ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug935

Comprehensive Study of the Phytochemistry, Bioactivities, and Green Nanoparticle Synthesis Using *Calotropis gigantea* Latex

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Publication Date: 2026/08/25

Abstract: The present study investigates the phytochemical composition and pharmacological potential of *Calotropis gigantea* latex, a traditionally used medicinal plant. Preliminary phytochemical screening revealed the presence of bioactive constituents such as flavonoids, alkaloids, tannins, saponins, steroids, and carbohydrates, which have been associated with diverse biological activities. Functional assays demonstrated appreciable *in vitro* anti-inflammatory activity through human red blood cell (HRBC) membrane stabilization and protein denaturation inhibition, and anticoagulant activity via prolonged clotting time. Antioxidant activity assessed using hydrogen peroxide scavenging assay showed concentration-dependent H_2O_2 scavenging activity. Furthermore, the latex exhibited antimicrobial and antifungal activities against standard bacterial and fungal strains, indicating its broad-spectrum antimicrobial potential. Green synthesis of silver nanoparticles (L-AgNPs) using the plant latex was also successfully achieved, and the resulting preparation was evaluated for antimicrobial activity. These findings provide preliminary experimental support for the reported biological potential of *C. gigantea* latex and providing a basis for further investigation of its biological and nanotechnological applications.

Keywords: *Calotropis Gigantea, Phytochemical Screening, Anti-Inflammatory Activity, Antioxidant Activity, Antimicrobial Activity, Anticoagulant Activity, Silver Nanoparticles, Green Synthesis.*

How to Cite: Sushmita S. Pawar; Vaibhav Sapkal; Pranav Lokhande; Dinesh Narwaiye; Sharad Tanpure (2026) Comprehensive Study of the Phytochemistry, Bioactivities, and Green Nanoparticle Synthesis Using *Calotropis gigantea* Latex. *International Journal of Innovative Science and Research Technology*, 11(8), 1658-1671. https://doi.org/10.38124/ijisrt/26aug935

I. INTRODUCTION

Since antiquity, plants have played a crucial role in human health, serving both nutritional and therapeutic purposes. Traditional medicine, which heavily relies on plant-based remedies, continues to be a primary healthcare resource for a significant portion of the global population. According to the research, over 80% people worldwide depend on traditional medicine to meet their basic health needs [22].

Over time, numerous herbal remedies have demonstrated their effectiveness, contributing to the growing acceptance of plant-derived drugs in modern science. These natural products often contain bioactive compounds, some with known chemical structures, others still under investigation that have shown clinical benefits across a range of diseases. In many rural and underserved regions, traditional plant-based medicines remain popular due to their affordability and accessibility compared to conventional pharmaceuticals [3].

Plants possess a remarkable ability to synthesize a wide array of chemically diverse compounds, many of which have significant medicinal potential. These include alkaloids, steroids, tannins, glycosides, volatile and fixed oils, resins, phenols, and flavonoids. Such compounds are typically concentrated in specific plant parts like leaves, flowers, bark, seeds, fruits, and roots [22]. In addition to their therapeutic value, these substances also function as natural defense mechanisms, protecting plants from microbial infections, insect predation, and herbivores [31].

One such plant is *Calotropis gigantea* L., a perennial shrub widely distributed across tropical and subtropical regions of Asia and Africa. Its milky latex has been employed for centuries in traditional medicine systems Ayurveda, Unani

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug935

and Siddha for a spectrum of ailments including rheumatism, skin disorders, and gastrointestinal disturbances [8, 9].

Ethnobotanical surveys in India and Pakistan consistently report topical application of *C. gigantea* latex for wound healing and parasitic skin infections, as well as oral use for asthmatic and febrile conditions [12, 26].

Phytochemical investigations have revealed that *C. gigantea* latex is rich in cardenolides, phenolic acids, flavonoids and a variety of proteolytic enzymes [23]. Cardenolides are steroidal glycosides known for their profound bioactivities but narrow therapeutic index, whereas the phenolics and flavonoids contribute antioxidant potential. Qualitative phytochemical screening typically detects saponins, tannins, and alkaloids alongside the major cardiac glycosides, suggesting a multifaceted matrix of secondary metabolites [3].

Inflammation is a protective response of living tissues to injury, infection, or irritation. During inflammation, the release of lysosomal enzymes from activated inflammatory cells can contribute to tissue damage by degrading cellular macromolecules and promoting membrane lipid peroxidation. Stabilization of lysosomal and erythrocyte membranes is therefore considered an important mechanism for limiting inflammatory damage. The HRBC membrane stabilization assay is commonly used as an in vitro model for evaluating the membrane-stabilizing and potential anti-inflammatory effects of plant extracts [7, 24].

Various studies have employed antioxidant assays such as DPPH, ABTS, FRAP, and H_2O_2 scavenging methods to assess the efficacy of natural antioxidant compounds [16]. These techniques provide valuable insights into the free radical scavenging potential of plant-derived substances and their possible therapeutic applications.

The antimicrobial potential of *C. gigantea* latex has been evaluated against a range of Gram-positive and Gram-negative bacteria, as well as dermatophytic fungi. Agar-well diffusion assays reveal moderate to strong activity against *Staphylococcus aureus, Escherichia coli, and Candida albicans*, with minimum inhibitory concentrations (MICs) in the 0.5–2.0 mg/mL range [5]. Mechanistic studies suggest that latex glycoproteins disrupt microbial cell membranes, while cardenolides may interfere with ATP-dependent transport systems.

Nanotechnology has emerged as a powerful tool across various fields, particularly in health and medicine, due to the unique properties of materials at the nanoscale. Silver nanoparticles (AgNPs), among the most studied nanomaterials, exhibit remarkable antimicrobial properties and have found extensive use in pharmaceutical and biological applications. Green synthesis methods utilizing plant extracts, fungi, algae, and latex have gained attention for being eco-friendly, cost-effective, and safer than conventional chemical or physical methods [10, 11]. These biosynthesized AgNPs typically possess high surface area, enhanced dispersion, and strong bactericidal activity, even at concentrations below their cytotoxic limits, making them suitable for use as antimicrobial agents. Recent studies have highlighted the potential of plant latex-based synthesis of AgNPs, which not only simplifies the process but also imparts additional bioactivity, including antimicrobial and cytotoxic effects.

The present study is designed to achieve the following objectives:

- To perform preliminary phytochemical screening of *Calotropis gigantea* latex collected from the Chhatrapati Sambhajinagar region of Maharashtra.
- To evaluate the *in vitro* anti-inflammatory, anticoagulant, and antioxidant activities of the plant latex extract.
- To investigate the antibacterial and antifungal properties of *C. gigantea* latex against selected microbial strains.
- To synthesize silver nanoparticles using *C. gigantea* latex and evaluate their antimicrobial activity.

By bridging phytochemistry with pharmacology and microbiology, this work seeks to substantiate the therapeutic potential of *C. gigantea* latex and guide future development of novel phytopharmaceuticals.

