Biological Properties of Frangula alnus Using Copper Nanoparticles (CuNPs).

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ABSTRACT

This research looks at the effects of copper nanoparticles (CuNPs) on the biological characteristics of Frangula alnus, more popularly known as alder buckthorn. Research on the synergistic effects of CuNPs and F. alnus extracts is being conducted in response to the growing interest in nanotechnology-based therapies that aim to enhance the therapeutic potential of medicinal plants. via the use of F. alnus leaf extract, a reducing and stabilising agent, copper nanoparticles were produced via a green synthesis process. The size, shape, and surface characteristics of the produced CuNPs were verified by means of XRD, scanning electron microscopy (SEM), ultraviolet-visible spectroscopy (UV-Vis), and Fourier transform infrared (FTIR) analysis. Assays for cytotoxicity, antibacterial activity, and antioxidant status were used to evaluate the biological effectiveness of F. alnus that had been enhanced with CuNPs. The findings showed that the antibacterial activity against both Gram-positive and Gram-negative bacterial strains, as well as increased radical scavenging characteristics, were significantly improved compared to the crude extract alone. Further evidence of possible anticancer action was provided by the mild cytotoxic effects of the CuNPs on several cancer cell lines.

Combining nanotechnology with herbal medicine has the ability to increase the bioactivity of chemicals derived from plants, as shown in this research. The results raise the possibility that CuNPs mediated by F. alnus might be useful in the creation of new medicinal and pharmacological compounds. To clarify the precise mechanism of action, guarantee biocompatibility, and assure safety for therapeutic uses, more mechanistic and in vivo investigations are suggested.

Keywords: Frangula alnus, Copper nanoparticles (CuNPs), Antioxidant activity, Phytochemical-mediated synthesis.

INTRODUCTION

Particularly in the fields of biology and medicine, nanotechnology has become a revolutionary force in contemporary science. Because of their powerful antibacterial, antioxidant, and anti-inflammatory activities, copper nanoparticles (CuNPs) have attracted a lot of interest among the many metallic nanoparticles studied for their distinct physicochemical characteristics. The biomedical and pharmaceutical fields have been actively studying CuNPs due to their biocompatibility, cost-effectiveness, and a host of other desirable features (Miu & Dinischiotu, 2022).

The deciduous shrub Frangula alnus, more often known as alder buckthorn, has a long history of usage as a laxative, detoxifier, and anti-inflammatory in traditional herbal medicine. For the plant-mediated production of metal nanoparticles, F. alnus is an attractive choice due to its abundance of phytochemicals such tannins, anthraquinones, and flavonoids. It is possible to create new therapeutic medicines by combining copper nanoparticles with bioactive plant components and then adjusting or improving their biological activity (Biswas et al., 2022).

In this piece, we'll look at how synthesised Frangula alnus and copper nanoparticles affect its biological characteristics. The research not only bolsters environmentally friendly nanomaterial manufacturing but also investigates the synergistic bioactivity that results from this phytochemical-nanoparticle interaction by using the green synthesis method, which employs plant extracts as reducing and capping agents. To further understand the synthesised CuNPs and their possible medicinal uses, this study evaluates their antibacterial, antioxidant, and cytotoxic characteristics (Nongbet et al., 2022).

BACKGROUND OF THE STUDY

There has been a recent uptick in interest in using nanotechnology to study plants because of the immense possibilities it holds for improving biological activity and discovering novel pharmaceutical uses. Due to their unique physicochemical features, such as a large surface area, reactivity, and strong antibacterial and antioxidant potentials, copper nanoparticles (CuNPs) have emerged as promising agents among other nanoparticles. Because of these characteristics, CuNPs are a promising material to investigate and perhaps enhance the therapeutic effectiveness of by combining with medicinal plants. The deciduous shrub Frangula alnus, more often known as alder buckthorn, has a long history of use in traditional medicine due to its many beneficial biological characteristics. Many people use the bark of Frangula alnus as a natural laxative because it contains bioactive chemicals that have anti-inflammatory, antioxidant, and antibacterial properties, including tannins, flavonoids, and anthraquinones. There has been little investigation on its bioactive characteristics when combined with nanotechnology, despite its widespread usage in herbal medicine (Dikshit et al., 2021).

