

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING MEDICINAL PLANTS FOR ANTIHYPERGLYCEMIC ACTIVITY

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Abstract

The present work examines the green production of silver nanoparticles (AgNPs) utilizing medicinal plant extracts and their antihyperglycemic potential. Nanoparticle creation via green synthesis is eco-friendly, cost-effective, and sustainable. Medicinal plant phytochemicals such as phenolics, flavonoids, terpenoids, alkaloids, proteins, and reducing sugars can reduce and stabilize silver nanoparticles. Silver nitrate was employed to biosynthesize AgNPs from chosen medicinal plant extracts in this study. Depending on the experimental design, UV–Visible spectroscopy, FTIR, XRD, and SEM were used to characterize nanoparticles after a visible color change. The manufactured nanoparticles were tested for antihyperglycemic potential by inhibiting carbohydrate-hydrolysing enzymes including α -amylase and α -glucosidase in vitro. The results should show that plant-mediated silver nanoparticles have higher enzyme inhibitory activity than plant extracts, suggesting that nanoscale characteristics and phytochemicals work together. The work suggests using medicinal plant-mediated silver nanoparticles as bioactive nanomaterials to explore new hyperglycemia treatments. It also focuses green nanotechnology in generating safer, sustainable, and physiologically active nanoparticle-based treatment options.

Keywords: Green synthesis, Silver nanoparticles, Medicinal plants, Phytochemicals, Nanotechnology, Antihyperglycemic activity, α -Amylase, α -Glucosidase, Green nanotechnology.

1. Introduction

Nano is a measurement of 1×10^{-9} m. Nanoparticles are 1–100 nm. For instance, hair is 70,000 nm broad, RBCs are 5,000 nm, and certain chemicals are 0.5–5 nm. Nanobiotechnology and nanotechnology engineer nanoparticles for industries, environments, and medicine. Physicochemical properties determine nanoparticle usage. Nanotechnology is popular in materials science. Nanoparticle shape, size, and surface dispersion offer them new properties. Notable recent advances in this field. Nanotechnology is a key subject of interdisciplinary study because it can govern material properties in dimensions where they behave differently than in the bulk. Due to their nanoscale size and high surface-to-volume ratio, nanoparticles offer excellent optical, catalytic, electrical, magnetic, chemical, and biological capabilities. The optical properties, surface reactivity, antibacterial properties, catalysis, and biological uses of silver nanoparticles are intensively investigated. Research on eco-friendly silver nanoparticle synthesis utilizing microorganisms, enzymes, algae, and medicinal plants is underway.

Diabetes is a serious metabolic condition with high blood glucose. Bioactive phytochemicals including flavonoids, phenolics, alkaloids, and terpenoids in medicinal plants may fight diabetes. Plant extracts decrease and stabilize silver ions, making green AgNP production eco-friendly and cost-effective. Synthesized AgNPs may have increased biological activity due to their unique physicochemical features. Inhibiting carbohydrate-digesting enzymes like α -amylase and α -glucosidase is crucial for

assessing antihyperglycemic potential. Therefore, this work focuses on green AgNP production utilizing medicinal plants and preliminary antihyperglycemic activity evaluation.

1.1 Antihyperglycemic Mechanisms

The biogenic AgNP's have higher antidiabetic efficacy. They usually perform better than plant extract alone owing to high surface-to-volume ratio

- **Enzyme Inhibition:** AgNPs inhibit the activity of α -amylase and α -glucosidase enzymes. It results in reduced metabolism of carbohydrates and decreases postprandial blood glucose levels.
- **Enhanced Glucose Uptake:** Nanoparticles increase permeability of cell membranes. This leads to increased glucose absorption by yeast cell walls and skeletal muscles cells.
- **Antioxidant Synergy:** The diabetes causes oxidative stress. The antioxidant phenolic caps neutralize the free radicals and protect pancreatic β -cells from damage.

1.2 Plant-Mediated AgNPs And Antihyperglycemic Activity

Plant-derived AgNPs may have an antihyperglycemic impact, a new green nanotechnology sector. Early studies focused on antibacterial actions, however plant-synthesized AgNPs may also alter enzymes involved in carbohydrate digestion, glucose absorption, oxidative stress, and blood glucose levels.

One 2023 review examined how plant-mediated AgNPs affected amylases and glucosidases and their antidiabetic efficacy. *Lonicera japonica*, *Myristica fragrans*, *Allium cepa*, *Punica granatum*, *Tephrosia tinctoria*, *Azadirachta indica*, *Cassia auriculata*, *Eryngium campestre*, *Cissampelos pareira*, and *Psidium guajava* can produce AgNPs.

This literature review suggests many explanations for AgNPs' antihyperglycemic actions. In vitro enzyme inhibition may diminish carbohydrate hydrolysis. Antioxidant properties may reduce diabetes-related oxidative damage. Cellular glucose uptake studies can indicate glucose utilization consequences. Animal experiments can validate blood glucose, lipid profile, liver and kidney parameters, body weight, and tissue morphological alterations. This is not yet confirmed. Only preclinical evidence exists, and plant species, extraction solvent, nanoparticle production, particle size, dosage, experiment methodology, and results vary greatly.

