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"Biogenic Synthesis Of Antimicrobial Silver Nanoparticles Utilizing Flavonoid Rich Cacabek Thevetia Leaf Extract: Formulation Optimization And Characterization"

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Abstract
*Drug delivery has remained a dynamic and continuously progressing area within pharmaceutical science, especially after the introduction of synthetic therapeutic agents and modern medical devices. Over the years, a wide range of approaches and materials have been investigated to administer drugs through intravenous, intramuscular, oral, transdermal, and other routes in both humans and animals. Although conventional drug-delivery methods are still routinely used for managing various common health conditions, they often demand repeated dosing or higher quantities of medication, which may lead to unwanted side effects. The emergence of targeted and controlled drug-delivery systems has significantly transformed the management of complex diseases—including cancer, tuberculosis, autoimmune disorders, and cardiovascular abnormalities—by enabling the precise and sustained release of therapeutic agents through specialized carrier systems. The present study reports the biogenic synthesis of antimicrobial silver nanoparticles (AgNPs) using flavonoid-rich Cascabela thevetia leaf extract. Fresh leaves were collected from Bhopal, authenticated, and extracted through aqueous maceration. The extract was evaluated for phytochemical constituents and quantified for total phenolic (58.85 ± 1.604 µg GAE/mg) and flavonoid content (20.71 ± 1.601 µg QE/mg). The extract served as a natural reducing and stabilizing agent for AgNP synthesis, initially confirmed by a characteristic color transition from yellow to brown. Nanoparticle formulation was optimized by varying extract concentration, silver nitrate concentration, and incubation temperature. Optimal conditions—7.5% v/v extract, 0.002 M $AgNO_3$, and 40 °C—yielded nanoparticles with a mean size of **213.8 nm**, PDI **0.321**, and zeta potential **–28.8 mV**, indicating good stability. UV–Vis spectroscopy showed an absorption band at **280–300 nm**, while SEM imaging revealed predominantly spherical particles with rough surfaces. FTIR analysis confirmed the involvement of amine, hydroxyl, and amide groups in nanoparticle formation. The biosynthesized AgNPs exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria, suggesting that the phenolic and flavonoid compounds in Cascabela thevetia play a crucial role in nanoparticle synthesis and enhanced antimicrobial efficacy. This study highlights the potential of Cascabela thevetia as an eco-friendly and effective source for green nanoparticle production.*

Keywords: *Cascabela Thevetia, Silver nanoparticles (AgNPs), Green synthesis, Flavonoids, Phenolic compounds, Bioreduction, Nanoparticle optimization, UV–Vis spectroscopy, FTIR analysis, SEM characterization, Antibacterial activity, Phytochemical screening.*

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I. Introduction

The word nanotechnology—originally introduced by Taniguchi in 1974—describes the creation, manipulation, and investigation of materials whose structural dimensions fall within the nanoscale range of approximately 1–100 nm. Over the last few decades, nanotechnology has emerged as one of the most rapidly expanding and multidisciplinary scientific domains, influencing fields such as energy systems, electronics, environmental science, and biomedical engineering. Its ability to reshape modern technology stems from the unique behaviors expressed by matter at the nanoscale, enabling breakthroughs in diagnostics, therapeutics, sensing, and material design. Nanoparticles represent the basic building blocks of nanoscale architectures. They occupy a size regime smaller than objects characterized by classical Newtonian mechanics, yet remain significantly larger than atoms or simple molecules governed by the rules of quantum mechanics. Although the scientific study of nanoscale materials is relatively recent, humans have unknowingly utilized nanomaterials for centuries. A well-known example is colloidal gold, which was intentionally studied more than a century ago and even found therapeutic applications in the mid-20th century. Historical artifacts, such as the Roman Lycurgus Cup, further reveal the early use of nanoscale metallic particles as pigments to produce vibrant optical effects. The conceptual foundation for manipulating matter at extremely small scales was prominently articulated by

Richard Feynman in his landmark 1959 lecture "There's Plenty of Room at the Bottom." However, the major acceleration in nanomaterials research began only in the last quarter-century, driven by advancements in high-resolution imaging techniques and nanoscale fabrication tools. These improvements allowed scientists to visualize individual atoms and design structures that exploit size-dependent behaviors emerging at a few nanometers. The exceptional properties of nanoparticles arise primarily from two major factors. First, reducing particle size dramatically increases the surface-area-to-volume ratio. A larger fraction of atoms reside on the particle's surface, many of which are coordinatively unsaturated. This increase in reactive surface sites often enhances catalytic performance compared to their bulk material counterparts. The high surface energy associated with nanoscale dimensions can also alter lattice parameters, crystal configurations, and physical characteristics—including the reduction of melting points.

II. Types Of Nanoparticles:

Nanoparticles encompass a wide and diverse group of materials that exhibit unique physicochemical properties due to their nanoscale dimensions. They vary in origin, composition, structure, and functional behavior, making them suitable for applications ranging from medicine and biotechnology to electronics, environmental remediation, and energy systems. Broadly, nanoparticles can be classified into several categories based on their material type and synthesis approach.