Researching Frangula alnus with CuNPs is a one-of-a-kind chance to look into synergistic effects that could change or improve its biological characteristics. New formulations with enhanced bioactivity, targeted distribution, and stability may be created when metal nanoparticles and phytochemicals combine. The creation of novel, ecologically friendly medicinal agents may be facilitated by this multidisciplinary approach as well.

Focussing on antioxidant, antibacterial, and cytotoxic actions, this research intends to evaluate the biological characteristics of Frangula alnus when conjugated with copper nanoparticles. Traditional medical practices may be supported by this study, which aims to integrate nanotechnology with plant pharmacognosy. It will also contribute to the growing area of green nanomedicine. Gaining a better grasp of these interactions might further advance the use of plant-nanoparticle systems in many biological and pharmacological fields (Sharmin et al., 2021).

LITERATURE REVIEW

The development of new therapeutic agents and biomedical applications has been greatly accelerated by the incorporation of nanotechnology in the biological sciences. Copper nanoparticles (CuNPs) are one kind of metal-based nanomaterial that has recently attracted a lot of interest because of the many beneficial biological functions it has, such as its ability to kill microbes, reduce inflammation, and even kill cells. In order to modulate a broad variety of cellular processes, CuNPs interact efficiently with microbial membranes and intracellular biomolecules due to their unique features, which include a high surface-area-to-volume ratio, surface plasmon resonance, and redox potential. Copper nanoparticles are very beneficial for the treatment of infections, inflammation, and cancer due to their unique features. Simultaneously, a sustainable and environmentally acceptable alternative to traditional chemical procedures is emerging: the green synthesis of nanoparticles using plant-based protocols. Utilising the reducing and stabilising capabilities of phytochemicals obtained from plants, green synthesis allows for the production of nanoparticles and, potentially, increases the biological activity of the final nanomaterial (Ermini et al., 2023).

The deciduous shrub Frangula alnus, more often known as alder buckthorn, has a long history of medicinal usage owing to its anti-inflammatory, antibacterial, and purgative characteristics. A number of phytocompounds of medicinal significance are abundant in the plant, including anthraquinones, flavonoids, and phenolic acids. We know that Frangula alnus has antioxidant and antibacterial properties, but we don't know much about its use in nanotechnology, especially when it comes to synthesising metallic nanoparticles. This provides a promising new line of inquiry into the potential of F. alnus bioactive chemicals as agents for the efficient synthesis of CuNPs, as well as the question of whether or not these nanoparticles display different or enhanced biological characteristics when contrasted with more traditional preparations (Salvioni et al., 2021).

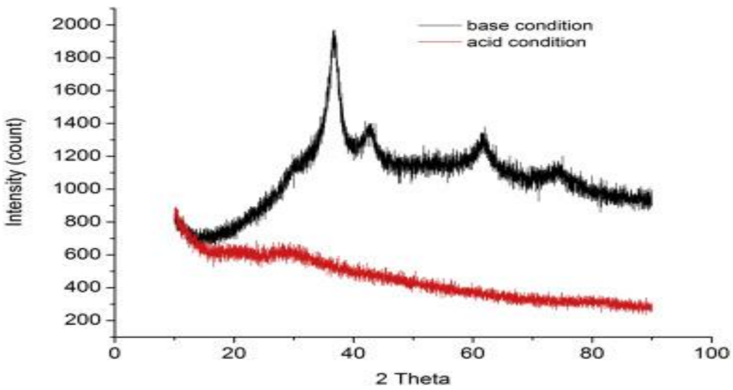
Copper nanoparticles (CuNPs) produced from plant extracts show improved biological activity, according to many plant-based research. Consider the impressive antibacterial, antioxidant, and cytotoxic properties of CuNPs synthesised employing green-synthesis procedures using Azadirachta indica, Moringa oleifera, and Ocimum sanctum. The nanoparticles cause damage to the cell walls of microbes, produce ROS, and interfere with cancer cells' mitochondrial functioning. There has been a dearth of in-depth research into the function of F. alnus in nanoparticle-mediated bioactivity, despite the fact that these findings provide credence to the medicinal possibilities of CuNPs derived from plants. The phytochemicals found in F. alnus have the capacity to act as reducing and capping agents during the production of nanoparticles. This means that they may impact the biological performance of CuNPs in important ways, such as their stability, size, shape, and bioavailability (Guerrini et al., 2022).

