RESEARCH PAPER

Plant-Mediated Green Synthesis of Silver Nanoparticles Using Clitoria ternatea L. and Their Broad-Spectrum Bioactivities

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ABSTRACT

Green nanotechnology, which emphasises environmentally benign processes and improved biocompatibility, has emerged as a viable substitute for traditional physicochemical nanoparticle manufacturing. Clitoria ternatea L. is a medicinal plant that has drawn a lot of attention because of its rich phytochemical profile, which includes terpenoids, anthocyanins, phenolics, and flavonoids that help reduce and stabilise silver nanoparticles (AgNPs). The current research highlights the physicochemical properties, molecular routes, and multifunctional bioactivities of C. ternatea extracts used in the phytochemical-assisted green production of silver nanoparticles. Plant-derived metabolites produce stable, evenly dispersed nanoparticles with regulated size and shape by acting as natural reducing and capping agents. Surface functionalisation and nanoparticle production are confirmed by characterisation methods such UV-Vis spectroscopy, FTIR, XRD, SEM, and TEM. Biologically synthesized CT-AgNPs demonstrate broad-spectrum antimicrobial, antioxidant, anti-inflammatory, anticancer, antidiabetic, and wound-healing activities through mechanisms involving reactive oxygen species modulation, membrane disruption, and apoptotic signaling pathways. Despite promising therapeutic potential, safety concerns including dose-dependent cytotoxicity, oxidative stress induction, organ bioaccumulation, and environmental toxicity require systematic investigation. Variability in synthesis protocols and lack of standardized dosing further limit translational applications. Overall, C. ternatea-mediated AgNPs represent a promising nanobiotechnological platform with significant biomedical and environmental relevance. To close the gap between lab results and practical applications, however, thorough toxicological investigation, mechanistic validation, and clinical evaluation are crucial.

Keywords: C. ternatea, Nanotechnology, Silver, Nootropic, toxicity

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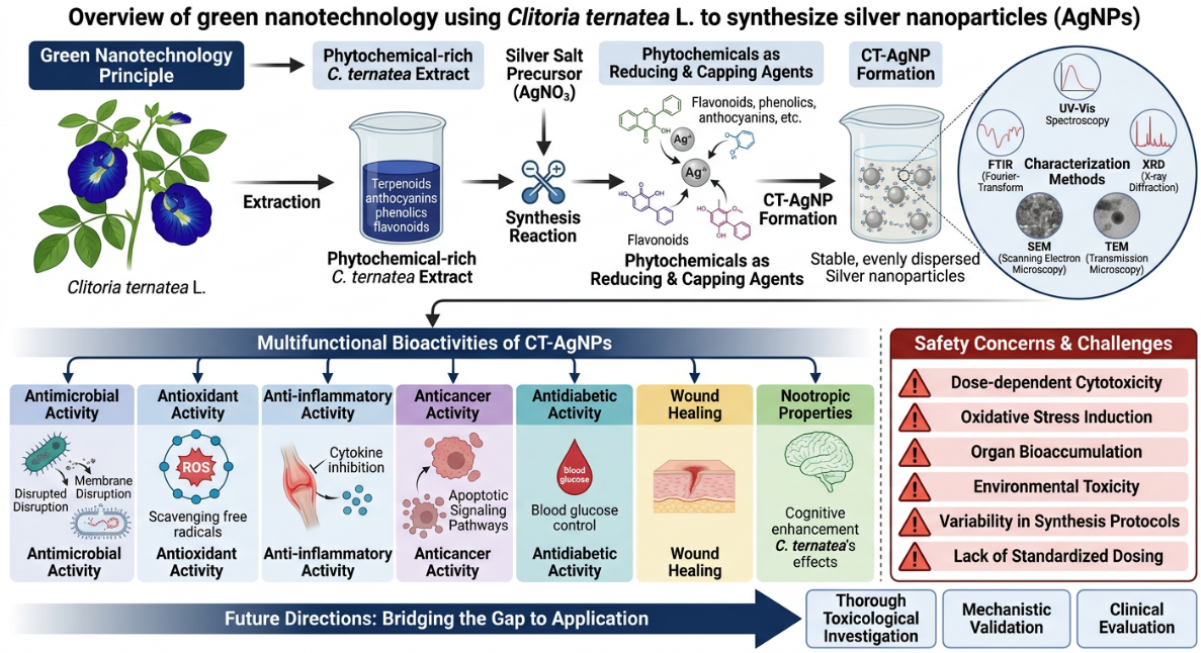

INTRODUCTION

Nanobiotechnology represents a rapidly expanding interdisciplinary domain integrating principles of chemistry, physics, materials science, engineering, and life sciences to develop functional nanomaterials for diverse technological and biomedical applications [1]. Nanoparticles (NPs), typically defined as materials with dimensions below 100 nm, possess a high surface-area-to-volume ratio and unique physicochemical properties that distinguish them from their bulk counterparts [2]. These attributes confer enhanced reactivity, optical behavior, catalytic efficiency, and biological interactions, enabling their application in drug delivery, diagnostics, antimicrobial formulations, biosensing, catalysis, nutraceuticals, and environmental remediation [3]. Among various nanomaterials, silver nanoparticles (AgNPs) have garnered particular attention due to their broad-spectrum antimicrobial activity, antioxidant potential, anticancer efficacy, and catalytic performance [4].