Metal Nanoparticles:

These nanoparticles are composed of pure metals such as silver, gold, platinum, copper, and iron. Their remarkable optical, catalytic, and antimicrobial properties arise from surface plasmon resonance and high surface activity. Silver nanoparticles, in particular, are widely explored for biomedical and antimicrobial applications.[3]

Metal Oxide Nanoparticles:

Nanoparticles derived from metal oxides—such as zinc oxide, titanium dioxide, iron oxide, and cerium oxide—exhibit distinctive magnetic, photocatalytic, and semiconducting behaviors. They are frequently used in sensors, drug delivery, imaging, sunscreens, and environmental purification.[5]

Polymeric Nanoparticles:

Formed from natural or synthetic polymers, these nanoparticles include nanospheres and nanocapsules. They offer excellent biocompatibility and controlled drug-release properties, making them valuable carriers in targeted drug delivery, vaccine formulations, and tissue-engineering systems.[7]

Lipid-Based Nanoparticles:

This group includes liposomes, solid lipid nanoparticles, and nanostructured lipid carriers. Their amphiphilic nature allows efficient encapsulation of both hydrophilic and hydrophobic drugs. They are especially relevant in pharmaceutical formulations, nutraceutical delivery, and gene therapy.[6]

Carbon-Based Nanoparticles:

Carbon nanomaterials such as fullerenes, carbon nanotubes, graphene, and carbon quantum dots possess exceptional electrical, mechanical, and thermal characteristics. Their structural versatility enables applications in electronics, biosensing, energy storage, and regenerative medicine.[28]

Semiconductor Nanoparticles (Quantum Dots):

Quantum dots are nanoscale semiconductor crystals that display size-dependent fluorescence due to quantum confinement effects. They are highly useful in biological imaging, diagnostic assays, optoelectronics, and LED technologies.

Ceramic Nanoparticles:

These are inorganic, nonmetallic particles such as silica and alumina nanoparticles. They offer thermal stability, chemical resistance, and tunable surface functionalities, making them suitable for catalysis, coatings, and biomedical formulations.

Biological Or Biogenic Nanoparticles:

These nanoparticles are synthesized using biological sources such as plant extracts, microbes, enzymes, or proteins. Biogenic nanoparticles—especially silver and gold—are preferred for biomedical and environmental applications due to their eco-friendly, non-toxic, and sustainable method of production.

Table:1 Type Of Nanoparticle & Size Range:

S. No.	Type of Nanoparticle	Typical Size Range	Key Features / Remark
1	**Metal Nanoparticles** (Ag, Au, Cu, Pt)	1–100 nm	Strong optical, catalytic, antimicrobial properties
2	**Metal Oxide Nanoparticles** (ZnO, TiO_2, Fe_3O_4)	5–150 nm	Magnetic, photocatalytic, semiconducting behavior
3	**Polymeric Nanoparticles** (nanospheres, nanocapsules)	10–200 nm	Biocompatible, suitable for controlled drug release
4	**Lipid-Based Nanoparticles** (liposomes, SLNs, NLCs)	50–400 nm	Good drug-loading capacity for hydrophilic & lipophilic drugs
5	**Carbon-Based Nanoparticles** (CNTs, graphene, fullerenes)	1–100 nm (diameter)	Exceptional mechanical, electrical, and thermal properties
6	**Quantum Dots** (Semiconductor Nanoparticles)	2–10 nm	Strong fluorescence due to quantum confinement
7	**Ceramic Nanoparticles** (silica, alumina)	5–200 nm	High thermal stability and chemical resistance
8	**Biogenic / Green Synthesized Nanoparticles**	5–100 nm	Eco-friendly, plant- or microbe-derived synthesis

Metal-based nanoparticles have emerged as promising carriers for drug delivery because they can enhance the bioavailability of therapeutic agents and reduce the frequency of dosing. Over the past decade, nanoscale formulations—ranging from anticancer and antimicrobial agents to antituberculosis drugs, peptides, and proteins—have been widely investigated for their improved therapeutic potential.

Despite the extensive research activity in this area, only a limited number of sustained-release products employing polymeric nanocarriers have reached the market, most of which are available as topical preparations such as ointments or wound-healing dressings.[20] Among various metal nanoparticles, silver nanoparticles are particularly valued for their strong antibacterial properties and notable antioxidant potential. In recent years, "green" or biological synthesis has gained prominence as a sustainable approach for producing nanoparticles. Plant-based extracts are frequently used because they offer several advantages—such as simplicity of preparation, environmental safety, availability of natural reducing agents, and reduced cost—while also yielding high-quality nanoparticles.

Properties Of Nanoparticles:

1. Unlike bulk materials, which generally exhibit un iform physical behavior regardless of their dimensions, materials at the nanoscale often display size-dependent characteristics. As the particle size approaches a few nanometers, the fraction of atoms located on the surface becomes significant, contributing to marked changes in physical and chemical properties.
2. In bulk solids larger than one micrometer, only a negligible proportion of atoms exist on the surface compared with the interior of the material. At the nanoscale, however, the extremely high surface-to-volume ratio dominates the material's behavior, often leading to properties that differ radically from those of their larger counterparts.
3. Nanoparticles often exhibit unusual optical, thermal, and electronic responses. For example, gold nanoparticles—which appear yellow in bulk—display red hues at the nanoscale. Their melting point also drops substantially; 2.5 nm gold nanoparticles melt near 300°C, far below the 1064°C melting point of bulk gold. Similarly, nanostructured materials demonstrate enhanced absorption of solar radiation compared to continuous thin films, with smaller particles showing higher absorption efficiency.
4. Nanoparticles can remain suspended in liquids because their surface interactions with the solvent are sufficiently strong to counterbalance density-driven sedimentation or flotation. This behavior enables stable colloidal dispersions that are not typically seen with larger particles.
5. Due to quantum confinement, nanoparticles frequently possess unexpected optical properties. Gold nanoparticles, for instance, appear deep red or even black in solution because their electrons are confined within a very small volume, altering their interaction with light.
6. The exceptionally high surface area-to-volume ratio also increases the driving force for atomic diffusion, especially at elevated temperatures, influencing reactivity, sintering, and phase transitions.
7. Nanoparticles introduce enhanced functionalities in many everyday products. Titanium dioxide nanoparticles contribute to the well-known self-cleaning or "lotus effect" on surfaces, while zinc oxide nanoparticles exhibit superior UV-blocking efficiency compared to their bulk form—an advantage that makes them suitable for photostable sunscreen formulations.

Characterizing nanoparticles is essential for understanding their physicochemical behavior, optimizing synthesis parameters, and ensuring their suitability for specific biomedical and industrial applications. A range of sophisticated analytical tools—primarily originating from materials science—are used to evaluate particle size, morphology, surface chemistry, crystallinity, dispersion stability, and functional properties. Electron microscopy techniques such as **Transmission Electron Microscopy (TEM)** and **Scanning Electron Microscopy (SEM)**

provide high-resolution images that reveal particle shape, internal structure, and size distribution. **Atomic Force Microscopy (AFM)** offers topographical information at the nanoscale by scanning the particle surface with a fine probe. Dynamic Light Scattering (**DLS**) is widely employed to estimate the hydrodynamic diameter and polydispersity index of nanoparticles in suspension. Surface elemental composition and chemical states are commonly examined using **X-ray Photoelectron Spectroscopy (XPS)**. The crystalline nature of nanoparticles is determined using **Powder X-ray Diffraction (XRD)**, which identifies phase purity and lattice structure. Chemical bonding, functional groups, and possible interactions with stabilizers or biomolecules are evaluated by **Fourier Transform Infrared Spectroscopy (FTIR)**. High-molecular-weight biomolecule conjugation and structural integrity can be assessed using **MALDI-TOF mass spectrometry**. Additionally, **UV–Visible spectroscopy** is used to monitor optical properties, particularly the surface plasmon resonance of metal nanoparticles. Techniques such as **dual-polarization interferometry** further allow the study of molecular interactions and surface-associated changes.[14]

Advantages Of Nanoparticles:

1. Nanoparticles allow easy tuning of particle size and surface chemistry, enabling both passive and active drug targeting following parenteral administration.
2. They can modulate drug release profiles, ensuring controlled and sustained delivery during transit and at the target site. This often results in improved therapeutic efficacy, altered organ distribution, reduced drug clearance, and minimized side effects.
3. The degradation rate and controlled-release behavior of nanoparticles can be tailored by selecting appropriate matrix materials. They also allow high drug-loading efficiency, and medications can be encapsulated without requiring chemical modification—an important factor in maintaining drug stability and activity.
4. Surface functionalization with targeting ligands or the use of magnetic guidance systems enables precise site-specific drug delivery.
5. Nanoparticles offer flexibility for multiple administration routes, including oral, nasal, parenteral, intraocular, and others.

Shortcomings Of Nanoparticles:

1. Their extremely small size and high surface energy often promote particle–particle aggregation, complicating handling, processing, and storage in both liquid suspensions and dry powder forms.
2. The same high surface area that enables enhanced functionality may also result in low drug-loading capacity and cause an initial "burst release" of the drug, which can be undesirable for controlled-release applications.

III. Methodology:

Solvent Evaporation Method:

In this widely used technique, a polymer is first dissolved in an organic solvent, which also serves to solubilize a hydrophobic drug. The resulting solution is emulsified in an aqueous phase containing a suitable surfactant, forming an oil-in-water emulsion. Once the emulsion is stabilized, the organic solvent is eliminated either by continuous stirring or by applying reduced pressure. The size of the nanoparticles obtained is influenced by several factors, including homogenization speed, the concentration and nature of the stabilizer, and the amount of polymer present.[15]

Spontaneous Emulsification / Solvent Diffusion Method:

This process represents a refined version of the solvent evaporation technique. Here, a partially water-miscible solvent—typically mixed with a small proportion of an immiscible organic solvent—acts as the dispersed oil phase. Upon contact with the aqueous medium, rapid solvent diffusion generates interfacial turbulence, which promotes the formation of much smaller droplets and, consequently, finer nanoparticles. Increasing the proportion of the diffusing solvent generally results in further reduction in particle size.[14]

Polymerization Method:

In this approach, nanoparticles are synthesized directly by polymerizing monomers within an aqueous medium. Drugs can be incorporated either by adsorption onto the surface of the freshly formed nanoparticles or by dissolving them in the reaction medium after polymerization is complete. The resulting dispersion is purified through ultracentrifugation to remove unreacted surfactants and stabilizers, and the nanoparticles are subsequently re-suspended in a surfactant-free isotonic medium. This method is particularly suitable for preparing poly(alkyl cyanoacrylate) and poly(butyl cyanoacrylate) nanoparticles. The particle size is largely dictated by the concentration of the stabilizers and surfactants used.[14]