II. MATERIALS AND METHODS

A. Collection of Calotropis Gigantea Latex

Healthy plants of *Calotropis gigantea* were identified near Chhatrapati Sambhajinagar, Maharashtra, India. The plant was authenticated from Botany Department, Deogiri College, Chhatrapati Sambhajinagar. The plant latex was collected early in the morning by making small incisions on the stem and mature leaves of the plant using a sterilized blade. The exuding white latex was allowed to drip directly into clean, dry, sterile containers. Care was taken to avoid contamination during collection. The collected latex was immediately transported to the laboratory and stored at 4°C until further use in experimental procedures.

B. Phytochemical Screening

Phytochemical screening involves preliminary qualitative tests used to detect the presence of primary and secondary metabolites in the plant latex. The following tests were performed to detect the presence of steroids, carbohydrates, proteins, anthocyanin, phenols, alkaloids, flavonoids, tannins, and saponins.

➢ *Detection of Steroids:*

2 mL of plant latex was dissolved in 2 mL of chloroform, and shaken thoroughly. A few drops of concentrated sulfuric acid were added into this mixture from the side of the test tubes. The formation of a red color in the lower chloroform layer indicates the presence of steroids.

➢ *Detection of Carbohydrates (Molisch's Test):*

2 mL of the plant latex was treated with few drops of Molisch's reagent and the resulting mixture shaken properly. A few drops of concentrated H_2SO_4 were then added carefully down the side of the test tube. The formation of a violet ring at the interface indicates the presence of carbohydrates.

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➢ *Detection of Proteins (Ninhydrin Test):*

2 mL of plant latex was transferred into a test tube and treated with few drops of ninhydrin reagent. The mixture was then placed in water bath and allowed to boil for a few minutes. Formation of blue to purple color indicates the presence of proteins.

➢ *Detection of Anthocyanins:*

2 mL of plant latex was transferred into a test tube and treated with 2 mL of 2N HCl and NH_3. The color change from pink-red to blue-violet indicates the presence of anthocyanin.

➢ *Detection of Phenols:*

2 mL of plant latex was transferred into a test tube and treated with few drops of 10% alcoholic $FeCl_3$ solution. The appearance of green, blue or violet color indicates the presence of phenolic compound.

➢ *Detection of Tannins:*

2 mL of plant latex was taken in test tube and treated with few drops of 5% aqueous $FeCl_3$. The formation of a green precipitate was an indication for the presence of tannins.

➢ *Detection of Alkaloids (Mayer's Test):*

2 mL of plant latex was mixed with 2 mL of 1% HCl on a steam bath and used for detection of alkaloids. The acidic mixture was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow-colored precipitate indicates the presence of alkaloids.

➢ *Detection of Saponins (Foam Test):*

5 mL of plant latex was shaken vigorously. The formation of stable foam for at least ten minutes is an indication for the presence of saponins.

➢ *Detection of Flavonoids (Alkaline Reagent Test):*

2 mL of plant latex was treated with 10% of NaOH solution. The formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

C. Anti-Coagulation Activity:

Blood sample was collected from healthy volunteer and transferred it in three separate test tubes (2 mL each). 0.2 mL of whole aqueous plant latex (100%) was transferred in one test tube; 0.2 mL of water was transferred in second test tube and third test tube containing untreated blood was considered as blank. The clotting activity of each tube was measured using a stop watch and noted the clotting time. Same procedure was performed with 50% and 10% aqueous plant latex.

D. Anti-Inflammatory Activity:

Anti-inflammatory activity of plant latex was carried out using various *in vitro* models such as HRBC membrane stabilization activity and protein denaturation inhibitory activity. Standard drug was also used in order to compare the efficacy of anti-inflammatory activity of plant extract.

➢ *Membrane Stabilization*

- A total of 10 mL of fresh whole human blood was collected from healthy volunteers and transferred into centrifuge tubes. An equal volume of Alsever solution (composition: 2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl) was added to the blood sample to prevent coagulation. The mixture was centrifuged at 3,000 rpm for 5 minutes. The resulting pellet was washed three times with isotonic saline, and a 10% erythrocyte suspension was subsequently prepared using the same isotonic saline.
- To evaluate the membrane stabilization potential, 0.5 mL of 10% *C. gigantea* latex was mixed with 0.5 mL of the 10% erythrocyte suspension. For the control, 0.5 mL of isotonic saline was used in place of the latex. Diclofenac served as the standard reference drug.
- All test tubes, including control and standard, were incubated in a water bath at 50°C for 30 minutes. After incubation, the tubes were cooled, and 0.5 mL of isotonic saline was added to each tube.
- The reaction mixtures were then centrifuged at 3,000 rpm for 5 minutes. The supernatant from each tube was carefully collected, and the absorbance was measured at 560 nm using a UV-Visible spectrophotometer.
- The percentage of membrane stabilization (protection) was calculated using the following formula:

% Protection = 100 − [(OD of sample / OD of control) × 100]

- This procedure was repeated for latex concentrations of 50% and 100% to assess dose-dependent activity.

➢ *Protein Denaturation Inhibitory Activity*

- A reaction mixture containing 0.5 mL of 1% bovine serum albumin prepared in phosphate-buffered saline (pH 6.4) and 0.5 mL of 10% *C. gigantea* latex was prepared. 0.5 mL of a 1% aqueous solution of Bovine Serum Albumin was considered as a Control.
- The reaction mixture was incubated at 37°C for 20 min and then heated to 50°C for 20 min. The reaction mixture was cooled to room temperature, and turbidity was measured spectrophotometrically at 660 nm.
- The percent inhibition was calculated by using following formula;

% Inhibition = [{Abs control- Abs sample}/Abs control] × 100

E. H_2O_2 Assay for Antioxidant Activity:

The H_2O_2 assay evaluates the ability of a *C. gigantea* latex to scavenge hydrogen peroxide, a harmful reactive oxygen species. Hydrogen peroxide can cause oxidative damage to cells and tissues. This assay measures the reduction in hydrogen peroxide concentration after exposure to the *C. gigantea* plant latex, indicating its antioxidant potential.

The scavenging activity of natural antioxidants found in *C. gigantea* plant latex against hydrogen peroxide (H_2O_2) is

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commonly assessed by detecting the decrease in H_2O_2 concentration in an incubation system containing both H_2O_2 and the antioxidant (scavenger). This is typically done using a classical UV spectrophotometric method.

➢ *Chemicals and Reagents:*

- Phosphate buffer (0.2 M, pH 7.4), Hydrogen peroxide (H_2O_2), Ascorbic acid (10% w/v), Distilled water, *C. gigantea* plant latex (1%, 5% and 10% v/v).

