Research Article

Phytoremediation, Antioxidant, Antimicrobial Activity of *Callisia repens* Silver Nanoparticles

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ABSTRACT

The present work investigates the antioxidant, antibacterial, and photocatalytic properties of silver nanoparticles. At first, an apparent colour change was seen as proof that the AgNPs had been generated. The formation, size, and shape of the produced AgNPs were characterised using UV-vis spectroscopy and scanning electron microscopy. The existence of phytocompounds that were in charge of AgNP synthesis, bioreduction, stabilisation, and capping. The AgNPs' and each leaf extract's antioxidant potential was assessed using the TPC, FRAP, and phosphomolybdate tests. The antioxidant assay findings revealed that the AgNPs had higher antioxidant properties than the corresponding leaf extracts. In the TPC leaf, the extract showed greater activity than the respective AgNPs. Employing the well diffusion method in agar, the antimicrobial activity was performed on both bacterial and fungal strains. The photocatalytic activity of the synthesized nanoparticles was examined using the methylene blue degradation process. Green synthesized *C. repens* silver nanoparticles efficiently decreased the dye by approximately 68.77 percent at 72 hr after exposure.

Keywords: Antimicrobial activity, Antioxidant activity, *Callisia repens,* Methylene blue degradation, Silver nanoparticles.

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INTRODUCTION

Metal nanoparticles have a wide range of previously unexpected advantages, according to the latest discoveries by researchers in nanotechnology. Most of them are prepared from noble metals, which include silver, gold, platinum, and palladium.[1]

Silver nanoparticles are one of them and have a great range of potential for biological applications, such as antimicrobial activity[2] and they are non-toxic to humans.[3] Recent research trends highly advise the utilization of nanoparticles, and this is attributed to their nano size, which is one-billionth (10^{-9}). It is well known that nanoparticles have at least one dimension between 1–100 nanometers (nm) in size.[4]

The manufacture of silver nanoparticles has been accomplished by a number of techniques, including physical, chemical, and biological ones. In the beginning, dangerous and poisonous substances were used in the manufacture of silver nanoparticles. As a result, "green synthesis," or environmentally friendly processes are used to create silver nanoparticles. The benefits of green synthesis over conventional synthesis include its single-step process, economic effectiveness, ease of scaling up for large-scale synthesis, and lack of the need for harmful chemicals, high pressure, or temperatures.[5] The use of nanoparticles of silver in the delivery of drugs, discovery of drugs, and new drug therapy has declared war on many terrifying diseases because they employ the body's natural transport channel as well as the infected cell's own mechanisms for absorbing the medication.[6]

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There have been reports of numerous medical applications for nanoparticles. Studies such as the anti-microbial activity of nanoparticles that are green synthesized have been reported[7] the antifungal activities of silver nanoparticles were also demonstrated.[8] In addition, nanoparticles have anti-inflammatory,[9] anti-angiogenesis,[10] and antiviral activity.[11] Due to their abundance in bioactive components,[12] plant extracts have lately been used to create green nanoparticles. Accordingly, this study attempted to create nanoparticles from extracts of plants and evaluate how well they worked in various scenarios.

The goal of the current study is to use a leaf extract from the Commelinaceae family plant *Callisia repens*, often known as the turtle vine plant, for the green synthesis of silver nanoparticles. The current study concentrates on the green synthesis of nanoparticles of silver using *Callisia repens* leaf extract and explores its phytoremediation, antioxidant, antibacterial, as well as antifungal properties. Using the TPC, FRAP, and phosphomolybdate tests, the antioxidant activity will be evaluated. *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus,* and *Bacillus cerus*, respectively, will be used to test the antibacterial action against a Gram-negative and Gram-positive bacterium, and the removal of methylene blue dye will be employed to analyze the photocatalytic activity. The AgNPs can help tend to diseases caused by free radicals and improve the environment by eliminating dangerous azo dyes by satisfying all the goals.

MATERIALS AND METHODS

Reagents

Silver Nitrate ($AgNO_3$), Methanol (CH_3OH), Folin-Ciocalteu phenol reagent, Sodium Carbonate (Na_2CO_3), Sodium Acetate (CH-COONa), Acetic Acid (CH_3COOH), 2,4,6 - Tris(2-pyridyl)-s-triazine (TPTZ), Ferric Chloride ($FeCl_3$), Sulphuric Acid (H_2SO_4), Sodium Phosphate (Na_3PO_4), Ammonium Molybdate($(NH_4)_2MoO_4$), Methylene Blue ($C16H18CIN_3S$), Nutrient agar, Mueller Hinton agar powder, Potato dextrose agar powder, and saline.

Sample collection

Callisia repens plant was collected from a nursery in Kukatpally, Hyderabad, India. Leaves were cleaned to eliminate the dust; the leaf material was rinsed with running tap water and sun-dried. The dried-out leaf material was finely powdered and put into a container for further processing.