Coacervation / Ionic Gelation Method:

Extensive research has explored the development of nanoparticles using biodegradable hydrophilic polymers such as chitosan, gelatin, and sodium alginate. In this technique, two aqueous solutions are brought together—typically chitosan (a cationic polymer) and sodium tripolyphosphate (a polyanion). Electrostatic interactions between the positively charged amino groups of chitosan and the negatively charged tripolyphosphate result in the formation of nanoscale coacervates. Ionic gelation occurs as these interactions induce a transition from a liquid state to a gel-like network at room temperature, generating stable nanoparticles without requiring harsh processing conditions.[14]

Supercritical Fluid Technology:

Conventional nanoparticle-fabrication methods—such as solvent extraction, solvent diffusion, and phase separation—often rely on organic solvents, which pose environmental and safety concerns. Supercritical fluid technology has therefore emerged as a greener alternative for producing biodegradable micro- and nanoparticles. A supercritical fluid is defined as a substance maintained above its critical temperature, where it exists as a single phase regardless of pressure. **Supercritical carbon dioxide (CO_2)** is the most widely used in pharmaceutical processing because of its mild critical parameters (Tc = 31.1°C, Pc = 73.8 bar), non-flammable and non-toxic nature, and relatively low cost. This technique enables particle formation without relying on harmful organic solvents, promoting both safety and sustainability.[20]

IV. Materials:

Reagents And Chemicals:

All reagents and chemicals used in the study were obtained from reputable commercial suppliers and were utilized directly without any additional purification or drying procedures.

Table: 2 List Of Reagents/Chemicals:

S. No.	Reagent/Chemical	Source
1	*Cascabela thevetia* leaves	Collected locally from Bhopal region
2	Silver nitrate	Loba Chemie
3	Sodium hydroxide	Qualigens Chemicals
4	Dragendorff's reagent	Oxford Fine Chemicals
5	Acetic anhydride	SD Fine Chemicals
6	Hydrochloric acid	Rankem Chemicals
7	Sulphuric acid	Finar Chemicals
8	Ferric chloride	Loba Chemie
9	Distilled water	Freshly prepared in laboratory
10	Nutrient agar	SRL Diagnostics
11	Folin–Ciocalteu reagent	Oxford Fine Chemicals
12	Aluminium chloride	Loba Chemie
13	Sodium nitrate	Loba Chemie
14	Chloroform	Finar Chemicals

Instruments And Equipment:

All analytical instruments and laboratory equipment were operated using the manufacturer-provided calibration settings.

Table: 3 Instruments/Equipment Used:

S. No.	Instrument/Equipment	Model/Make
1	Centrifuge	Remi RC-12
2	UV–Visible Spectrophotometer	Labtronics LT-2201
3	FT-IR Spectrophotometer	Shimadzu IRAffinity-1S
4	pH meter	Labtronics LT-53
5	Magnetic stirrer	BioTechnics
6	Hot air oven	BioTechnics
7	Rotary vacuum evaporator	Kshitij R&D

8	Desiccator	Polylab
9	Orbital shaker	Labtronics

Collection Of Plant Material:

Fresh leaves of Cascabela thevetia were collected from the natural vegetation surrounding Bhopal. Botanical authentication was carried out by Dr. Shashibala Mishra, RB Science, Bhopal.

Preparation Of Plant Material:

The collected leaves were thoroughly washed with distilled water to eliminate dust and particulate matter, then air-dried at room temperature in a shaded area to avoid photodegradation. Once dried, the material was pulverized using a slow-speed grinder to obtain a coarse powder. The powder was stored in airtight containers until extraction. [SELF]

Extraction Of Cascabela Thevetia Leaves:

Approximately 85 g of dried leaf powder was macerated in 450 mL of distilled water for 24 hours with continuous shaking at 100 rpm using an orbital shaker. The mixture was filtered, and the filtrate was concentrated using a rotary vacuum evaporator. The semisolid extract was then lyophilized and stored in a desiccator containing anhydrous calcium chloride. The yield of the dried extract was recorded.

Qualitative Phytochemical Screening:

A preliminary phytochemical evaluation was performed to identify major classes of secondary metabolites. A sample solution was prepared by dissolving 0.5 g of the extract in 5 mL of distilled water, which was then filtered and used for testing.

Table: 4 Phytochemical Tests, Procedures, And Positive Indicators:

Phytochemical	Test / Reagent	Procedure (Summary)	Positive Indicator
Alkaloids	Dragendorff's reagent	Sample acidified with 1% HCl and treated with Dragendorff's reagent	Orange to reddish-orange precipitate
Cardiac Glycosides	Keller–Killani test	Formation of color layers at interface	Brown ring at interface; violet/greenish layer
Tannins	Ferric chloride test	Heated extract cooled and treated with $FeCl_3$	Green precipitate
Flavonoids	Ammonia + H_2SO_4 test	Extract mixed with dilute ammonia then conc. sulphuric acid	Yellow coloration
Saponins	Froth test	Persistent foam observed for ≥15 minutes	Stable froth/foam
Steroids	Acetic anhydride + H_2SO_4	Extract treated with reagents	Violet → blue/green color change
Terpenes / Terpenoids	Salkowski test	Conc. H_2SO_4 added to extract	Greyish coloration

Total Phenolic Content:

Phenolic content was estimated using the Folin–Ciocalteu method. The extract solution (1 mg/mL) was mixed with Folin reagent and sodium carbonate. After incubation in the dark, absorbance was measured at 765 nm.

