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LIESTR

Exploring the pharmacological potentials of green synthesized nanoparticles of

Clerodendrum infortunatum Linn.

Hiba Muhammed 1,*, Aiswarya Lakshmi A. G ² and Shamiya Shirin K. B ¹

¹M. Pharm Student, Department of Pharmacology, Kerala University of Health Sciences, College of Pharmaceutical Sciences, Government Medical College, Kannur, Kerala, India. ²Assistant Professor, Department of Pharmacology, Kerala University of Health Sciences, College of Pharmaceutical

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Sciences, Government Medical College, Kannur, Kerala, India.

Abstract

Clerodendrum infortunatum Linn., a widely cultivated shrub known for its extensive medicinal properties, has emerged as a valuable resource for the green synthesis of metallic nanoparticles. This review discusses the synthesis of silver, zinc oxide, and gold nanoparticles using extracts from various parts of the plant. The nanoparticles were comprehensively characterized, revealing unique structural and morphological features. Evaluations of their pharmacological activities demonstrated remarkable antibacterial efficacy for silver nanoparticles, potent anthelminthic activity for both silver and gold nanoparticles, and significant anticancer potential for gold nanoparticles against leukemic cells. Additionally, zinc oxide nanoparticles exhibited strong antioxidant and antifungal properties. These findings highlight the versatility and therapeutic potential of nanoparticles synthesized from *Clerodendrum infortunatum,* emphasizing its importance in green nanotechnology and drug development.

Keywords: *Clerodendrum infortunatum*; Green synthesis approach; Silver nanoparticles; Zinc oxide nanoparticles; Gold nanoparticles

1. Introduction

Clerodendrum infortunatum Linn. commonly known as hill glory bower or bhandirah in sanskrit is a commonly grown shrub in various parts of the world like Asia, Africa, America, Australia etc. This plant belongs to the family Lamiaceae or Verbanaceae and is considered as one of a prodigious treasure of Indian traditional medicine used against fresh wounds, cirrhosis, jaundice, bronchitis, asthma, fever, inflammation, burning sensation, epilepsy, hepatic disorders etc. Additionally, it has free radical scavenging property, anti-hyperglycaemic activity, and anti-microbial activity [1]. The plant also has thirst relieving property and used to treat burning sensation, foul odour, disease of blood etc. Medical physicians of bhadra uses the tender leaf paste of *Clerdodendrum infortunatum* for curing leprosy and cut wounds [2]. The tribal region of Chotanagpur, Jharkhand, India prepares expectorant pills using the leaves of this plant [3].

The shrub has a height of 2-4 feet, the leaves are oblong, elliptic and serrate. The flowers of the plant are bluish purple in colour often in white pyramid shaped terminal panicles. Fruits has four lobes and is succulent with each lobe containing pyrene [4]. The microscopic study of the leaves showed that both the upper and lower epidermis is similar with glandular and covering trichomes. The upper epidermis cells are larger compared with the cells of the lower epidermis. The vascular bundles occupied at the central portion are conjoint, collateral and closed. The sclerenchymatous fibres surrounds the vascular bundles containing calcium oxalate crystals. The lower surface of the leaf consists of anisocytic type of stomata and prisms of calcium oxalate crystals in the mesophyll cells [1].

^{*} Corresponding author: Hiba Muhammed

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The phytochemical analysis has revealed that the plant has the presence of sterols, tannins, flavonoids, saponins, carbohydrates, diterpenes, triterpenes, phenolics, volatile oil, steroids, alkaloids, glycosides making the plant a prominent model with various pharmacological activities. It has been screened that the plant contains sterols, the root has beta sitosterol and steroidal glycosides in the aerial part, the leaves contain diterpene clerodin and the flower is rich for beta sitosterol, lupeol, cleridine, hentriacontane and fumaric acid esters of caffein acid [5]. The flavonoids isolated have been reported to have several properties including anti-inflammatory, oestrogen activity, antimicrobial activity, anti-cancer, antioxidant, cytotoxic and anti-tumour activity [6]. The qualitative assessment of phytochemical parameters of the flower extract of *Clerodendrum infortunatum* reveals the presence of secondary metabolites like alkaloids, flavonoids, saponins, phenols and tannins [7]. The different parts of the plant such as the stem, leaves and flowers possess various secondary metabolites like triterpenes, flavanoids, steroids and is used in conditions like scorpion sting, snake bite, smallpox, skin diseases etc [8,9]. The leaves of the plant *Clerodendrum infortunatum* has the following phytoconstituents such as saponin, phenol, lipid, tannin, clerodin, fixed oils (glycerides of linoleum, oleic, static, lignoceric), alkyl sterols, glycopyranoside, triterpenes, steroids and flavonoids. The root of the plant has Lupeol and beta sitosterol as the phytoconstituents [10,11].