Preparation of leaf extract using distilled water

In a 250 mL beaker, 10 g of powder 1 dissolved in 100 mL of double-distilled water, and the solution boiled for 5 min at 60°C, and then set aside overnight before filtering the reaction mixture through Whatman No 1 filter paper. The filtrate was stored in the refrigerator. (Concentration - 0.1g/mL).

Optimization and pH of leaf extract

1.5 mM $AgNO_3$ with different volumes was added with different volumes of leaf extract having different pH in a series of three tubes and stored in a dark place, and the color change in each tube was examined and reported after overnight incubation.

Synthesis and purification of silver nanoparticles

Aliquot 60 mL of the aqueous leaf extract was added to 40 mL of 1.5mM $AgNO_3$ and stored in the dark at room temperature. Nanoparticle formation is visually identified by a change in colour. Centrifugation of the above reaction mixture is done at 5000 rpm for 15 min. The pellet was extracted and rinsed with 5 mL of ethanol for removal of any plant material that may act as a contaminant before centrifugation for 15 min, after which the pellet was collected from the tube and preserved for drying. After drying the pellet, a powder obtained which is 120 mg, 3 mL of DMSO is added which should be stored in the bottle that will be used for further assays. (Concentration - 40mg/mL).

Characterization of AgNPs

The AgNPs obtained by using leaf extract and 1.5 mM $AgNO_3$ at a ratio of 6:4 with pH 8 and overnight incubation time at room temperature were characterized.

UV-visible spectroscopy

Sample (1 mL) of the suspension were collected periodically to monitor the completion of Ag^+ bio-reduction in an aqueous solution, then diluted with 1 mL of deionized water and distilled water served as a blank subsequently scanned from wavelengths of 420 and 520 using UV-vis spectrophotometer. UV-vis spectra were recorded at 0-24 hr intervals.

SEM Analysis

The Scanning Electron Microscope (SEM) is one of the most widely used techniques for characterising nanomaterials and nanostructures. Electron sample interactions provide signals that provide information about the sample, including its chemical composition and surface texture. The produced silver nanoparticles were characterised using SEM images from every stage of the integrative bioconversion process. After drying by air, the samples were placed on aluminium sample

stubs. Before SEM analysis, a thin film of gold was coated onto the mounted samples in the sputter coater's chamber.

Antioxidant activity

Total Phenolic content

The Folin-Ciocalteu method was used to spectrophotometrically determine the total phenolic contents.[13] Gallic acid was used in the construction of the standard curve. 100 µL each of leaf extracts and AgNPs were added to 500 µL of 10% Folin-Ciocalteau reagent, and the mixture was then left to sit at room temperature for 5 min. After 90 min of incubation at room temperature with the addition of 1.5 mL of sodium carbonate, the mixture's total phenols were measured. The resulting blue color's reaction mixture absorbance was measured at 760 nm. Calculations were made in relation to the gallic acid standard curve. Gallic Acid Equivalents (GAE) are phenolic component concentrations given as mg per gram dry weight in the samples.[14]

FRAP Assay

The antioxidant capacity was determined spectrophotometrically using Benzie and Strain's method.[15] The process is based on the reduction of Fe^{3+} TPTZ complex (an inert compound) to Fe^{2+}-tripyridyl triazine (a blue-colored compound), which was created at a low pH by the activity of antioxidants by donating electrons. By analysing the change in absorbance at 420 nm, this process is being monitored. By combining 0.25M acetate buffer, 10mM TPTZ, and 20 mM $FeCl_3.6H_2O$ in a ratio of 10:1:1 at 37°C, the Ferric Reducing Antioxidant Power (FRAP) reagent was prepared.

100 µL of the leaf extract and AgNPs were thoroughly combined with 3 mL of freshly made functioning FRAP reagent. After 5 min of incubation at 37°C, the ferric tripyridyl triazine (Fe3+ TPTZ) complex was reduced to ferrous (Fe2+) form, resulting in the formation of a strong blue colour complex. The absorbance at 593 nm was measured against a reagent blank (3 mL FRAP reagent + 400 µL distilled water). Plotting the absorbance at 573 nm vs. various $FeSO_4$ concentrations produced the calibration curve, Fe^{2+} equivalents (M) or FRAP values were used to express the FRAP value.[14]

Phosphomolybdate Assay

The overall capacity of the extract of the leaf and AgNPs as an antioxidant was calculated using the Prieto-explained phosphomolybdenum technique.[16] 1 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added to an aliquot of 100 µL of the leaf extract and AgNPs. Ascorbic acid was employed as a standard, along with 1 mL of reagent solution and 1 mL of distilled water as a blank. 90 min were spent heating this reaction mixture to 95°C. At 695 nm, the mixture's absorbance was measured after it had cooled to room temperature. Ascorbic Acid Equivalent (AAE) per gramme of sample is used to express the results.