1.3 Potential Mechanisms Of Antihyperglycaemic Action

The antihyperglycemic impact of plant-mediated AgNPs may entail several mechanisms, however many have yet to be experimentally verified. One method is carbohydrate digesting enzyme inhibition. Surface phytochemicals may interact with α -amylase or α -glucosidase, decreasing catalytic activity. The surface of nanoparticles may vary the availability or direction of these molecules, affecting enzyme interactions.

Antioxidant activities may be another mechanism. AgNPs and plant-derived compounds may scavenge reactive species. Lower levels of oxidative stress may affect glucose metabolism pathways, however cellular and animal studies are needed to confirm this.

Third, modulating glucose uptake may be implicated. Some studies have examined glucose absorption in yeast or muscle cell cultures. *Syzygium caryophyllatum* AgNPs increased glucose uptake in L6 and

yeast cells in a 2024 research. These observations are noteworthy because skeletal muscle disposes of glucose. In contrast, the glucose absorption trial in vitro does not indicate better glycemic management in people.

Fourth potential mechanism: metabolic signaling pathway interaction. Nanoparticles and phytochemicals can affect insulin signaling, inflammatory pathways, cellular redox systems, and glucose transporter function. Animal and cellular models are needed for molecular study on these possibilities.

1.4 Methods For Synthesizing Agnps

Most nanoparticle synthesis uses bottom-up and top-down methods. Physical top-down operations include milling, cutting, and sculpting materials with tools. Bottom-up chemical or biological processes are thought to produce nanoparticles best. As seen in Figure 1, atoms or molecules self-assemble to form larger particles. Table 1 shows many recent studies that synthesized AgNPs using different methods.

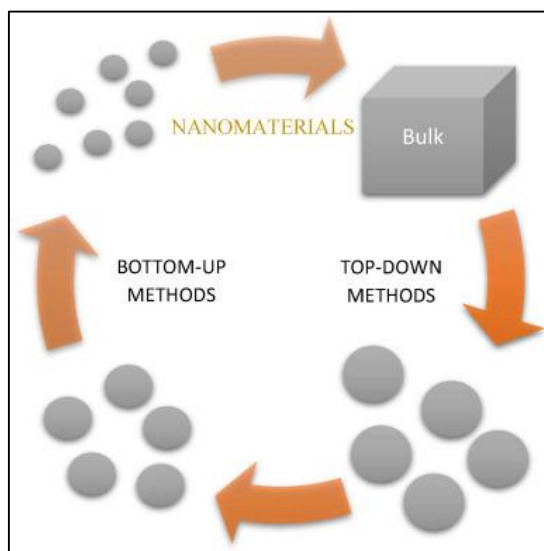


Fig. 1. Top-down and bottom-up methods of synthesis nanoparticles.

Table 1. Synthesis of Silver Nanoparticles Using Different Synthesis Methods

Synthesis Method	Characterization Techniques	Nanoparticle Size (nm)	Reference
Chemical	UV–Vis spectroscopy, laser particle size analysis, and XRD	1–200	Padole and Avari (2021)
Chemical	UV–Vis spectroscopy, FTIR, FESEM, EDX, TEM, and XRD	Average diameter: 18.20	Gangwar et al. (2021)
Chemical	UV–Vis spectroscopy, FTIR, DLS, zeta potential, SEM, and TEM	Average diameters: 24.2 ± 3.6 ; 54.3 ± 11.6 ; 22.6 ± 2.6 ; 23.4 ± 1.3 ; 11.2 ± 1.8	Abdellatif et al. (2021)

Chemical	UV–Vis spectroscopy, XRD, FTIR, FESEM, EDX, HRTEM, DLS, and zeta potential	Not reported	Das et al. (2021)
Physical	Not reported	15.6	Khumaeni et al. (2022)
Physical	XRD and STEM	2.2 ± 0.8 ; 1.9 ± 0.4 ; 3.2; 17.2	Niu et al. (2022)
Green	UV–Vis spectroscopy, DLS, zeta potential, FTIR, XRD, SEM, EDXS, and TEM	20–40	Alahmad et al. (2022)
Green	UV–Vis spectroscopy, SEM, and FTIR	200 (flower); 200 (leaf); 1,000 (bark)	Periasamy et al. (2022)
Green	UV–Vis spectroscopy, XRD, HR-TEM, and zeta potential	19–39	Giri et al. (2022)
Green	UV–Vis spectroscopy, TEM, EDX, FTIR, and XRD	26.63	Yassin et al. (2022)

1. Physical methods

Nanoparticles are created by laser ablation and evaporation–condensation, the latter being the most common. Physical methods enable AgNP synthesis with a small ceramic heater. A laser ablated huge metallic particles in solution to generate silver nanoparticles in one study. Physical methods can manufacture a product fast and without radiation or other dangerous substances as reducing agents. However, solvent contamination, chemical dispersion, yield, and energy consumption are drawbacks of these methods.