Nanoparticles have been used lately and have seen wide applications in medicine, energy, nutrition etc [12]. Recent years has shown that green synthesised nanoparticles have potential applications in various field like treatment, diagnosis, development of surgical nano devices etc [13]. Nanomedicines have huge applications over healthcare sector and is considered in the forthcoming generations to control various diseases [14]. Nanoparticles are materials with a minimum size of 100 nm and are considered the best for site specific delivery of drugs as they improve drug efficiency, bioavailability, stability, promote targeted drug delivery and prolongs drugs action within the target tissue [15,16,17].

Plant extracts contains many secondary metabolites like phenolics, flavonoids, alkaloids and terpenoids and these primary and secondary metabolites are involved in redox reactions to synthesise environment friendly nano sized particles. The obtained nanoparticles are capped with the similar biological active components and inherits the pharmacological activity of the plant [30].

The nanoparticles biosynthesised through this route has found effective over oxidative stress, apoptosis related changes and genotoxicity, improved water solubility, biocompatibility with less toxicity [18,19]. The medicinal plants with higher number of secondary metabolites like alkaloids, phenolics etc. could indeed hasten the transformation of metal ions into biologically active nanoparticles in an eco-friendlier manner [20]. Several microorganisms like fungi, bacteria, yeasts have the potential to reduce metallic salts into nanoparticles [21-23]. These microorganisms as they produce proteins, enzymes, reducing cofactors, peptides play a significant role in reducing metallic salts to nanoparticles as they in and itself serve as either a reducing agent or capping agent [24].

The various pharmacological applications of metallic nanoparticles are anti-bacterial activity, anti-fungicidal activity, anti-plasmodial activity, anti-inflammatory activity, anti-cancer activity, anti-diabetic activity, anti-viral activity, antioxidant activity, targeted drug delivery, anti-mycotic activity etc [25-31].

1.1 Description of the plant

Table 1 Description of *Clerodendrum infortunatum* Linn.

1.2 Plant Profile

Table 2 Plant profile of *Clerodendrum infortunatum*

 Figure 1 *Clerodendrum infortunatum* Linn.

2. **Different types of metallic nanoparticles studied using** *Clerodendrum infortunatum*

2.1 Silver nanoparticles

Silver is considered as a dynamic metal due to its ability to exert excellent biological activity such as anti-fungal activity, anti-bacterial activity, anti-viral activity, wound healing activity, anti-inflammatory activity etc. [33,34]. Silver nanoparticles have been used in garment industry and wastewater treatment [35].

When silver nanoparticles are prepared using green synthesis route, the plant extract plays as a reducing and stabilising agent for the formation of silver nanoparticles. And it is known that the presence of poly phenolic, aldehydes or ketones are accountable for the reduction of silver nitrate to silver nanoparticles. Silver nanoparticles possess several pharmacological applications such as anti-cancer, anti-angiogenic, anti-microbial, neurodegenerative, anti-diabetic, anti-inflammatory, wound healing and anti-coagulant properties. Along with this the silver nanoparticles have various diagnostic applications like bio-sensing and bio-imaging properties as well [36].

Figure 2 Applications of silver nanoparticles

2.2 Zinc oxide nanoparticles

Zinc as a metal has numerous biological applications such as enzyme function, wound healing activity, hormone regulation etc. Zinc oxide is a semiconducting metal oxide of the n-type and the nanoparticles synthesised from this holds extensive applications in the fields of biological systems, optics, and electronics. Thereby, zinc oxide nanoparticles (NP) have garnered attention in the last two to three years. Zinc oxide nanoparticles (ZnO NPs) are the most interesting metal oxides because of their low cost of production, safety, and ease of preparation. ZnO NPs have remarkable semiconducting capabilities, including strong catalytic activity, optical activity, UV filtering activity, anti-inflammatory activity, and wound healing activity. It has been widely utilised in cosmetics like sunscreen lotions because of its UV filtering qualities. Its anti-microbial, anti-fungal, anti-cancer, anti-diabetic, and agricultural qualities are just a few of its many biomedical uses. Studies have demonstrated that ZnO NPs exhibit a stronger antibacterial impact than ZnO NPs that are chemically synthesised, with the antibacterial action occurring at very low concentrations of both gram-positive and gram-negative bacteria. Moreover, they have been used in the production of rubber, paint, water purification, protein adsorption, and dentistry applications.

