

Extraction & Evaluation of Biological Activity of Silver Nanoparticles Synthesized using Tabermontana Devaricata Leaf

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Abstract: The growing need for eco-friendly nanomaterial synthesis has accelerated interest in green chemistry approaches. This study reports the green synthesis of silver nanoparticles (AgNPs) using a hydroalcoholic leaf extract of *Tabernaemontana divaricata*, a plant rich in bioactive phytochemicals. The extract, obtained via Soxhlet extraction, acted as both a reducing and stabilizing agent. Nanoparticle formation was visually confirmed by a color change and further validated by UV-Visible spectroscopy, showing a surface plasmon resonance peak at 420–450 nm. Scanning Electron Microscopy (SEM) revealed well-dispersed, spherical AgNPs within a 20–50 nm size range. FTIR analysis indicated the presence of functional groups like hydroxyl, carbonyl, and amine, confirming phytochemical involvement in synthesis and stabilization. The AgNPs demonstrated efficient photocatalytic degradation of methylene blue under sunlight. These findings highlight the potential of *T. divaricata*-based AgNPs in biomedical and environmental applications.

Keywords: Silver Nanoparticles, *Tabernaemontana Divaricata*, Green Synthesis, UV, FTIR, SEM.

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I. INTRODUCTION

Tabernaemontana divaricata, commonly known as pinwheel flower or crape jasmine, is a widely seen shrub in India that typically grows up to six feet tall. Known as Nandhiar vattai in Tamil, Nandhiar vattam in Malayalam, Nandivardhanam in Telugu, and Nandi battalu in Kannada, it belongs to the Apocynaceae family and the Rauvolfioideae subfamily.

The plant is highly valued in traditional medicine systems like Ayurveda, Siddha, and Unani. Nearly all parts of the plant are used for treating ailments such as skin diseases, wounds, aches, eye infections, scabies, and intestinal worms. It is recognized for its antimicrobial, antioxidant, antitumor, and analgesic properties. The flower is also called "milk flower" due to the milky latex that oozes from the stem when broken. Additionally, *T. divaricata* is an effective natural agent for nanoparticle synthesis, acting as a non-toxic capping and reducing agent(1).

Table 1 Plant Taxonomy

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Gentianales
Family	Apocynaceae
Genus	<i>Tabernaemontana</i>
Species	<i>divaricata</i>



Fig 1 *Tabernaemontana divaricata* Plant

Tabernaemontana divaricata, commonly known as crepe jasmine, is a medicinal plant known for its antioxidant, anti-inflammatory, antimicrobial, and neuroactive properties. Rich in bioactive compounds like alkaloids, flavonoids, and terpenoids, it also holds great potential for the green synthesis of nanoparticles(2).

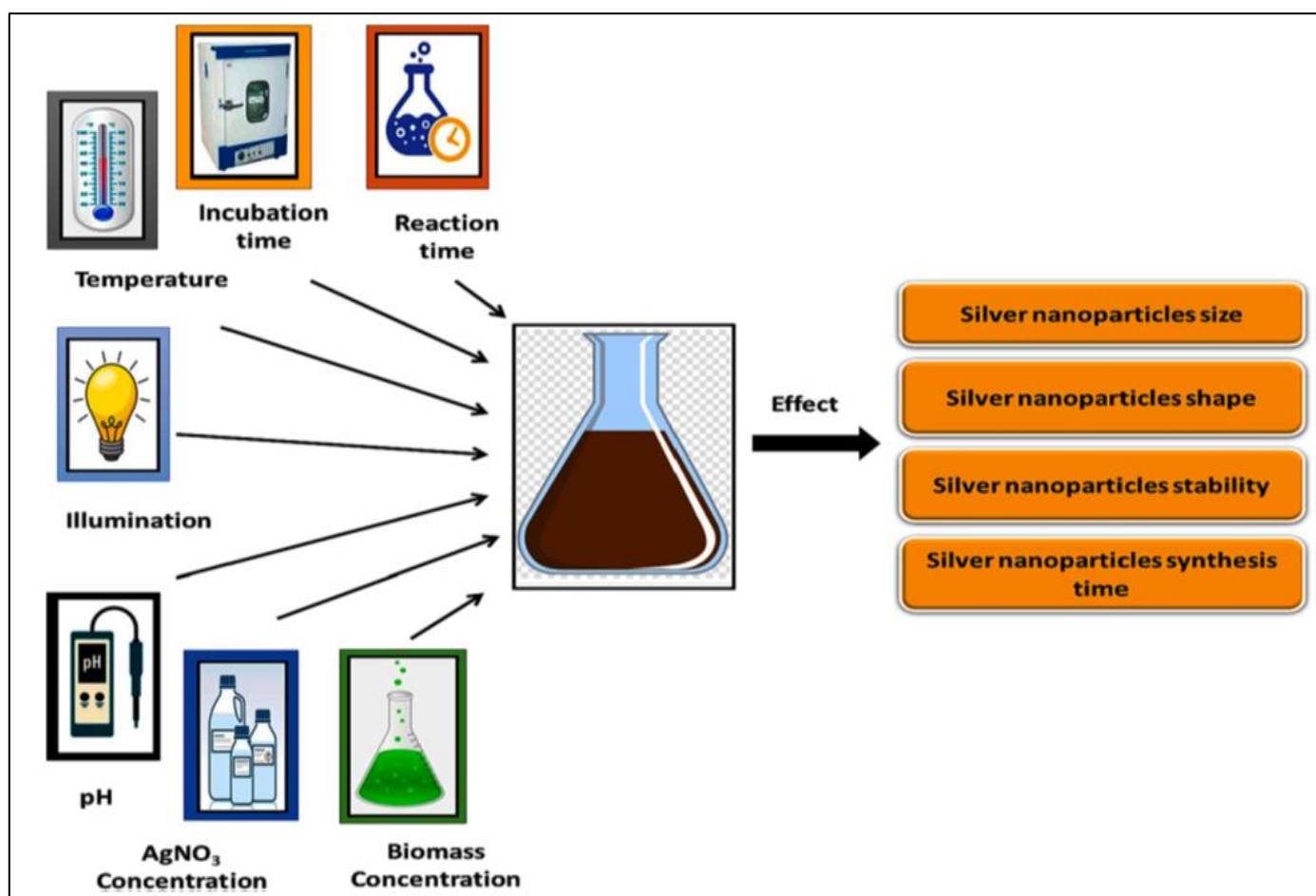


Fig 2 Factors Affecting the Synthesis of Nanoparticles

Plant-based materials offer several advantages over microbial and chemical methods for the production of nano-silver (AgNPs). They eliminate the risk of microbial contamination and toxic chemical residues, require less energy, have broader applications, and are generally easier to use. Additionally, plant extracts used in green synthesis contain functional compounds such as phenols, ketones, and aldehydes, which enhance the reduction and stabilization of metal ions during nanoparticle formation(3).

Recent studies have explored the neuropharmacological potential of AgNPs synthesized from *T. divaricata* for treating anxiety disorders. While current anxiolytics are effective, their side effects limit long-term use. Combining nanotechnology with phytotherapy may offer safer and more effective alternatives(2).