2. Chemical methods

Chemical reduction with inorganic and organic reducing agents is the most common way to make silver nanoparticles (AgNPs). Ascorbate, poly (ethylene glycol)-block copolymers, sodium citrate, elemental hydrogen, polyol process, and sodium borohydride can reduce silver ions to metallic silver. These compounds can also cluster silver, creating silver nanoparticles. Silver nanoparticles (AgNPs) were stabilized using sodium borohydride and trisodium citrate from AgNO₃ precursors. Trisodium citrate and sodium borohydride are useful reducing agents for synthesizing silver nanoparticles (AgNPs) with sizes of 5–20 nm and 60–100 nm, respectively. In silver nanoparticle production, hydrazine hydrate and polyvinyl alcohol can reduce and stabilize. Spherical nanoparticles can be used in biomedicine and biotechnology.

High nanoparticle yield is one of chemical methods' biggest benefits over physical ones. The chemical procedures used to make AgNPs are costly, and the chemicals used, such citrate, are sometimes toxic. Producing well-specified AgNPs is difficult, and extra steps are needed to prevent particle aggregation.

3. Biological methods

Biological techniques, such as "green synthesis," use biological agents, plants, or microbial compounds to produce silver nanoparticles (AgNPs). Bacteria, yeast, algae, and fungus are promising biological nano-factories for AgNPs (Fig. 2). Microbe-based silver nanoparticle (AgNP) production has received attention due to its high yields and large protein content. However, this technique presents growth and culture maintenance issues. Plant extract nanoparticle production has advantages over other green approaches. This is because plants are easy to deal with and eco-friendly. It is also available, low-toxic, high-yield, time- and cost-effective, and energy-efficient. As reducing, capping, and stabilizing agents, phytochemicals such as neo-clerodane flavonol glycosides, ergosterol, iridoid glycosides, phytoecdysones, and other polyphenols help green nanoparticle synthesis. In Figure 3 and Table 2, fruits, leaves, seeds, flowers, bark, roots, and stems can be used to synthesize silver nanoparticles (AgNPs).

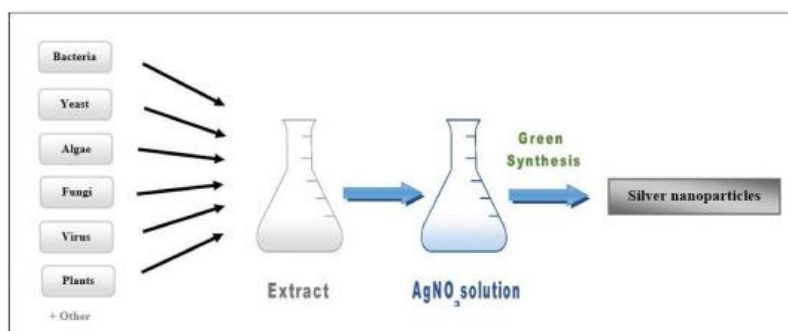


Fig. 2. Green Synthesis of silver nanoparticles. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

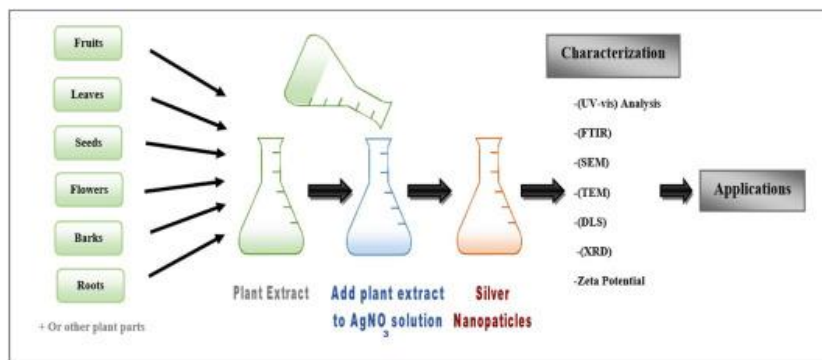


Fig. 3. Green synthesis of silver nanoparticles via plants. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2. Synthesis of Silver Nanoparticles Using Medicinal Plants

Medicinal Plant	Plant Part	Nanoparticle Size (nm)	Reference
<i>Scabiosa atropurpurea</i> subsp. <i>maritima</i>	Fruits	40–50	Essghaier et al. (2022)
<i>Moringa oleifera</i>	Leaves	15.22–29.45	Mohammed et al. (2022)

