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Green Synthesis of Silver Nano Particles From *Acalypha Indica.L* Leaf Extracts And Evaluation Of Their Antimicrobial Activity

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ABSTRACT

Silver nanoparticles (AgNPs) have gained significant attention due to their potent antimicrobial properties. In the present study, the biosynthesis of silver nanoparticles using the traditionally used medicinal plant *Acalypha indica* and their antimicrobial activity against selected bacterial and fungal pathogens were investigated.The test organisms included Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and fungal species (*Aspergillus niger*, *Aspergillus fumigatus*, and *Candida albicans*), which were isolated from soil samples.For the synthesis of AgNPs, 10 ml of aqueous, ethanol, and acetone leaf extracts of *Acalypha indica* were added to 90 ml of 1 mM silver nitrate ($AgNO_3$) solution. The reaction mixture was incubated at 37°C under static conditions. A visible color change within 30 minutes indicated the formation of silver nanoparticles.

The green synthesized AgNPs were characterized using UV–Visible spectroscopy. The formation of nanoparticles was confirmed by an absorption peak at 338.45 nm. Antimicrobial activity was evaluated, and all extracts showed effectiveness against Gram-positive organisms, with minimum inhibitory concentration (MIC) values ranging from

0.138 to 3.1 mg/ml.Among the different extracts, the acetone leaf extract-mediated AgNPs of *Acalypha indica* exhibited the most effective antimicrobial activity against the tested pathogens, comparable to standard values.

Keywords: Silver nanoparticles (AgNPs), *Acalypha indica*, Synthesis, Antimicrobial, UV-Vis Spectroscopy.

INTRODUCTION

The increasing demand for eco-friendly methods in nanoparticle synthesis has led to the emergence of a novel approach known as "Green Nanotechnology," which eliminates the use of toxic chemicals. The development of reliable green processes for the synthesis of silver nanoparticles (AgNPs) has become a key focus in current nanotechnology research.Various nanomaterials, including silver (Ag), gold (Au), platinum (Pt), and palladium (Pd), have been synthesized using different methods such as hard template techniques and biological approaches involving bacteria, fungi, and plants (Husseiny *et al.*, 2007).

Among these, silver nanoparticles have attracted significant attention due to their unique physical, electronic, and optical properties, as well as their wide range of applications. These include cancer treatment, medical devices, antimicrobial coatings in paints and textiles, and biological and chemical sensing. Additionally, AgNPs exhibit anti-inflammatory, antiviral, antifungal, and antiplatelet activities.Plant-mediated synthesis of silver nanoparticles is an important branch of biosynthesis. It has long been recognized that plants possess the ability to reduce metal ions both on their surfaces and within various tissues, even at sites distant from ion penetration (Makarov *et al.*, 2014).

Acalypha indica, a common weed plant, is widely distributed across India, Sri Lanka, Thailand, and Pakistan, and is known for its significant medicinal value. The leaves of *Acalypha indica* contain bioactive compounds with antibacterial, antifungal, and antioxidant properties, which are beneficial in protecting the skin from environmental hazards. The plant has also been traditionally used in the treatment of pneumonia, asthma, rheumatism, and other ailments (Rimi *et al.*, 2020). Therefore, the present study was undertaken to synthesize silver nanoparticles using *Acalypha indica* leaf extracts and to evaluate their antimicrobial activity against selected pathogens.

MATERIALS AND METHODS

COLLECTION OF SAMPLE

Healthy leaves of *Acalypha indica* were collected from the campus of STET Women's College (Autonomous), Sundarakottai, Thiruvarur District, Tamil Nadu, India. A herbarium specimen was prepared and deposited in the PG and Research Department of Microbiology, STET Women's College (Autonomous), Sundarakottai, Thiruvarur District, Tamil Nadu, India.

PREPARATION OF LEAF EXTRACT (Jebakumar Solomon *et al* 2005)

Fresh, mature leaves of *Acalypha indica* L. were thoroughly washed under running tap water to remove debris and other contaminants, followed by rinsing with double-distilled water. The cleaned leaves were air-dried at room temperature for one week. After complete drying, the leaves were ground into a fine powder using an electric grinder and stored in airtight plastic bags for further use.