Photocatalytic degradation of dye

Usually, 500 mL of distilled water with 5 mg of methylene blue dye added (0.01 mg/mL) is used as the working solution. Biosynthesized AgNPs were taken in aliquots ranging from 20 to 100 L in a series of tubes. Methylene blue dye (3 mL) was added to each tube. Additionally, a control was kept without the inclusion of AgNPs. The reaction suspension must be well mixed to create the working solution's equilibrium prior to exposure to radiation. The dispersion was then exposed to sunlight and was observed for 72 hr. At predetermined intervals, aliquots of a 2-3 mL suspension were obtained to measure the dye's photocatalytic breakdown. The absorbance spectrum was determined using a UV-vis spectrophotometer. The amount of dye present during dye degradation is determined by the absorbance at 490 nm. The following formula was used to estimate the percentage of dye degradation:

$$\%\text{Degradation} = \%\text{Degradation} = 100 * C_o\text{-}C\ /C \quad (1)$$

Where C_o is the initial concentration of dye solution and C is the concentration of dye solution after photocatalytic degradation.[17]

Antimicrobial activity

Disc diffusion assay is used for both the antibacterial and antifungal activities of the biosynthesized silver nanoparticles, leaf extract, $AgNO_3$ and standard antibiotics.

Antibacterial assay

Varying concentrations of the leaf extract and biosynthesized AgNPs tested for bioactivity against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus and Bacillus cereus*. Pure cultures of bacteria were sub-cultured on a Nutrient broth medium at 37°C and incubated overnight. Prepare Muller-Hinton agar medium. Autoclave the medium and pour it into Petri plates while still it is in a molten state. Allow it to solidify in a cool place. Puncture the plates with the help of borer aseptically and the extracts added. Leaf extracts (5 mg/50 µL, 10 mg/100 µL), AgNPs (1 mg/25 µL,

2 mg/50 µL, 3 mg/75 µL, and 4 mg/100 µL), $AgNO_3$, DMSO. Inoculate the plates by swabbing with bacteria. To determine the sensitivity of each bacterial species, antibiotics (ciprofloxacin-10 µg/mL) are used as a positive reference control. Overnight incubation of the plates at 37°C was done. The diameters of zones of inhibition were measured using an ordinary scale.

Antifungal assay

Varying concentrations of the leaf extracts and AgNPs were determined against the *Aspergillus*. Prepare Potato dextrose agar medium. Autoclave the medium and pour it into Petri plates while still it is in a molten state. Allow it to solidify in a cool place. Puncture the plates with the help of borer aseptically and the extracts added. Leaf extracts (5 mg/50 µL, 10 mg/100 µL), and AgNPs (1 mg/25 µL, 2 mg/50 µL, 3 mg/75 µL, and 4 mg/100 µL). Inoculate the plates by swabbing with *Aspergillus*. Determination of the antifungal activity is done after 1 week of incubation at 37°C. The diameters of the zone of inhibition are measured using an ordinary scale.

Statistical analysis

Quantitative data is presented in tables, and the data is calculated in MS Excel. The data was subjected to descriptive statistics and stated as Mean ± Standard Deviation (SD) (n=3).

RESULTS

Optimization and pH of leaf extract

The leaf extract turned to dark brown color after overnight incubation with the addition of silver nitrate at a concentration of 6:4 ratio of leaf extract and silver nitrate. Change in color indicates the formation of AgNPs. At pH 8 color change was observed when compared with pH 7 and pH 9.

Biosynthesis of silver nanaoparticles

Initial preparation of silver nanoparticles involved reduction of Ag+ into Ag^0 with leaf extract, at room temperature. Change in colour from visible yellow to dark brown with overnight incubation is indicative of silver nanoparticles formation.

CHARACTERISATION OF SILVER NANOPARTICLES

UV-visible Spectrophotometer Analysis

The absorbance of AgNPs is indicated by a peak in the surface plasmon resonance of AgNPs that is centred at 490 nm.

SEM Analysis

Silver nanoparticles that have been bio-synthesized are visible in size and shape in SEM images. At various magnifications, the nanoparticles' size was observed. The size range of 93 to 130 nm was noted for the spherical form of nanoparticles with significant aggregation (Figure 3). Due to the aggregation of smaller nanoparticles, some of the nanoparticles in this SEM image have large dimensions.

Antioxidant activity

Total Phenolic content

By using the Folin-Ciocalteu (F-C) method and gallic acid as the standard, the total phenolic contents of the *Callisia repens* aqueous extract and its nanoparticles were determined. Gallic acid absorbance measurements at various concentrations were utilised to create the calibration curve (Figure 7a). In comparison to AgNPs (0.303 0.003 GAE/gm), leaf extract (0.357 0.002 GAE/gm) had the highest amount of phenolic content.

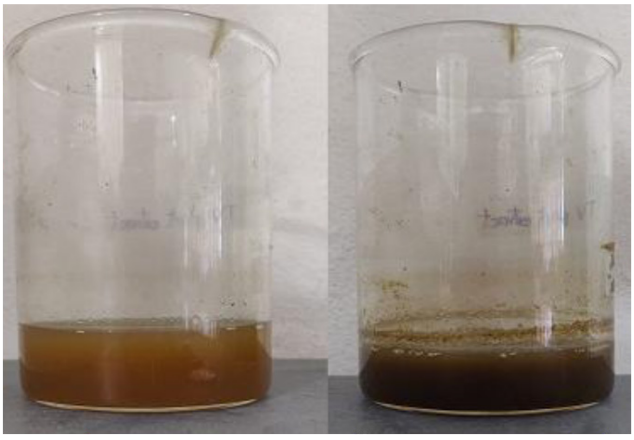


Figure 1: Biosynthesis of AgNPs using *Callisia repens*.