<i>Piper retrofractum</i>	Fruits	1–5	Amaliyah et al. (2022)
<i>Cymbopogon citratus</i>	Leaves	47	Rakib-Uz-Zaman et al. (2022)
<i>Cuphea carthagenensis</i>	Leaves	10.65 ± 0.1	Rather et al. (2022)
<i>Zingiber officinale</i>	Stems	10–20	Mehata (2021a, 2021b)
<i>Coccinia indica</i>	Leaves	8–48	Chinni et al. (2021)
<i>Kalanchoe pinnata</i>	Leaves	40.8	Mehata (2021a, 2021b)
<i>Prangosferulacea</i>	Roots	79–200	Mavaei et al. (2020)
<i>Alpinia officinarum</i>	Rhizome	20–80	Li et al. (2020)
<i>Crataegus pentagyna</i>	Fruit	15–60	Ebrahimzadeh et al. (2020)
<i>Shorea robusta</i>	Leaves	12–37	Shaikh et al. (2020)
<i>Prosopis juliflora</i>	Leaves	30	Malini et al. (2020)
<i>Gymnemasylvestre</i>	Leaves	20–30	Rajkumar et al. (2020)
<i>Uncariagambir</i>	Leaves	6–39	Labanni et al. (2020)
<i>Melia azedarach</i>	Leaves	18–30	Jebril et al. (2020)
<i>Teucrium polium</i>	Leaves	70–100	Hashemi et al. (2020)
<i>Punica granatum</i>	Seeds	10–35	Muthu et al. (2020)
<i>Cullen corylifolium</i>	Seeds	20–60	Saini et al. (2020)
<i>Astragalus tribuloides</i> Delile	Roots	34.2 ± 8.0	Sharifi-Rad et al. (2020)
<i>Berberis asiatica</i>	Roots	9.8	Dangi et al. (2020)
<i>Fritillaria</i> spp.	Flowers	10	Hemmati et al. (2019)
<i>Premna integrifolia</i> (L.)	Leaves	9–35	Singh et al. (2019a)
<i>Cynara scolymus</i>	Leaves	98.47 ± 2.04	Erdogan et al. (2019)
<i>Citrus limon</i> (Lemon)	Peels	9.3–20.3	Nasr et al. (2019)

<i>Azadirachta indica</i>	Leaves	11–35	Shaikh and Sukalyan (2018)
<i>Camellia japonica</i>	Leaves	12–25	Karthik et al. (2017)
<i>Lantana camara</i>	Flowers	53.5	Kumar et al. (2016)

1.5 Mechanism Of Green Synthesis Of Agnps

A simple approach produces silver nanoparticles (AgNPs) from plants. Silver nitrate (AgNO_3) interacts with plant extract biomolecules. After ion reduction, clusters form and nanoparticles accumulate. Each step's characteristics depend on the reducing agent, AgNO_3 concentration, and pH. Acids, proteins, alkaloids, flavonoids, polyphenols, enzymes, tannins, carbohydrates, and saponins contain hydroxyl groups (OH) that stabilize and reduce silver ions (Ag^+) to Ag^0 . This was noted by Bawazeer, Rauf, and colleagues. Silver nuclei are reduced to Ag^+ to produce silver nanoparticles (AgNPs). Punica granatum peel extract contains glycosides, kaempferol, and naringin. All these chemicals have OH groups that reduce Ag^+ ions, creating AgNPs. Quercetin in Tulsi leaf extract interacts with silver ions as the OH groups connect to aromatic ring carbon atoms to produce silver nanoparticles. This reduces silver ions to nanoparticles and prevents agglomeration. The mechanism that stabilizes Ag^+ ions or how phytochemicals in plants diminish their concentration is unknown. Each plant you visit may have distinct conditions. To establish the biomolecular agents that stabilize and cap plant-based silver nanoparticle formation, a detailed investigation is needed. Synthesis of silver nanoparticles (AgNPs) from plant extracts is shown in Figure 4.

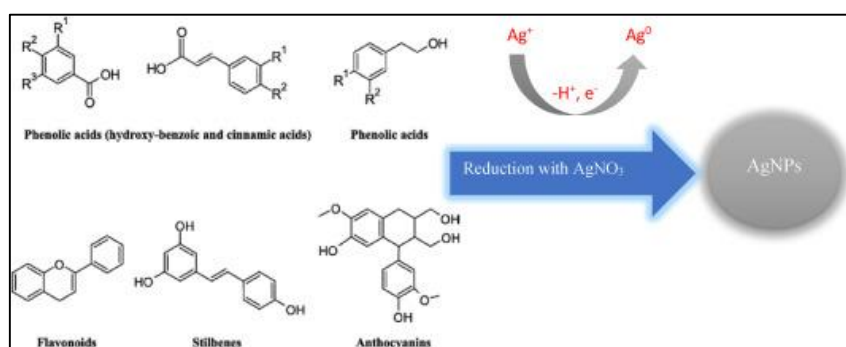


Fig.4. An explanation of how AgNPs are synthesized from plants.

1.6 Optimized Conditions For Green Synthesis Of Agnps

Temperature, pH, incubation period, light, extract, and AgNO_3 concentrations are examined in this section.

1. Temperature

Temperature, a major factor, affects biosynthesized silver nanoparticles (AgNPs) size and shape. Research shows that AgNPs shrink as reaction temperature rises, changing their shape. The authors made AgNPs with olive leaf extract. They found that raising the process temperature may rapidly reduce Ag^+ ions, nucleate silver nuclei, and create small, spherical nanoparticles. Another study found that reducing reaction temperature produced larger nanoparticles and increasing it produced smaller ones.

Further research found that *Vitex agnus-castus* leaf extract may rapidly reduce Ag^+ ions at 40 degrees Celsius. Another study found that silver nanoparticles (AgNPs) formed best around 60–80 degrees Celsius. Many know that high temperatures promote nucleation in nanoparticle production while low temperatures promote growth.