Conventional physical and chemical methods for nanoparticle synthesis, although effective, are often associated with high energy requirements, toxic reagents, hazardous byproducts, and limited environmental sustainability [5]. These drawbacks have driven increasing interest in green nanotechnology approaches that emphasize eco-friendly, cost-effective, and scalable production strategies [6]. Plant-mediated synthesis has emerged as a promising alternative, utilizing phytochemicals as natural reducing and stabilizing agents [7]. Secondary metabolites such as flavonoids, phenolics, tannins, alkaloids, terpenoids, and anthocyanins play a crucial role in the bioreduction of metal ions and subsequent stabilization of the formed nanoparticles[8,9]. The presence of functional groups including hydroxyl, carbonyl, and carboxyl moieties facilitates electron transfer, enabling efficient conversion of Ag^{+} to Ag^{0} while simultaneously preventing agglomeration [10].

C. ternatea (butterfly pea), a perennial climber belonging to the Fabaceae family, is widely distributed across tropical Asia, Africa, Australia, and the Americas [11]. Traditionally valued in Ayurvedic and ethnomedicinal systems, it is recognized for its cognitive-enhancing, anti-inflammatory, antidiabetic, antipyretic, and analgesic properties [12]. The plant's distinctive blue hue and strong antioxidant activity are caused by its abundance of anthocyanins, particularly ternatins. The plant is a good option for nanoparticle manufacturing since it includes anthocyanins as well as flavonoids, phenolic acids, triterpenoids, and alkaloids. Various parts of *C. ternatea*, including flowers, leaves, stems, and roots, have been explored as bioreductors for metallic nanoparticle fabrication [13].

Recent investigations have demonstrated the successful synthesis of AgNPs and gold nanoparticles using *C. ternatea* extracts [14]. However, certain studies reported limitations like large particle size distribution and low zeta potential values, which may predispose nanoparticles to agglomeration and reduced stability.

For stable, evenly distributed nanoparticles with improved biological performance, reaction parameters such as extract concentration, metal precursor concentration, pH, temperature, and reaction duration must be optimised.

Beyond antimicrobial and antioxidant activities, the catalytic degradation of hazardous dyes and environmental pollutants using plant-mediated AgNPs represents an emerging area of interest [15]. In this context, *C. ternatea*-derived AgNPs offer dual advantages: intrinsic phytochemical bioactivity and nanoscale physicochemical functionality [16].

Therefore, the present review comprehensively discusses the phytochemical-assisted green synthesis of AgNPs utilising *C. ternatea*, highlighting mechanistic insights, optimization strategies, physicochemical characterization, and their broad-spectrum bioactivities including antimicrobial, antioxidant, anticancer, and catalytic applications. This synthesis approach exemplifies a sustainable, rapid, and environmentally benign platform for developing multifunctional nanomaterials with significant biomedical and environmental relevance.

MORPHOLOGY

The butterfly pea flower (*C. ternatea* L.) is a perennial twining climber characterized by pinnately compound leaves bearing five to nine elliptic to oblong leaflets. The leaflets are smooth, thin, and arranged alternately along a slender rachis. The plant bears single, axillary, papilionaceous blooms that are usually deep blue, though there are also white-flowered variations. The corolla exhibits the characteristic unequal petal arrangement of the Fabaceae family, with a prominent standard petal and a distinct bearded style located beneath the stigma. The fruit is a flat, linear pod measuring approximately 5–7 cm in length, containing 6–10 dark brown to black seeds. Flowering generally occurs during the rainy season, while fruiting is commonly observed in the winter months [17].

According to Suarna and Wijaya [18], BPFs have two different kinds of corollas (the group of petals in the flower) and stamen: a normal corolla has four petals, where two petals at the lateral area called wings, and other two petals at the posterior area called carina with diadelphous stamen. In contrast, a multiple-layer corolla, which comprises five corollas, has ten single stamens and one petal, the largest, in the anterior region. Luengwilai and Havananda [19] stated that the reproduction of BPFs is conducted through fruit seeds, and it quickly shows excellent regrowth after cutting or grazing and produces a high yield of blooming flowers.

PHYTOCHEMICAL CONSTITUENTS

C. ternatea is enriched with diverse bioactive phytoconstituents that contribute to its pharmacological significance. Among the main substances found in the plant are pentacyclic triterpenoids like taraxerol and taraxerone. Its strong antioxidant activity is attributed to the presence of flavonoids, terpenoids, tannins, and steroids, which are revealed by ethanolic and aqueous extracts. Comprehensive phytochemical investigations have further confirmed the occurrence of anthocyanins (ternatins), alkaloids, saponins, carbohydrates, proteins, resins, and starch, particularly in the roots and flowers [20].

Important elements like calcium, magnesium, potassium, zinc, sodium, and iron are particularly abundant in the blooms. In addition to phytosterols, flavonoid glycosides, mucilage, and derivatives of cinnamic acid, seeds also contain important fatty acids such palmitic, stearic, oleic, linoleic, and linolenic acids. The synergistic interaction of these phytochemicals underlies the plant's wide spectrum of biological activities and supports its suitability as a natural reducing and stabilizing agent in green nanoparticle synthesis [12].

Mukherjee [21] and Kazuma [22] reported that the phenolic compounds present in the flowers of *C. ternatea* are predominantly ternatin anthocyanins along with various flavanol glycosides of kaempferol, rutin, quercetin, and myricetin, which are mainly isolated from hydrophilic extracts. On the other hand, a number of fatty acids, such as palmitic acid, stearic acid, petroselinic acid, linoleic acid, arachidic acid, behenic acid, and phytanic acid, are present in lipophilic floral extracts. The lipophilic fraction has also been shown to contain several phytosterols such campesterol, stigmasterol, β-sitosterol, and sitostanol, as well as tocols like α-tocopherol and γ-tocopherol.