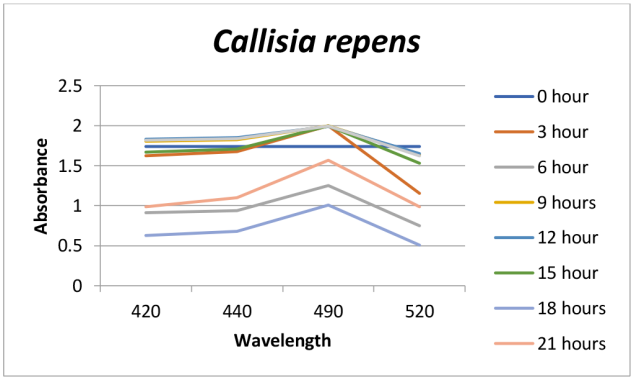


Figure 2: UV-vis spectrum of silver nanoparticles synthesized by leaf extract of *Callisia repens*.

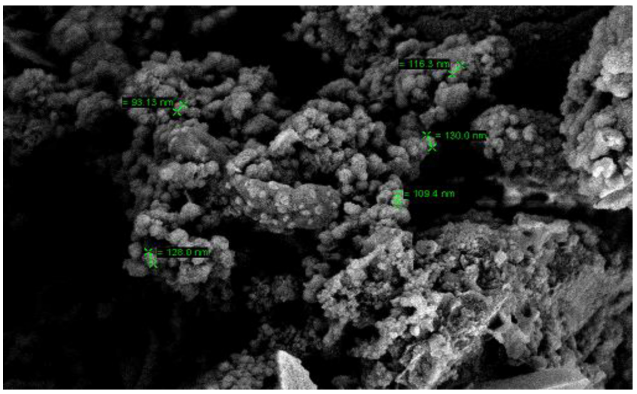
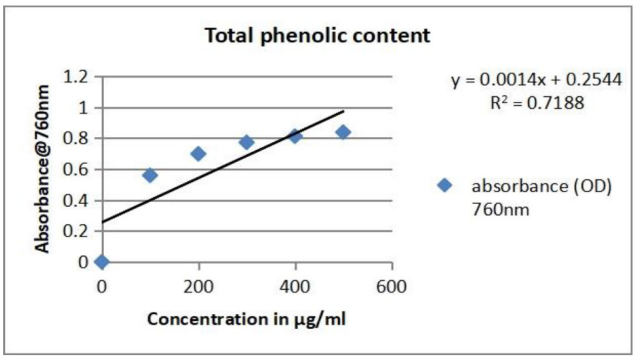
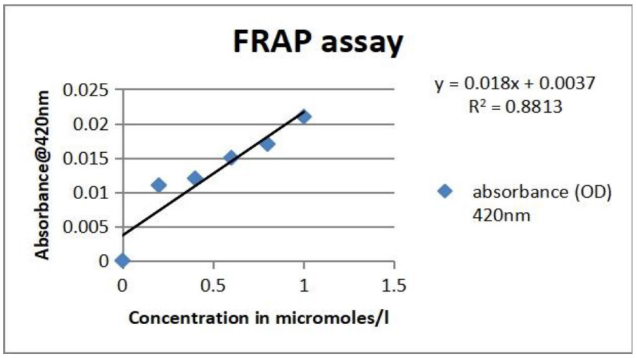


Figure 3: SEM images of silver nanoparticles synthesized from leaf extract of *Callisia repens*.



(a)



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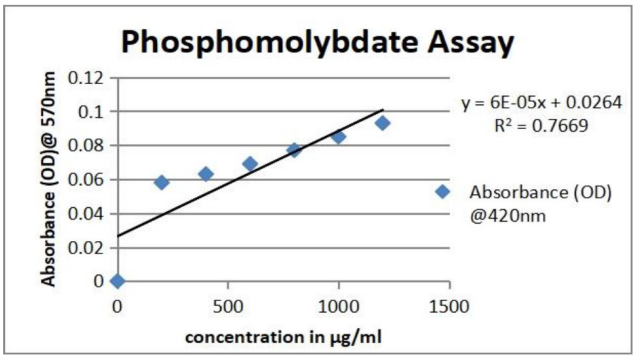

(c)

Figure 4: Standard curve for Antioxidant assays. (a) Standard curve for TPC. (b) Standard cure for FRAP. (c) Standard curve for Phosphomolybdate assay.

FRAP ASSAY

The reducing power of Fe^{2+} by selected plants was evaluated. All of the extracts from the chosen plant *Callisia repens* demonstrated concentration-dependent reduction power, similar to the radical scavenging activity. The maximum reducing antioxidant power was found in AgNPs (0.738 ± 0.003 µM/L) than the leaf extract (0.587 ± 0.002 µM/L) as compared to standard ferric sulfate (Figure 7b).

