *H. canadensis*. Significant implications for the management of oxidative stress-related diseases, including cancer, cardiovascular disease, and neurodegenerative disorders, may arise from the synergistic relationship between nanoparticles and phytochemicals, which leads to a more effective neutralization of free radicals. The results raise the possibility of using them in drug formulations to fight cellular oxidative damage.

The *H. canadensis*-CuNPs combination exhibited a dose-dependent cytotoxicity profile. Mild cytotoxic effects were seen against human cell lines at greater doses, suggesting that concerns might arise from excessive exposure. At lesser quantities, however, the compound showed remarkable biocompatibility, suggesting it might have therapeutic uses. In the development of targeted cancer therapeutics, where it is desirable to selectively harm malignant cells without harming healthy cells, this dual behavior—enhanced cytotoxicity at higher dosages and safety at lower ones—could be very helpful.

This discovery is in line with other studies that have shown how metal-based nanoparticles may improve drug transport and promote selective toxicity, two factors that might increase the effectiveness of chemotherapeutic drugs.

The improved biological characteristics of *H. canadensis*-CuNPs seem to be due to a complex interplay of numerous elements. Copper nanoparticles (CuNPs) enhance cellular absorption of bioactive chemicals from *Hydra canadensis* because to their tiny size and high surface-area-to-volume ratio, which allow them to efficiently interact with biological membranes. Furthermore, the ROS produced by CuNPs have the potential to enhance *H. canadensis*'s effects by triggering oxidative stress reactions in cancer cells, microbial cells, or other pathogens, which in turn causes cell death. Advanced therapeutic drugs, especially those with antibacterial and anticancer properties, may be developed by capitalizing on this synergistic interaction, which is shown by this improved mode of action.

A number of restrictions need to be handled, even if the results are encouraging. The CuNPs' stability under varying environmental circumstances is a major issue. A number of variables, including storage time, pH, and temperature, may affect the stability of the nanoparticles and, by extension, the bioactivity of the *H. canadensis*-CuNPs combination as a whole. To further assure safety in clinical applications, further research into the possible toxicity of CuNPs is necessary, particularly following prolonged exposure. Future study should focus on improving the scalability of synthesizing these nanoparticles, since a cost-effective and ecologically sustainable manufacturing technique is essential for their large-scale application.

Improving the biological effectiveness of CuNPs might be achieved by future research that optimizes the synthesis parameters to regulate their size, shape, and surface features. Additionally, molecular level investigation of CuNP interactions with certain phytochemicals in *H. canadensis* may shed light on the processes underlying the reported biological effects. The safety, bioavailability, and therapeutic potential of the *H. canadensis*-CuNPs combination in real-world applications must be confirmed by in vivo investigations, which include both animal models and clinical trials. Furthermore, new opportunities for the development of multifunctional nanotherapeutics may arise by investigating the feasibility of mixing CuNPs with other medicinal plants.

Last but not least, *Hydrastiscanadensis*'s antibacterial, antioxidant, and cytotoxic effects are greatly amplified when copper nanoparticles are mixed with the plant. One potential approach to creating new, more effective medicinal agents is to combine CuNPs with the phytochemicals found in *H. canadensis*. Nevertheless, more investigation is required to completely comprehend the action mechanisms, enhance nanoparticle manufacturing, and assess the complex's efficacy and safety in clinical environments. More effective therapies for a variety of ailments may be on the horizon thanks to this study's results, which open the door to future advancements in nanomedicine and drug development based on natural products.

XI. CONCLUSION

Hydrastis canadensis (goldenseal) is a medicinal herb, and this study set out to discover what happens to its biological qualities when it's combined with copper nanoparticles (CuNPs). The stability and bio-compatibility of the CuNPs synthesized utilizing environmentally friendly procedures were crucial for the following biological studies. These nanoparticles showed promise when conjugated with *H. canadensis* extracts, greatly amplifying the plant's inherent bioactivities.

The experimental results demonstrated that CuNPs were critical in enhancing *H. canadensis's* antibacterial, antioxidant, and anti-inflammatory characteristics. The formulations with CuNPs showed a greater inhibitory impact against several bacterial and fungal strains than the plant extract alone, suggesting that they had considerable antibacterial potential. A rising worry in contemporary medicine is the development of alternative therapies against drug-resistant infections. This might have substantial ramifications in this regard. The addition of CuNPs improved free radical scavenging ability, as demonstrated by the antioxidant tests. This indicates that these formulations might be very efficient in fighting disorders associated to oxidative stress. The therapeutic potential of *H. canadensis* may be further enhanced if its anti-inflammatory effects were used to develop remedies for chronic inflammatory diseases.