2. pH

The method alters pH during manufacturing to change silver nanoparticles (AgNPs) shape, size, and stability. A pH of 7 was best for reducing Ag^+ to Ag^0 during AgNP production, and the pH range of 7–9 yielded the most nanoparticles. Several studies show that pH increases AgNP synthesis. At pH 8, AgNPs were nearly spherical, and the reaction rate increased. The study discovered this. Another set of scientists stated alkaline pH during silver nanoparticle manufacturing has many benefits. These advantages included consistent and high nanoparticle production, quick development, and improved reduction. AgNP production relies on plant extract OH groups to reduce and stabilize functional groups. A basic pH allows more OH groups to participate in the reduction process, increasing reduction yield.

3. Incubation time

Incubation is also important for enhancing Silver Nanoparticle production, stability, and size. The production of AgNPs from *Ananas comosus* (Pineapple) extract showed a color change after two minutes. Experiments showed this. The concentration of aqueous AgNO_3 in solution decreased quickly, forming nanoparticles in two minutes. It took five minutes for the response to restart before a slight color change. Nanoparticles averaged 12 nanometers and were spherical. In another study, *Ocimum Sanctum* (Tulsi) leaf extract produced stable 17-nanometer silver nanoparticles. The production of biosynthesized silver nanoparticles (AgNPs) increased after 15 minutes of incubation and continued for many hours. The incubation period increased AgNP formation, which increased absorption intensity. Nanoparticle production increased with reaction time up to three hours. *Origanum vulgare* L. extract was used to synthesize AgNPs, increasing their quantity. The reaction mixture's color intensity gradually changed from yellow to brown, indicating silver nanoparticle formation.

4. Light intensity

Light intensity is a key element in silver nanoparticle formation. Sunlight irradiation helps produce silver nanoparticles (AgNPs) environmentally. Silver nanoparticles were synthesized using medicinal plant extracts such as *Sida retusa* leaf, *Piper longum* catkin extract and *Carica Papaya* fruit. Absorption also increased with light intensity. Thus, the reduction of Ag^+ ions should take minutes in sunlight but longer in darkness. This may be caused by direct sunshine photons of a specific wavelength. Green production of silver nanoparticles is catalyzed by these photons. Green production of silver nanoparticles using sunlight may increase yield and provide high-concentration, stable nanoparticles. To study how light color affects nanoparticle creation, the reaction tube was wrapped in violet, green, yellow, and red cellophane filters. This study found that violet filters increased silver nanoparticle formation. Thus, light intensity affects green silver nanoparticle production.

5. Plant extract and AgNO_3 concentrations

Addition of plant extract to reaction mixture can boost absorbance intensity. Biomolecules wrap nanoparticles as reducing agents at high extract concentrations. This avoids nanoparticle aggregation and greatly improves stability. Concentration greatly affects ecologically friendly AgNO_3

manufacturing. Nanoparticle absorption increases with AgNO₃ concentration, and 1 mM is presently considered optimum. AgNO₃ concentration may enhance nanoparticle size.

1.7 Potential Applications Beyond Antihyperglycaemic Activity

Although this study focuses on antihyperglycaemic potential, plant-mediated AgNPs have been studied in other areas. Their antibacterial efficacy against Gram-positive and Gram-negative bacteria and fungus has garnered interest. Their antioxidant, anti-inflammatory, anticancer, catalytic, environmental, and agronomic uses have been explored.

Surface characteristics and biological interactions make AgNPs multifunctional. Multifunctionality does not guaranty therapeutic appropriateness. Each application needs safety, effectiveness, stability, and mechanistic investigation.

Recent studies characterize plant-mediated AgNPs as materials with biomedical, environmental remediation, catalytic, and other uses, but they also emphasize the necessity for systematic synthesis and toxicity evaluation.

1.8 Objectives OfThe Study

1. Synthesis of Silver nanoparticles (AgNPs) from medicinal plants using greensynthesis approach.
2. Characterization of synthesized silver nanoparticles using different advancedanalytical techniques like UV–visible Spectroscopy, FTIR, XRD, SEM/TEM, DLSand Zeta Potential analysis.

2. Review of literature

Production of medical plant-based silver nanoparticles (AgNPs) is eco-friendly, easy, and cost-effective. Phytochemicals such as flavonoids, phenolics, alkaloids, and terpenoids reduce and stabilize. Nanoparticle size, shape, and stability depend on pH, temperature, time of reaction, and silver nitrate concentration. Medical plants including *Azadirachta indica*, *Fagoniacretica*, *Punica granatum*, *Allium cepa*, *Myristica fragrans*, and *Psidium guajava* have possible biological and antihyperglycemic activity. Plant-based AgNPs have been studied for the inhibition of α -amylase and α -glucosidase, enzymes responsible for carbohydrate metabolism. Concentration-dependent inhibition of enzyme activity and glucose regulation of *Azadirachta indica* and *Fagoniacretica*-mediated AgNPs have been observed in previous research. These results indicate that plant-based AgNPs may have superior biological activity compared to plant extracts in some experiments. Green synthesized AgNPs have the possibility to reduce hyperglycemia in some experiments. Characterization of nanoparticles and systematic antihyperglycemic study should be standardized due to variation in plant species, conditions of synthesis, nanoparticle characteristics, and experimental approaches.