A standard calibration curve was prepared using gallic acid (10–100 ppm), and the results were expressed as µg gallic acid equivalents (GAE) per mg of extract.

Total Flavonoid Content:

Flavonoid quantification was performed using the aluminium chloride assay. After reacting the extract with $NaNO_3$, $AlCl_3$, and NaOH, the absorbance was recorded at 510 nm. A quercetin-based standard curve (10–100 ppm) was used, and values were expressed as µg quercetin equivalents (QE) per mg extract.

Biosynthesis Of Silver Nanoparticles:

For nanoparticle synthesis, 95 mL of 0.001 M $AgNO_3$ solution was mixed with 5 mL of plant extract (1 mg/mL). The mixture was stirred for 24 hours, during which a colour shift from yellow to brown indicated nanoparticle formation. The suspension was centrifuged at 6000 rpm for 30 minutes, washed thrice with distilled water, and dried at 40°C.[56]

Table: 5 Optimization Parameters:

S.NO	Parameter	Levels
1	Extract	2.5%, 5.0%, 7.5% w/v
2	Silver nitrate	0.001, 0.002, 0.003 M
3	Drying temperature	40°C

V. Characterization Of Silver Nanoparticles:

UV–Visible Spectroscopy:

The dried nanoparticles were dispersed in distilled water, and absorption spectra were recorded between 200–700 nm to identify the characteristic surface plasmon resonance band.[58]

Morphology And Size Analysis:

Particle size distribution and polydispersity index were determined using a dynamic light scattering analyzer. Surface morphology was examined through scanning electron microscopy after gold-sputter coating the samples.

FT-IR Analysis:

FT-IR spectra were obtained to determine functional groups responsible for the reduction and stabilization of silver nanoparticles.

Antibacterial Activity Of AgNPs:

Test organisms (*Escherichia coli* MTCC 40 and *Staphylococcus aureus* MTCC 3160) were obtained from IMTECH, Chandigarh.

VI. Preparation Of Nutrient Broth:

Nutrient broth was prepared according to standard composition and sterilized. Bacterial cultures were revived by adding 0.3 mL broth to lyophilized cultures.

Preparation Of Nutrient Agar:

Nutrient agar was prepared and sterilized, and plates were poured and solidified under aseptic conditions before use.

Table: 6 Composition Of Nutrient Agar Powder (Ph 6.8 ± 0.2):

Ingredients	Amount (g/L)
Beef Extract	3.0
Peptone	5.0
Sodium Chloride	8.0
Agar	15.0
Distilled Water	**1000 mL**

A total of 23 g of nutrient agar powder was dissolved in 1000 mL of distilled water with the aid of gentle heating. The medium was sterilized using an autoclave at 121°C and 15 lbs pressure for 15 minutes. Sterilized nutrient agar plates were prepared by pouring the hot medium into pre-sterilized Petri dishes, appropriately marked and labeled. The plates were allowed to solidify inside a laminar airflow cabinet, then stored under sterile conditions until required for microbial culturing and antimicrobial screening.[60]

Antibacterial Screening:

The cup–plate method was used. Agar plates were inoculated with bacterial suspensions, wells were created, and various concentrations of AgNPs (50–150 µg/mL) were added. Plates were incubated at 37°C for 24 hours, and the zone of inhibition was measured.

VII. Results & Discussion:

Plant extracts are naturally rich in a wide range of phytonutrients, including diverse biomacromolecules and secondary metabolites. These phytochemicals have been extensively utilized for the *ex vivo* fabrication of metal nanoparticles. As reported by Mohamad et al. [58], functional groups such as hydroxyl and carbonyl moieties present in bioactive plant constituents serve as inherent reducing agents, facilitating nanoparticle formation, surface capping, and stabilization. Hence, plant-derived extracts represent an excellent natural source for green nanoparticle synthesis, offering a process that is simple, cost-effective, efficient, and environmentally friendly [59].

Qualitative phytochemical analysis of the aqueous extract of Cascabela leaves confirmed the presence of major bioactive groups, including alkaloids, flavonoids, terpenes, and saponins (Table 7).

Table: 7 Result Of Phytochemical Screening (Qualitative):

CLASS	TEST	INFERENCE
Alkaloids	Dragendorff	+
Carbohydrates (Molish test)	Molisch	-
Glycosides	Keller-Kiliani	-
Flavonoids	Ammonia	+
Terpenoids	Liebrman	+
Tannins	Ferric Chloride	-
Saponins	Foam	+
Phytosterols	Salkowski	-

Total Phenolic Content:

A standard calibration curve of gallic acid was prepared in distilled water, and the absorbance values obtained were used to construct the plot (TABLE 8). Based on the calibration data, the Beer–Lambert law range and regression coefficient were determined. The resulting linear regression equation for gallic acid was **y = 0.005x – 0.0016**, with a correlation coefficient of **R^2 = 0.999** (Figure 1), indicating excellent linearity. The total phenolic content of the extract was quantified using the **Folin–Ciocalteu method**. The aqueous extract of *Cascabela thevetia* exhibited a total phenolic concentration of **58.85 ± 1.604 µg GAE/mg extract**.