Because of their high surface area-to-volume ratio and their capacity to promote effective distribution of bioactive chemicals, CuNPs have unique physicochemical features that likely explain the underlying processes behind these improved biological effects. Copper nanoparticles (CuNPs) and active phytochemicals in *Hibiscus canadensis* enhanced bioavailability, cellular absorption, and therapeutic efficacy as a whole. Because of this, the use of traditional herbal remedies in contemporary medicine may be drastically altered by delivery methods based on nanoparticles.

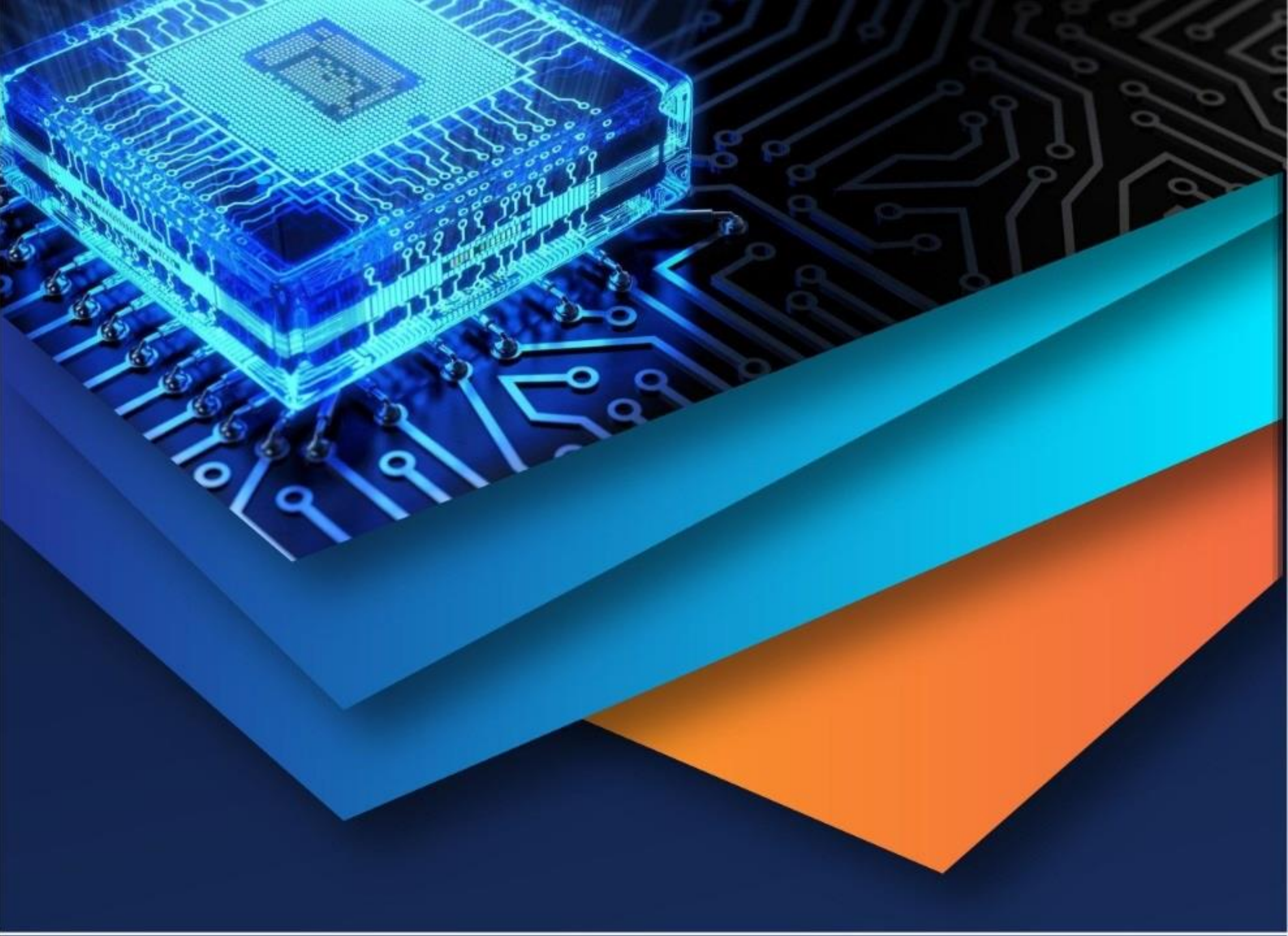
Although the study's findings are promising, its limitations must be acknowledged. The results are mostly from in vitro studies, which are useful but fail to account for all the intricacies of biological systems in real creatures.