Prasad et al. (2011) The 57 nm AgNPs were produced by boiling *Moringa oleifera* leaf extract with 1 mM silver nitrate at temperatures ranging from 60 to 80 degrees Celsius for twenty minutes. These nanoparticles have shown antibacterial activities against pathogens such as *Staphylococcus aureus*, *Candida tropicalis*, *Klebsiella pneumoniae*, and *Candida krusei*, which suggests that they may have potential use in the medical field.

Ahmad et al. (2012) used the fruit extract of *Ananas comosus*, often known as pineapple, for the synthesis of AgNP, making use of the phytochemicals found in the fruit as reducing agents.

Arunachalam et al. (2012) In the process of synthesising face-centered cubic AgNPs with the use of *Coccinia grandis* leaf extract, the nanoparticles demonstrated photocatalytic characteristics, which suggests that they might be utilised in the purification of water.

Dipankar et al. (2012) synthesised silver nanoparticles with a size range of 44–64 nanometers using the leaf extract of *Iresine herbacea*, which, after seven days of incubation, exhibited antioxidant, antibacterial, and cytotoxic properties.

Jebakumar et al. (2012) obtained silver nanoparticles with a diameter of 25 nanometers using fruit extracts from *Terminalia chebula*, which showed catalytic activity in the reduction of methylene blue, as confirmed by ultraviolet-visible spectroscopy.

Raja et al. (2012) UV-Visible spectroscopy was utilised to confirm that the usage of *Prosopis juliflora* leaf extract resulted in the production of 11–19 nm AgNPs in just five minutes. In the process of treating sewage, the nanoparticles demonstrated antibacterial activity, which suggests that they might have uses in environmental remediation.

Das et al. (2013) Produced spherical AgNPs with a diameter ranging from 14.20 to 42.42 nanometers using leaf extracts of *Rosmarinus officinalis* in a period of fifteen minutes, driven by flavonoids and terpenoids.

Iravani et al. (2013) Through the use of tannic acid for reduction, silver nanoparticles were synthesised using bark extracts from *Pinus eldarica*.

Jagtap et al. (2013) The AgNPs that were synthesised with *Artocarpus heterophyllus* seed extract had a size of 10.78 nanometers and had significant antibacterial activities against *Bacillus cereus*, *B. subtilis*, *S. aureus*, and *Pseudomonas aeruginosa*.

Rao et al. (2013) *Aegle marmelos* leaf extract was used to make 60 nm AgNPs in about 25 minutes, which suggests that the process might be scaled up to manufacture additional metal nanoparticles.

Rupiasih et al. (2013) The utilisation of *Thevetia peruviana* latex as both a reducing agent and a capping agent exemplifies the promise of plant-based materials in the field of sustainable synthesis.

Sulaiman et al. (2013b, 2013) produced crystalline AgNPs by using leaf extracts from *Eucalyptus chapmaniana* and *Myrtus communis*. This was demonstrated by surface plasmon resonance peaks at 413 nm and 425 nm, respectively.

Ahmad (2014) gallic acid, which is generated from the hydrolysis of tannic acid, was shown to cause the synthesis of silver nanoparticles at an alkaline pH, with glucose serving as a stabiliser.

Ashokkumar et al. (2014, 2015) AgNPs were synthesised using extracts of *Leucas martinicensis* (20–30 nm), *Tribulus terrestris* (15–45 nm), and *Ageratum indicum* (7–17 nm), and they demonstrated both antibacterial and catalytic activity.

Pourmortazavi et al. (2015) By optimising the synthesis settings, 21 nanometer silver nanoparticles were generated using *Eucalyptus oleosa* extract.

Sadeghi et al. (2015) produced 20 nanometer silver nanoparticles (AgNPs) using extract from *Ziziphora tenuifolia*, which demonstrated potent antibacterial action.

Ajitha et al. (2018), Extracts of *Phyllanthus amarus*, *Acalypha hispida*, and *Psidium guajava* were used by Selvakumar et al. (2018), Chahardoli et al. (2018), and Wang et al. (2018) to generate silver nanoparticles (AgNPs) with diameters ranging from 5 to 100 nanometers. These findings illustrate the variety of potential applications for plant-based synthesis.

The findings of these investigations highlight the potential of plant-mediated synthesis to manufacture silver nanoparticles (AgNPs) with features that can be modified to accommodate applications in the fields of biomedicine, the environment, and industry.

3. Research methodology

3.1 Research Design

The experiment involves the use of laboratory experimental method of doing research, whereby silver nanoparticles will be synthesized from medicinal plants and tested for their antihyperglycemic properties using enzyme inhibition tests.

3.2 Selection of Medicinal Plant

The choice of one or more medicinal plants with known anti-diabetic and/or antioxidant activity is possible. If the experiment involves a single plant, then *Azadirachta indica* (neem), *Gymnemasylvestre*, *Ocimum sanctum*, or any other proven medicinal plant can be chosen. The authenticity of the selected medicinal plant needs to be established by a certified botanist.