It is considered that the prototypic zinc oxide nanoparticles are being used as delivery agents for vaccines and anticancer drugs [37]. Zinc oxide nanoparticles are used for its anti-microbial activity, as a photo catalyst and an additive to industrial products [38]. The green synthesised nanoparticles of Zinc oxide (ZnO NANPs) were found to be non- toxic, bio safe, biocompatible and had potent inhibition against various microorganisms when compared with the chemically derived nanoparticles [39]. Moreover, the US FDA has classified ZnO as a Generally Recognised as Safe (GRAS) compound [40].

Figure 3 Applications of zinc oxide nanoparticles

2.3 Gold nanoparticles

Gold metal has wide range of applications both in biologics and other fields. In biologics they are used in the treatment of rheumatoid arthritis, cancer etc. Gold is a well-known biocompatible metal, and in the past, people utilised colloidal gold as a drinking solution with healing effects on a variety of illnesses. Gold nanoparticles (Au NPs) are distinct among other nanoparticles due to their distinct surface morphologies, stable behaviour, and regulated geometry. Additionally, a few disorders are detected, diagnosed, and treated with Au NPs [41]. The bactericidal action of AuNPs changes depending on the size and shape of the particle, and they are highly effective against a wide variety of microbes. The distribution of proteins, nucleic acids, gene therapy, in vivo delivery, targeting, etc. is facilitated by AuNPs. Over the past ten years, gold nanoparticles have the distribution of proteins, nucleic acids, gene therapy, in vivo delivery, targeting, etc. is facilitated by AuNPs. Gold nanoparticles have been extensively used for its application as anti-bacterial, antiinflammatory, anti-fungal agent, eye treatments, wound dressing, anti-microbial properties etc. [25].

When the metal is nano sized, the particles easily penetrate the cell wall and the cell membrane of the microbes and used for inhibiting its actions.

Figure 4 Applications of gold nanoparticles

3. Green synthesis of nanoparticles and its characterization findings

The biological method used for producing nanoparticles are also known as green synthesis route for nanoparticles formulation. This is considered as a more economical and environment friendly method for nanoparticles formulation. The various biological sources used for the preparation includes microbes (both extracellular and intracellular), algae and plant sources (extracts of leaves, stem, root, bark etc.) [42]. These biological resources can produce nanoparticles that possess various activities like anti-cancer activity, anti-bacterial activity, antioxidant activity etc.

3.1 Green synthesis of silver nanoparticles from the plant *Clerodendrum infortunatum*

The aqueous extract of the leaf extract of *Clerodendrum infortunatum* was prepared initially and to this 0.0 1M aqueous solution of silver nitrate was added and stirred using a magnetic stirrer upon which there is a transformation of colour from colourless to light yellow and then later to dark brown colour. The colour change confirms the formation of silver nanoparticles. The formed silver nanoparticles were separated from the reaction vessel using centrifugation later the nanoparticles were lyophilized and stored in an airtight container away from moisture [43].

The bio reduction mechanism for the preparation of silver nanoparticles are as follows: the plant extract reacts with silver nitrate leading to the formation of silver nanoparticles. The reaction is as follows: [44]

Silver nitrate + plant broth/plant extract ——————> silver nanoparticles + byproducts

3.2 Green synthesis of zinc oxide nanoparticles from flowers of *Clerodendrum infortunatum*

50 ml extract of *Clerodendrum infortunatum* was heated at 60°C and to this 5g in 50 ml aqueous solution of zinc nitrate was added slowly. The mixture was heated till a deep yellow suspension is formed. After few hours, the paste was transferred into a ceramic crucible and heated in a furnace at 100°C for 2 hours thus forming a light white coloured powder. Later the characterization findings were carried out [45,46].