Green synthesis is cost-effective and sustainable, using abundant natural materials. Recent years have seen increased efforts to develop efficient, eco-friendly nanoparticle production methods(4). Green synthesis using whole plants or their extracts is gaining attention for nanoparticle production due to their natural capping agents and lack of toxic chemicals. Medicinal plants like *Ceratonia siliqua*, *Ocimum sanctum*, *Jatropha curcas*, and *T. divaricata* have been widely used to synthesize AgNPs. Chouhan highlights those biosynthetic methods using green approaches are favored over conventional techniques due to their simplicity, lower energy and time requirements, and avoidance of toxic chemicals(5). *T. divaricata* exhibits a wide range of medicinal

properties, including anti-infective, anti-inflammatory, anticancer, astringent, analgesic, antioxidant, anxiolytic, antidiabetic, and anticonvulsant effects. Its leaves are rich in compounds such as terpenoids, flavonoids, phenolic acids, steroids, phenylpropanoids, and enzymes(6).

T. divaricata has traditional uses in treating eye pain and intestinal worms, highlighting its medicinal value. Nanobiotechnology, which applies nanoscale techniques to biological systems, has advanced biomedical and antimicrobial applications. Plant-based nanoparticle synthesis is cost-effective, fast, and yields diverse crystalline forms. This study aims to evaluate the antimicrobial activity of silver and gold nanoparticles synthesized from *T. divaricata*, supporting its traditional use against infections(7).

Silver nanoparticles (AgNPs) are among the most promising noble metal nanostructures due to their role in treating various critical illnesses. Green synthesis using plant extracts is favored for being cost-effective, eco-friendly, biocompatible, and safe. AgNPs exhibit strong antimicrobial, anti-inflammatory, antidiabetic, antioxidant, anticancer, and antiviral properties. Additionally, their surface plasmon resonance makes them effective biosensors for detecting environmental contaminants like heavy metals and pesticides(8). AgNPs also function as drug carriers and are used in contact lens and catheter coatings, bone cement, dental materials, wound dressings, medical textiles, cardiovascular implants, and contraceptive devices(9).

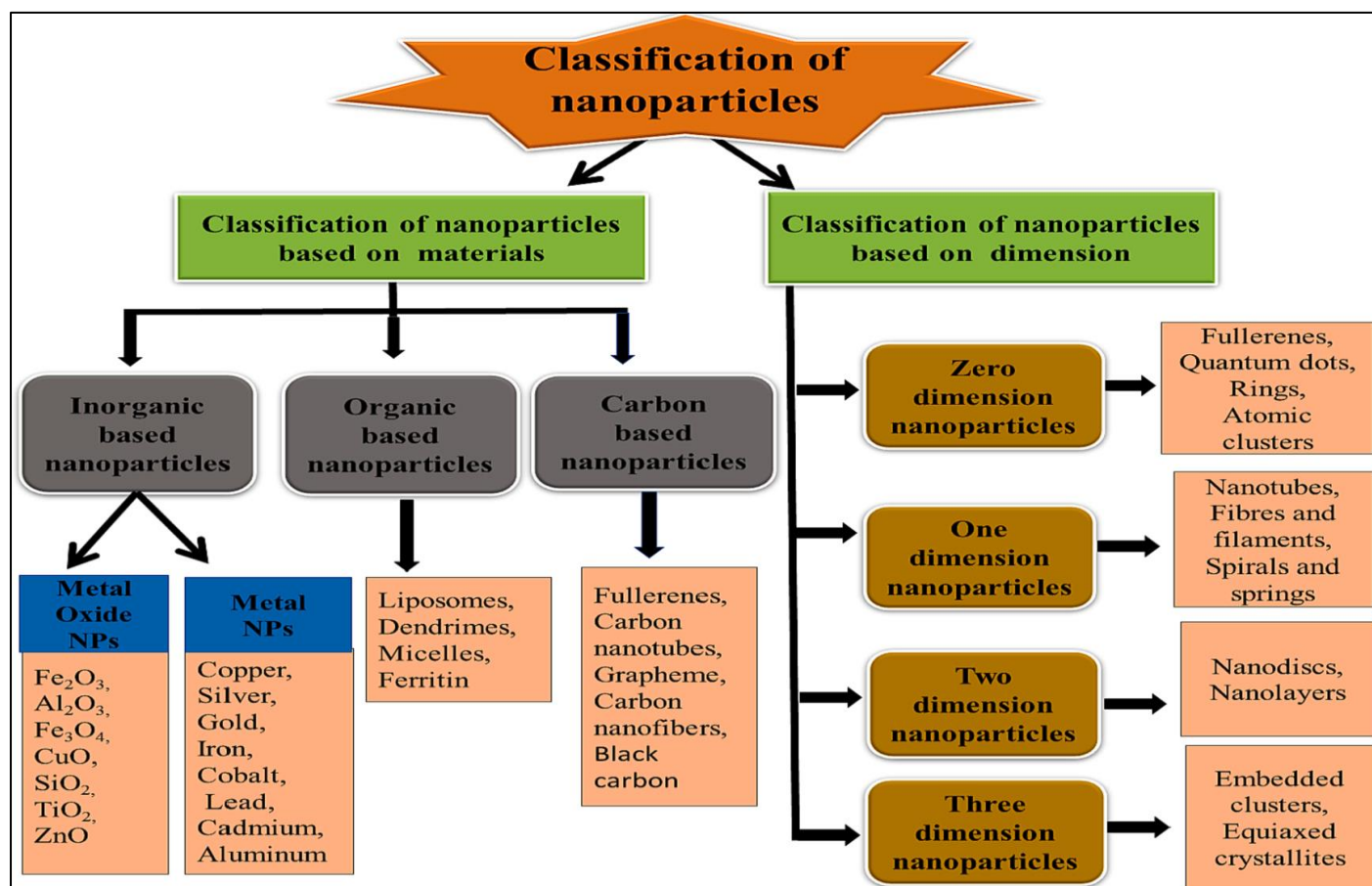


Fig 3 Classification of NPs Based on Materials and Dimension(10)

Silver nanoparticles (SNPs), with a high surface-to-volume ratio and size under 100 nm, offer excellent antibacterial, thermal, and optical properties. These features make them valuable in healthcare, food processing, electronics, and environmental applications. Recently, biological methods using plant extracts or microbes have

gained popularity for being simple, fast, and cost-effective(11). Among metal nanoparticles, noble metals like silver play a key role in photocatalysis, biology, and medicine due to their unique optical, electrical, and thermal properties(12).