➢ *Method:*

- A dilution series of the *C. gigantea* plant latex was prepared.
- 600 µL of H_2O_2 was added to each test tube (Control, Sample, and Standard).
- 100 µL of either the *C. gigantea* plant latex or standard (Ascorbic acid) was added to the respective tubes.
- The volume in each tube was adjusted to 4 mL using phosphate buffer (pH 7.4).
- The ascorbic acid series without plant extract served as the standard (containing phosphate buffer, H_2O_2, and ascorbic acid).
- All test tubes were incubated at room temperature for 10 minutes. The absorbance of the H_2O_2 solution was measured at 300 nm against a blank.
- H_2O_2 scavenging activity percentage was calculated relative to the standard, using the following formula:

$$\text{Scavenging Activity (\%)} = \left(\frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}}\right) \times 100$$

F. Antimicrobial Activity:

➢ *Antibacterial and Anti-Yeast Activity:*

- The antimicrobial potential of *C. gigantea* latex was evaluated against selected bacterial strains using the agar well diffusion method [30] with slight modifications.
- The microbial strains included the bacteria *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhimurium*, and *B. subtilis*, and the yeast *C. albicans*. Pure cultures of each organism were maintained on nutrient agar slants and sub-cultured prior to experimentation.
- Bacterial and yeast cultures were grown in nutrient broth for 18-24 hours at 37°C. The turbidity of each culture was adjusted to approximately 10^7 to 10^8 CFU/mL based on optical density.
- Sterile nutrient agar medium was poured into Petri dishes and allowed to solidify. Once solidified, the surface of each agar plate was uniformly spreaded with 100 µL of the standardized microbial suspension using a sterile spreader by the spread plate technique.
- Using a sterile cork borer, wells of 6 mm diameter were punched into the agar. Each well was then filled with 20 µL of *C. gigantea* latex at varying concentrations: 100%, 50%, 10%, and 1%, prepared using sterile distilled water as the diluent.
- All procedures were performed aseptically inside a laminar air flow chamber. The inoculated plates were incubated in an upright position at 37°C for 24–48 hours.
- Following incubation, the antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition around each well using a transparent ruler.
- The results were recorded in millimeters (mm).

➢ *Antifungal Assay:*

- The antifungal activity of *C. gigantea* latex was assessed using a plate-based method under aseptic conditions.
- The following materials were used for conducting the antifungal assay of *Calotropis gigantea* latex: Potato Dextrose Agar (PDA) plates**,** Fresh latex of *C. gigantea***,** Sterile cotton buds**,** Nichrome wire loop**,** Fungal cultures: *Aspergillus niger* and Inhouse isolated fungi (white fungus, and brown fungus).
- Sterile PDA plates were prepared and supplemented with 1% (v/v) fresh *C. gigantea* latex.
- A sterile cotton swab was used to collect fungal cultures (from *A. niger*, white fungus, and brown fungus). The fungal inoculum was evenly spread on the surface of the latex-containing PDA plates using a gentle zigzag pattern.
- Control plates were prepared using PDA without latex. These plates were also inoculated using the same fungal cultures and swabbing pattern.
- All plates (test and control) were incubated at room temperature for 3 days (72 hours).
- After the incubation period, plates were observed for signs of fungal growth inhibition.

G. Green Synthesis of Calotropis gigantea Latex Silver Nanoparticles (L-AgNPs):

- The biosynthesis of silver nanoparticles using *C. gigantea* latex was performed by following the method described by Saeed and Nejad [30], with slight modifications.
- Initially, 5 mL of *C. gigantea* latex was mixed with 5 mL of 2 mM silver nitrate ($AgNO_3$) solution in a clean reaction vessel.
- The mixture was heated in a water bath at 60°C for 15 minutes to initiate the reduction reaction. Subsequently, the temperature was gradually increased to 80°C, during which a visible color change from pale yellow to dark brown was observed, indicating the formation of silver nanoparticles.
- The reaction mixture was then cooled to room temperature and incubated at 37°C to allow the nanoparticles to settle. After incubation, the supernatant was carefully decanted, and the pale white precipitate was collected.
- The precipitate was subjected to two washing cycles with distilled water (centrifuged at 5000 rpm for 15 minutes each) followed by a wash with ethanol to remove any unreacted components.
- Finally, the purified nanoparticle pellet was dried at 80°C for 7 to 8 hours to obtain a fine powder of latex-synthesized silver nanoparticles (L-AgNPs).

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H. Antimicrobial Activity of Calotropis Gigantea Latex Silver Nanoparticles (L-AgNPs):

- Antimicrobial activity of *Calotropis gigantea* Latex Silver Nanoparticles (L-AgNPs) performed by well diffusion method as per the procedure elaborated under "antimicrobial activity".

III. RESULTS AND DISCUSSION

➢ *Collection of Calotropis gigantea Latex*

- Fresh white latex was successfully obtained from the authenticated plants of *C. gigantea*. The collected latex appeared white and free from visible impurities or contamination. These observations confirmed the suitability and quality of the collected latex for subsequent phytochemical and biological investigations. A representative photograph of the authenticated *C. gigantea* plant and freshly collected latex is presented in Figure 1.

Fig 1 *Calotropis gigantea* Plant and Latex Collection

➢ *Phytochemical Screening:*

- The preliminary phytochemical screening of Calotropis gigantea latex revealed the presence of several key bioactive compounds, as summarized in Table 1.

Table 1 Phytochemical Screening Details

Phytoconstituent		**Test Results**
Steroids		Positive
Carbohydrates		Positive
Proteins		Positive
Anthocyanin		Negative
Phenols		Negative
Alkaloids		Positive
Flavonoids		Positive
Tannins		Positive
Saponins		Positive

- The results indicated that *C. gigantea* latex contains a rich variety of phytochemicals including steroids, carbohydrates, proteins, alkaloids, flavonoids, tannins, and saponins, all of which are known for their diverse biological activities. The detected steroids may contribute to the biological activities observed; however, their specific contribution cannot be established from qualitative phytochemical screening alone, while alkaloids are widely reported for their antimicrobial and pharmacological effects.

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- Flavonoids and tannins, both of which were found to be present, are recognized for their strong antioxidant properties, which may contribute to the plant's traditional medicinal applications.
- The detection of saponins implies possible antimicrobial and antifungal activities, and carbohydrates and proteins are essential for cellular metabolism and may support overall bioactivity. Although phenols were not detected by the qualitative assay used, the presence of flavonoids and tannins may contribute to the observed antioxidant activity. However, the contribution of individual constituents cannot be confirmed without quantitative phytochemical analysis and compound characterization.
- These findings support the traditional use of *C. gigantea* latex in folk medicine and warrant further investigation into its pharmacological potential, particularly its antioxidant, anti-inflammatory, and antimicrobial properties. Future studies could focus on isolating and characterizing the individual bioactive compounds to better understand their mechanisms of action.