Phosphomolybdate Assay

This assay relies on the generation of a green phosphate, which can be detected spectrophotometrically, as a result of the reduction of phosphomolybdate ions in the presence of an antioxidant. demonstrates *Callisia repens*'s ability to be an antioxidant. However, because of its antioxidant capabilities, ascorbic acid, a popular antioxidant, was chosen as the positive control.

The maximum antioxidant capacity was found in AgNPs (24.543 ± 0.32 AAE per gram sample) than the leaf extract (19.777 ± 0.23 AAE per gram sample) as compared to standard ascorbic acid (Figure 7c).

Photocatalytic Degradation of Dye

Visual Observation

Methylene blue is broken down photocatalytically under sunlight using greenly synthesised silver nanoparticles. Colour shifts in dye degradation serve as the earliest indicator. After being exposed to solar light for 72 hours while being incubated with silver nanoparticles, the dye's initial deep blue colour transformed to a light blue hue. After that, the dark blue turned light blue. At 72 hr, the degradation process was finally finished, and the change in the reaction mixture allowed for its identification (Figure 5).

UV-visible spectrophotometer

AgNPs, a complex dye, were exposed to sunlight for longer periods, and their oxidation percentage increased. Methylene blue dye's peak absorption was reduced, and AgNPs' 490 nm absorption was enhanced (Figure 6).

Table 1: Total Phenolic content, FRAP assay, Phosphomolybdate assay of leaf extracts and Nanoparticles.

Extract	Total phenolic content (GAE/ gm)	FRAP value of sample (µM/l)	Ascorbic acid equivalent (AAE) per gram sample
Callisia repens leaf extract	0.357 ± 0.002	0.587 ± 0.002	19.777 ± 0.23
Callisia repens Nanoparticles	0.303 ± 0.003	0.738 ± 0.003	24.543 ± 0.32

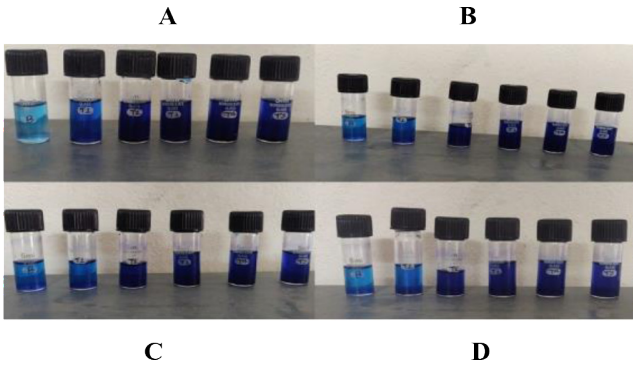

Figure 5: Visual observation of color change from deep blue to light blue indicates degradation of methylene blue at different time intervals (*Callisia repens*).
(A) 0 hr (B) 0-24 hr (C) 0-48 hr (D) 0-72 hr.

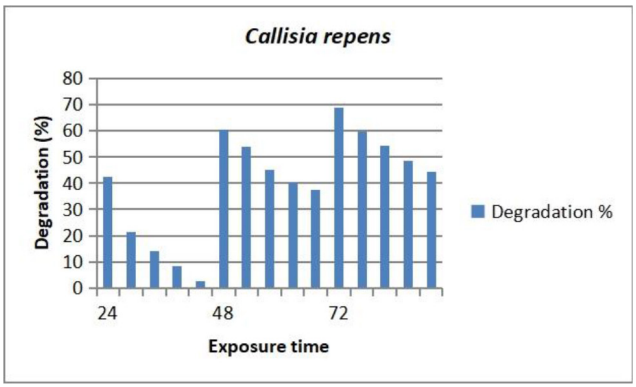

Figure 6: Percentage of dye degradation by 10mg of synthesized silver nanoparticles of *Callisia repens* at different functional time intervals.

Table 2: Results of Antibacterial assay of leaf extract and Nanoparticles.

Organism	Nanoparticles 25µL in mm	Nanoparticles 50µL in mm	Nanoparticles 75µL in mm	Nanoparticles 100µL in mm	Leaf extract 100µL in mm	Antibiotics in mm	$AgNO_3$ in mm	DMSO in mm
E. coli	0	0	0	9.16 ± 0.15	11.9 ± 0.4	18.06± 0.3	0	0
Bacillus cerus	0	0	0	7.960 ± 0.15	8.03 ± 0.25	15.1 ± 0.26	0	0
Klebsiellapneumoniae	0	0	6.43 ± 0.25	12.06 ± 0.20	10.03 ± 0.35	15.03± 0.15	0	0
Staphylococcus aureus	0	0	8.03 ± 0.41	10.02 ± 0.15	9 ± 0.3	20.06± 0.2	0	0

Table 3: Results of Antifungal assay of leaf extract and Nanoparticles.