THERAPEUTIC AND TRADITIONAL USES

Numerous conditions, including infections, physical discomfort, and urogenital problems, have been reported to benefit from the usage of *C. ternatea*. Infections and bodily aches and pains are also treated using the roots and leaves. Additionally, the roots of *C. ternatea* contain laxative, diuretic, and purgative properties. It also helps in the treatment of different ailments, including dyspepsia, constipation, pain, fever, eye conditions, enlarged abdominal organs, and

skin and throat irritation diseases [21]. It also acts as a mental tonic. It greatly improves children's mental health, physical stamina, and mental capacity. There have also been reports of the emmenagogue qualities of flowers and roots. Rheumatism and ear issues can be treated with the root powder or decoction. Powdered seeds and ginger are used as laxatives, although the action is accompanied by lower abdominal gritting [23]. The seeds are also utilized for swelling joints, colic, dropsy, and the expansion of the abdominal viscera [21]. Seeds are said to have vermifugal, laxative, and somewhat emetic qualities. Examples include the use of seeds as a refrigerant, a diuretic, an antidote for poisons, and an anti-helminthic. Green manure is another use for *C. ternatea*. Because of its beautiful flower colours, it is frequently planted as an ornamental plant [24]. Young shoots, leaves, blooms, and delicate pods are eaten as vegetables in the Philippines. In Malaysia, the blossoms are used to give rice cakes a striking blue colour, and the leaves are used to give meals a green tint. Throughout the year, the climber produces useful green fodder, especially during the dry season, as well as dry feed [23].

MEDICINAL USES

Anti-Cholesterol activity

Using 50 µL of 2.5 µL/mL *C. ternatea* flower crude lyophilised extracts (CLE) and partially purified extract (PPE), respectively, the inhibitory effect on the oxidation of human copper-induced low-density lipoprotein (LDL) cholesterol was investigated. Following a few hours of incubation, PPE exhibited more inhibition than CLE. Both demonstrated the phenolic compound's protection against human LDL cholesterol oxidation [25]. In López Prado et al. [26], the extraction was obtained using distilled water, methanol, and a combination of both (1:1) after 6, 12, and 24 h soaking times. The *C. ternatea* flower extract was employed to prevent cholesterol oxidation in an emulsion model, and the results were obtained after 24 and 48 hours. The combined solvents blocked 89.8% of the formation of 7-ketocholesterol in the emulsion and produced 63.9 µg/mL of anthocyanin in the extract after a 6-hour soaking period. The study indicated that *C. ternatea* flower extracts can increase the health benefits, especially anti-cholesterol and antilipidemic [26].

Anti-Inflammatory activity

C. ternatea flower, root, and leaf extracts have been shown to have anti-inflammatory, analgesic, and antipyretic properties [27]. Using healthy albino rats of either gender and the paw oedema method, a study assessed the anti-inflammatory properties of petroleum ether extract of *C. ternatea* flower. Rats administered 200 and 400 mg/kg of the extract showed a significant reduction in paw oedema when compared to an untreated control group. The study demonstrated the possibility that the extract may have protective benefits against the release of prostaglandins, kinnins, and other chemicals in carrageenan-induced edema [28]. Another study reported that carrageenin-induced rat paw oedema and acetic acid-induced vascular permeability in rats were considerable reduced after oral administration of methanolic root extracts of *C. ternatea* [29]. It was discovered that the extract's antipyretic effectiveness was on par with paracetamol. Leaf extracts from *C. ternatea* have recently been connected to analgesic effects [30]. The well-known rat tail flick pain experiment was used to examine the effects of pre-treatment with both ethanolic and petroleum *C. ternatea* extract. After 1 h treatment, *C. ternatea* leaf extract had a favorable analgesic effect comparable to diclofenac sodium (10 mg/kg) [30].

Nootropic activity

According to certain research, *C. ternatea* has nootropic effects. It is reported when *C. ternatea* extracts were given to experimental animals, it improved their cognitive performance [31]. According to a study, rats given "medhya rasayana," a 1:1 mixture of crushed *C. ternatea* whole plant and jaggery, for 60 days exhibited far less autophagy in their brains. According to this study, *C. ternatea* protects the brain by affecting the autophagy-directed pathway [32]. Another subsequent study examined rats that were orally dosed with 300 mg/kg ethanolic extracts derived from *C. ternatea* roots or aerial tissues, and they were shown to attenuate electric shock-induced amnesia better than the controls [33]. In another study, the memory retention and spatial learning performance of 7-days old newborn rats orally dosed with 100 mg/kg aqueous *C. ternatea* root extract improved 48 h and 30 days after treatment [34].

Antidiabetic activity

Researchers also concentrated on the effects of *C. ternatea* flower extracts on human glycemic response and antioxidant capability [35]. A small clinical research study involving 15 healthy males found that when 1 or 2 g of *C. ternatea* flower extract were combined with 50 g of sugar, plasma glucose and insulin levels were reduced [35]. These investigations generally suggested that the extract's flavonol glycosides, anthocyanins,

and alkaloids may have hypoglycemic effects. These compounds may increase the transport of blood glucose from plasma to peripheral tissues or involve the possibility of insulin secretion from ß-cells. *C. ternatea* leaf extracts have recently shown potential as an anti-diabetic [36]. Wistar rats given 400 mg ethanolic leaf extracts of *C. ternatea* per kg of body weight per day for 28 days demonstrated considerably lower blood glucose, insulin, glycosylated hemoglobin, urea, and creatinine levels than diabetic control [36].

Antioxidant potential

Oxidative stress is known to be one of the main causes of many chronic and degenerative disorders. Numerous research have documented the *C. ternatea* flower's antioxidant properties. Phenolic compounds, flavonoids, and anthocyanins, which were isolated in the water extract of *C. ternatea* flower, were found to effectively prevent the hemolysis and oxidative damage that 2,20-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) induces in canine erythrocytes [37]. A study conducted by Zakaria et al. [38] showed that human HaCaT keratinocytes pre-treated with the polyacylated anthocyanins and flavonol glycosides, two key components of *C. ternatea* flower water extracts, showed a reduction in UV-induced mitochondrial DNA damage. Likewise, it showed antioxidant properties that protect skin cells from oxidative stress imposed over by H2O2 and UV radiation on skin cell [38]. Another study demonstrated that the acute administration of *C. ternatea* flower extract/beverage was observed to boost plasma antioxidant capacity in a randomized crossover study, and the effect was strengthened when ingested with sucrose in healthy men [35].