The bio reduction mechanism for the preparation of zinc oxide nanoparticles are as follows: the flower extract reacts with zinc nitrate hexahydrate leading to the formation of zinc oxide nanoparticles. The reaction is as follows:

Zinc nitrate hexahydrate + plant broth/plant extract ——————> zinc oxide nanoparticles + byproducts

3.3 Green synthesis of gold nanoparticles from leaves of *Clerodendrum infortunatum*

The synthesis of gold nanoparticles was carried out by mixing 9ml of aqueous solution of chloroauric acid (HAuCl4) and 1 ml aqueous solution of the extract of the plant which was added drop wise to the chloroauric acid mixture under vigorous condition at varying temperatures ranging from 26 °C- 80 °C. The colour change is monitored, and the resultant mixture was taken for centrifugation and washed with double distilled water twice. The obtained pellet was resuspended in MilliQ water and taken for characterization [47].

The bio reduction mechanism for the preparation of gold nanoparticles are as follows: the leaf extract reacts with chloroauric acid leading to the formation of gold nanoparticles. The reaction is as follows:

Chloroauric acid + plant broth/plant extract ——————> gold nanoparticles + byproducts

3.4 Characterization of silver nanoparticles

Using UV-Vis spectrophotometric analysis it was found that the dark brown colour indicates the formation of nanoparticles occurred at a wavelength of 400-500 nm due to the stimulation of surface plasmon vibrations which is specific to silver nitrate. Through FTIR analysis it can be concluded that the various functional groups like amide, amine, alcohol, ketones, and carboxylic acids were present because of bio-reduction reaction [48]. X-ray diffraction analysis was carried out to determine the average crystal size with the help of Debye - Scherrer formula and the average crystal size of the nanoparticles was found to be 25-35 nm [49,50]. The SEM analysis concluded that the surface morphology of the nanoparticles was granular and spherical shaped with diameters ranging between 20-50 nm. [49].

3.5 Characterization of zinc oxide nanoparticles

The UV-Vis spectrophotometric analysis showed the maximum absorption peak of the zinc oxide nanoparticles between 300-370 nm [51]. The XRD analysis of the ZnO nanoparticles using Debye Scherer's formula was found to be in the range of 25-32 nm and the crystal structure of the ZnO nanoparticles was found to be spherical shaped or hexagonal wurtzite in shape. The SEM analysis showed that the synthesised nanoparticles were homogenous in nature and equally distributed over the surface with a size range between 25-55 nm [52].

3.6 Characterization of gold nanoparticles

The UV Visible absorption spectra appeared to have a surface plasmon resonance peak at 500-560 nm and hence validate the formation of the gold nano suspension. The XRD analysis of the gold nanoparticles using Debye Scherer's formula was found to be in the range of 15-25 nm and the crystal structure of the ZnO nanoparticles was found to be spherical shaped. The TEM analysis confirmed the morphology of the nanoparticles as mono dispersed spherical shaped particles, and the average size of the synthesised nanoparticle was found to be between 20-50 nm which is an optimum size range for effective and enhanced permeation and retention [53].

Table 3 Characterization findings and pharmacological applications of green synthesised nanoparticles

Table 4 Different parts of *Clerodendrum infortunatum* used for the preparation of nanoparticles

4. Pharmacological applications

4.1 Antibacterial activity

According to Kamrun Nahar *et al.* (2020), silver nanoparticles have wide applications in pharmaceuticals, food storage, and waste treatment due to their antibacterial properties. These properties were evaluated against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Bacillus subtilis*. The green-synthesized silver nanoparticles exhibited inhibition zones with diameters ranging from 10-17 mm, while the leaf extract alone showed inhibition zones of 6-9 mm. In contrast, silver nitrate alone showed no visible inhibition zone. This led to the conclusion that the prepared silver nanoparticles had significant antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus*, with the least activity observed against *Bacillus subtilis* [43].

Sahil Kumar et al. (2024) performed the phyto-mediated synthesis of zinc oxide nanoparticles from *Clerodendrum infortunatum* L. leaf extract and evaluated their antibacterial potential against *E. coli, S. aureus,* and *K. pneumoniae* using the agar well diffusion method. The best inhibition was observed against *E. coli* at a minimum inhibitory concentration (MIC) of 0.156 mg/ml, suggesting potential for biomedical and therapeutic applications [54,55].

Zinc oxide nanoparticles exhibit antibacterial activity through the formation of reactive oxygen species, which disrupt the bacterial cell structure. Additionally, the release of zinc ions can damage the metabolic pathways of the bacteria, further contributing to their antibacterial efficacy.