Fig 2 Phytochemical Screening of *C. gigantea* Latex

➢ *Anti-Coagulation Activity:*

- The anticoagulant activity of *C. gigantea* latex was evaluated by observing the clotting time of blood in the presence of various concentrations of the latex. As shown in figure 3, the latex exhibited a concentration-dependent prolongation of clotting time, indicating its potential anticoagulant effect.
- The untreated blood control showed a clotting time of 4 min. In contrast, 10% latex delayed clot formation to 8 minutes, 100% latex extended this duration to more than 30 minutes.

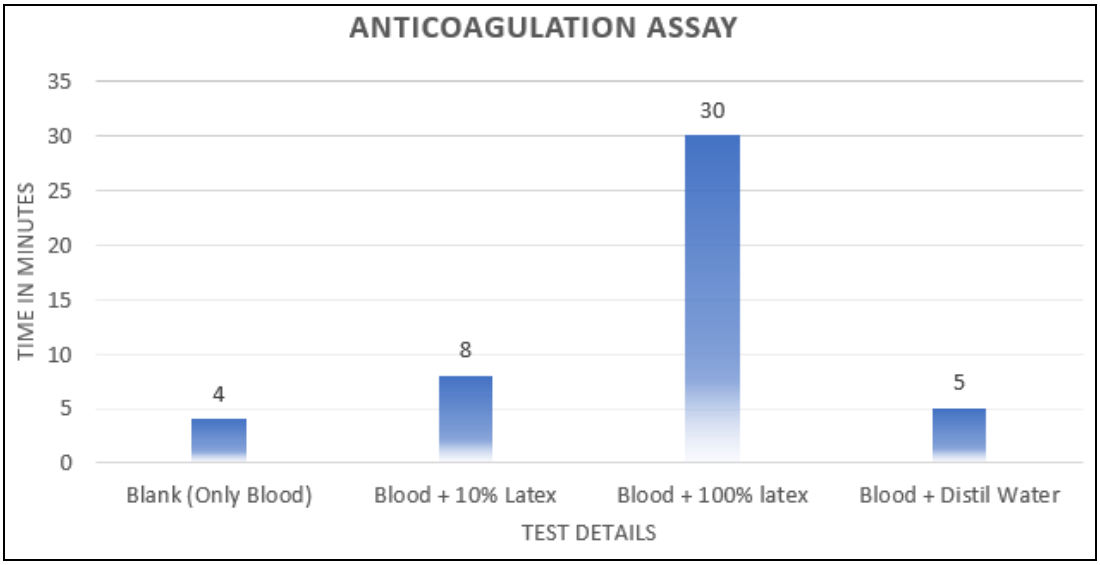

Fig 3 Anticoagulant Activity of *C. gigantea* Latex

- The prolonged coagulation time may be associated with one or more phytochemical constituents detected in the latex. The prolonged clotting time suggests that components of the latex may interfere with one or more processes involved in coagulation; however, the specific mechanism remains to be determined.

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Blank	Blood + 10% Latex	Blood + 100% Latex	Blood + D/W
			

Fig 4 Blood with Different Concentrations of Latex

- This activity may be beneficial in the development of herbal formulations for cardiovascular conditions where blood clot prevention is crucial. However, further mechanistic studies and toxicity evaluations are essential to validate these findings for therapeutic applications.

➢ *Anti-Inflammatory Activity:*

- The anti-inflammatory potential of *C. gigantea* latex was assessed using two *in vitro* models: the Human Red Blood Cell (HRBC) membrane stabilization assay and the protein denaturation inhibition assay. The results presented in graphically in figure 5, demonstrate a concentration-dependent activity, indicating the efficacy of *C. gigantea* latex in reducing inflammation.
- In the HRBC membrane stabilization assay, which models the ability of substances to protect erythrocyte membranes against heat-induced lysis, Under the experimental conditions used, the 100% latex preparation showed a higher percentage of membrane stabilization than the diclofenac reference. This indicates that the plant latex can effectively stabilize cell membranes and prevent inflammatory damage at higher concentrations.
- Similarly, in the protein denaturation assay, *C. gigantea* latex demonstrated inhibition of denatured protein formation a process associated with inflammatory responses. The 100% concentration of latex inhibited protein denaturation by 82%, which is notably higher than the inhibition observed with diclofenac (65%). Both assays highlight the dose-dependent anti-inflammatory effects of *C. gigantea* latex, which may be attributed to its rich phytochemical composition, including flavonoids, tannins, and alkaloids known for their membrane-stabilizing and protein-interaction properties. These findings support the traditional use of *C. gigantea* in managing inflammatory conditions and suggest its potential as a natural alternative or adjunct to synthetic anti-inflammatory drugs.
- Further *in vivo* studies and isolation of active compounds are recommended to better understand its therapeutic potential.