IMPORTANCE OF PHYTOCHEMICALS IN NANOPARTICLE SYNTHESIS

Diverse phytochemicals found in *C. ternatea* are essential to the environmentally friendly production of silver nanoparticles (AgNPs). During the creation of nanoparticles, major bioactive components such as flavonoids, anthocyanins (ternatins), phenolic acids, tannins, glycosides, alkaloids, and terpenoids work in concert as reducing, stabilising, and capping agents. These secondary metabolites help reduce silver ions (Ag^{+}) into metallic silver (Ag_2) nanoparticles by giving them electrons. Because they include hydroxyl and carbonyl functional groups that facilitate effective redox reactions, flavonoids and phenolic substances are regarded as the primary reducing agents. Beyond reduction, phytochemicals also regulate nucleation, growth kinetics, particle morphology, and surface stabilization, thereby influencing the size distribution and physicochemical properties of the synthesized nanoparticles. Agglomeration is inhibited and colloidal stability is improved by the bioorganic molecules adsorbed onto the surface of the nanoparticles. Moreover, the phytochemical corona surrounding AgNPs contributes to enhanced biological performance through synergistic interactions, leading to improved antimicrobial, antioxidant, anti-inflammatory, and anticancer activities. Owing to its rich phytoconstituent profile and absence of toxic residues, *C. ternatea* serves as an efficient and sustainable bioresource for eco-friendly nanotechnological applications [39,40].

Green synthesis of silver nanoparticles (AgNPs)

Using biological systems including plants, algae, fungi, yeast, and bacteria to produce nanomaterials in an environmentally safe manner is known as green synthesis. This approach aligns with the principles of green chemistry by employing renewable resources, minimizing hazardous waste generation, reducing energy consumption, and utilizing non-toxic solvents such as water or ethanol [5]. Based on their phytochemical makeup, different plant parts such as leaves, flowers, stems, roots, seeds, and rhizomes are used in plant-mediated synthesis. The intrinsic metabolites present in plant extracts function as natural bioreductants and stabilizers, eliminating the need for synthetic chemicals and harsh reaction conditions [4].

Green synthesis of AgNPs using *C. ternatea*

Biosynthesis of AgNPs utilising *C. ternatea* typically involves preparation of an aqueous plant extract followed by its reaction with a silver salt precursor, commonly silver nitrate. A typical procedure involves combining the plant extract in an ideal ratio (e.g., 1:5) with a 3 mM silver nitrate solution. Sodium hydroxide is used to bring the reaction mixture's pH down to an alkaline level (around pH 10), and it is then incubated for 15 minutes at about 50 °C.

The reaction mixture's clear colour shift from blue to brown, which corresponds to the surface plasmon resonance (SPR) of silver nanoparticles, visibly indicates the creation of AgNPs. This rapid, cost-effective, and environmentally sustainable approach demonstrates the dual functionality of *C. ternatea* phytochemicals in mediating reduction and stabilization, thereby producing biofunctionalized

nanoparticles suitable for biomedical and environmental applications [41].

COMPARISON OF *C. TERNATEA* -DERIVED SILVER NANOPARTICLES WITH OTHER PLANT-BASED AGNP

Silver nanoparticles synthesized using *C. ternatea* extract demonstrate notable advantages over many other plant-mediated AgNP systems, primarily due to the plant's rich and diverse phytochemical profile. The abundance of anthocyanins (ternatins), flavonoids, and phenolic compounds provides strong reducing capability and efficient surface stabilization during nanoparticle formation. These bioactive molecules facilitate controlled nucleation and growth, leading to the formation of relatively smaller (approximately 10–30 nm), predominantly spherical, and well-dispersed nanoparticles with significant colloidal stability. Reported zeta potential values ranging from −20 to −30 mV further indicate enhanced electrostatic repulsion and reduced aggregation, while distinct UV–Vis surface plasmon resonance (SPR) peaks observed between 420–450 nm confirm the formation of stable AgNPs [42,43].

In comparison, AgNPs synthesized utilising extracts of Aloe vera, Zingiber officinale, and Curcuma longa have frequently been reported to exhibit relatively larger particle sizes and broader size distributions, which may compromise uniformity and biological performance. While AgNPs derived from Camellia sinensis and Azadirachta indica also display promising antimicrobial and antioxidant properties, *C. ternatea*-mediated AgNPs demonstrate comparable or superior multifunctional activity, particularly in terms of enhanced antibacterial, antioxidant, and anticancer potential [44,45].

The superior performance of *C. ternatea*-derived AgNPs can be attributed to synergistic interactions between nanoparticle surfaces and surface-bound phytochemicals, which amplify bioactivity while maintaining stability. Consequently, *C. ternatea* emerges as a highly promising and efficient botanical source for the sustainable production of homogeneous, stable, and bioactive silver nanoparticles with broad biomedical and environmental applications [46,47].