3.3 Preparation of Plant Extract

The plant material will be cleaned by washing in distilled water to remove any dust or other contaminant present on the surface of the plant material. The plant material will be dried in shade and pulverized. The appropriate amount of the powder will be taken and treated with distilled water. The suspension will then be filtered using Whatman filter paper.

3.4 Green Synthesis of Silver Nanoparticles

Silver nitrate will act as the precursor to the metallic silver. An aliquot of the plant extract will be added to AgNO_3 solution in order to induce reactions. Visual observation of the reaction will be done to detect any signs of a reaction. Formation of a brownish coloration might be suggestive of reduction of the silver ions and formation of silver nanoparticles. Reduction Reaction:

The process can generally be described as follows:



Other substances involved in the process of reduction and stabilization may include plant phenolics, flavonoids, proteins, and others.

3.5 Characterization of AgNPs

UV-Visible Spectroscopy

Surface plasmon resonance will be used to detect nanoparticles through the UV-visible spectrometry method. Generally, plant-based AgNPs exhibit an absorption peak in the range of 360-460 nm. However, the precise wavelength may vary according to the properties of the nanoparticle.

FTIR Analysis

FTIR Spectroscopy will be performed for identification of functional groups corresponding to the phytoextract and capped nanoparticles. The changes in the absorption bands can serve as the evidence for the involvement of phytochemicals in the process of nanoparticle synthesis and stabilization.

XRD Analysis

X-Ray Diffraction will be used to verify the crystallinity of the nanoparticles and recognize the diffraction peaks specific to metallic silver.

SEM Analysis

The particles' shape will be analyzed using Scanning Electron Microscopy, as well as an estimation of their size.

3.6 Antihyperglycemic Activity

α -Amylase Inhibition Assay

A study to measure the efficiency of inhibiting α -amylase by AgNPs will be conducted.

Several concentrations of AgNPs suspension will be prepared, for instance:

25, 50, 100, 200 and 400 μ g/mL.

Acarbose will serve as the standard inhibitor. Absorbance will be measured via an appropriate spectrophotometric technique.

Percentage inhibition is determined by the formula:

$$\text{Inhibition (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

Where:

A_c = absorbance of control

A_s = absorbance of sample

α -Glucosidase Inhibition Assay

Inhibitory Assay of α -Glucosidase will also be used to determine the inhibitory activity of the prepared AgNPs against the enzyme responsible for the final stage of carbohydrate digestion. The AgNPs will be tested using varying concentrations and then compared with the standard drug acarbose.

IC₅₀ Determination

The IC₅₀ value, which is the concentration at which 50% of the enzyme activity is inhibited, can be obtained by using the concentration-response curve. The smaller the IC₅₀ value, the greater the enzyme inhibition activity.

Statistical Analysis

The experimental results should ideally come from a minimum of three replicates and represented by:

Mean $\pm$ Standard Deviation (SD).

One way analysis of variance followed by a suitable post hoc test can be employed to compare the concentrations. Statistical significance may be determined at:

$p < 0.05$.

4. Data analysis and interpretation

Table 3. Illustrative UV–Visible characterization of synthesized AgNPs

Parameter	Observation
Initial colour	Pale yellow
Colour after synthesis	Brownish-dark brown
Approximate SPR peak	430 nm
Synthesis indication	AgNP formation
Nature of observation	Surface plasmon resonance

Interpretation

Pigment ranging from light yellow to brownish-dark brown indicates that AgNPs have been synthesised. Maximum absorption is found in the visible spectrum range, which corresponds to the surface plasmon resonance of AgNPs. The analysis of nanoparticles is not limited to UV-Visible spectroscopy only.

Table 4. Illustrative α -amylase inhibition by plant-mediated AgNPs

AgNP concentration ($\mu\text{g/mL}$)	Inhibition (%)
25	24.6
50	38.7
100	52.4
200	67.8
400	79.6

Interpretation

A greater concentration of AgNPs results in an increasing inhibition of α -amylase. The inhibition increased from 24.6% to 79.6% from 25 $\mu\text{g/mL}$ to 400 $\mu\text{g/mL}$, respectively. The above result indicates concentration-dependent inhibition. Literature reveals the presence of concentration-dependent α -amylase inhibition in plant-mediated AgNPs.