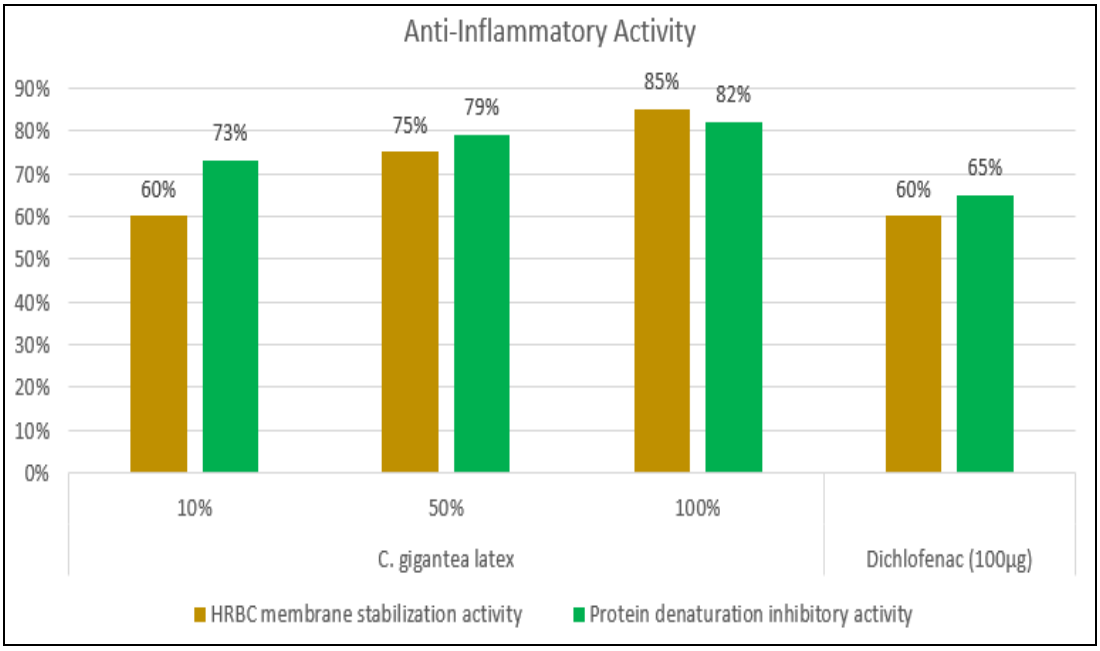

Fig 5 Anti-Inflammatory Activity of *C. gigantea* Latex

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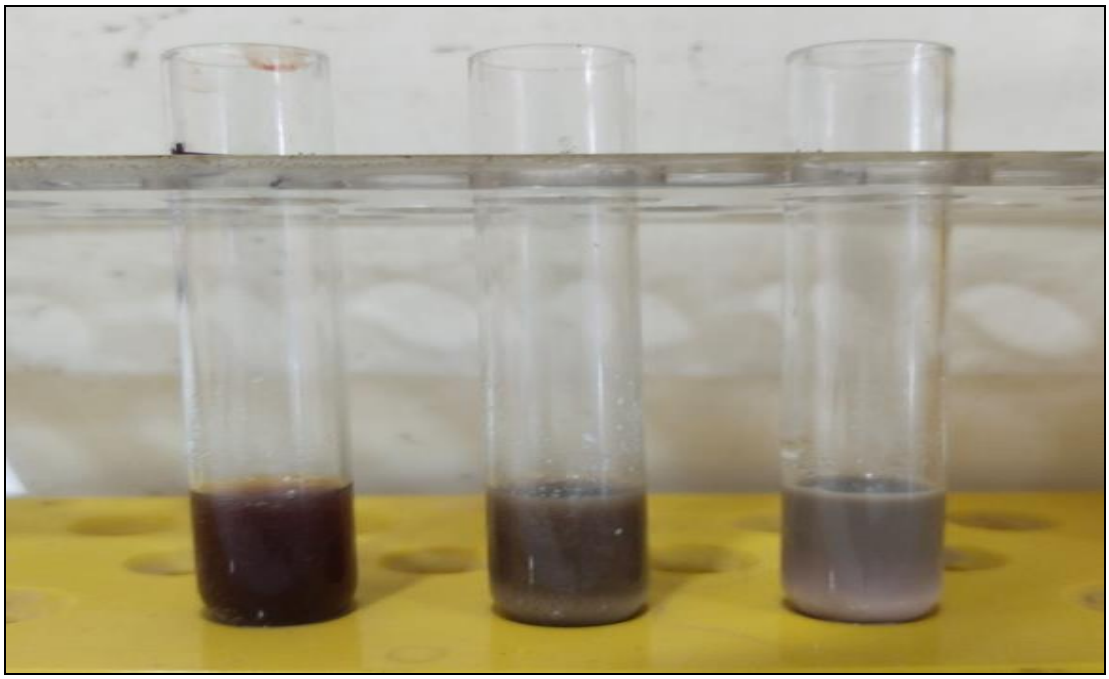

Fig 6 Membrane Stabilization Activity Using 10%, 50% and 100% Latex

➢ *Antioxidant Activity:*

- The antioxidant potential of *C. gigantea* latex was evaluated using a hydrogen peroxide (H_2O_2) scavenging assay. The results, presented graphically in figure 7, demonstrate a concentration-dependent increase in H_2O_2 scavenging activity. This indicates the plant latex's potential to neutralize reactive oxygen species (ROS) and protect biological systems from oxidative stress.
- As shown in the figure 7, H_2O_2 scavenging activity increased from 53% at 10% latex to 76% and 87% at 50% and 100%, respectively. This concentration-associated increase suggests that the antioxidant compounds present in the latex are more effective at higher concentrations.

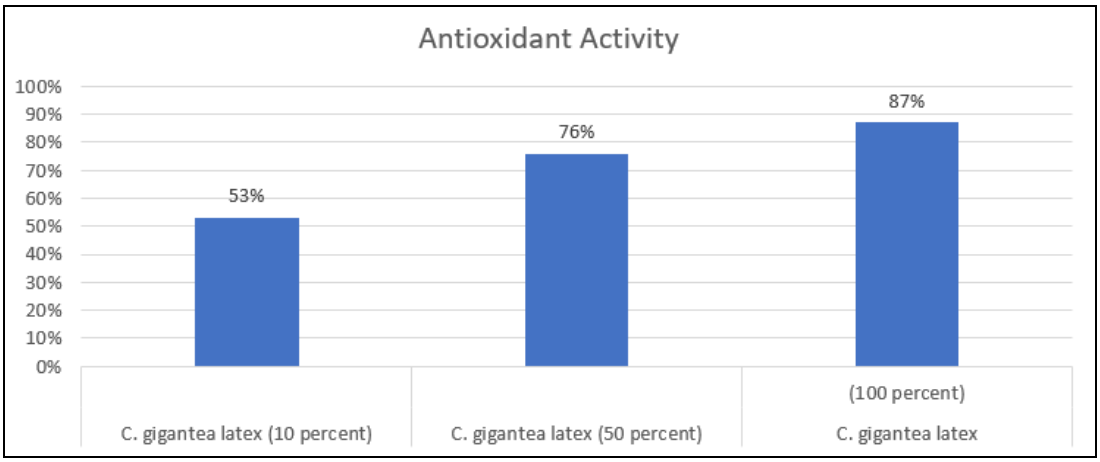

Fig 7 Antioxidant Activity of *C. gigantea* Latex

- The antioxidant effect of *C. gigantea* latex is likely attributed to its phytochemical constituents such as flavonoids, tannins, alkaloids, and saponins, all of which are known for their ROS-scavenging potential. These compounds may contribute to neutralizing H_2O_2 by donating electrons or hydrogen atoms, thereby reducing its reactivity and preventing cellular damage.
- The high antioxidant activity at 100% latex concentration (87%) indicates that *C. gigantea* latex has considerable potential as a natural source of antioxidants. This supports its ethnomedicinal use in managing oxidative stress-related disorders and encourages further studies for isolation and characterization of the bioactive compounds responsible for this activity.

➢ *Green Synthesis of Calotropis gigantea Latex Silver Nanoparticles (L-AgNPs):*

- The biosynthesis of silver nanoparticles (AgNPs) using *Calotropis gigantea* latex was successfully achieved through a green, eco-friendly approach. Upon mixing the latex with a 2 mM silver nitrate ($AgNO_3$) solution and subjecting the reaction mixture to gradual heating, a distinct color change from pale yellow to dark brown was

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observed. The observed color change from pale yellow to dark brown was consistent with the reduction of silver ions and suggested the formation of silver nanoparticles.

- Phytoconstituents such as flavonoids, alkaloids, and saponins are likely responsible for facilitating this bio reduction process. Following incubation and washing, a pale white precipitate of silver nanoparticles was recovered, providing preliminary evidence of silver nanoparticle formation.
- This green synthesis method provides a sustainable alternative to conventional chemical and physical nanoparticle synthesis techniques. Moreover, using *C. gigantea* latex not only simplifies the process but also enhances the biological potential of the nanoparticles due to effects of the plant's inherent antimicrobial and antioxidant properties.