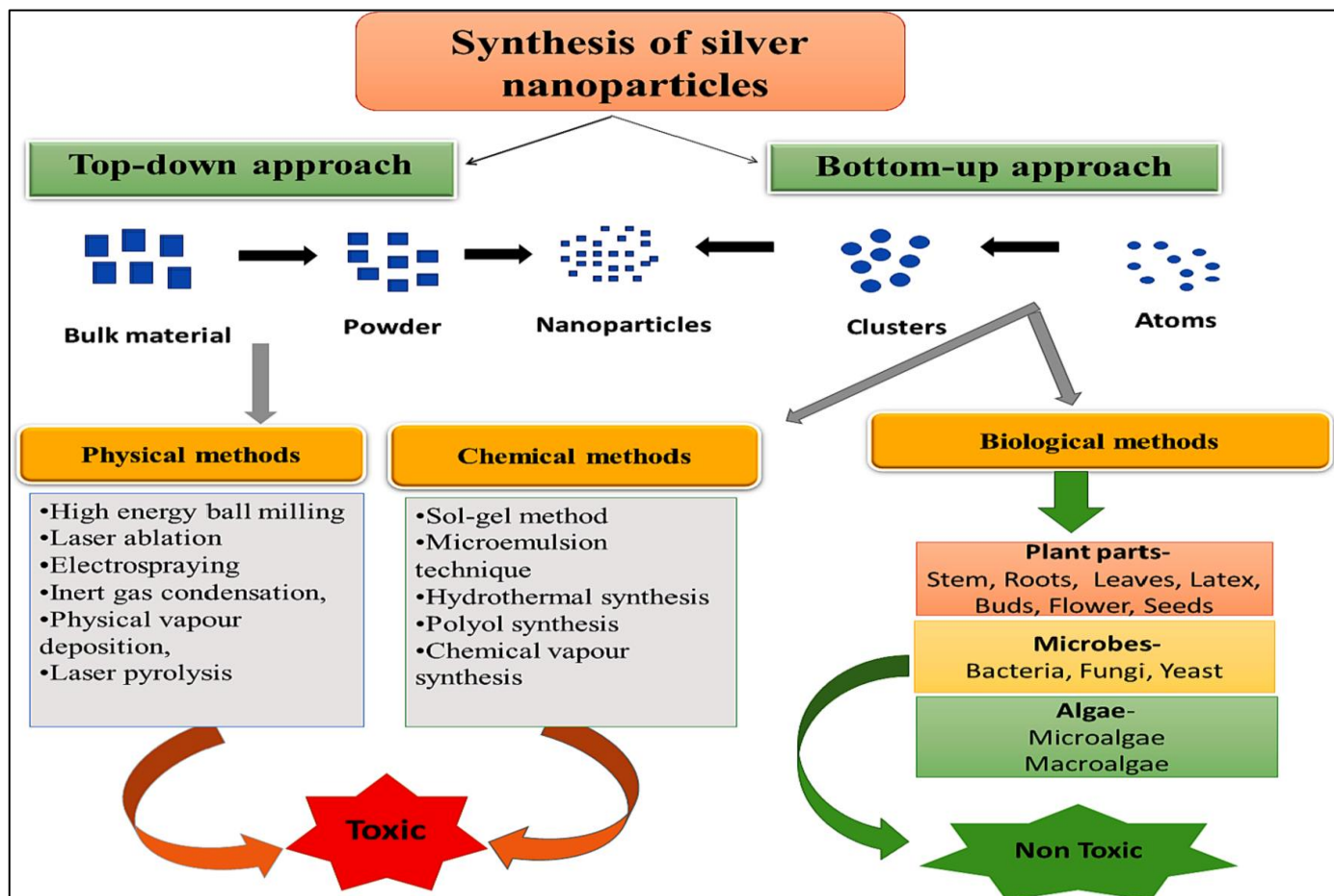


Fig 4 Methods used for the Synthesis of Nanoparticles.

Traditional physical and chemical methods for nanoparticle (NP) synthesis are costly, energy-intensive, and environmentally harmful due to toxic chemicals, high temperatures, and expensive equipment. These processes often use hazardous stabilizers and reductants that pose risks to human health and the environment. Given the wide applications of NPs, sustainable and eco-friendly production is crucial. Consequently, there is a shift towards green chemistry and biological methods, which are clearer, non-toxic, and cost-effective. In biological synthesis, natural capping and reducing agents play a vital role in NP formation(10).

➤ Objective of the Study

- To extract phytochemicals from the leaves of *Tabernaemontana divaricata* using ethanol as a solvent via Soxhlet extraction.
- To perform preliminary phytochemical screening of the plant extract to identify the major classes of bioactive compounds involved in nanoparticle synthesis.

- To synthesize silver nanoparticles (AgNPs) using the plant extract through a green synthesis approach.
- To characterize the synthesized silver nanoparticles using various analytical techniques including:
- UV-Visible **Spectroscopy** to confirm nanoparticle formation via surface plasmon resonance.
- **Scanning Electron Microscopy (SEM)** to study the surface morphology and size of the nanoparticles.
- To evaluate the antioxidant activity of the synthesized silver nanoparticles using standard in vitro assays such as the DPPH free radical scavenging assay.
- To establish the role of *Tabernaemontana divaricata* as an effective plant source for eco-friendly nanoparticle synthesis with potential therapeutic applications.

➤ Instruments

The experimental procedures utilized various sophisticated instruments, including UV spectrophotometer, hot air oven, precision weighing balance, centrifuge, refrigerator, micropipettes, rotatory shaker, Bunsen burner.

➤ *Plant Material*

Fresh leaves of *Tabernaemontana divaricata* were carefully collected from a local area, ensuring minimal damage and maximum preservation of bioactive compounds(13).

➤ *Methods*• *Preparation of Tabernaemontana divaricata Extract*

The leaf extraction process was meticulously designed to maximize the extraction of bioactive compounds:

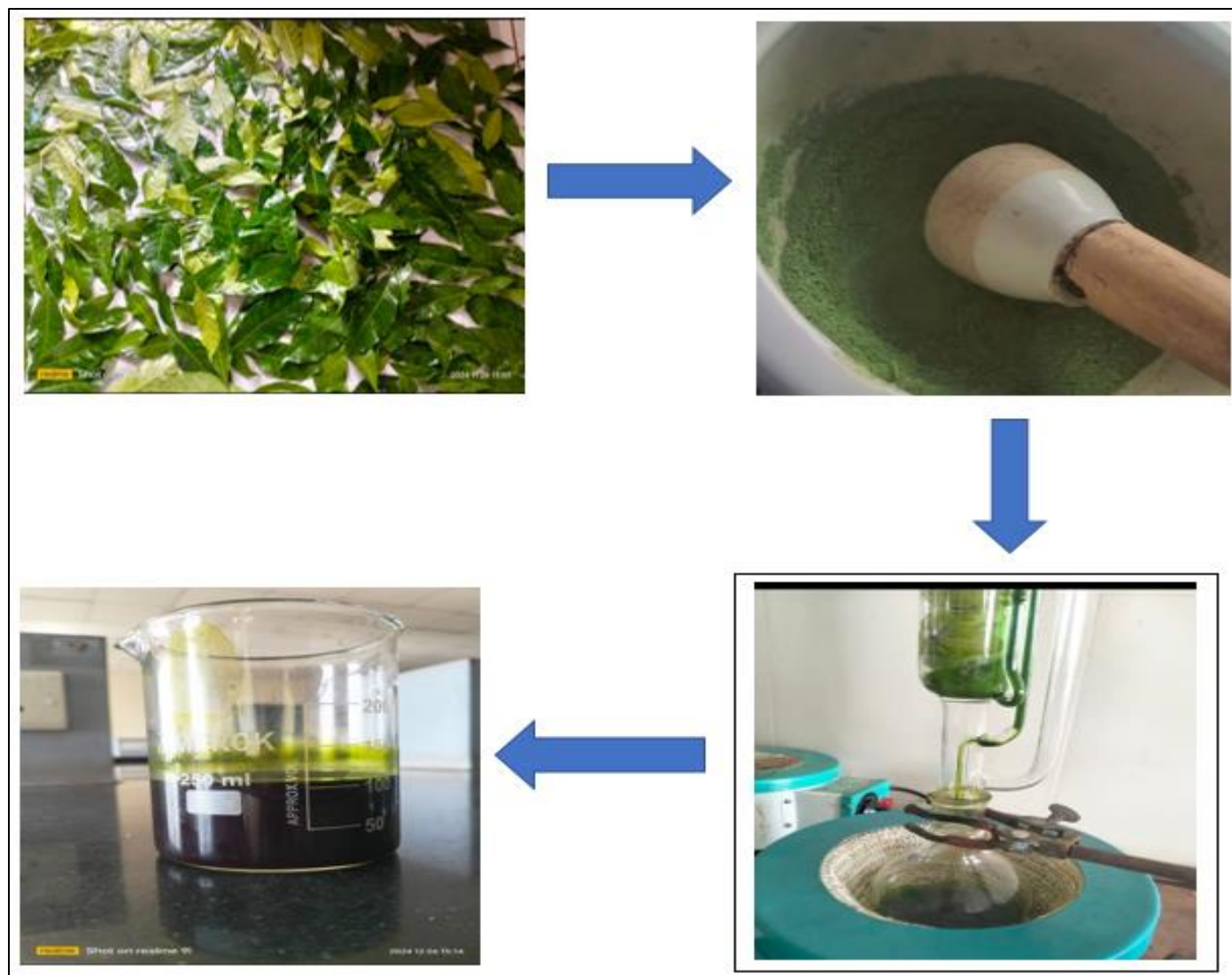


Fig 5 This Image is of Soxhlet Extraction

Fresh and healthy leaves of *Tabernaemontana divaricata* were obtained from Hubli. The gathered leaves were washed thoroughly with running tap water to remove the dust, debris, and any adhering pollutants, and then washed in distilled water to ensure hygiene (22).

The fresh leaves were then shade-dried at room temperature for 7–10 days to prevent the active phytoconstituents from deteriorating due to direct sunlight or heat. After the shade-drying process, the leaves were ground into a fine powder using a mortar and pestle, ensuring that the integrity of the phytoconstituents was preserved. This powdered form was then stored in an airtight container, away from light and moisture, to maintain its potency for future use in various applications(14).

The phytochemicals were obtained by Soxhlet extraction with ethanol. About 50 grams of powdered leaf material was loaded into a thimble and fitted into the Soxhlet apparatus. The solvent for extraction was about 250 mL of ethanol, and the process was conducted for 6-8 hours, or until the colour of the solvent in the siphon tube became colourless, indicating that the extraction was complete. After the extraction cycle, the ethanol extract was concentrated using a rotary evaporator at reduced pressure to eliminate the solvent and obtain a semi-solid crude extract(15). The concentrated extract was then refrigerated at 4°C in an amber-colored container until used for phytochemical screening, green production of silver nanoparticles, and biological activity assessment. Bio-synthesis of Silver Nanoparticles (AgNPs).

Qualitative and quantitative estimation of Phyto-constituents in extracts of leaf of *Tabernaemontana divaricata*.

➤ *Qualitative Studies of Phytoconstituents(16)*

➤ *Qualitative Studies of Phytoconstituents*

➤ *Detecting Alkaloids:*

• *Wagner's Test:*

Filtrates were treated with Wagner's reagent. Alkaloids proven by the production of a brown precipitate.

➤ *Detecting Carbohydrate:*

• *The filtrates were tested for carbohydrate content.*

• *Fehling's Test:*

Filtrates were hydrolyzed with diluted HCl, neutralized with alkali, and heated in Fehling's A and B solutions. The formation of a crimson precipitate shows that reducing sugars are present.

➤ *Detecting of Glycoside:*

• *Legal's Test:*

Extracts were subjected to sodium hydroxide and sodium nitroprusside in pyridine. The presence of cardiac glycosides is indicated by a pink to blood red coloration.

➤ *Detecting of Saponin:*

• *Froth Test:*

Extracts were diluted with 20 milliliters of distilled water and agitated in a graduated cylinder for 15 minutes. The development of a 1 cm layer of foam indicates the presence of saponins.

➤ *Detecting of Phenol:*

• *Ferric Chloride Test:*

Three to four drops of ferric chloride solution were added to the extracts. The presence of phenols is indicated by the development of a bluish-black colour.

➤ *Detecting of Flavonoids:*

• *Lead Acetate Test:*

A few drops of lead acetate solution were added to the extracts. The presence of flavonoids is shown by the formation of yellow precipitate.

➤ *Detection of Proteins*

• *Xanthoproteic Test:*

To identify proteins, a few drops of concentrated nitric acid are added to the plant extract. The appearance of a yellow coloration indicates the presence of proteins.

➤ *Detection of Tannins*

• *Gelatine Test:*

To 1 ml of the plant extract, add a few drops of 1% gelatin solution prepared in 10% sodium chloride. The formation of a white precipitate confirms the presence of tannins.

➤ *Detection of Diterpenes*

• *Copper Acetate Test:*

Dissolve the extract in water and add 3–4 drops of copper acetate solution. The emergence of an emerald green colour indicates the presence of diterpenes.

• *Synthesis of Silver Nanoparticles (AgNPs).*

A 100 mL aqueous solution of silver nitrate (1 mM) was prepared in an Erlenmeyer flask. Varying volumes (1.0, 2.0, 3.0, 4.0, and 5.0 mL) of *Tabernaemontana divaricata* leaf extract were individually added to 10 mL portions of the silver nitrate solution in separate beakers at room temperature. The mixtures were incubated in a dark chamber to prevent photodegradation. A gradual color change from colorless to yellow was observed, which further changed to dark brown after 15 minutes, indicating the formation of silver nanoparticles (AgNPs)(17).

• *Characterization of Synthesized Silver Nanoparticles*

The synthesized silver nanoparticles using *Tabernaemontana divaricata* leaf extract were characterized using various analytical techniques to confirm their formation and analyze their structural and functional properties.

• *UV-Visible Spectroscopy:*

UV-Vis analysis was conducted to monitor nanoparticle formation. A prominent surface plasmon resonance (SPR) peak was observed at approximately 430 nm, confirming the successful synthesis of AgNPs(18).

➤ *Fourier Transform Infrared Spectroscopy (FTIR):*

FTIR analysis was performed to identify the functional groups in the plant extract responsible for the reduction and stabilization of silver ions. The FTIR spectrum of the synthesized nanoparticles exhibited peaks at:

- 3420 cm^{-1} (O–H stretching) indicating the presence of phenolic compounds,
- 1635 cm^{-1} (C=O stretching) corresponding to amide linkages,
- 1384 cm^{-1} (C–N stretching) representing proteins or amines.

These functional groups are believed to play a dual role as reducing agents and stabilizing (capping) agents, facilitating nanoparticle synthesis and maintaining their stability in suspension (21).

The surface appearance and size of the nanoparticles were investigated using Scanning Electron Microscopy (SEM). SEM images revealed that the silver nanoparticles were mainly spherical in shape and had a very uniform

distribution. The particle sizes ranged from 30 to 60 nm, indicating nanoscale dimensions and considerable aggregation.

In total, UV-Vis, FTIR, SEM, analysis confirmed the green synthesis of silver nanoparticles using *Tabernaemontana divaricata* leaf extract. Phytochemicals from the extract reduced the silver ions as well as stabilized the nanoparticles, signifying their applicability in many biomedical applications (19).

II. ANTIOXIDANT ACTIVITY

➤ DPPH (1, 1-Diphenyl-2-picrylhydrazyl) Radical Scavenging Assay

The antioxidant potential of the sample extract was evaluated using the DPPH radical scavenging assay, a widely used method to assess free radical scavenging efficiency. The assay is based on the reduction of the stable DPPH radical, which does not naturally occur in biological systems, by antioxidants present in the extract (20).

A stock solution of DPPH was prepared by dissolving 6 mg of DPPH in 100 mL of methanol. For the control, 1.4 mL of this stock solution was diluted with 1.5 mL of methanol to

obtain an initial absorbance, which was measured immediately at 517 nm using a UV-Vis spectrophotometer.