Bharathkumar Nagaraj et al. (2022) studied the antimicrobial activity of gold nanoparticles phytofabricated using clerodin-enriched *Clerodendrum infortunatum* ethanolic leaf extract. The antimicrobial properties were assessed against pathogens such as *S. aureus, B. cereus, K. pneumoniae, E. coli*, and *P. aeruginosa* using the Resazurin assay. The green-synthesized gold nanoparticles demonstrated increasing inhibitory activity in the range of 28%-98%, whereas pure clerodin from the plant extract showed an inhibitory activity range of 30%-100%. This indicated that the gold nanoparticles could inhibit microorganisms at much lower concentrations compared with the pure clerodin from the plant extract [56].

The antibacterial effects of gold nanoparticles occur in two main steps: reduction of adenosine triphosphate (ATP), leading to changes in membrane potential, and disruption of the ribosome subunit responsible for tRNA binding, thereby disrupting biological mechanisms. The nanoparticles disrupt bacterial proteins found within the cell wall and cytoplasm, which are essential for normal bacterial functioning. Gold nanoparticles have an affinity for the thiol groups of enzymes such as nicotinamide adenine dinucleotide (NADH) dehydrogenases, disrupting respiratory chains and causing the release of reactive oxygen species. This release produces oxidative stress, that results in significant disruption and damage to the bacterial cell structure [57,58].

4.2 Anthelminthic activity

Rima Majumdar et al. (2023) conducted a study on the biosynthesis, characterization, and anthelminthic activity of silver nanoparticles derived from *Clerodendrum infortunatum* isolate. They tested its efficacy against *Raillietina* species, a significant threat to the livestock industry. Upon exposure to the test medium containing green-synthesized silver nanoparticles, the parasites' movements transitioned from vigorous to a relaxed state, ultimately resulting in death. At a concentration of 125 micrograms/ml, the maximum time for paralysis was 1.07 hours, and death occurred within 0.43 hours [59].

The silver nanoparticles demonstrated excellent anthelminthic activity through transcuticular diffusion, which is a predominant route for the uptake of broad-spectrum antibiotics in nematodes [60].

4.3 Anticancer activity

Ranjan K. Sahoo et al. (2019) characterized and evaluated the anticancer activity of nano silver particles supported by *Clerodendrum viscosum* vent leaf extract. The silver nanoparticles were tested against human cervical cancer (HeLa) cell lines, showing higher anticancer activity with an IC50 value of $8.41 \pm 1.54 \,\mu g/ml$. Additionally, the green-synthesized silver nanoparticles ascertained to be nontoxic to normal cell lines in a concentration-dependent manner [61]. Although the exact mechanism of action of silver nanoparticles on cancerous cells is not well established, it is believed that increased cellular internalization, interaction with macromolecules like DNA and proteins, production of ROS, mitochondrial dysfunction, chromosomal aberrations, and lipid peroxidation can ultimately lead to cell death [62].

Bharathkumar Nagaraj investigated the anticancer activity of gold nanoparticles phytofabricated using clerodinenriched *Clerodendrum infortunatum* ethanolic leaf extract. The cytotoxicity was analysed against human monocytic leukemic cells (THP-1) and healthy peripheral blood mononuclear cells using an MTT assay. The results revealed a direct, concentration-dependent reduction in THP-1 cell viability due to clerodin-gold nanoparticles (CGNPs). There was a significant decrease in the survival of THP-1 cells from 79% to 5% as the CGNP concentration increased from 0.0125 to 0.5 mM. Pure clerodin exhibited a similar cytotoxic trend against THP-1 cells, but the concentration of CGNPs required to achieve comparable cell viability reduction was substantially lower, approximately tenfold, than that of the pure compound. These findings designate a strong in vitro cytotoxic effect of CGNPs against human leukemic cells [56].

The gold nanoparticles showed increased production of reactive oxygen species (ROS), inducing oxidative stress, protein and lipid oxidation, and mitochondrial dysfunction, eventually leading to cell death [63].