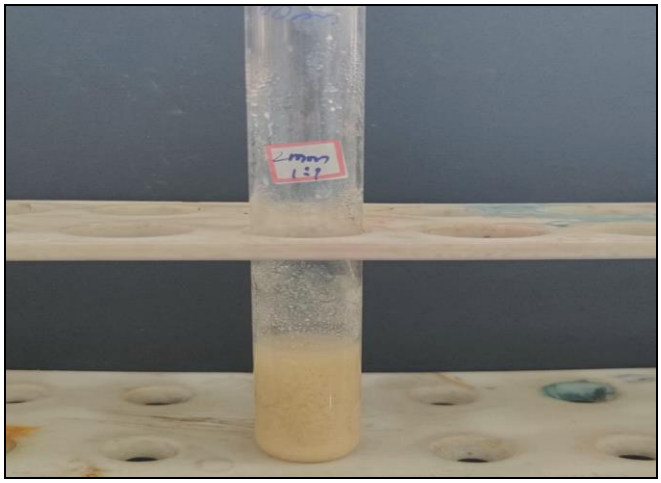

Fig 8 Synthesis of L-AgNPs

➢ *Antimicrobial Activity:*

- The antimicrobial activity of different concentrations of *C. gigantea latex* and L-AgNPs was performed against standard bacterial and yeast strains using well diffusion method and the results are summarized in figure 9 and 10.
- The activity increased with increasing latex concentration, with the highest inhibition observed at 100% latex concentration. *Staphylococcus aureus* ATCC 6538 exhibited the greatest sensitivity, showing a maximum zone of inhibition of 39 mm, followed by *B. subtilis* ATCC 6633 with 28 mm. Moderate inhibition was observed against *P. aeruginosa* ATCC 9027 and *E. coli* ATCC 8739 with zones measuring 22 mm, and *S. typhimurium* ATCC 14028 with zones measuring 21 mm each at 100% concentration.
- A noticeable decrease in activity was observed at lower concentrations, especially at 1%, indicating the latex's potent but concentration-dependent antibacterial properties. These findings suggest the presence of bioactive compounds in *C. gigantea* latex that can effectively suppress pathogenic bacterial growth, aligning with previous reports on the plant's antimicrobial potential.

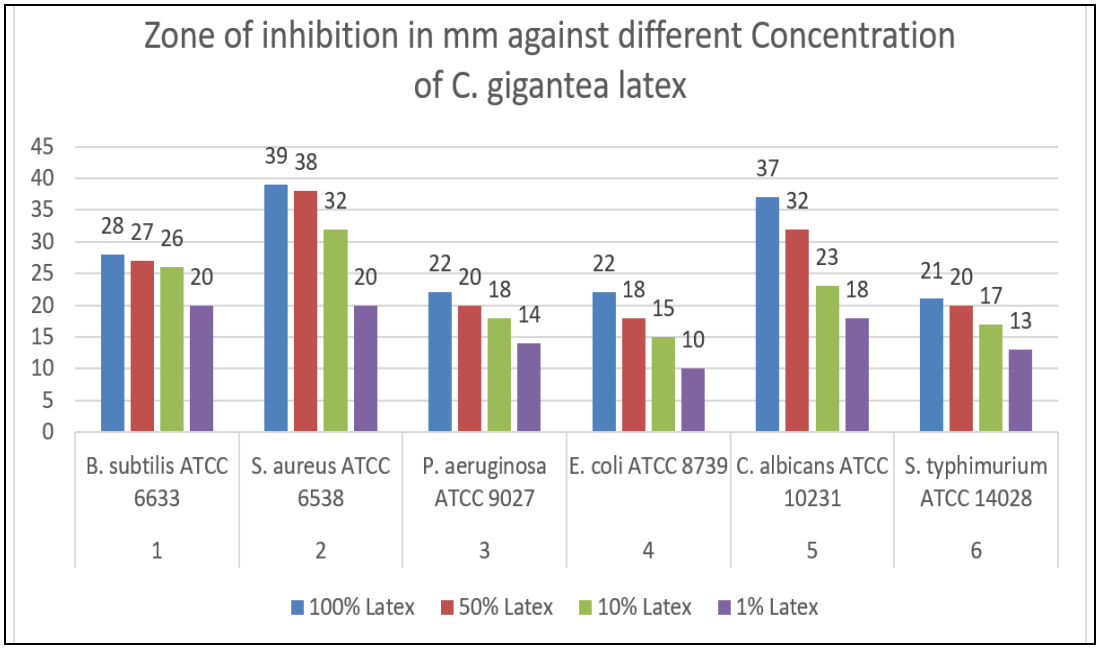

Fig 9 Antimicrobial Activity Using Different Concentration of Latex

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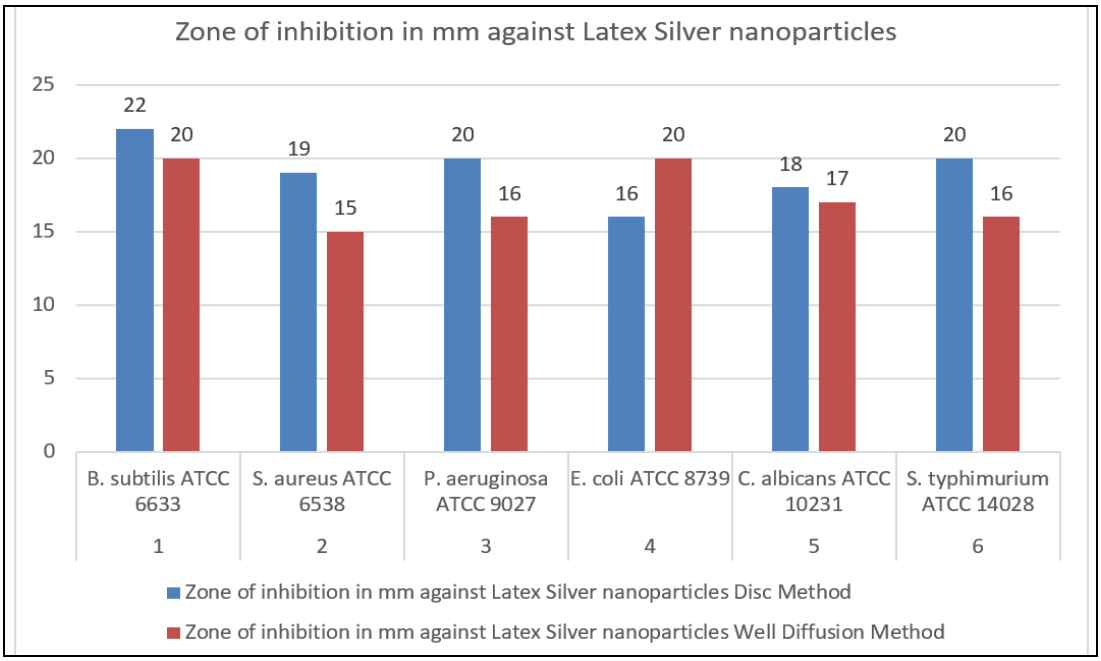
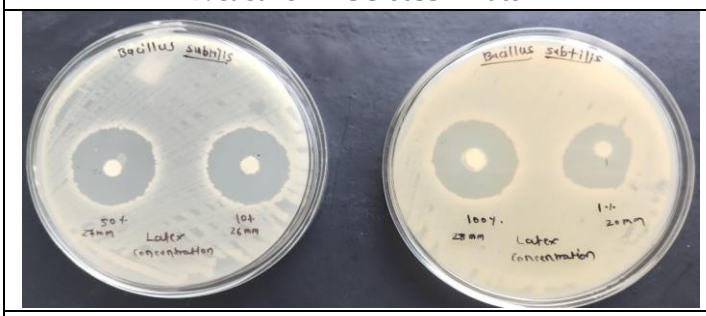