Table 1: Comparison table of *C. ternatea* Silver Nanoparticles with Other Plant-Based AgNPs

Plant Source	Key Phytochemicals Responsible for Reduction/Capping	Typical AgNP Size (TEM/SEM)	Shape	UV–Vis SPR Peak	Stability (Zeta Potential)	Biological Activities
C. ternatea	Flavonoids, anthocyanins (ternatins), phenolics, alkaloids	10–30 nm	Mostly spherical	420-450nm	−20 to −30 mV	Strong antibacterial, antioxidant, anticancer
Azadirachta indica	Quercetin, nimbin, nimbolide, terpenoids	15–50 nm	Spherical/polydispersed	410–430 nm	−22 to −34 mV	Antibacterial, antifungal
Camellia sinensis	EGCG, catechins, tannins	20–40 nm	Spherical	420-440nm	-33mV	Antioxidant, antimicrobial
Aloe vera	Aloin, tannins, polysaccharides, phenols	30-80nm	Spherical /irregular	430-460nm	-18 to -25mV	Antibacterial,wound healing
Zingiber officinale	Gingerols, shogaols, terpenoids	50-100nm	Spherical	430-450nm	-17mV	Anti-inflammatory, antibacterial

MULTIFUNCTIONAL BIOACTIVITIES OF *C. TERNATEA* -DERIVED SILVER NANOPARTICLES

Silver nanoparticles synthesized using *C. ternatea* extract (CT-AgNPs) exhibit broad-spectrum biological and environmental functionalities, attributable to the synergistic interaction between nanosilver and surface-bound phytochemicals such as anthocyanins and flavonoids.

Antimicrobial and antibacterial activity

Strong antibacterial action is shown by CT-AgNPs against both Gram-positive and Gram-negative organisms, such as Staphylococcus aureus and Escherichia coli. There have been reports of significant zones of inhibition and lower minimum inhibitory concentration (MIC) values against common clinical isolates. Membrane disruption, the production of reactive oxygen species (ROS), denaturation of proteins, and interference with DNA replication are all part of the antibacterial process. Recent advancements incorporate CT-AgNPs into topical gels, nanofibers, and wound dressings to enhance infection control and prevent biofilm formation [48].

Antioxidant activity

DPPH and related antioxidant experiments show that CT-AgNPs maintain strong free radical scavenging activity because of the presence of anthocyanins and polyphenols. The phytochemical coating enhances cytoprotective and anti-inflammatory potential, supporting their application in therapeutic formulations aimed at oxidative stress-related disorders [16].

Wound-healing and tissue-regenerative potential

Formulations such as nanogels, hydrogels, and polymeric beads containing CT-AgNPs have shown promising results in accelerating wound closure *in vitro*. These systems promote fibroblast proliferation, collagen deposition, and reduced microbial load, making them attractive candidates for advanced wound management and regenerative medicine [49].

Anticancer and cytotoxic activity

Recent preclinical studies (2024–2025) indicate that flower-derived CT-AgNPs exhibit dose-dependent cytotoxicity against selected cancer cell lines. The proposed mechanisms include ROS-mediated apoptosis, mitochondrial dysfunction, and cell cycle arrest. Although promising, these findings remain largely confined to *in vitro* investigations, necessitating further mechanistic and *in vivo* validation [50].

Food packaging and intelligent indicators

Intelligent and biodegradable packaging methods can be developed thanks to *C. ternatea* pH-sensitive anthocyanins. When integrated with AgNPs, these materials function as colorimetric freshness indicators and optical sensors, exploiting visible spectral shifts to detect food spoilage or environmental changes [51].

Antidiabetic potential

Enzymes that break down carbohydrates, such α-glucosidase and α-amylase, are inhibited by the anthocyanins and flavonoids included in CT-AgNP formulations. This inhibition delays carbohydrate digestion and glucose absorption, thereby aiding postprandial glycemic control and suggesting potential adjunct applications in diabetes management [52].

Catalytic degradation of dyes

CT-AgNPs function as efficient catalysts in the reduction of toxic dyes, including Congo red, Methyl orange, and Methylene blue. The nanoparticle surface facilitates electron transfer from reducing agents such as $NaBH_4$ to dye molecules, leading to rapid degradation and potential application in wastewater treatment [53].

Larvicidal activity

CT-AgNPs exhibit significant larvicidal efficacy against mosquito vectors such as Aedes aegypti and Culex quinquefasciatus. The nanoparticles penetrate the larval cuticle, induce oxidative stress, disrupt gut epithelial integrity, and ultimately cause mortality, highlighting their relevance in eco-friendly vector control strategies [54].

SAFETY, TOXICITY, AND LIMITATIONS OF *C. TERNATEA* –DERIVED SILVER NANOPARTICLES

Despite their promising multifunctional applications, silver nanoparticles synthesized using *C. ternatea* extract (CT-AgNPs) present several safety considerations and translational limitations that must be carefully evaluated prior to clinical or environmental deployment.

Dose-dependent cytotoxicity

CT-AgNPs generally exhibit low cytotoxicity at minimal concentrations; however, toxicity increases in a dose-dependent manner. Elevated nanoparticle exposure may induce oxidative stress, mitochondrial membrane depolarization, disruption of cellular respiration, and apoptosis or necrosis. The narrow therapeutic window necessitates precise dose optimization for biomedical applications [55].

Oxidative stress generation

Reactive oxygen species (ROS) can be produced in excess when plant-mediated AgNPs accumulate excessively or uncontrollably, despite the fact that they are somewhat more biocompatible than their chemically synthesised counterparts. Increased ROS levels may result in DNA strand breaks, lipid peroxidation, protein denaturation, and activation of inflammatory pathways, thereby compromising cellular integrity [53].

Hemocompatibility concerns

Hemolysis assays have demonstrated that higher concentrations of AgNPs may cause mild to moderate red blood cell membrane disruption. Establishing standardized hemolytic thresholds and conducting comprehensive hemocompatibility assessments are essential before considering intravenous or systemic applications [56].

***In Vivo* organ accumulation**

According to preclinical research, after systemic exposure, silver nanoparticles can build up in important organs such the liver, spleen, kidneys, and lungs. Persistent bioaccumulation raises concerns regarding long-term toxicity, altered organ function, and potential inflammatory responses, especially in chronic exposure scenarios [57].