Aqueous Leaf Extract

Ten grams of the powdered leaf material were mixed with 100 ml of double-sterilized distilled water in an Erlenmeyer flask. The mixture was boiled for 5 minutes, then allowed to cool and filtered using Whatman filter paper. The filtrate obtained was stored at 4°C for further use.

Acetone Leaf Extract

Ten grams of dried leaf powder were extracted with 250 ml of acetone using a Soxhlet apparatus at a boiling temperature range of 55.5–56.5°C for 8 hours. The extract was then collected and stored at 4°C for subsequent use.

Ethanol Leaf Extract

Ten milligrams of dried and powdered leaf material were mixed with 3 ml of 50% (v/v) ethanol. The mixture was agitated on a rotary shaker for 3 days. After extraction, the samples were centrifuged, and the supernatant was collected and used for nanoparticle synthesis.

SYNTHESIS OF SILVER NANO PARTICLES (Sakthivel and Anitha 2016)

Ten ml of the plant extract was added with 90 ml of 1 mM $AgNO_3$ solution and it was incubated in the dark at 37˚C in static condition until a visible colour change was observed. A similar control set up was prepared with 10 ml of double distilled water in 90 ml of $AgNO_3$ (1 mM) solution.

For synthesis of silver nanoparticles, 10ml acetone extract of *Acalypha indica* was added to 90ml 1mM solution of silver nitrate in 250ml conical flask and kept at room temperature for 1hour. 0.2ml of the ethanol extract was mixed with 2ml of $AgNO_3$ solution under magnetic stirring at 90˚C for one hour to reduce Ag^+ to Ag^0 and was then left at 28˚C until the colour of the mixture changed.

UV -VISIBLE ABSORBANCE SPECTROSCOPY (Sakthivel and Anitha 2016)

The 90-ml silver nitrate solution was added to 10 ml of leaf extract and was put in the shaker until a visible color change was observed. The readings were recorded at regular intervals of time and the absorbance was scanned using the UV-Vis spectra, at a wavelength of 350-800 nm. Further, the mixture was double centrifuged for pellet collection at 10,000 rpm for 5 min; the pellet was collected in vials and stored for cryopreservation at -4˚C. The powdered nanoparticles obtained were stored in plastic airtight containers in the refrigerator for further use.

SERIAL DILUTION (Aneja 2002)

Sterilized tubes were taken and labeled 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} about 1g of sample was added to the first tube (10^{-1}) containing 10 ml of distilled water and shacked well. The sample was serially diluted up to 10^{-5} dilution to 10^{-6} dilution.1 ml of sample suspension were inoculated separated petri plates.

SPREAD PLATE METHOD

Nutrient agar medium was used for spread plate method. It contains Peptone (5g), Beef extract (3g), NaCl (5g), Agar (15g) and distilled water 1000ml. Medium was sterilized at 121°C for 15 minutes. Petri plates were sterilized and labeled as control and 1ml of sample from 10^{-3} dilution was transferred into there's respective plates. Finally, the cooled medium was poured into the samples were incubated at 37°C for 24 hours and the colonies were counted.

GRAM'S STAINING (Han's Christian Gram, 1884)

A thin smear of each of the pure 24 h old culture was prepared on clean grease-free slides, fixed by passing over gentle flame. Each heat-fixed smear was stained by addition of 2 drops of crystal violet solution for 60 sec and rinsed with water. The smear were again flooded with iodine for 30 sec and rinsed with water, decolourized with 70% alcohol for 15 sec and were rinsed with distilled water.

They were then counter stained with 2 drops of Safranin for 60 sec and finally rinsed with water, then allowed to air dry. The smears were mounted on a microscope and observed under oil immersion objective lens. Gram negative cells appeared pink or red while gram positive organisms appeared purple.