Fig 10 Antimicrobial Activity Using Latex Silver Nanoparticles

➢ *Antifungal Activity:*

- The antifungal activity of *C. gigantea* latex was assessed by incorporating 1% v/v of the latex into Potato Dextrose Agar (PDA) media and comparing the fungal growth against control plates lacking the latex.
- The results demonstrated a clear inhibitory effect of all tested fungal strains including *A. niger*, white fungus, and brown fungus on the latex-supplemented media (figure 13). Visual observation of the culture plates after three days of incubation showed a clear inhibitory effect, as evidenced by reduction in number of fungal colonies in the test plates compared to the vigorous growth observed in the control. These findings support the antifungal potential of *C. gigantea* latex, possibly due to the presence of bioactive phytochemicals such as alkaloids, flavonoids, and saponins, known for their antimicrobial activity.

***B. subtilis* ATCC 6633 + Latex**	***S. aureus* ATCC 6538 + Latex**
	
***C. albicans* ATCC 10231 + Latex**	***E. coli* ATCC 8739 + Latex**
	
***P. aeruginosa* ATCC 9027 + Latex**	***S. typhimurium* ATCC 14028 + + Latex**

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug935

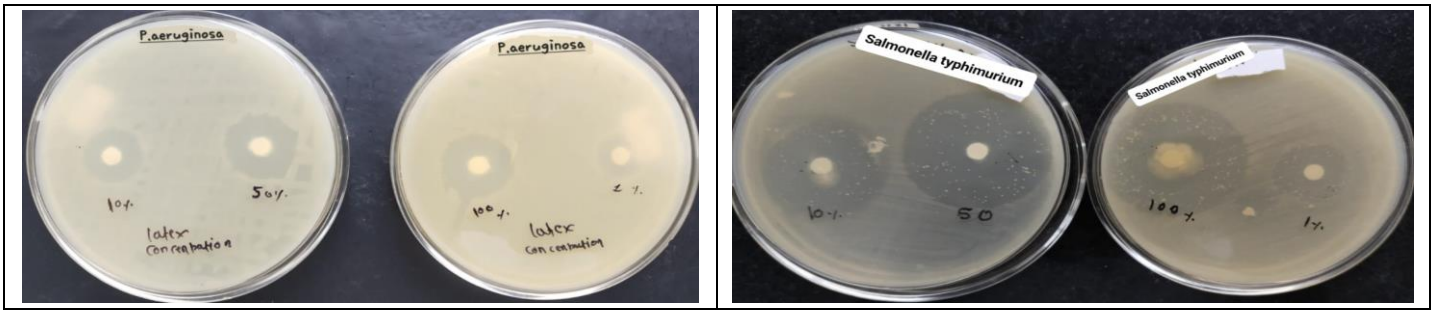


Fig 11 Representative Photographs of Antimicrobial Activity of *C. gigantea Latex*

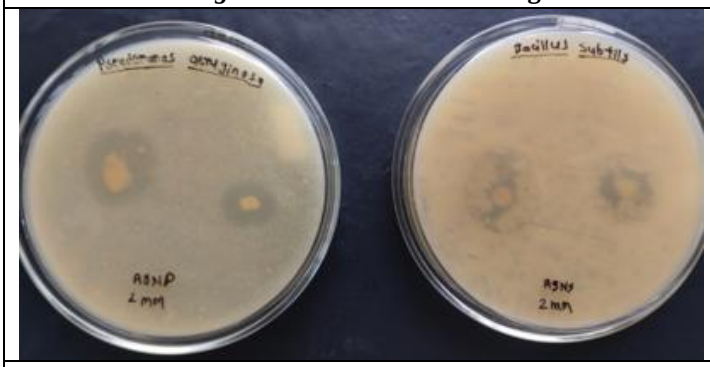
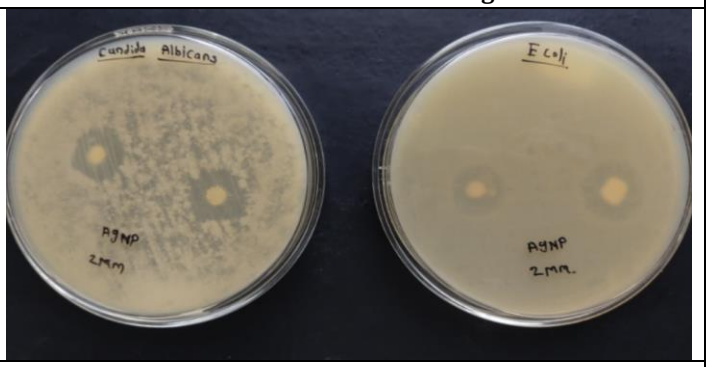
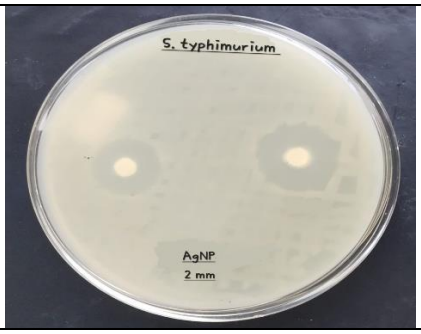
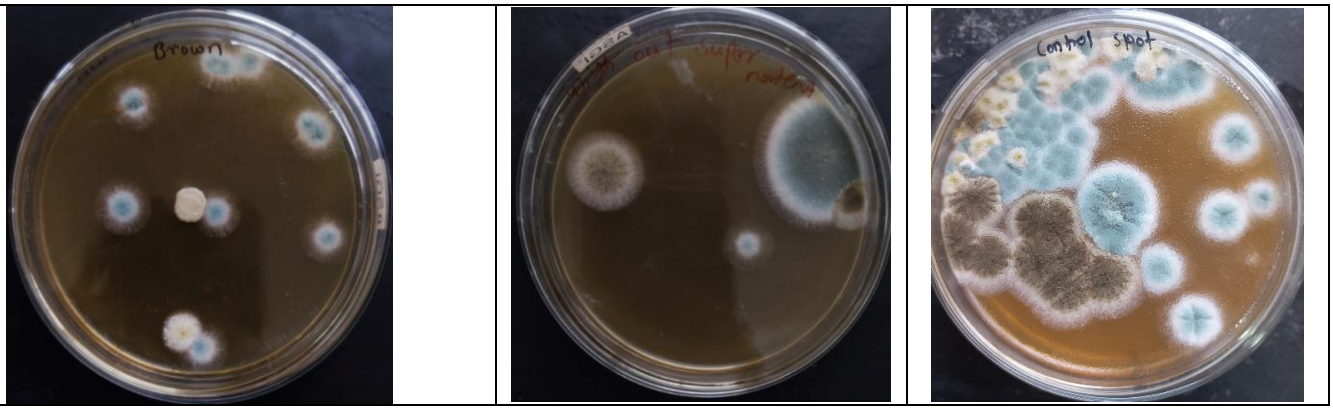
***P. aeruginosa and B. subtilis* + L-AgNPs**	***C. albicans and E. coli* + L-AgNPs**
	