Lack of standardized dosing and characterization

Currently, no universally accepted safe dosage guidelines exist for CT-AgNPs. Variability in synthesis protocols including differences in particle size, morphology, surface charge, and phytochemical capping can result in inconsistent toxicity profiles. This heterogeneity complicates reproducibility and regulatory standardization [58]

Environmental toxicity

The release of AgNPs into aquatic or terrestrial ecosystems may adversely affect beneficial microorganisms, soil microbiota, and aquatic species. The release of silver ions from nanoparticles can upset ecological balance and microbial enzymatic systems. The long-term environmental fate and ecotoxicological consequences remain insufficiently characterized [59].

Limited clinical evidence

Most available safety evaluations are restricted to *in vitro* cell culture systems or small animal models. To date, no well-designed human clinical trials have been conducted to establish safety, pharmacokinetics, biodistribution, or therapeutic efficacy of *C. ternatea*-derived AgNPs [60].

Stability and aggregation issues

Environmental factors like pH, ionic strength, and temperature affect the stability of nanoparticles. Under unfavorable conditions, AgNPs may aggregate, leading to altered surface properties, reduced bioactivity, unpredictable biodistribution, and potentially increased toxicity [61].

FUTURE PROSPECTIVE

Standardised green synthesis techniques should be the main focus of future studies in order to produce CT-AgNPs with consistent surface chemistry, morphology, and particle size. Advanced mechanistic studies employing omics technologies, molecular docking, and pathway analysis are needed to elucidate precise molecular targets. Long-term *in vivo* toxicity, pharmacokinetics, and biodistribution studies must be prioritized to establish safety margins. Development of targeted drug delivery systems, nano-formulations, and synergistic combinations with conventional therapeutics may enhance clinical applicability. Furthermore, large-scale production strategies and regulatory compliance frameworks should be designed to facilitate industrial translation. Comprehensive ecotoxicological assessments are also required to ensure environmental sustainability.

CONCLUSION

In conclusion, *C. ternatea* -derived silver nanoparticles offer a green, cost-effective, and multifunctional nanoplatform with substantial therapeutic and antimicrobial potential. Phytochemical components contribute to increased bioactivity and biocompatibility by acting as effective reducing and stabilising agents. Nevertheless, challenges related to toxicity profiling, standardization, stability, and regulatory validation remain significant barriers to clinical translation. The safe and long-term integration of CT-AgNPs into pharmaceutical and biomedical applications will be made possible by addressing these constraints through interdisciplinary research.

Declarations

Ethical Approval and Consent to Participate

Not applicable. This study did not involve human participants or animals.

Consent for Publication

Not applicable.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article. Additional data may be obtained from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Authors' Contributions

All authors contributed equally to the conception, design, experimental work, data analysis, and manuscript preparation. All authors have read and approved the final manuscript.

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References

1. Bayda S, Adeel M, Tuccinardi T, et al. The History of Nanoscience and Nanotechnology: From Chemical–Physical Applications to Nanomedicine. *Molecules* 2019; 25: 112.
2. Altammar KA. A review on nanoparticles: characteristics, synthesis, applications, and challenges. *Front Microbiol*; 14. Epub ahead of print 17 April 2023. DOI: 10.3389/fmicb.2023.1155622.
3. Jafarzadeh S, Oladzadabbasabadi N, Dheyab MA, et al. Emerging Trends in Smart and Sustainable Nano-Biosensing: The Role of Green Nanomaterials. *Ind Crops Prod* 2025; 223: 120108.
4. Fahim M, Shahzaib A, Nishat N, et al. Green synthesis of silver nanoparticles: A comprehensive review of methods, influencing factors, and applications. *JCIS Open* 2024; 16: 100125.
5. Raut SS, Sharma A, Yadav R, et al. Biological green synthesis of nanoparticles: A sustainable platform for agricultural, environmental, food, and cosmetic applications. *J Environ Chem Eng* 2026; 14: 121102.
6. Cerbu C, Trif E. Advantages and drawbacks of

eco-friendly nano-delivery systems. *Ther Deliv* 2025; 16: 415–417.