MOTILITY TEST (Bailey and Scott, 1966)

A sterile needle was used to pick a loop of a 24 h old culture and was stabbed onto nutrient agar in glass vials. The vials were incubated at 37°C for 24- 48 h. Non-motile bacteria had growth confined to the stab line with definite margins without spreading to surroundings area while motile bacteria gave diffused growth extending from the surface. A positive motility test was indicated by red turbid area extending away from the line of inoculation. A negative test was indicated by red growth along the inoculation line.

INDOLE TEST

5 mL of Tryptone broth was placed into different test tubes after which a loop full of the isolated bacterial isolates was inoculated into the test tubes, leaving one of the test tubes un inoculated to serve as control. The test tubes were then incubated at 37°C for 48 hours.

After incubation, 0.5 mL of Kovac's reagent was added and shaken gently; it was allowed to stand for 20 min to permit the reagent to rise. A red or red-violet colour at the top surface of the tube indicates a positive result while yellow colouration indicates a negative result.

METHYL RED (MR) TEST

5 ml of glucose phosphate broth (1 g glucose, 0.5% Dipotassium phosphate, 0.5% peptone and 100 mL distilled water) were dispensed into clean test tubes and sterilized. The tubes were then inoculated with the isolated test organisms and incubated at 37°C for 48 hours after which few drops of methyl red solution were added to each test and colour change was observed. A red colour indicates a positive reaction.

VOGES-PROSKAEUR (VP) TEST

5 ml of glucose phosphate broth (1 g glucose, 0.5% Dipotassium phosphate, 0.5% peptone and 100 mL distilled water) were dispensed in clean test tubes and sterilized. The tubes were then inoculated with the test organisms and incubated at 37 °C for 48 h. After incubation, 6% α-naphhtol and 6% sodium hydroxide were added to about 1 mL of the broth culture of the isolated organisms. A strong red colouration formed within 30 min indicates positive reaction.

CITRATE UTILIZATION TEST

Citrate utilization test was performed to determine the ability of the microorganisms to utilize citrate as a source of carbon. Simmons's citrate agar medium was prepared and sterilized then a slant was prepared in a test tube. The bacterial culture was inoculated by stabbing to the base and streaking on the surface of slant and it was incubated at 37°C for 48 hours.

After incubation observe the colour change. A positive test is demonstrated by growth with a color change from green to intense blue along the slant. A negative test is demonstrated by no growth and no color change, and the color of the slant remains green.

CATALASE TEST

Catalase test was performed to detect the ability of the organisms to produce the enzyme catalase which degrades hydrogen peroxide. Trypticase soy agar was prepared, sterilized and slants were made. They were inoculated at 37°C for 24 hours. After incubation, 3% hydrogen peroxide was added to tube.

Nutrient agar medium was prepared sterilized and poured in to the petri plates. The isolated organisms were grown on the agar surface. Then 2 or 3 drops of paraaminodimethylalanine oxalate to the surface of the inoculated plates. Formation of purple indicates positive where no colour change indicates a negative result.

UREASE TEST

5ml of prepared urea agar base transferred into each test tube and sterilized by using autoclaving at 121°C for 5 minutes. Slant was made and each tube was inoculated with different bacterial culture. Later, it incubated at 37°C for 24 hours observed the colour change.

TRIPLE SUGAR IRON TEST

The TSI Agar slope was prepared and a loop full of colony was streaked on TSI Agar slope surface in the butt portion, incubated at 37°C for 24 hours colour change of the slant and butt or both indicates the sugar utilization.

IDENTIFICATION OF FUNGI

Fungal testing typically includes a microscopic examination of the sample on a slide, sometimes using a preparation or stain to aid in detection of fungal elements.

DILUTION METHOD (National Committee for Clinical Laboratory Standards 2000)

For determination of minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC), the broth twofold macrodilution method in Muller-Hinton broth was applied. Briefly, test strains were grown in a nutrition medium containing progressively lower dilutions of the test extract and incubated at 37°C. The last two tubes were free of test extract and served as growth control in broth and respective solvent.