***S. aureus* ATCC 6538 + L-AgNPs**	***S. typhimurium* ATCC 14028 + L-AgNPs**
	

Fig 12 Representative Photographs of Antimicrobial Activity of *C. gigantea* L-AgNPs



ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug935

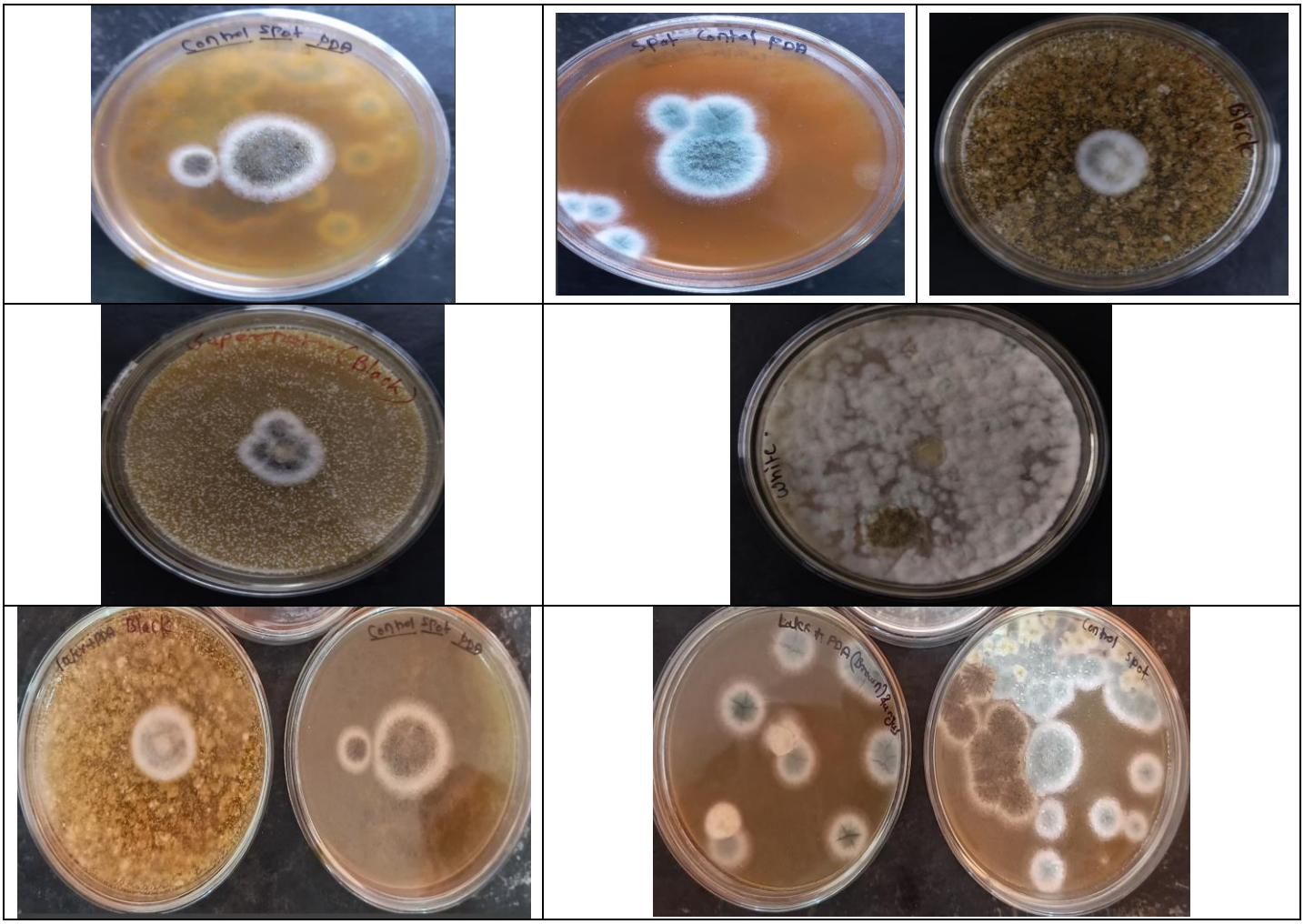


Fig 13 Representative Photograph Antifungal Activity of *C. gigantea* Latex against Different Fungi

IV. CONCLUSION

The present study evaluated the phytochemical composition and selected biological activities of *Calotropis gigantea* latex. Qualitative phytochemical screening revealed the presence of several bioactive compounds, including steroids, alkaloids, flavonoids, tannins, saponins, carbohydrates, and proteins, whereas phenols and anthocyanins were not detected under the experimental conditions.

The latex demonstrated concentration associated anticoagulant, membrane stabilizing, protein denaturation inhibitory, and H_2O_2 scavenging activities. Antimicrobial evaluation demonstrated inhibitory effects against the selected bacterial and yeast strains, while incorporation of latex into PDA resulted in a visible reduction in fungal growth.

The observed membrane stabilization and protein denaturation inhibitory activities suggest the potential of *C. gigantea* latex to exhibit anti-inflammatory properties. Similarly, the prolonged clotting time observed in the anticoagulation assay indicates a possible effect on the blood coagulation process. The latex also demonstrated appreciable H_2O_2 scavenging activity, indicating its antioxidant potential. Furthermore, both the latex and latex-mediated silver nanoparticles (L-AgNPs) exhibited antimicrobial activity against selected microorganisms, with notable activity observed against *S. aureus*, *E. coli*, and *C. albicans.*

The green synthesis of silver nanoparticles using *C. gigantea* latex provides an eco-friendly approach for the preparation of biologically active nanomaterials and extends the potential applications of the plant latex. Overall, the findings provide preliminary experimental support for the reported biological potential of *C. gigantea* latex. However, the specific compounds responsible for these activities and the mechanisms underlying their effects require further investigation.

Further studies involving *in vivo* evaluation, isolation and characterization of active compounds, physicochemical characterization of L-AgNPs, and comprehensive toxicological assessment are recommended to elucidate their mechanisms of action and assess their potential for future pharmaceutical applications.

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