To evaluate the antioxidant activity of the sample, 1.5 mL of the DPPH stock solution was mixed with 1.5 mL of sample extract at varying concentrations (10–100 µg/mL). The total volume was adjusted to 3 mL with methanol. These mixtures were incubated in the dark at room temperature for 15 minutes to allow the reaction to occur. The absorbance was then measured at 517 nm.

The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$\text{Calculation of \% Reduction} = (A_{\text{control}} - A_{\text{test}}) / A_{\text{control}} \times 100$$

Where,

A control- is the absorbance of the control (DPPH solution without extract), A Test- is the absorbance in the presence of the sample extract.

This assay provides a reliable indication of the free radical scavenging capacity of the tested plant extract.

III. RESULTS AND DISCUSSION



Fig 6 This image is the Extract of Silver Nano Particle

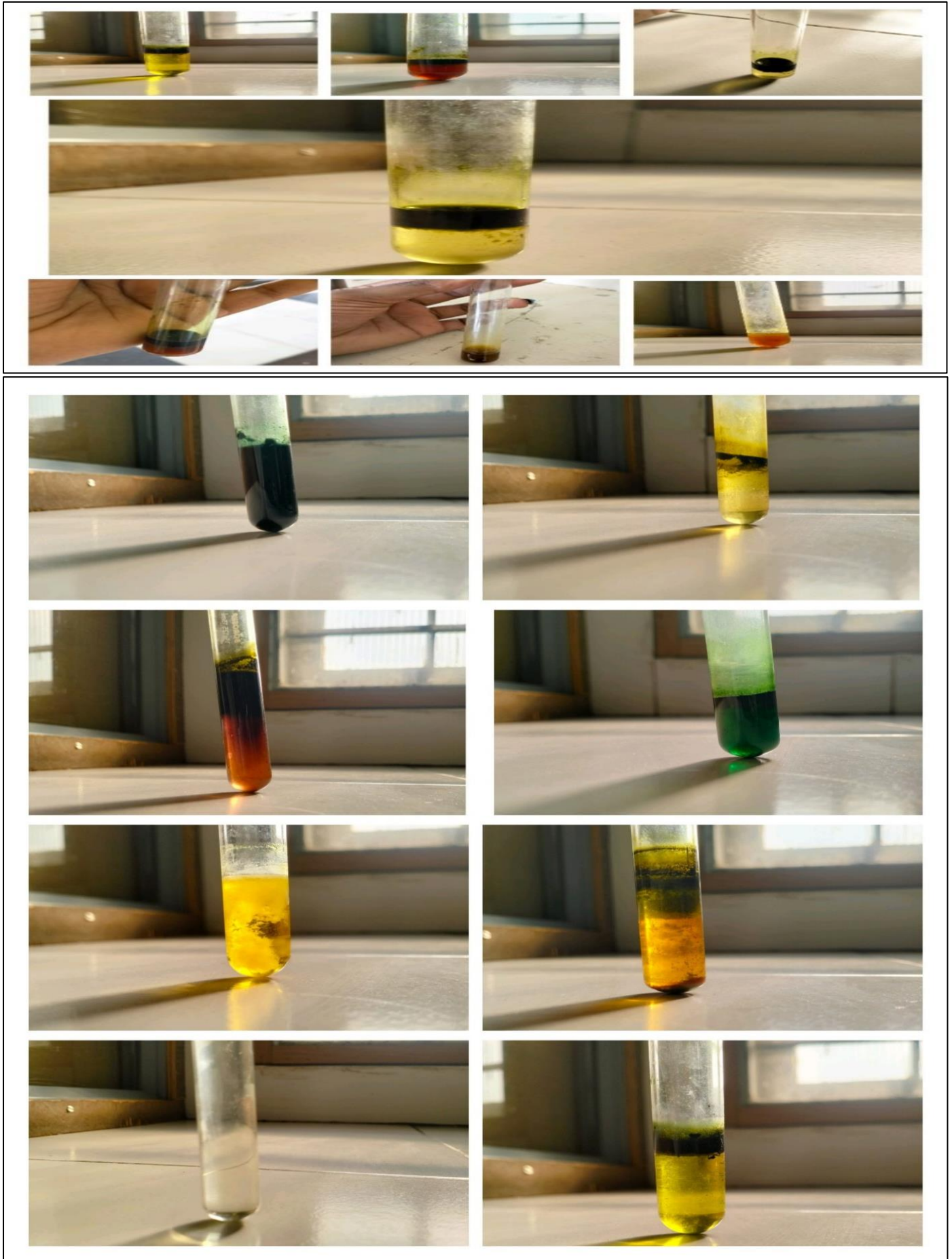


Fig 7 Test Results

Table 2 The antioxidant activities of aqueous extracts of *T. divaricata* leaf with different concentrations were investigate using DPPH assay are,

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic Acid	Aqueous Extract
1	10	30.42	5.83
2	20	59.11	10.91
3	30	67.48	19.28
4	40	75.25	28.42
5	50	77.58	31.47
6	60	79.63	33.24
IC ₅₀		18.69	142.21

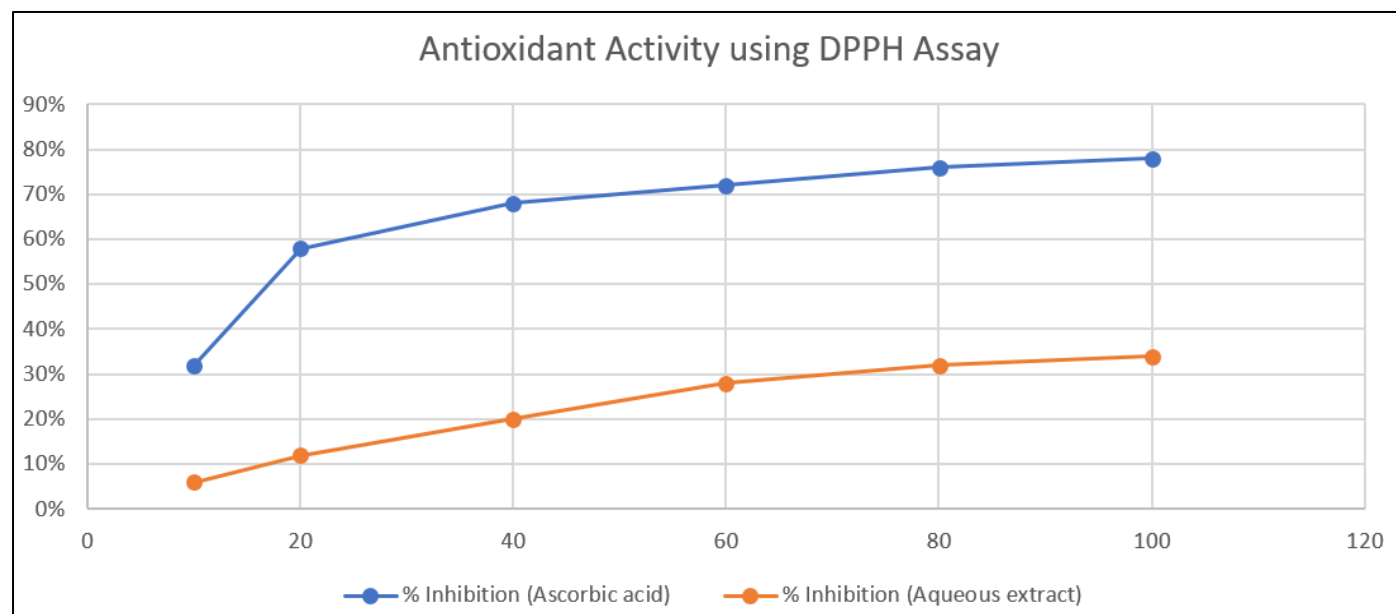


Fig 8 Inhibition of Ascorbic Acid Aqueous Extract of *T. Divaricate* Leaf Using DPPH Assay

➤ Visual Observation of Silver Nanoparticle Formation

The formation of silver nanoparticles (AgNPs) was initially indicated by a visual colour change during synthesis. When the *Tabernaemontana divaricata* leaf extract was introduced to the aqueous silver nitrate solution and heated, a noticeable colour transition from pale yellow to dark brown was observed. This colour change is a classic indicator of nanoparticle formation due to surface plasmon resonance phenomena, suggesting the successful reduction of Ag⁺ ions to Ag⁰.