7. Singh H, Desimone MF, Pandya S, et al. Revisiting the Green Synthesis of Nanoparticles: Uncovering Influences of Plant Extracts as Reducing Agents for Enhanced Synthesis Efficiency and Its Biomedical Applications. *Int J Nanomedicine* 2023; Volume 18: 4727–4750.
8. Thatyana M, Dube NP, Kemboi D, et al. Advances in Phytonanotechnology: A Plant-Mediated Green Synthesis of Metal Nanoparticles Using Phyllanthus Plant Extracts and Their Antimicrobial and Anticancer Applications. *Nanomaterials* 2023; 13: 2616.
9. Omkar Tipugade, Jyotiram Sawale NJ. Network Pharmacology and Molecular Docking-Based Exploration of Rubiaceous Plants for Breast Cancer: Phytochemicals, Preclinical Studies, And Regulatory Perspectives. *Asian J Pharm Clin Res* 2025; 52–71.
10. Hanna DH, Nady DS, Wasef MW, et al. Plant-derived nanoparticles: Green synthesis, factors, and bioactivities. *Next Mater* 2025; 9: 101275.
11. Oguis GK, Gilding EK, Jackson MA, et al. Butterfly Pea (Clitoria ternatea), a Cyclotide-Bearing Plant With Applications in Agriculture and Medicine. *Front Plant Sci*; 10. Epub ahead of print 28 May 2019. DOI: 10.3389/fpls.2019.00645.
12. Multisona RR, Shirodkar S, Arnold M, et al. Clitoria ternatea Flower and Its Bioactive Compounds: Potential Use as Microencapsulated Ingredient for Functional Foods. *Appl Sci* 2023; 13: 2134.
13. Du X, Wang Y, Chen H, et al. Botany, phytochemistry, pharmacology and application of Clitoria ternatea L.: a review. *Rec Nat Prod* 2025; 4–4.
14. Rathikota D, Nannepaga JS, Yeguvapalli S. Green synthesis of gold nanoparticles using Clitoria ternatea: in-vitro and in-silico antioxidant activity of its phytochemicals. *Inorg Nano-Metal Chem* 2025; 55: 1031–1039.
15. Maduraimuthu V, Ranishree JK, Gopalakrishnan RM, et al. Antioxidant Activities of Photoinduced Phycogenic Silver Nanoparticles and Their Potential Applications. *Antioxidants* 2023; 12: 1298.
16. Maia NMA, Andressa I, Cunha JS, et al. Clitoria ternatea: Perspectives on Its Application in Foods and Potential Health Benefits. *Plants* 2025; 14: 3322.
17. Hasanah NN, Mohamad Azman EM, Rozzamri A, et al. A Systematic Review of Butterfly Pea Flower (Clitoria ternatea L.): Extraction and Application as a Food Freshness pH-Indicator for Polymer-Based Intelligent Packaging. *Polymers (Basel)* 2023; 15: 2541.
18. Suarna IW, Wijaya IMS. Butterfly Pea (Clitoria ternatea L.: Fabaceae) and Its Morphological Variations in Bali. *J Trop Biodivers Biotechnol* 2021; 6: 63013.
19. Havananda T, Luengwilai K. Variation in floral antioxidant activities and phytochemical properties among butterfly pea (Clitoria ternatea L.) germplasm. *Genet Resour Crop Evol* 2019; 66: 645–658.
20. Sahu D, Sahu JK, Kumar V, et al. Phytochemicals and Medicinal Uses of Clitoria ternatea. *Int J Plant Soil Sci* 2023; 35: 942–951.
21. Mukherjee PK, Kumar V, Kumar NS, et al. The Ayurvedic medicine Clitoria ternatea—From traditional use to scientific assessment. *J Ethnopharmacol* 2008; 120: 291–301.
22. Kazuma K, Noda N, Suzuki M. Flavonoid composition related to petal color in different lines of Clitoria ternatea. *Phytochemistry* 2003; 64: 1133–1139.
23. Ashraf K, Adlin NF, Basri AN, et al. The Traditional Uses, Phytochemistry, and Pharmacological Effects of Clitoria ternatea: A Review. *Indian J Pharm Educ Res* 2023; 58: 1–14.
24. . SMG, . AK. Butterfly Pea (Clitoria ternatea): A Nutritive Multipurpose Forage Legume for the Tropics - An Overview. *Pakistan J Nutr* 2003; 2: 374–379.
25. Escher GB, Marques MB, do Carmo MAV, et al. Clitoria ternatea L. petal bioactive compounds display antioxidant, antihemolytic and antihypertensive effects, inhibit α-amylase and α-glucosidase activities and reduce human LDL cholesterol and DNA induced oxidation. *Food Res Int* 2020; 128: 108763.
26. López Prado AS, Shen Y, Ardoin R, et al.

Effects of different solvents on total phenolic and total anthocyanin contents of Clitoria ternatea L . petal and their anti-cholesterol oxidation capabilities. *Int J Food Sci Technol* 2019; 54: 424–431.