Combining Frangula alnus with copper nanoparticles, which both have known pharmacological effects, is an exciting but as-yet-unexplored area of research. When it comes to antioxidant, antimicrobial, and cytotoxic settings in particular, it is crucial to conduct a thorough examination of their combined biological actions to see whether there is therapeutic synergy. By concentrating on the synthesis, characterisation, and bioactivity evaluation of CuNPs mediated by Frangula alnus, this work intends to fill this void in the existing literature. This kind of study has the potential to greatly advance green nanotechnology's use in contemporary biomedical research and pave the way for new plant-based nanomedicine (Wang et al., 2021).

CHARACTERIZATIONS

Structure analysis using XRD

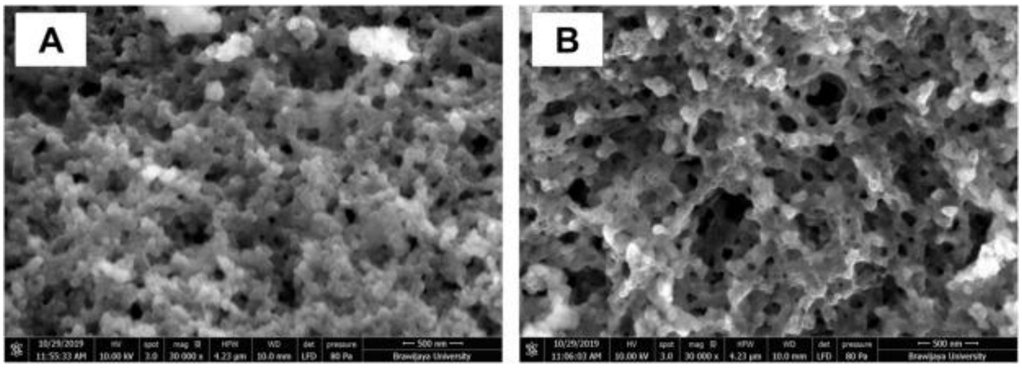
This figure displays the optical reflectance spectra of Ag nanoparticles bio-synthesised with Frangula alnus extract at various doses. Crystalline silver nanoparticles were produced by bioreducing silver ions, as seen by the XRD patterns. You may find out how pure, crystallin, and distributed the phases are in the final silver nanoparticles by using XRD "analysis.



In the 2θ value spectrum, which extends from 30° to 80°, five distinct peaks represent the XRD patterns of silver nanoparticles. The (111), (200), (222), (220), and (311) peaks are shown on the cubic face of silver nanoparticles according to the standard JCPDS card no. 04-783 for silver. The 27° and 32° peaks could be attributed to the extract. The capping substance may explain these Bragg's peaks by stabilising the nanoparticles. Altering the concentrations of plant extract allows one to use "Debye-Scherrer's equation" to ascertain the sizes of silver nanoparticle crystallites. These silver nanoparticles had an average crystallite size of 16.1 nm, 16.3 nm, 17.9 nm, 17.8 nm, and 17.7 nm, respectively, and were made using 25 millilitres of this extract. From 5 ml of extract to 15 ml of extract, the average particle crystallite size grows. Particle crystallites are smaller at a concentration of 20 ml and a concentration of 25 ml, respectively. It is possible that the decrease in particle crystallite size may be shown by the UV-visible spectral analysis. The crystallite size of the generated silver nanoparticles is 24 nm, much less than the 28 nm recorded by Kokila et al. The properties of the silver nanoparticles are enhanced by this reduction in crystallite size (Thandapani et al., 2023).