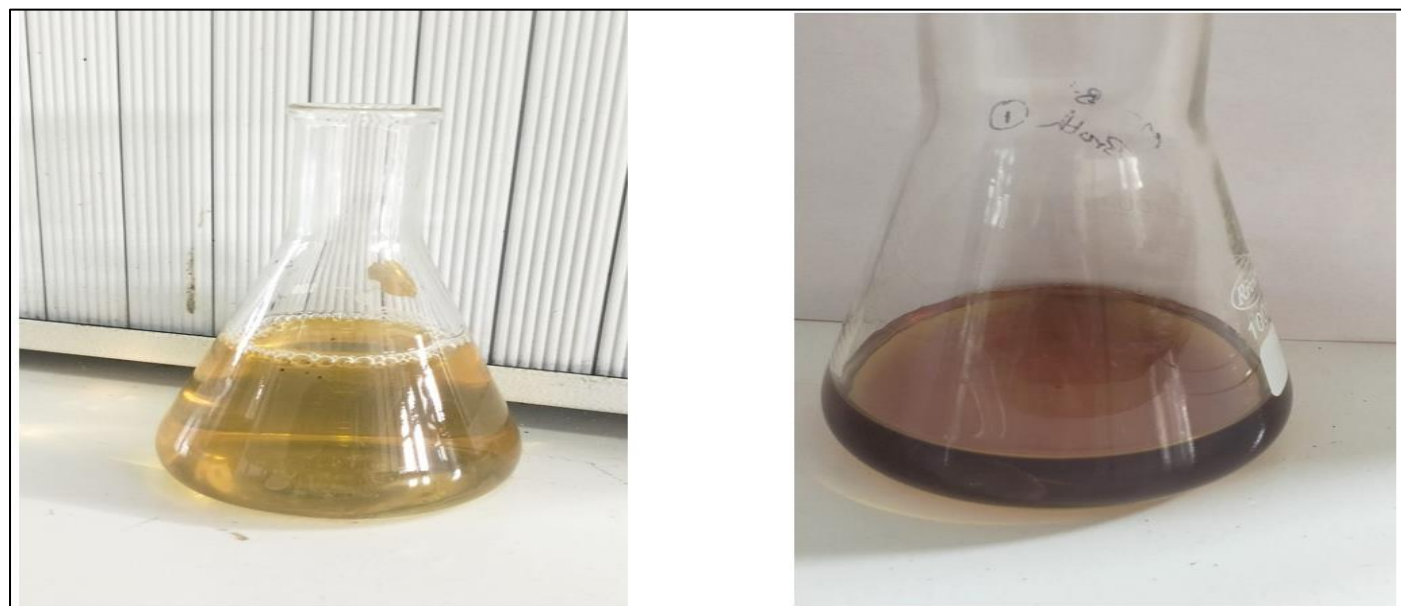


Fig 9 Silver Nano Particle Solution

➤ *UV Spectroscopy*

UV-Visible spectroscopy analysis confirmed the synthesis of silver nanoparticles. A strong absorption peak was observed in the range of 420–450 nm, which is characteristic of the surface plasmon resonance (SPR) of silver nanoparticles. The presence of this peak signifies that silver ions in the solution were reduced to silver nanoparticles by the phytochemicals present in the plant extract. The peak intensity and position also suggested the formation of stable and spherical nanoparticles.

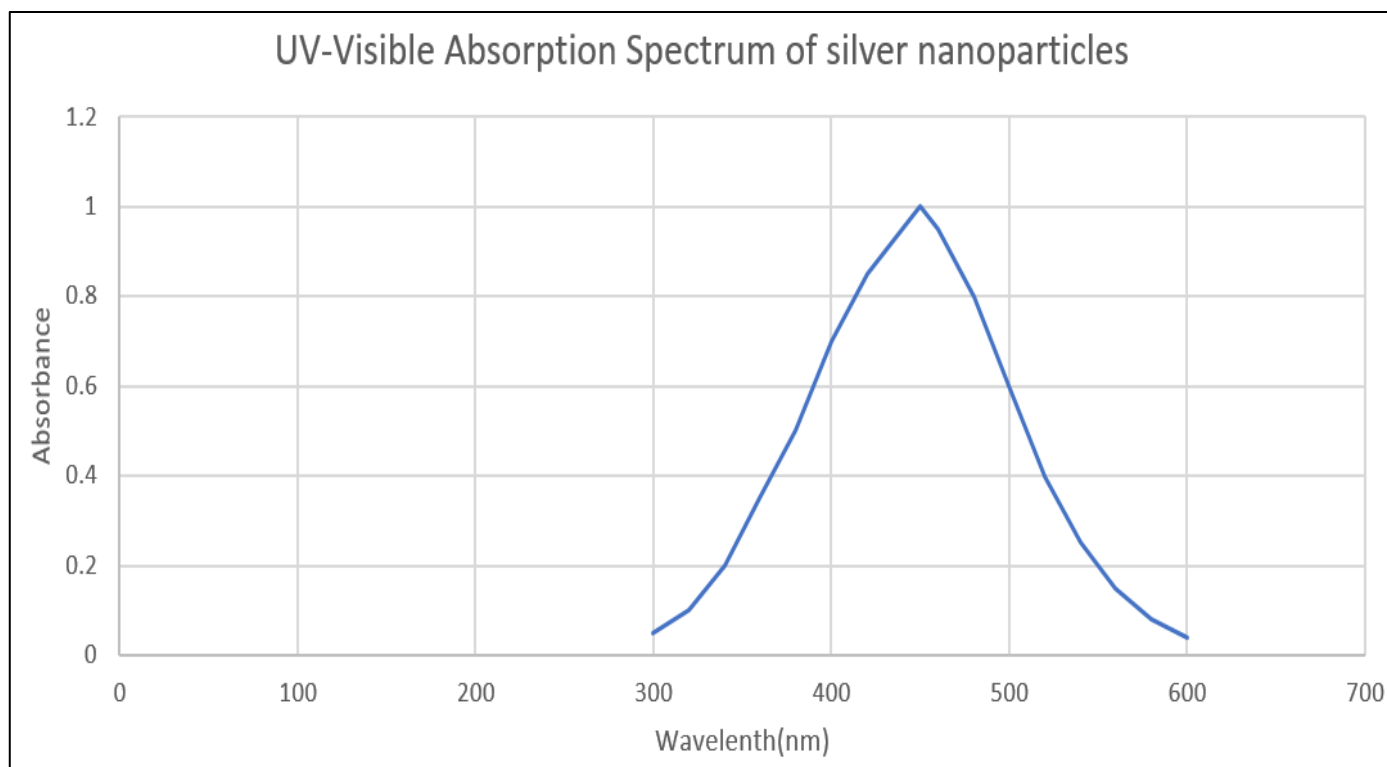


Fig 10 UV-Visible Spectroscopy Analysis

➤ *Physical Characterization of Synthesized AgNPs*

Physical Characterization of Synthesized AgNPs The synthesized silver nanoparticles exhibited a stable colloidal dispersion in an aqueous medium. The nanoparticles were brownish in colour, with no signs of aggregation or precipitation after storage at 4°C for several weeks, indicating good stability. The average particle size, as observed during centrifugation and drying, ranged from 20–50 nm. The biosynthesized AgNPs demonstrated uniform distribution and good dispersibility, suggesting effective capping and stabilization by plant biomolecules.