27. Singh NK, Garabadu D, Sharma P, et al. Anti-allergy and anti-tussive activity of Clitoria ternatea L. in experimental animals. *J Ethnopharmacol* 2018; 224: 15–26.
28. Shyamkumar, I.B.; Ishwar B. Anti-Inflammatory, Analgesic, and Phytochemical Studies of Clitoria ternatea Linn Flower Extract. *Int Res J Pharm* 2013; 3: 208–210.
29. Devi BP, Boominathan R, Mandal SC. Anti-inflammatory, analgesic and antipyretic properties of Clitoria ternatea root. *Fitoterapia* 2003; 74: 345–349.
30. Bhatia, M.; Chahal, J.; Gupta S. Analgesic and Anti-Inflammatory Activities of Clitoria ternatea Linn. Leaves Extract on Rat Model. *Int J Pharm Sci Res* 2005; 6: 600.
31. Jain NN, Ohal C., Shroff S., et al. Clitoria ternatea and the CNS. *Pharmacol Biochem Behav* 2003; 75: 529–536.
32. Raghu KS, Shamprasad BR, Kabekkodu SP, et al. Age dependent neuroprotective effects of medhya rasayana prepared from Clitoria ternatea Linn. in stress induced rat brain. *J Ethnopharmacol* 2017; 197: 173–183.
33. Taranalli AD, Cheeramkuzhy TC. Influence of Clitoria Ternatea Extracts on Memory and Central Cholinergic Activity in Rats. *Pharm Biol* 2000; 38: 51–56.
34. Rai KS, Murthy KD, Karanth KS, et al. Clitoria ternatea (Linn) root extract treatment during growth spurt period enhances learning and memory in rats. *Indian J Physiol Pharmacol* 2001; 45: 305–13.
35. Chusak C, Thilavech T, Henry CJ, et al. Acute effect of Clitoria ternatea flower beverage on glycemic response and antioxidant capacity in healthy subjects: a randomized crossover trial. *BMC Complement Altern Med* 2018; 18: 6.
36. Kavitha R. Biochemical Studies on The Effect of Ethanolic Extracts of Trichosanthes Dioica and Clitoria ternatea in Streptozotocin Induced Diabetic MaleWistar Rats. *Int J Pharm Sci Res* 2018; 9: 4682.
37. Phrueksanan W, Yibchok-anun S, Adisakwattana S. Protection of Clitoria ternatea flower petal extract against free radical-induced hemolysis and oxidative damage in canine erythrocytes. *Res Vet Sci* 2014; 97: 357–363.
38. Zakaria NNA, Okello EJ, Howes M, et al. In vitro protective effects of an aqueous extract of Clitoria ternatea L. flower against hydrogen peroxide-induced cytotoxicity and UV-induced mtDNA damage in human keratinocytes. *Phyther Res* 2018; 32: 1064–1072.
39. Radulescu D-M, Surdu V-A, Ficai A, et al. Green Synthesis of Metal and Metal Oxide Nanoparticles: A Review of the Principles and Biomedical Applications. *Int J Mol Sci* 2023; 24: 15397.
40. Jeyaraj EJ, Lim YY, Choo WS. Antioxidant, cytotoxic, and antibacterial activities of Clitoria ternatea flower extracts and anthocyanin-rich fraction. *Sci Rep* 2022; 12: 14890.
41. Abada E, Mashraqi A, Modafer Y, et al. Review green synthesis of silver nanoparticles by using plant extracts and their antimicrobial activity. *Saudi J Biol Sci* 2024; 31: 103877.
42. Vanlalveni C, Lallianrawna S, Biswas A, et al. Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: a review of recent literature. *RSC Adv* 2021; 11: 2804–2837.
43. Suci A, Akhmad S, Masruri M, et al. Characterization and antibacterial application of biosynthesized silver nanoparticles using Piper retrofractum Vahl fruit extract as bioreductor. *J Appl Pharm Sci*. Epub ahead of print 5 March 2022. DOI: 10.7324/JAPS.2022.120311.
44. Golpour M, Ebrahimnejad P, Gatabi ZR, et al. Green tea-mediated synthesis of silver nanoparticles: Enhanced anti-cancer activity and reduced cytotoxicity melanoma and normal murine cell lines. *Inorg Chem Commun* 2024; 161: 111989.
45. Singh P, Kim Y-J, Zhang D, et al. Biological Synthesis of Nanoparticles from Plants and Microorganisms. *Trends Biotechnol* 2016; 34: 588–599.
46. Hu D, Gao T, Kong X, et al. Ginger (Zingiber officinale) extract mediated green synthesis of silver nanoparticles and evaluation of their

antioxidant activity and potential catalytic reduction activities with Direct Blue 15 or Direct Orange 26. *PLoS One* 2022; 17: e0271408.

47. Dalal R, Singh S, Sangwan S, et al. Biochemical and Molecular Mechanism of Plant-mediated Synthesis of Silver Nanoparticles – A Review. *Mini Rev Org Chem* 2022; 19: 939–954.
48. Veetil VT, Jayakrishnan V, Aravindan V, et al. Biogenic silver nanoparticles incorporated hydrogel beads for anticancer and antibacterial activities. *Sci Rep* 2024; 14: 27269.
49. Jain R, Singh R, Badhwar R, et al. Development and optimization of Clitoria teratea synthesized silver nanoparticles and its application to nanogel systems for wound healing. *Drug Dev Ind Pharm* 2024; 50: 181–191.
50. Singh SR, Kittur B, Bhavi SM, et al. The effect of Clitoria ternatea L. flowers-derived silver nanoparticles on A549 and L-132 human cell lines and their antibacterial efficacy in Caenorhabditis elegans in vivo. *Hybrid Adv* 2025; 8: 100359.
51. Omar SR, NOH AA, ROHAIZAN AN. The Potential Use of Anthocyanin in Butterfly Pea (Clinoteria ternatea) Petals as Colorimetric Indicator in Intelligent Food Packaging Article. *Malaysian J Sci Heal Technol* 2022; 8: 71–76.
52. V.Bindu, K.R.Padma, M.Reshma Anjum, M.Sankari TD. Green Synthesis of Silver Nanoparticles from Butterfly Pea and Ginger: A Review on Their Anti-Diabetic and Anti-Obesity Potential. *J Chem Heal Risks* 2025; 15: 161–168.
53. Khwannimit D, Maungchang R, Rattanakit P. Green synthesis of silver nanoparticles using Clitoria ternatea flower: an efficient catalyst for removal of methyl orange. *Int J Environ Anal Chem* 2022; 102: 5247–5263.
54. Jeyaraj EJ, Nathan S, Lim YY, et al. Antibiofilm properties of Clitoria ternatea flower anthocyanin-rich fraction towards Pseudomonas aeruginosa. *Access Microbiol*; 4. Epub ahead of print 29 April 2022. DOI: 10.1099/acmi.0.000343.
55. Rajakumar G, Abdul Rahuman A. Larvicidal activity of synthesized silver nanoparticles using Eclipta prostrata leaf extract against filariasis and malaria vectors. *Acta Trop* 2011; 118: 196–203.
56. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *J Colloid Interface Sci* 2004; 275: 177–182.
57. Kim YS, Kim JS, Cho HS, et al. Twenty-Eight-Day Oral Toxicity, Genotoxicity, and Gender-Related Tissue Distribution of Silver Nanoparticles in Sprague-Dawley Rats. *Inhal Toxicol* 2008; 20: 575–583.
58. Fröhlich E, Roblegg E. Models for oral uptake of nanoparticles in consumer products. *Toxicology* 2012; 291: 10–17.
59. Marambio-Jones C, Hoek EM V. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *J Nanoparticle Res* 2010; 12: 1531–1551.
60. Chen X, Schluesener HJ. Nanosilver: A nanoproduct in medical application. *Toxicol Lett* 2008; 176: 1–12.
61. Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: Green synthesis and their antimicrobial activities. *Adv Colloid Interface Sci* 2009; 145: 83–96.