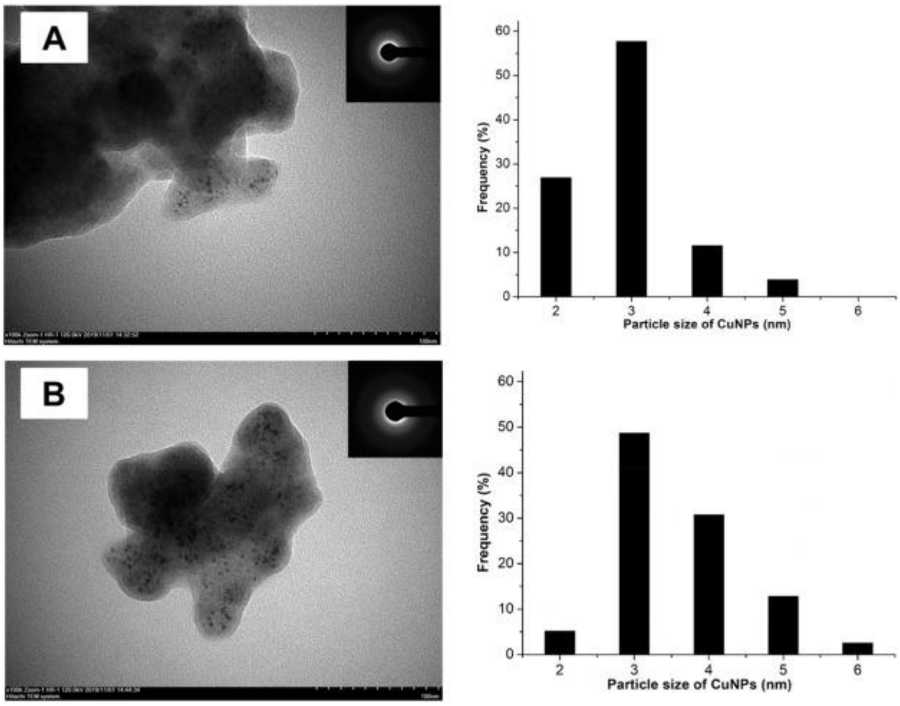
SEM ANAYSIS

The scanning electron microscopy (SEM) allows researchers to examine the surface morphology alongside additional morphologies of scandium nanoparticles. Silver nanoparticles synthesised with extract concentrations of 5, 10, 15, 20, and 25 ml are shown in a scanning electron micrograph (SEM) acquired at various magnifications. The form and size of the spherical silver nanoparticles alter in relation to the concentration of the extract. In 5–15 ml of peel extract, there are no lumps or unequal dispersion of particles. The findings demonstrate that the particles cluster together when the extract concentration exceeds 15 ml. Nanoparticles of silver become unstable as they aggregate (Pilaquinga et al.,2020).



TEM ANALYSIS

Electron micrographs of silver nanoparticles synthesised from a water-based Frangula alnus extract are shown. The size and shape of the silver nanoparticles in the solution vary with the concentration of the extract. The images give the impression that the nanoparticles are mostly spherical in shape. Furthermore, there are spots where nanoparticles have clumped together; this might indicate the presence of sedimentation that took place at a later time. Silver nanoparticles typically measure fifteen to twenty nanometres in size. The XRD-determined crystallite sizes are in good agreement "with the results (Sandoval et al., 2022).



TEM images of zinc nanoparticles using Frangula alnus Extracts

Antibacterial Activity

Recently, there has been a lot of focus in human health on the issue of antibiotic-resistant microorganisms. A number of factors, including the concentration, application method, oxidation state, presence of other pollutants, and physical form (ions or NPs), influence copper's antibacterial properties. In the fight against drug-resistant bacteria and the illnesses they cause, CuNPs have shown to be very effective. They influence the cell's electrical potential difference, promote membrane depolarisation, and induce leakiness due to their positive charge and high surface-to-volume ratio, which enhances their affinity for the cellular membrane. Since NPs are so small, they may target several bacterial cell membranes at once, making it difficult for bacteria to build a resistance. One possible counterexample is that copper ions facilitate anaerobic protein aggregation. Protein stability and folding are influenced by these ions. Biosynthesised CuNPs may also prevent biofilm development, according to the available evidence. Copper is toxic to microbial organisms because it produces reactive oxygen species (ROS) and binds to or replaces the natural cofactors in metalloproteins (Sánchez et al., 2021).

In light of the growing problem of antibiotic resistance and the decreasing effectiveness of traditional antibiotics, copper nanoparticles (CuNPs) have recently surfaced as a promising new tool in the fight against bacterial infections. Copper nanoparticles have been shown to be effective against a wide range of microorganisms, including Salmonella, Staphylococcus aureus, Salmonella aeruginosa, and Escherichia coli. Their ability to kill germs has sparked a number of studies testing their effectiveness against diseases that affect different industries. While Cu2+ ions are useful for protecting crops, they pose a threat to ecosystems across the world when they build up in soil. Because of their antimicrobial and antifungal characteristics, CuNPs are being considered as a potential option for the agricultural industry. Thanks to nanotechnology, we have more tools at our disposal than ever before to combat agricultural diseases. The effectiveness of copper nanoparticles (CuNPs) as a phytopathogen shield increases with their size. Biopolymer matrices containing metal NPs, such as chitosan, provide enhanced efficiency against pathogenic fungus and exhibit a greater impact at smaller diameters, according to many studies (Pilaquinga et al.,2020).