After incubation, approximately the 10 µl of content of each test-tube was transferred with a loop onto Muller Hinton agar. Agar plates were incubated for an appropriate time under aerobic conditions at 37°C. MIC was defined as the lowest concentration of extract that allows no more than 20% bacterial growth and MMC as the lowest extract concentration from which the microorganisms did not recover and grow when transferred to fresh medium.

DIFFUSION METHOD (Kirby et al. 1966)

Antimicrobial activity was carried out using disc-diffusion method. Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted at three different concentrations of the crude extract (5, 2.5 and 1.25 mg per disc) with three replicates.

The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Streptomycin (10µg/disc) was used as positive control. The plates were incubated for 24 h at 37°C. Zone of inhibition was recorded in millimeters and the experiment was repeated three replicates. Bacteria and fungi each three pathogens were subjected to antibacterial activity.

STATISTICAL ANALYSES

Random sampling was used for the entire test. The data of all the parameters were statistically analyzed and expressed as mean + SD by using the formula given by Gupta (1977).

$$\text{Mean } X = \frac{\Sigma x}{N}$$

Where, Ex = Sum of all the values of variable

N = Number of observation, SD = N

Where, (x- x) 2 = the sum of the square of the deviation of each value from the mean.

RESULTS

Green Synthesis of Silver Nanoparticles

In recent years, eco-friendly approaches for the synthesis of silver nanoparticles (AgNPs) have gained importance, with green synthesis being preferred over conventional chemical methods due to its non-toxic, cost-effective, and environmentally safe nature. In the present study, silver nanoparticles were synthesized using leaf extracts of *Acalypha indica* L. Healthy leaves were collected from STET Women's College (Autonomous), Sundarakottai, Thiruvarur District, Tamil Nadu, India, and a herbarium specimen was deposited in the PG and Research Department of Microbiology.

Synthesis and Visual Observation

The formation of silver nanoparticles was confirmed by a visible color change due to the reduction of silver ions (Ag^+). The aqueous extract turned **reddish-brown**, the acetone extract turned **yellowish-brown**, and the ethanol extract turned **intense brown**, whereas the control ($AgNO_3$ without extract) showed **no color change**.

UV–Visible Spectroscopy Analysis

UV–Visible spectroscopy confirmed nanoparticle formation, with characteristic absorption peaks observed at **347.9 nm (aqueous extract)**, **367.95 nm (acetone extract)**, and **379.5 nm (ethanol extract)**, indicating surface plasmon resonance of silver nanoparticles.

Identification of Bacterial Isolates

The bacterial isolates were identified as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* based on morphological and biochemical characteristics. These included Gram-positive cocci (≈1 µm) arranged in clusters for *S. aureus*, and Gram-negative rod-shaped motile organisms for *E. coli* and *P. aeruginosa*, with positive biochemical tests such as catalase, oxidase, indole, and citrate reactions.

Identification of Fungal Isolates

Fungal isolates were identified as *Aspergillus niger*, *Aspergillus fumigatus*, and *Candida albicans*. *A. niger* showed black powdery colonies within **2–6 days**, *A. fumigatus* exhibited green conidia measuring **2.5–3 µm**, and *C. albicans* appeared as Gram-positive budding yeast with pseudohyphae.

Dilution Method (MIC and MMC)

Antimicrobial activity was evaluated using the dilution method. The **minimum inhibitory concentration (MIC)** of AgNPs was recorded at **17 µg/ml** against *Escherichia coli* and *Pseudomonas aeruginosa*. The **minimum microbicidal concentration (MMC)** of $AgNO_3$ was observed at **25 mg/ml** against *E. coli* and *Staphylococcus aureus*. Among the extracts tested at different doses, ethanol and acetone extracts showed higher activity at **20 ± 1.0 mg/disc** and **25 ± 1.0 mg/disc**, while aqueous extract showed activity at **17 ± 0.0 mg/disc**.