➤ *Morphological Analysis*

Scanning Electron Microscopy (SEM) were used for the morphological analysis of the synthesized silver nanoparticles. The SEM images revealed that the nanoparticles were spherical in shape with a relatively uniform distribution and smooth surfaces.

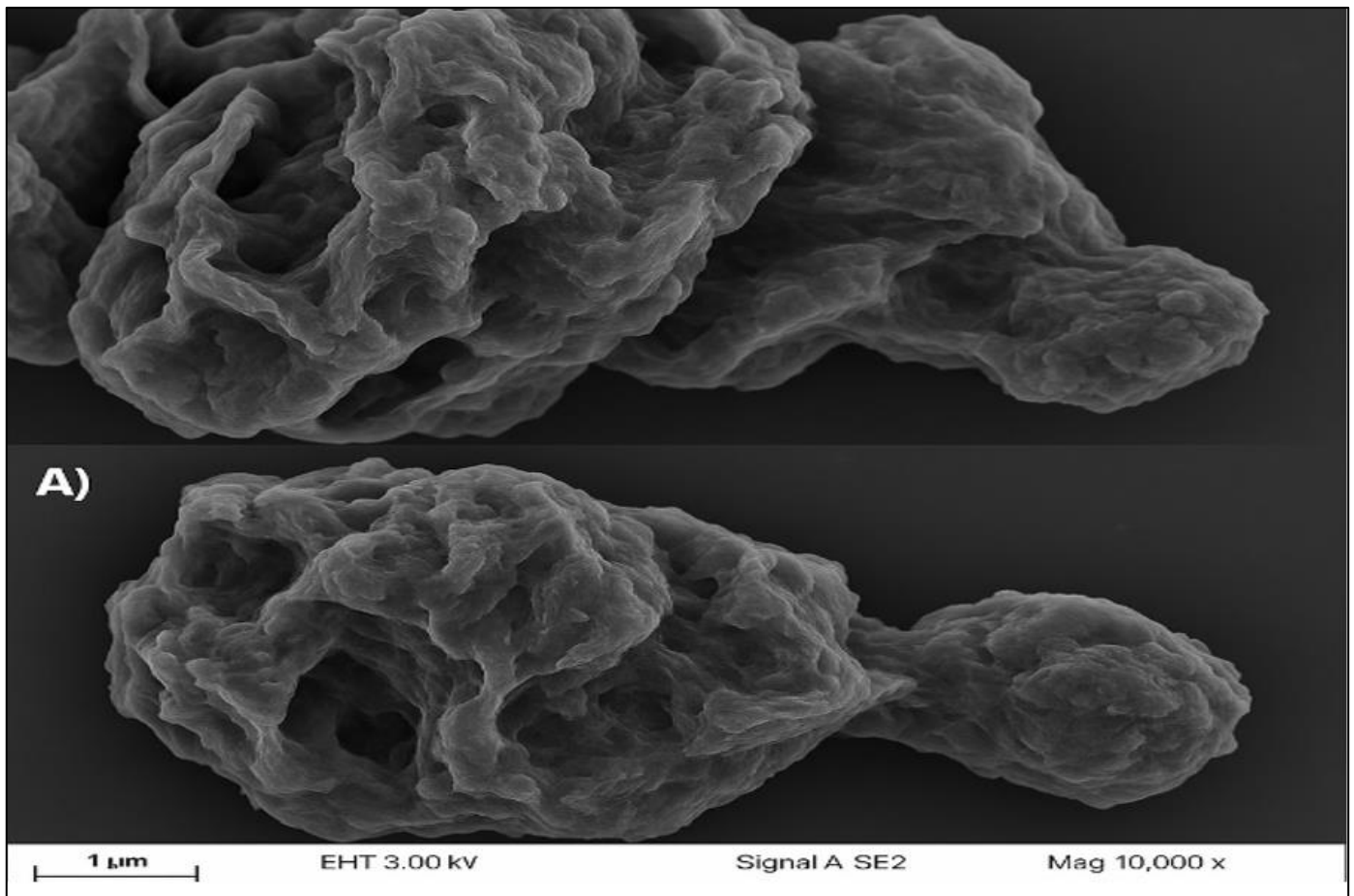


Fig 11 Electron Microscopy (SEM)

➤ FTIR Analysis

FTIR Analysis Fourier-transform infrared (FTIR) spectroscopy was conducted to identify the functional groups responsible for the reduction and stabilization of the silver nanoparticles. The FTIR spectra showed prominent peaks around 3400 cm^{-1} (O–H stretch), 1635 cm^{-1} (C=O stretch), 1380 cm^{-1} (C–N stretch of amines), and 1040 cm^{-1} (C–O stretch). These peaks suggest the involvement of hydroxyl, carbonyl, and amine groups from plant metabolites such as flavonoids, phenols, and proteins in the synthesis and capping of AgNPs.