Table: 8 Absorbance Data Of Gallic Acid (At 765 Nm):

Concentration (µg/Ml)	Absorbance At 765 Nm
10	0.051
20	0.101
30	0.147
40	0.193
50	0.248
60	0.295
70	0.341
80	0.397

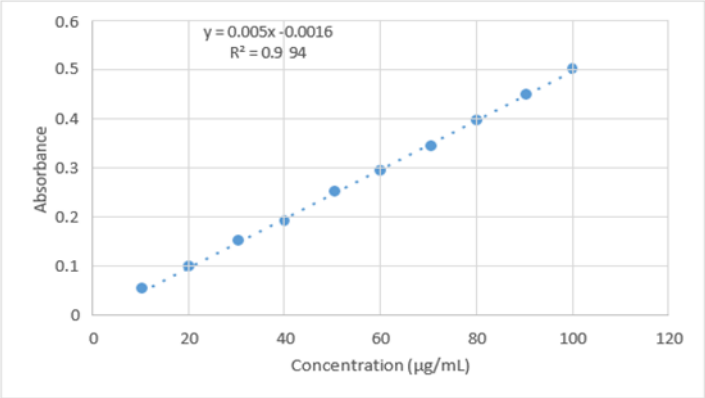

Figure:1 Calibration Curve Of Gallic Acid

Total Flavonoid Content Determination:

A standard calibration curve of quercetin was prepared, and the corresponding absorbance values were used to construct the plot (TABLE 9). From the calibration data, the Beer–Lambert law range and regression coefficient were established. The linear regression equation for quercetin was determined to be y = 0.0058x + 0.0052, with a correlation coefficient of R^2 = 0.998 (Figure 2), indicating excellent linearity. The total flavonoid content of the extract was quantified using the aluminum chloride colorimetric method. The aqueous extract of *Cascabela thevetia* exhibited a total flavonoid content of 20.71 ± 1.601 µg QE/mg extract.

Table: 9 Calibration Curve Data Of Quercetin:

Concentration Ppm	Absorbance At 620 Nm
10	0.061
20	0.127

30	0.182
40	0.233
50	0.294
60	0.352
70	0.411
80	0.485
90	0.522
100	0.587

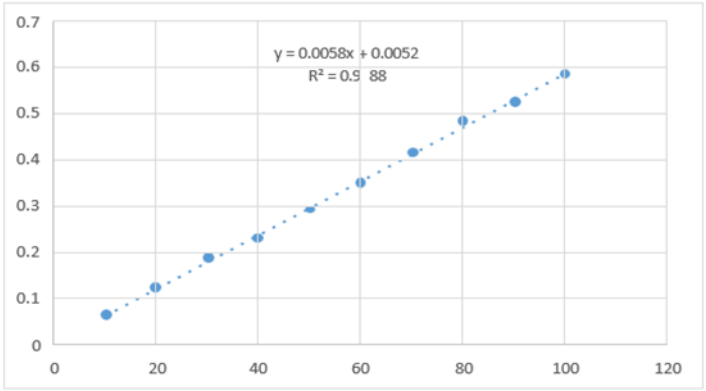

Figure:2 Calibration Curve Of Quercetin

Silver Nanoparticles Biosynthesis:

On addition of extract to the silver nitrate solution, the color of solution was yellow which started to turn brown on continual reduction of the silver ions suggesting formation of silver nanoparticles (Figure 3).

Figure:3 Color Of Solution (A) Addition Of Extract (B) Formation Of Silver Nanoparticles

The optimization of parameters for biogenic synthesis of silver nanoparticles using Cascabela thevetia leaf extract was done by analysis of particle size and polydispersity index of the particles. The optimized conditions were selected which were able to reduce the particle size and PDI of the nanoparticles (TABLE 10).

Table:10 Effect Of Optimization Variables On Particle Size And Pdi Of Silver Nanoparticles:

Experiment Name	Extract (%V/V)	Silver Nitrate (Moles)	Particle Size (Nm)	Pdi
F1	2.5	0.001	228.9	0.893
F2	5	0.001	201.6	0.717
F3	7.5	0.001	157.2	0.515
F4	2.5	0.002	288.4	0.427
F5	5	0.002	259.1	0.393
F6	7.5	0.002	213.8	0.321
F7	2.5	0.003	418.5	0.316
F8	5	0.003	382.1	0.343
F9	7.5	0.003	357.4	0.311

UV Spectral Study:

UV–Vis spectroscopy serves as a key analytical tool for confirming the formation of silver nanoparticles (AgNPs) by examining their optical characteristics and electronic transitions. When nanoparticles interact with incident light, the surface electrons oscillate collectively, absorbing radiation at specific frequencies. This collective oscillation, known as **surface plasmon resonance (SPR)**, produces a characteristic absorption band detectable by a UV–Vis spectrophotometer [60]. The UV spectrum of the optimized formulation displayed a distinct and narrow absorption peak, indicating the presence of smaller-sized nanoparticles and a high yield of AgNPs in the reaction mixture. The elevated absorbance intensity corresponds to the greater number of nanoparticles generated through the reduction of silver ions by the phytochemicals present in the plant extract [55]. The absorption maxima appeared within the range of **280–300 nm**, which is consistent with the presence of phenolic and flavonoid compounds in the extract (Figure 4). Furthermore, the sharpness of the peak suggests that the synthesized nanoparticles were predominantly **spherical in shape**.