4.4 Antiplasmodial activity

Ranjan K. Sahoo et al. (2019) characterized and evaluated the antiplasmodial activity of nanosilver particles supported by *Clerodendrum viscosum* Vent leaf extract. The antiplasmodial activity of the green-synthesized nanoparticles was assessed in vitro using the schizont maturation inhibition assay method. The results revealed that the green-synthesized silver nanoparticles exhibited higher antiplasmodial activity against the 3D7 strain of *P. falciparum*, with an IC50 value of $2.30 \pm 1.8 \,\mu$ g/ml, compared to the leaf extract alone and pure silver nanoparticles [62].

The mechanism of action of silver nanoparticles as an antiplasmodial agent is likely due to their adhesion to plasmodial cells, penetration into parasite-infected red blood cells, and the release of silver ions. This release can lead to the production of reactive oxygen species (ROS), ultimately contributing to their antiplasmodial effects [64].

4.5 Antioxidant activity

Shakeel Ahmad Khan et al. (2018) conducted the green synthesis of ZnO and Cu-doped ZnO nanoparticles using leaf extracts from various plants, including *Clerodendrum infortunatum*, and investigated their antioxidant potential through two assays: DPPH free radical scavenging assay and Ferric Thiocyanate (FTC) assay. The DPPH assay results indicated that the lowest IC50 value of 41 μ g/ml was obtained at a concentration of 1000 μ g/ml, compared to the lowest concentration of 60 μg/ml, which showed an IC50 value of 85 μg/ml for the green-synthesized zinc oxide nanoparticles.

A lower IC50 value implies higher scavenging activity, indicating that the green-synthesized zinc oxide nanoparticles exhibited significant DPPH free radical scavenging activity. The antioxidant activity was also determined using the linoleic acid oxidation method. The maximum percentage inhibition of linoleic acid oxidation was $63 \pm 0.21\%$ at the highest concentration of 1000 μg/ml, while the minimum percentage inhibition recorded was 18 ± 0.23% at the lowest concentration of 60 μg/ml. This further demonstrated the potential of the green-synthesized zinc oxide nanoparticles in inhibiting linoleic acid oxidation [65].

In a healthy organism, the production of reactive oxygen species (ROS) such as NO, OH, and superoxide ions is balanced by the body's antioxidant defence mechanisms. Oxidative stress occurs when there is an imbalance between the generation of free radicals and the levels of antioxidants in the body [66].

4.6 Antifungal activity

Shakeel Ahmad Khan et al. (2018) conducted the green synthesis of ZnO and Cu-doped ZnO nanoparticles using leaf extracts from various plants, including *Clerodendrum infortunatum*. They investigated the antifungal activity of these nanoparticles against different fungal strains, such as *Aspergillus niger, Aspergillus flavus, and Trichoderma harzianum*. The green-synthesized zinc oxide nanoparticles showed effective antifungal activity against *Trichoderma harzianum*, with a zone of inhibition measuring 24 ± 0.05 mm and a minimum inhibitory concentration (MIC) of 0.08 mg/mL [65].

5. Conclusion

In this comprehensive exploration of *Clerodendrum infortunatum* Linn. as a source for green-synthesized metallic nanoparticles, we have uncovered a plethora of promising findings that underscore its potential significance in biomedical and pharmaceutical research. Through meticulous experimentation and characterization, it was demonstrated the successful synthesis of silver, zinc oxide, and gold nanoparticles using extracts derived from different parts of the plant.

The characterized nanoparticles exhibited distinct structural and morphological properties, as well as remarkable pharmacological activities. Silver nanoparticles displayed potent antibacterial effects, which could offer novel therapeutic strategies for combating bacterial infections. Furthermore, both silver and gold nanoparticles exhibited significant anthelminthic activity, suggesting their potential utility in addressing parasitic infections, which remain a significant public health concern globally.

The anticancer potential of gold nanoparticles against leukemic cells underscores their promise as future chemotherapeutic agents. Their ability to induce cytotoxicity in cancer cells while exhibiting minimal toxicity to healthy cells highlights their potential for targeted cancer therapy. Additionally, zinc oxide nanoparticles demonstrated potent antioxidant and antifungal properties, suggesting their potential utility in combating oxidative stress-related disorders and fungal infections, respectively.

These findings collectively highlight the multifaceted therapeutic potential of nanoparticles derived from *Clerodendrum infortunatum*. Moreover, the green synthesis approach offers numerous advantages, including sustainability, costeffectiveness, and environmental friendliness, positioning it as a viable alternative to conventional nanoparticle synthesis methods.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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