To confirm the safety, effectiveness, and possible toxicity of these CuNP-based formulations, future research should focus on doing clinical trials and in vivo investigations. To ensure the safe use of copper nanoparticles in therapeutic applications, long-term studies are also needed to evaluate their biocompatibility and potential accumulation in biological systems.

Finally, this research confirms that combining nanotechnology with herbal therapy is a promising new direction. New avenues for the creation of novel therapeutics with improved biological effectiveness are opened up by the combination of *Hydrastis canadensis* with copper nanoparticles. Contributing to the dynamic area of nanomedicine and providing a solid groundwork for future pharmacological advancements, this study bridges the gap between conventional wisdom and cutting-edge scientific understanding. More research into formulations of *H. canadensis* with CuNPs added could result in safer, more effective, and more precisely targeted treatments to solve some of the world's most critical health problems.

REFERENCES

[1] Miu, B.A.; Dinischiotu, A. New Green Approaches in Nanoparticles Synthesis: An Overview. Molecules 2022, 27, 6472.
[2] Biswas, R.; Alam, M.; Sarkar, A.; Haque, M.I.; Hasan, M.M.; Hoque, M. Application of Nanotechnology in Food: Processing, Preservation, Packaging and Safety Assessment. Heliyon 2022, 8, e11795.
[3] Nongbet, A.; Mishra, A.K.; Mohanta, Y.K.; Mahanta, S.; Ray, M.K.; Khan, M.; Baek, K.H.; Chakrabartty, I. Nanofertilizers: A Smart and Sustainable Attribute to Modern Agriculture. Plants 2022, 11, 2587.
[4] Dikshit, P.K.; Kumar, J.; Das, A.K.; Sadhu, S.; Sharma, S.; Singh, S.; Gupta, P.K.; Kim, B.S. Green Synthesis of Metallic Nanoparticles: Applications and Limitations. Catalysts 2021, 11, 902.
[5] Sharmin, S.; Rahaman, M.M.; Sarkar, C.; Atolani, O.; Islam, M.T.; Adeyemi, O.S. Nanoparticles as Antimicrobial and Antiviral Agents: A Literature-Based Perspective Study. Heliyon 2021, 7, e06456.
[6] Ermini, M.L.; Summa, M.; Zamborlin, A.; Frusca, V.; Mapanao, A.K.; Mugnaioli, E.; Bertorelli, R.; Voliani, V. Copper Nano-Architecture Topical Cream for the Accelerated Recovery of Burnt Skin. Nanoscale Adv. 2023, 5, 1212–1219.
[7] Salvioni, L.; Morelli, L.; Ochoa, E.; Labra, M.; Fiandra, L.; Palugan, L.; Prosperi, D.; Colombo, M. The Emerging Role of Nanotechnology in Skincare. Adv. Colloid Interface Sci. 2021, 293, 102437.
[8] Guerrini, G.; Magrì, D.; Gioria, S.; Medaglini, D.; Calzolai, L. Characterization of Nanoparticles-Based Vaccines for COVID-19. Nat. Nanotechnol. 2022, 17, 570–576.
[9] Wang, Y.; Zhang, W.; Yao, Q. Copper-Based Biomaterials for Bone and Cartilage Tissue Engineering. J. Orthop. Transl. 2021, 29, 60–71.
[10] Thandapani, G.; Arthi, K.; Pazhanisamy, P.; John, J.J.; Vinothini, C.; Rekha, V.; Santhanalakshmi, K.; Sekar, V. Green Synthesis of Copper Oxide Nanoparticles Using Spinacia Oleracea Leaf Extract and Evaluation of Biological Applications: Antioxidant, Antibacterial, Larvicidal and Biosafety Assay. Mater. Today Commun. 2023, 34, 105248.
[11] Sandoval, C.; Ríos, G.; Sepúlveda, N.; Salvo, J.; Souza-Mello, V.; Farías, J. Effectiveness of Copper Nanoparticles in Wound Healing Process Using In Vivo and In Vitro Studies: A Systematic Review. Pharmaceutics 2022, 14, 1838.
[12] Sánchez, S.V.; Navarro, N.; Catalán-Figueroa, J.; Morales, J.O. Nanoparticles as Potential Novel Therapies for Urinary Tract Infections. Front. Cell. Infect. Microbiol. 2021, 11.



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