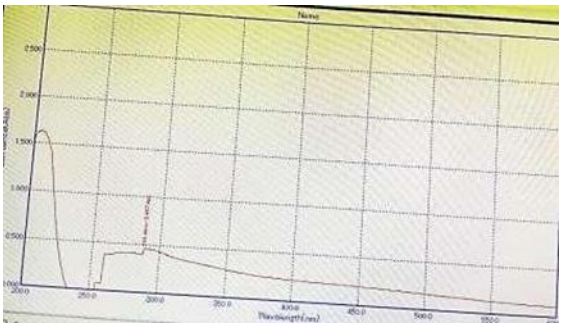

Figure: 4 Uv Spectrum Of Optimized Silver Nanoparticles

FTIR Spectral Study:

FTIR analysis was performed to identify the functional groups involved in the reduction of Ag^{+} ions and the stabilization of the synthesized AgNPs. The spectra of the *Cascabela thevetia* extract were compared with those of the resultant silver nanoparticles across the wavenumber range of **4000–500 cm^{-1}** (Figures 6.8 and 6.9). This comparison helps in determining the specific phytochemical moieties responsible for nanoparticle formation and capping.

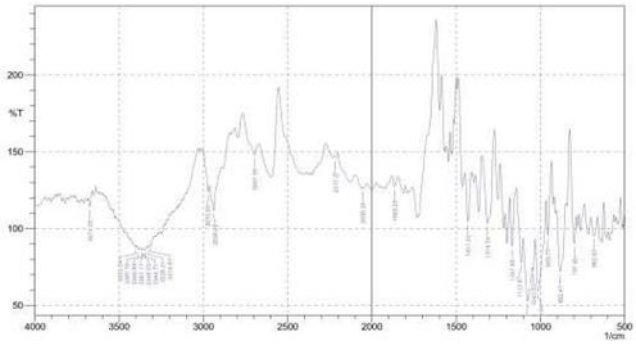

Figure: 5 Ftir Spectra Of Cascabela Thevetia Aqueous Extract

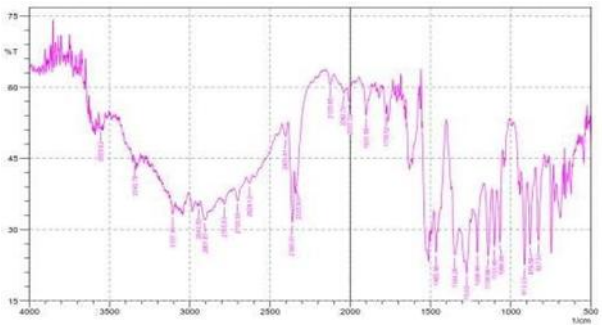

Figure: 6 Ftir Spectra Of Synthesized Silver Nanoparticles

Antibacterial Activity:

The antibacterial action of optimized nanoparticles was studied using disc diffusion method. The zone of inhibition obtained was taken as a measure of antibacterial activity. The biogenically synthesized silver nanoparticle was found to exhibit activity against both gram positive and gram negative bacteria (Figure 7).

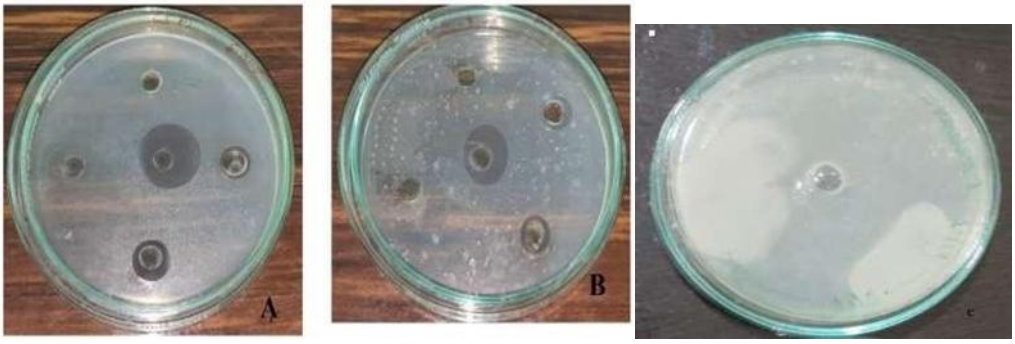

Figure:7 Antibacterial Action Of Silver Nanoparticles (A) *S. Aureus* (B) *E. Coli* (C) Control Plate