Extract name	Organism	Nanoparticles 25µl in mm	Nanoparticles 50µl in mm	Nanoparticles 75µl in mm	Nanoparticles 100µl in mm	Leaf extract 100µl in mm
Callisia repens	*Aspergillus*	2.36 ± 0.15	3.5 ± 0.2	5.96 ± 0.25	8.33 ± 0.15	6.93 ± 0.25

The photocatalytic degradation of dye was nearly complete because dye's absorption value's gradual decline toward the baseline and the enhanced peak for AgNPs.

Antimicrobial activity

Antibacterial assay

Inhibition of both gram-positive and gram-negative bacterial strains was observed significantly (Table 2). It was proved that AgNPs and leaf extract have excellent antibacterial activity against *Klebsiella pneumoniae* (12.06 ± 0.20mm) *Staphylococcus aureus* (10.02 ± 0.15mm) and *E. coli* (11.9 ± 0.4mm), *Klebsiella pneumoniae* (10.03 ± 0.35mm) (Figure 7a).

Antifungal Assay

The AgNPs and leaf extract have excellent antifungal activity (Table 3) against *Aspergillus* (8.33 ± 0.15mm) and (6.93 ± 0.25mm) (Figure 7b).

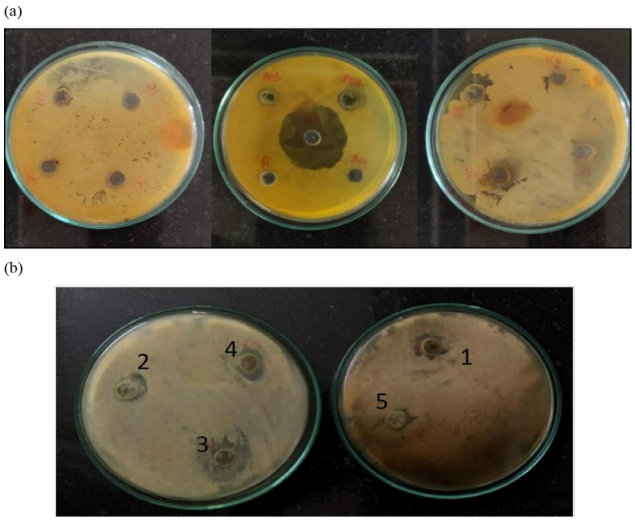

Figure 7: (a) Antibacterial assay *Callisia repens* AgNPs and leaf extract (b) Antifungal assay of *Callisia repens* AgNPs and leaf extract against *Aspergillus*.
1-25µL nanoparticles, 2-50µL nanoparticles, 3-75 µL nanoparticles, 4-100 µL nanoparticles, 5-100µL leaf extract.

DISCUSSION

This paper details the phytoremediation, antioxidant, and antibacterial properties of synthetic AgNPs made from *C. repens* leaf extract, which offers a straightforward, economical, and environmentally friendly way to make AgNPs.

After an overnight incubation, a colour change from yellow to dark brown was noticed when the leaf extract was introduced to the silver nitrate solution. The silver nitrate had been reduced into silver nanoparticles, as seen by the dark brown colour (Figure 1). Furthermore, the UV-Vis spectrophotometer (420-520 nm) indicated the creation of silver nanoparticles. The creation of silver nanoparticles was verified by the absorbance band at 490 nm, which was observed as a result of localised surface plasmon resonance (Figure 2).

Silver nitrates are reduced and capped into silver nanoparticles by the bioactive compounds present in *C. repens* leaves. AgNPs are stabilised by the capping agent**.** UV-vis and SEM were used to characterize the silver nanoparticles. The effective antioxidant, antibacterial, and photocatalytic degradation activity of the AgNPs produced in this manner was remarkable. These AgNPs may be employed in industries as an alternative material or as a silver dressing for wounds due to their benign, stable nature and antimicrobial properties.

SEM studies provided that AgNPs are spherical in shape with high agglomeration noted, which are formed due to dehydration during sample preparation. Due to the aggregation of smaller nanoparticles, some of the nanoparticles in this SEM image have large dimensions. The aggregation of nanoparticles is caused by insufficient capping agents in the leaf extract during nanoparticle manufacturing. Similar to this, M. Vanaja *et al.* reported earlier on the aggregation of nanoparticles[17] by reducing silver ions using a leaf extract of *Morinda tinctoria.*

It is well-recognised that free radicals play a significant role in a wide range of clinical manifestations. Antioxidants protect us from numerous diseases by eliminating free radicals. They exert their role either by scavenging the reactive oxygen species from the environment or by protecting the antioxidant defence mechanisms.[18]

Phenolic compounds, which are secondary metabolites and are the phytochemical compounds produced from phenylalanine and tyrosine, are widely distributed in plants.[19] that the phenolic compounds in the extracts directly function as an antioxidant through the chain reaction's reduction of an oxidised intermediate.[14] FRAP assay and Phosphomolybdate assay have confirmed that silver nanoparticles have antioxidant activities.