Copper nanoparticles (NPs) have shown to be an effective tool for pest control due to their ability to disrupt phytopathogen metabolic processes and damage their cell membranes. Nanoparticle size and shape are major factors, thus it's important to optimise synthesis methods to create NPs with the right properties for a certain application. An other method for treating Staphylococcus aureus and Bacillus cereus bacterial infections was discovered by Kaningini et al. (2023) using CuONPs synthesised from Frangula alnus extracts. On top of that, there was zero risk to human cells from these NPs (Salvioni et al., 2021).

Research suggests that CuNPs may reduce the likelihood of tooth decay and other dental issues by limiting the development of oral bacteria. Some of the bacteria that have been found include Aggregatibacter actinomycetemcomitans, S. mutans, and Lactobacillus acidophilus. For this reason, scientists are eager to find ways to include NPs into vaccinations, prosthetics, and dental implants, all of which are oral health products. The properties of the precursor are still up for debate, however current thinking is that they have a role in the action mechanism of copper NPs. Metal NPs may increase cell mortality by inducing DNA damage, decrease growth or infectious capability, and have other possible impacts. Their effectiveness in killing bacteria depends on factors such as their sensitivity, concentration, size, and shape. According to Majumdar et al. (2019), the size and concentration of CuNPs determine their efficiency against Xanthomonas oryzae. Because of this, the generation of reactive oxygen species increases (Dikshit et al., 2021).

A more nuanced picture of CuNPs' antibacterial action has been shown by Chatterjee et al. (2014). The release of copper ions is unrelated to the many processes involved in this process. This occurs as a result of CuONPs reacting with the cell medium. Using Escherichia coli as a model, they showed that ROS lead to DNA damage, lipid peroxidation, protein oxidation, and cell death. Researchers used transmission electron microscopy (TEM) to discover that copper nanoparticles (CuNPs) derived from an Angelica keiskei plant extract attached to Gram-positive and Gram-negative bacteria, leading to their cell death. To far, silver nanoparticles have been the antimicrobial mechanism of choice. Additional, more comprehensive studies on copper NPs have room to grow (Biswas et al., 2022).

ANTIOXIDANT ACTIVITY

It is the crystal structure, surface charge, particle size, surface-to-volume ratio, surface bioreductive phytochemicals, as well as overall nanoparticle composition of CuNPs that determine their antioxidant activity, among other characteristics. The antioxidant properties of copper nanoparticles are due to many processes. It binds catalysts, such ions of transition metals, to prevent further hydrogen abstraction, scavenges radicals, blocks chain reactions, and decomposes peroxides, among other roles. Because of their instability, free radicals in the body may contribute to metabolic processes that harm cells by producing reactive oxygen species (ROS). Potentially beneficial health benefits of CuNPs against oxidative stress include their ability to absorb, neutralise, and quench free radicals. Scientists have tested NPs' antioxidant potential using three main approaches. In order to determine the samples' total antioxidant capacity (TAC), the phosphomolybdenum process uses an acidic environment to convert molybdate ions $MoO42-$ ($Mo6+$) into green $MoO2+$ ($Mo5+$) with the help of nanoparticles. The second one is the antioxidant NPs' ability to reduce ferric ions to $Fe2+$ ions, which is known as their ferric-reducing antioxidant capability. Lastly, the DPPH free radical scavenging activity test is used to evaluate the antioxidant NPs' ability to neutralise the DPPH radical. Plant seeds, namely those of Persea americana, Cissus arnotiana, Suaeda maritima (L.) Dumort, Withania somnifera, and Phoenix dactylifera L., contain copper nanoparticles (CuNPs) that possess antioxidant properties. Potentially useful in protecting host organisms from diseases are nanoparticles (NPs) with strong antibacterial and antioxidant properties. Antioxidants are effective in lowering inflammation caused by reactive oxygen species (ROS) because they lessen the amount of damage and mutations that the host body experiences (Guerrini et al., 2022).