VIII. Conclusion:

Leaves of *Cascabela thevetia* were collected from the local region of Bhopal and authenticated by Dr. Shashibala Mishra, Botanist, RB Science, Bhopal. The powdered leaves were subjected to aqueous maceration for 24 hours, and the resulting extract was used for phytochemical analysis as well as for the determination of total phenolic and flavonoid content. The aqueous extract was also utilized for the green synthesis of silver nanoparticles (AgNPs), confirmed initially by the characteristic color change associated with the reduction of silver nitrate. Optimization of nanoparticle synthesis was carried out by varying extract concentration, incubation temperature, and silver nitrate concentration, with particle size and polydispersity index (PDI) serving as the primary evaluation parameters. Antibacterial activity of the synthesized AgNPs was assessed using the zone-of-inhibition method. The extraction yield was **9.58% (8.15 g)**. Qualitative phytochemical screening confirmed the presence of alkaloids, flavonoids, terpenes, and saponins in the aqueous leaf extract. Quantitative evaluation revealed total phenolic content of **58.85 ± 1.604 µg GAE/mg** and total flavonoid content of **20.71 ± 1.601 µg QE/mg**. Upon addition of the extract to silver nitrate solution, a progressive change from yellow to brown indicated the continuous reduction of Ag^+ ions and subsequent nanoparticle formation. The optimized conditions for AgNP synthesis were identified as **7.5% v/v extract**, **0.002 M silver nitrate**, and **40°C incubation temperature**. Dynamic light scattering (DLS) analysis showed that the optimized formulation had a particle size of **213.8 nm** with a **PDI of 0.321**. The zeta potential was measured at **–28.8 mV**, indicating good stability. UV–Vis spectroscopy demonstrated absorption in the **280–300 nm** range, consistent with the presence of phenolic and flavonoid constituents. SEM analysis revealed that the synthesized nanoparticles were **spherical with a rough surface morphology**. FTIR spectra confirmed functional groups such as amine, hydroxyl, and amide, suggesting their role in reduction and stabilization of AgNPs. The biogenically synthesized silver nanoparticles exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria. The phenolic and flavonoid constituents present in *Cascabela thevetia* extract are likely responsible for both the reduction of silver ions and the enhanced antimicrobial effects of the nanoparticles.

IX. Abbreviation:

ABB.	F. F	CAT.Y	ABB	F. F	CAT.Y
AFM	Atomic Force Microscopy	Instrumentation	NMR	Nuclear Magnetic Resonance	Spectroscopy
AgNPs	Silver Nanoparticles	Nanotechnology	PDI	Polydispersity Index	Nanoparticle Characterization
$AgNO_3$	Silver Nitrate	Chemical	PEG	Polyethylene Glycol	Polymer
ATR	Attenuated Total Reflectance	Spectroscopy	PPM	Parts Per Million	Unit
BC	Burst Release	Pharmaceutics	PVA	Polyvinyl Alcohol	Polymer
BSA	Bovine Serum Albumin	Biochemistry	PVP	Polyvinylpyrrolidone	Polymer
CNTs	Carbon Nanotubes	Nanomaterials	QE	Quercetin Equivalent	Phytochemical Analysis
CO_2	Carbon Dioxide	Chemical	QDs	Quantum Dots	Nanotechnology
CSE	Cascabela thevetia Extract	Plant Extract	RSM	Response Surface Methodology	Statistics
C.V.	Coefficient of Variation	Statistics	rpm	Revolutions Per Minute	Unit
DLS	Dynamic Light Scattering	Characterization	RT	Room Temperature	Condition

DNA	Deoxyribonucleic Acid	Biology	SA/V	Surface-Area-to-Volume Ratio	Nanoscience
DPPH	2,2-Diphenyl-1-picrylhydrazyl	Antioxidant Assay	SEM	Scanning Electron Microscopy	Imaging
EDX/EDS	Energy-Dispersive X-ray Spectroscopy	Instrumentation	SLNs	Solid Lipid Nanoparticles	Nanocarriers
FTIR	Fourier Transform Infrared Spectroscopy	Spectroscopy	SPM	Scanning Probe Microscopy	Instrumentation
GAE	Gallic Acid Equivalent	Phytochemical Analysis	SPR	Surface Plasmon Resonance	Optical Property
GCMS	Gas Chromatography–Mass Spectrometry	Instrumentation	TEM	Transmission Electron Microscopy	Imaging
GVE-SNP	Guava Phenolic Extract-Functionalized Silver Nanoparticles	Nanotechnology	TPP	Tripolyphosphate (Sodium Tripolyphosphate)	Chemical
HRTEM	High-Resolution Transmission Electron Microscopy	Imaging	TPTZ	2,4,6-Tripyridyl-s-triazine	Reagent
HPLC	High-Performance Liquid Chromatography	Analytical Instrument	UV–Vis	Ultraviolet–Visible Spectroscopy	Spectroscopy
IC_{50}	Half Maximal Inhibitory Concentration	Pharmacology	XPS	X-ray Photoelectron Spectroscopy	Surface Analysis
IM	Intramuscular	Administration Route	XRD	X-ray Diffraction	Crystallography
IV	Intravenous	Administration Route	v/v	Volume per Volume	Unit
LOD	Limit of Detection	Analytical Validation	mL	Millilitre	Unit
LOQ	Limit of Quantification	Analytical Validation	µg/mL	Micrograms per Millilitre	Unit
MIC	Minimum Inhibitory Concentration	Microbiology	µg	Microgram	Unit
MNPs	Metal Nanoparticles	Nanotechnology	nm	Nanometre	Unit
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide	Cytotoxicity Assay	M	Molar Concentration (Moles per Litre)	Unit
NPs	Nanoparticles	Nanotechnology	°C	Degrees Celsius	Unit
pH	Potential of Hydrogen	Unit	lbs	Pounds Pressure	Unit

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