The photocatalytic activity of silver nanoparticles made greenly is evaluated using the dye methylene blue. The primary absorption peak at 490 nm steadily dwindled with increasing exposure time, indicating photocatalytic breakdown of the methylene blue dye. The current study's findings suggest that the use of a naturally occurring, renewable as well environmentally friendly reducing agent for the synthesis of nanoparticles of silver produces excellent photocatalytic activity against molecules and can be used in the treatment of dye effluent and water purification systems.[17]

The first investigation into the composition of AgNPs obtained from *C. repens* leaves is this one. On gram-positive bacteria, gram-negative bacteria, and fungal species, leaf extracts and AgNPs' antimicrobial activity demonstrated good inhibition.

There are numerous potential modes of action for the AgNPs' ability to inhibit bacterial growth has been demonstrated although a proposed, the precise method is yet unclear. In general, it has been hypothesised that the silver cations from AgNPs bind to the negative-charged bacterial cell wall, rupturing which results in the denaturation of proteins and ultimately cell death.[20] When silver cations or nanoparticles attach to the cell wall, they result in an increase in the precursors of the envelope protein, which ultimately causes the loss of the proton motive force and cell death. AgNPs have also been shown to have the potential to rupture the outer and plasma membranes, which results in intracellular ATP reduction.[21]

The proposal of another mechanism is that reactions linked to produce R-S-S-R bond o. On the cell wall, there are sulphur groups with silver and oxygen, c Consequently, blocking respiration and resulting in cell death.[22]

Additionally, AgNPs display a strong antibacterial effect against the chosen microbes due to their small size and the presence of capping agents. These silver nanoparticles may be used as antibiotics since they are non-toxic, inexpensive, and environmentally friendly. Various plant and fruit extracts can be used to create Ag nanoparticles in a more cost- and environmentally-friendly manner.

CONCLUSION

The current study uses *C. repens* leaf extract to describe how silver nanoparticles develop. The bioactive elements in the leaves of *C. repens* are in charge of reducing silver nitrates into silver nanoparticles and capping them. The

AgNPs' stability is due to the capping agent. AgNPs created synthetically possess antioxidant properties. This activity is brought about by the functional groups that are present on the surface of AgNPs. Silver nanoparticles generated sustainably were tested for their photocatalytic activity using the colour methylene blue. The current study's findings suggest that reducing agents that are natural, sustainable, and environmentally friendly can be used to create silver nanoparticles that have outstanding photocatalytic properties against molecules and can be used to treat dye effluent and purify water. Furthermore, because of their tiny size and inclusion of capping agents, AgNPs exhibit potent antimicrobial and antifungal properties against the chosen pathogens. Given that silver nanoparticles are non-toxic, affordable, environmentally safe, and highly efficient against bacteria, upcoming antibiotics may contain them.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATIONS

nm: Nanometer; **mL:** Milliliters; **g:** Grams; **min:** Minutes; **C:** Celsius; **mM:** Millimolar; **g/mL:** Grams/milliliter; **mg:** Milligram; **mg/mL:** Milligram/milliliter; **hr:** Hour; **min:** Minutes; **µl:** Microliter; **M:** Molar; **mg/µL:** Milligrams/microliter; **µM/L:** Micromolar/liter; **AgNPs:** Silver nano particles; **TPC:** Total Phenolic Content; **FRAP:** Ferric Reducing Antioxidant Power; **Ag:** Silver; **AgNO3:** Silver Nitrate; **CH3OH:** Methanol; **Na2CO3:** Sodium Carbonate; **Na2COONa:** Sodium Acetate; **CH3COOH:** Acetic Acid; **TPTZ-2,4,6:** Tris(2-pyridyl)-s-triazine; **FeCl3:** Ferric Chloride; **H_2SO_4:** Sulphuric Acid; **Na3PO4:** Sodium Phosphate; **(NH4)2MoO4:** Ammonium Molybdate; **C16H18CIN3S:** Methylene blue; **MH Agar:** Mueller Hinton agar powder; **PDA:** Potato dextrose agar.

SUMMARY

The development of silver nanoparticles is described in the present study using *C. repens* leaf extract and describes the phytoremediation, antioxidant, and antibacterial activities of synthetic AgNPs. Synthesized nanoparticles were characterized using UV-vis spectroscopy and SEM. The TPC, FRAP, and phosphomolybdate tests were used to determine the antioxidant capacity of the AgNPs and each leaf extract. In comparison to AgNPs (0.303 0.003 GAE/gm), leaf extract (0.357 0.002 GAE/gm) had the highest amount of phenolic content. The maximum reducing antioxidant power was found in AgNPs (0.738 ± 0.003 µM/L) than the leaf extract (0.587 ± 0.002 µM/L) as compared to standard ferric sulfate. The maximum antioxidant capacity was found in AgNPs (24.543 ± 0.32 AAE per gram sample) than the leaf extract (19.777 ± 0.23 AAE per gram sample) as compared to standard ascorbic acid. Methylene blue is used to assess the photocatalytic activity. Extended exposure times, gradually diminished the principal absorption peak at 490 nm, showing photocatalytic degradation of the dye. Antimicrobial activity of the nanoparticles and leaf extract was tested and they demonstrated good inhibition.