Disc Diffusion Method

The antimicrobial activity of *Acalypha indica* leaf extracts was evaluated against bacterial and fungal pathogens using different solvents:

- **Aqueousextract:**

  S. aureus (14 ± 1.0 mm), *P. aeruginosa* (15 ± 1.0 mm), *E. coli* (11 ± 1.0 mm), *A. niger* (10 ± 1.0 mm), *A. fumigatus* (11 ± 1.0 mm), *C. albicans* (13.7 ± 1.0 mm)

- **Ethanolextract:**

  S. aureus (18.6 ± 1.0 mm), *P. aeruginosa* (17 ± 1.0 mm), *E. coli* (18 ± 1.0 mm), *A. niger* (18.2 ± 1.0 mm), *A. fumigatus* (17.4 ± 1.0 mm), *C. albicans* (14.2 ± 1.0 mm)

- **Acetoneextract:**

  S. aureus (20 ± 1.0 mm), *P. aeruginosa* (22 ± 1.0 mm), *E. coli* (18 ± 1.0 mm), *A. niger* (19 ± 1.0 mm), *A. fumigatus* (19.8 ± 1.0 mm), *C. albicans* (17.7 ± 1.0 mm)

The highest zone of inhibition was observed in **P. aeruginosa (22 ± 1.0 mm)** and **A. fumigatus (19.8 ± 1.0 mm)** in the acetone extract.

Among all the extracts, the acetone leaf extract of *Acalypha indica* exhibited the highest antimicrobial activity, followed by ethanol and aqueous extracts. The maximum inhibition was observed against *Pseudomonas aeruginosa*, indicating its strong antibacterial potential.

The results clearly demonstrate that plant-mediated silver nanoparticles possess significant antimicrobial properties and can be effectively utilized in biomedical and agricultural applications.

SUMMARY AND CONCLUSION

In the present study, green synthesis of silver nanoparticles (AgNPs) was carried out using leaf extracts of *Acalypha indica* L. The fundamental requirement for the green synthesis of AgNPs includes silver nitrate as the precursor and a natural reducing agent derived from plant extracts.Plant-mediated synthesis of silver nanoparticles is generally preferred over microbial methods, as it is simple, rapid, eco-friendly, and does not require the maintenance of microbial cultures.

Additionally, this method is cost-effective, less biohazardous, and suitable for large-scale production. The synthesized nanoparticles have potential applications in food industries and agricultural disease management.The antimicrobial activity of the synthesized AgNPs was evaluated against selected pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and fungal species such as *Aspergillus niger*, *Aspergillus fumigatus*, and *Candida albicans*.

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Table 1 Characterization of AgNPs of *A.indica* leaf Extracts using UV-Vis spectroscopy

EXTRACTS	PEAK (nm)
Aqueous	347.9
Ethanol	367.95
Acetone	379.5

Table 2 Minimum inhibitory concentration and minimum microbicidal concentration of pathogens

Name of the organisms	MIC (mg/ml			MMC (mg/Ml)		
	Aqueous	Acetone	Ethanol	Aqueous	Acetone	Ethanol
Staphylococcus aureus	10	13	09	18	20	15
Pseudomonas aeruginosa	11	17	14	13	20	20
Escherichia coli	8	10	11	15	22	17
Aspergillus niger	9	11	10	18	25	19

Aspergillus fumigates	13	15	12	20	23	18
Candida albicans	11	16	13	19	24	17

Table 3 Assessment of Antimicrobial Activity Against Bacterial Pathogens.

Name of the Organism	**Zone of Inhibition (Diameter in mm)**		
	ACETONE	**AQEOUES**	**ETHANOL**
E.coli	18.9±1.0	11.8±1.0	17±1.0
P. aeruoginosa	22 ±1.0	15±1.0	19±1.0
S.aureus	20±1.0	14±1.0	18.6±1.0

Value are triplicates and represented as Mean ± Standard deviation

Table 4 Antimicrobial susceptibility test for the fungal pathogen

Name of the Organism	**Zone of Inhibition (Diameter in mm)**		
	ACETONE	**AQEOUES**	**ETHANOL**
A.niger	19±1.0	10±1.0	18.2±1.0
A.fumigatus	19.8 ±1.0	11±1.0	17.4±1.0
C.albicans	27.7±1.0	13.7±1.0	14.±1.0

Values are triplicates and represented as Mean ± Standard deviation