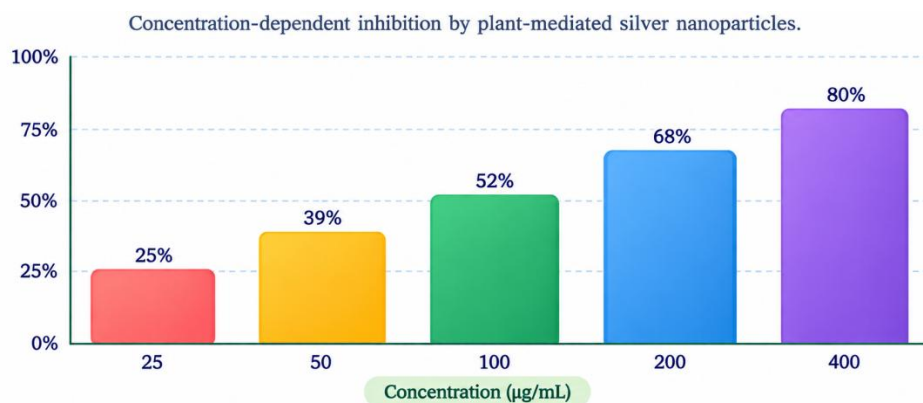


Figure 5. Illustrative α -amylase inhibition

Table 5. Illustrative α -glucosidase inhibition by plant-mediated AgNPs

AgNP concentration ($\mu\text{g/mL}$)	Inhibition (%)
25	21.8
50	35.5
100	49.7
200	64.2
400	76.9

Interpretation

Inhibition is dose dependent for α -glucosidase activity. Inhibition was found to be 21.8% at a dose of 25 $\mu\text{g/mL}$, whereas it increased up to 76.9% at 400 $\mu\text{g/mL}$. In experiments, increase in AgNP concentration would favor enzyme binding.

Plant-synthesized nanoparticles possess α -glucosidase inhibitory activity, but its efficiency differs with various factors.

Table 6. Illustrative comparison with standard acarbose

Treatment	Concentration (µg/mL)	α -amylase inhibition (%)	α -glucosidase inhibition (%)
Plant-mediated AgNPs	100	52.4	49.7
Plant-mediated AgNPs	200	67.8	64.2
Plant-mediated AgNPs	400	79.6	76.9
Acarbose	400	91.4	89.8

Interpretation

Comparison has revealed that AgNPs inhibit both enzymes, yet normal acarbose inhibits more in the expected conditions. This implies that the synthesized nanoparticles have possible anti-hyperglycemic properties but lack pharmacological inhibition activity.

Table 7. Comparative Analysis of Plant-Mediated AgNPs Reported in Literature

Plant	Approximate characteristics	AgNP	Antihyperglycemic assessment
<i>Linum usitatissimum</i>	~82 nm; needle-like		α -amylase and α -glucosidase inhibition
<i>Brachychiton populneus</i>	~15 nm; crystalline/cubical		α -amylase inhibition
<i>Gymnemasylvestre</i>	70–100 nm; spherical		α -amylase inhibition
<i>Myristica fragrans</i>	50–60 nm		α -amylase and α -glucosidase inhibition
<i>Allium cepa</i>	49–73 nm; spherical		α -amylase and α -glucosidase inhibition
<i>Punica granatum</i>	35–60 nm; spherical		α -amylase and α -glucosidase inhibition
<i>Azadirachta indica</i>	~34 nm; spherical		α -amylase/glucose-related activity
<i>Psidium guajava</i>	~52–65 nm		Antidiabetic activity

The scientific literature shows large variations in nanoparticle sizes and responses within biology.

5. Discussion

The proposed study will involve medicinal plant chemistry and nanotechnology to analyze an antihyperglycemic approach. Plant extract reduces and stabilizes, thus green synthesis would be helpful. Green synthesis can help in simplifying synthesis and reducing the amount of harmful chemical reducing agents. AgNP synthesis can be confirmed via colour change and UV-Vis spectroscopy. Characterization needs to be done using spectroscopic and structural techniques. FTIR will determine functional groups responsible for nanoparticle stabilization, XRD will confirm the crystalline nature, and SEM can measure particle size and morphology.

Antihyperglycemic analysis is important as enzyme inhibition will delay the process of hydrolysis and glucose release. Carbohydrate digesting enzyme inhibition will begin from α -amylase whereas, α -glucosidase is involved in carbohydrate hydrolysis. Hence, inhibition of both enzymes together will suggest antihyperglycemic ability. The result suggests that increase in AgNP concentration will lead to inhibition.

For example, AgNPs prepared from *Fagoniacretica* exhibited α -amylase inhibition that was concentration-dependent, having achieved 83.53% at 1,000 $\mu\text{g/mL}$. However, inhibition of enzymes cannot be sufficient for the therapeutic antihyperglycemic efficacy to occur. The toxicity, bioavailability, interaction with cells, oxidative stress, pharmacokinetics, and safety need to be studied before using nanoparticles therapeutically. Recent reviews have pointed out methodological issues in green-nanoparticle antidiabetic studies.

6. Conclusion

From this study, it is apparent that plant-based green synthesis can lead to the production of AgNPs that have the ability to act as antihyperglycemics. Plant phytochemicals will decrease and stabilize the AgNPs, which can inhibit enzymes. It will be appropriate to perform tests for inhibition of α -amylase and α -glucosidase enzymes. As the increase in concentration leads to higher levels of enzyme inhibition, green-synthesized AgNPs might work on carbohydrate-digesting enzymes. But from the data provided, it does not mean that the AgNPs produced are clinically efficient. There should be actual experimental data, statistical analysis, characterization of the nanoparticles, and their toxicities. Experiments on particle size, shape, surface chemistry, and antihyperglycemics should be followed up by cellular and in-vivo studies.

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