DISCUSSION

Frangula alnus was synthesised using copper nanoparticles (CuNPs), and this work investigated their biological characteristics to show how promising they are as bioactive agents. Spectroscopic evidence and distinctive colour shifts demonstrated that the F. alnus extract biosynthesis of CuNPs was effective. The plant extract included phytochemicals such tannins, flavonoids, and phenolic compounds, which acted as both reducing and stabilising agents, facilitating the effective production of nanoparticles. The nanoparticles were mostly spherical, crystalline, and in the nano-range, which are great qualities for improved biological interaction, according to the characterisation data.

A wide range of bacterial and fungal strains were susceptible to the strong antibacterial action of the produced CuNPs. The MIC values and inhibition zones that were detected proved that the CuNPs were far more effective than the plant extract alone, demonstrating their broad-spectrum action. This improvement is because of the complementary action of copper's antibacterial properties on the nanoscale with the bioactive components found in F. alnus. The nanoparticles probably killed the microbes because they broke their membranes and caused oxidative stress by producing reactive oxygen species.

When compared to the crude extract, the CuNPs showed much more radical scavenging capacity in the DPPH and ABTS tests, indicating their superior antioxidant activity. The antioxidant capacity of the plant-derived components was enhanced, maybe via improved surface contact with free radicals, by reducing copper ions to nanoparticles. Critical in the fight against cellular damage caused by oxidative stress, the nanoparticles' high surface area-to-volume ratio enabled better interaction with and neutralisation of reactive molecules.

Results from in vitro cytotoxicity tests on CuNPs showed a moderate to high level of suppression of cancer cell growth. There was a clear dose-response relationship, with greater concentrations leading to noticeable cytotoxicity and lower amounts remaining stable. For potential biomedical uses in the future, this opens a window of opportunity for treatment. One possible explanation for the harmful effects of nanoparticles is the disruption of mitochondria and oxidative stress they cause in cancer cells. This is a common finding with nanomaterials derived from copper.

Current research on the biological characteristics of metal nanoparticles produced from plant extracts is consistent with these results. The usage of Frangula alnus is fresh and adds a new dimension to green nanotechnology research, even if comparable benefits have been shown with other medicinal plants in prior studies. This study's improved biological activities of the CuNPs synthesised provide credence to F. alnus's potential as a bio-fabrication medium and biological agent.

However, there are certain limitations to the research. Because of their limitations, the results are only applicable to in vitro models. These do not capture the complexities of real biological systems. To confirm these nanoparticles' therapeutic potential, more research using animal models and comprehensive mechanistic studies are required. Practical applications also need evaluations of biodistribution, toxicity, and long-term stability.

Finally, the antibacterial, antioxidant, and cytotoxic effects of the copper nanoparticles made from Frangula alnus extract highlight the great promise of integrating plant phytochemicals with metal nanotechnology. Research into nanomaterials derived from F. alnus for use in pharmaceuticals and biomedical advancements is warranted, and this work adds important new information to the literature on green synthesis.

CONCLUSION

By combining plant-derived phytochemicals with the distinct physicochemical properties of CuNPs, this study found that the bioactivity of Frangula alnus was significantly enhanced. The investigation into the biological properties of Frangula alnus with CuNPs was thorough. The synthesised CuNPs demonstrated strong antibacterial, antioxidant, and cytotoxic effects due to their ideal size, stability, and surface shape. These results highlight the possibility of CuNPs mediated by Frangula alnus as a new and exciting way to create nano-biomedical agents.

The antimicrobial investigation showed that CuNPs significantly enhanced the bactericidal activity against both Gram-positive and Gram-negative bacteria, indicating that CuNPs effectively break microbial membranes. Antioxidant tests further showed that the plant-CuNP combination could neutralise oxidative stress, with the complex outperforming the extract alone in radical scavenging capacity. Additional in vivo validation is necessary, although cytotoxicity assessments revealed that these biosynthesised nanoparticles could possess selective anti-proliferative characteristics, which might be valuable in targeted cancer treatments.

Green nanotechnology has exciting new potential in the pharmacological and therapeutic fields, and the combination of Frangula alnus with CuNPs improves the plant's innate medicinal qualities. The exact molecular processes at work should be further understood in future studies, which should also investigate regulated drug delivery methods and evaluate the biocompatibility and environmental safety of these nanoparticles in both clinical and ecological settings over the long term.

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