REFERENCES

1. Roy N, Barik A. Green synthesis of silver nanoparticles from the unexploited weed resources. Int J Nanotechnol. 2010;4:95.
2. Sap-Iam N, Homklincha C, Larpudomle R, Warisnoich W, Sereemaspu A, Dubas ST. UV irradiation-induced silver nanoparticles as mosquito larvicides. J Appl Sci. 2010;10(23):3132-6. doi: 10.3923/jas.2010.3132.3136.
3. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv. 2009;27(1):76-83. doi: 10.1016/j.biotechadv.2008.09.002, PMID 18854209.
4. Edmundson M, Thanh NT, Song B. Nanoparticles based stem cell tracking in regenerative medicine. Theranostics. 2013;3(8):573-82. doi: 10.7150/thno.5477, PMID 23946823.
5. Saha J, Begum A, Kumar MA. S "A novel green synthesis of silver nanoparticles and their catalytic action in reduction of methylene blue dye,". Sustain Environ Res. 2017.
6. Balaji S. Nanobiotechnology. Chennai, India: MJP Publishers; 2010.
7. Mohamed TK, Widdatallah MO, Ali MM, Alhaj AM, Elhag DEAE. Green synthesis, characterization, and evaluation of the antimicrobial activity of *Camellia sinensis* silver nanoparticles. J Nanotechnol. 2021.
8. Kannan N. B, park HH. Antifungal activity of silver nanoparticles synthesized using turnip leaf extract (*Brassica rapa* L.) against wood-rotting pathogens. Eur J Plant Pathol. 2014;140(2):185-92. doi: 10.1007/s10658-014-0399-4.
9. Aparna Mani KM, Seethalakshmi S, Gopal V. Evaluation of *in vitro* anti-inflammatory activity of silver nanoparticles synthesised using *Piper nigrum* extract. J Nanomed Nanotechnol. 2015.
10. Baharara J, Namvar F, Mousavi M, Ramezani T, Mohamad R. Anti-angiogenesis Effect of Biogenic Silver Nanoparticles Synthesized Using *Saliva officinalis* on Chick Chorioalantoic Membrane (CAM). Molecules. 2014;19(9):13498-508. doi: 10.3390/molecules190913498, PMID 25255752.
11. Haggag EG, Elshamy AM, Rabeh MA, Gabr NM, Salem M, Youssif KA, *et al.* Antiviral potential of green synthesized silver nanoparticles of *Lampranthus coccineus* and *Malephora lutea*. Int J Nanomedicine. 2019;14:6217-29. doi: 10.2147/IJN.S214171, PMID 31496682.
12. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B Biointerfaces. 2010;76(1):50-6. doi: 10.1016/j.colsurfb.2009.10.008, PMID 19896347.
13. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. Methods Enzymol. 1999;299:152-78. doi: 10.1016/S0076-6879(99)99017-1.
14. Rajurkar NS, Hande SM. Estimation of phytochemical content and Antioxidant activity of some selected traditional Indian Medicinal Plants. Indian J Pharm Sci. 2011;73(2):146-51. doi: 10.4103/0250-474x.91574, PMID 22303056.

15. Benzie IF, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as measurement of "antioxidant power" The FRAP assay. Anal Biochem. 1996;239(1):70-6. doi: 10.1006/abio.1996.0292, PMID 8660627.
16. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999;269(2):337-41. doi: 10.1006/abio.1999.4019, PMID 10222007.
17. Vanaja M, Paulkumar K, Baburaja M, Rajeshkumar S, Gnanajobitha G, Malarkodi C, *et al*. Degradation of methylene blue using biologically synthesized silver nanoparticles. Bioinorg Chem Appl. 2014;2014:742346. doi: 10.1155/2014/742346, PMID 24772055.
18. Umamaheswari M, Chatterjee TK. *In vitro* antioxidant activities of the fractions of *Coccinnia grandis* L. leaf extract. Afr J Trad Complement Altern Med. 2008;5:61-73.
19. Naczk M, Shahidi F. Extraction and analysis of phenolics in food. J Chromatogr A. 2004;1054(1-2):95-111. doi: 10.1016/S0021-9673(04)01409-8, PMID 15553136.
20. Lin YE, Vidic RD, Stout JE, McCartney CA, Yu VL. In activation of *Mycobacterium avium* by copper and silver ions. Water Res. 1998;32(7):1997-2000. doi: 10.1016/S0043-1354(97)00460-0.
21. Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun HZ, *et al*. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J Proteome Res. 2006;5(4):916-24. doi: 10.1021/pr0504079, PMID 16602699.
22. Kumar VS, Nagaraja BM, Shashikala V, Padmasri AH, Madhavendra SS, Raju BD, *et al*. Highly efficient Ag/C catalyst prepared by electro-chemical deposition method in controlling microorganisms in water. J Mol Catal A Chem. 2004;223(1-2):313-9. doi: 10.1016/j.molcata.2003.09.047.

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