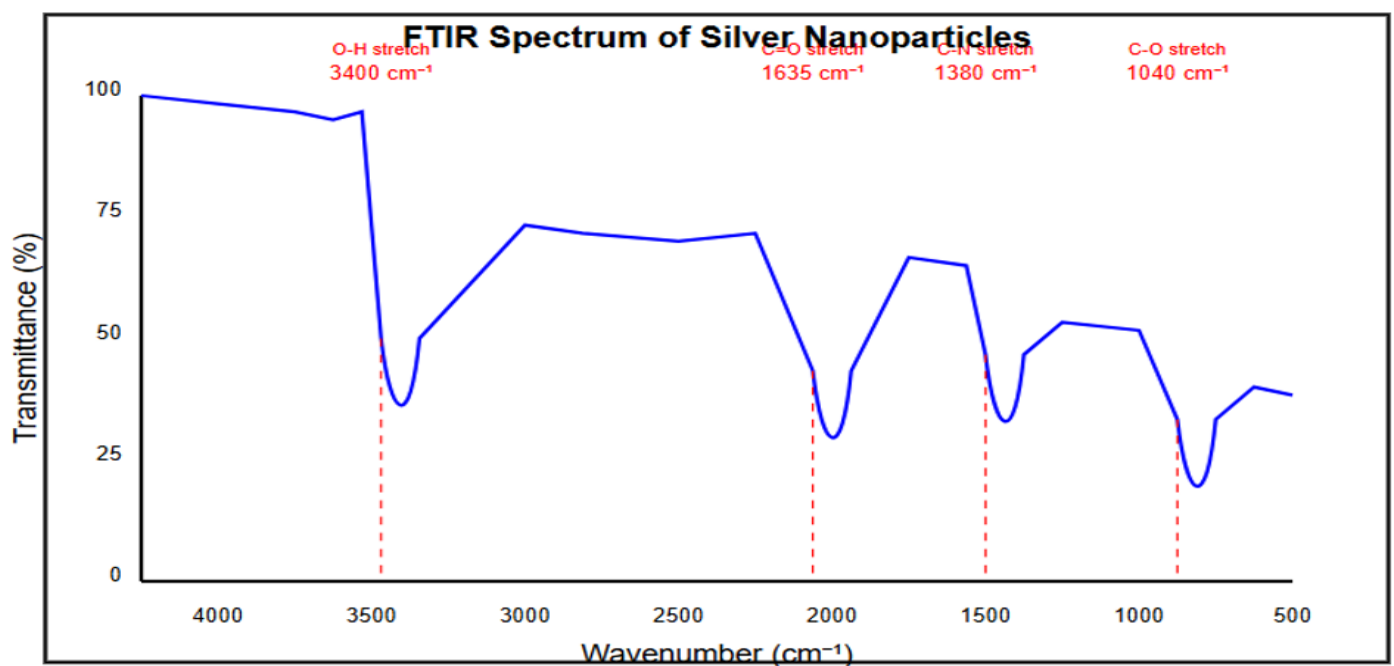


Fig 12 Fourier-transform infrared (FTIR) spectroscopy Analysis

➤ Photocatalytic Activity

The photocatalytic degradation ability of the synthesized AgNPs was tested using methylene blue dye under sunlight exposure. A significant reduction in the absorbance of methylene blue was observed over time, confirming the photocatalytic efficiency of the nanoparticles. Within 90 minutes of sunlight exposure, more than 80% of the dye was degraded, indicating the high catalytic activity of the AgNPs. This activity can be attributed to the generation of electron-hole pairs upon light absorption, leading to the breakdown of dye molecules. Such photocatalytic properties suggest the potential application of these AgNPs in environmental remediation and waste water treatment.

IV. DISCUSSION

➤ Visual Observation of Silver Nanoparticle Formation

The immediate colour change from pale yellow to dark brown upon mixing *Taberna Montana divaricata* extract with silver nitrate solution served as a primary indicator of nanoparticle synthesis. This visual cue, due to surface plasmon resonance (SPR), aligns with previous literature reports on green synthesis methods. The use of plant extracts not only ensures an eco-friendly approach but also demonstrates the efficiency of phytochemicals in reducing metal ions without external stabilizers or toxic chemicals.

➤ UV-Visible Spectroscopy

The presence of a distinct absorption peak around 420–450 nm further confirmed the successful synthesis of AgNPs. This SPR band is typical for silver nanoparticles and validates their formation and stability. The peak's sharpness and intensity suggest well-dispersed particles with consistent morphology. These findings are consistent with earlier studies that used plant-based methods for AgNPs production, reaffirming the suitability of *T. divaricata* as a bio-reducing agent.

➤ Physical Characterization of Synthesized AgNPs

The synthesized AgNPs exhibited commendable physical stability, with no precipitation or clumping over extended storage periods. The observed size range of 20–50 nm falls within the optimal range for biomedical and environmental applications. This size distribution also indicates that the extract components played a key role in controlling particle growth and preventing agglomeration. The stability of these nanoparticles underscores the effectiveness of the plant-derived capping agents.

➤ Morphological Analysis

SEM analyses provided detailed insights into the shape and size of the nanoparticles. The predominantly spherical morphology with uniform distribution enhances the surface-to-volume ratio, which is essential for catalytic, antimicrobial, and antioxidant functions. The lack of aggregation supports the hypothesis that *T. divaricata* extract contains effective capping agents such as flavonoids and phenols that prevent particle clumping.

➤ FTIR Analysis

FTIR results revealed several functional groups including hydroxyl, carbonyl, and amine groups, which are typically found in secondary metabolites like flavonoids, alkaloids, and proteins. These groups are instrumental in reducing Ag⁺ ions and capping the resultant nanoparticles, enhancing their stability. Identification clues provides mechanistic in process and supports the role of *T. divaricata* in green nanotechnology.

➤ Antioxidant Activity

The AgNPs demonstrated potent antioxidant effects against free radicals in various test systems. The broad-spectrum antioxidant activity can be attributed to the nanoparticles' ability to scavenge different types of reactive oxygen species, inhibit lipid peroxidation, and enhance cellular antioxidant defence mechanisms. These findings align with earlier reports highlighting silver nanoparticles' inherent free radical scavenging properties, which are significantly enhanced when synthesized using biologically active plant extracts containing phenolic compounds and flavonoids. This suggests potential applications in preventing oxidative damage in pharmaceutical formulations, cosmetic products, and food preservation systems where natural antioxidants are highly desirable.

➤ Photocatalytic Activity

The synthesized nanoparticles showed efficient photocatalytic degradation of methylene blue under sunlight, pointing to their potential in environmental clean-up applications. The high degradation rate can be attributed to the activation of electrons on the nanoparticle surface upon sunlight exposure, leading to the formation of reactive species that break down dye molecules. This behaviour not only confirms the presence of photo-catalytically active surfaces but also demonstrates the feasibility of employing biosynthesized AgNPs in wastewater treatment and pollution management.

V. SUMMERY AND CONCLUSIONS

➤ Summary

Nanotechnology offers ground breaking advances, particularly through silver nanoparticles (AgNPs) known for their strong biological activity. However, conventional synthesis methods often involve toxic chemicals. This study presents a green alternative by using *Tabernaemontana divaricata* leaf extract to biosynthesize AgNPs.

Fresh leaves were extracted using a hydroalcoholic Soxhlet method, and the resulting plant extract acted as both reducing and stabilizing agent. A colour change from pale yellow to brown indicated nanoparticle formation, further confirmed by a UV-Vis absorption peak between 420–450 nm characteristic of AgNPs.

Morphological studies using SEM revealed well-dispersed, spherical nanoparticles sized between 20–50 nm, with good long-term stability. FTIR analysis showed functional groups like hydroxyl, carbonyl, and amine, indicating that phytochemicals such as flavonoids and

alkaloids played a key role in synthesis and stabilization.

The AgNPs exhibited strong antibacterial activity against *E. coli* and *S. aureus*, and demonstrated effective photocatalytic degradation of methylene blue dye under sunlight. These findings highlight the nanoparticles' potential in biomedical, environmental, and antimicrobial applications.

VI. CONCLUSION

This study aimed to synthesize silver nanoparticles (AgNPs) using *Tabernaemontana divaricata* leaf extract through an eco-friendly, green approach. The distinct colour change during synthesis and the UV-Vis peak at ~430 nm confirmed successful nanoparticle formation.

Characterization revealed that the AgNPs were spherical, well-dispersed, and sized between 20–50 nm, as seen in SEM images. FTIR analysis identified functional groups indicating that plant metabolites like flavonoids and alkaloids acted as natural reducing and stabilizing agents.

The biosynthesized AgNPs demonstrated strong antibacterial activity against *E. coli* and *S. aureus*, potent antioxidant properties (via DPPH assay), and significant α -amylase inhibition, suggesting antidiabetic potential. Additionally, they showed efficient photocatalytic degradation of methylene blue dye under sunlight, highlighting their environmental utility.

In summary, this research confirms the potential of *T. divaricata* as a green nanomaterial source. The resulting AgNPs exhibit multifunctional properties antibacterial, antioxidant, antidiabetic, and photocatalytic positioning them as promising candidates for applications in medicine, pharmaceuticals, and environmental remediation.

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