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Phytochemical Profiling, Green Synthesis, and Bioactivity Evaluation of Silver Nanoparticles (AgNPs) Synthesized from *Ipomoea laxiflora* Extract

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ABSTRACT

This study focuses on the eco-friendly production of silver nanoparticles using leaf extract from Ipomoea laxiflora and assesses their antioxidant and hemolytic effects. To our knowledge, this is the first report on the synthesis of silver nanoparticles using this species. Green synthesis offers immense potential in both medical and environmental fields, aiming to utilize less hazardous chemicals. Plant-based synthesis, in particular, is considered safe and effective due to the presence of reducing and capping agents within plant extracts. Ipomoea laxiflora H.J. Chowdhery & Debta, belonging to the Convolvulaceae family, is an annual climber native to Tropical Africa and India. It has been traditionally used to treat fever, headaches, and stomach aches. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, phenols, tannins, terpenoids, steroids, glycosides, and cardio glycosides. Quantification of phytochemical contents, including total phenolic, flavonoid, and proanthocyanin content, was also performed. FT-IR spectroscopic analysis indicated characteristic peak values of major functional groups such as alkene, alkane, and carbonyl. Silver nanoparticles were synthesized by adding 10 mL of methanolic leaf extract to 90 mL of 1 mM aqueous silver nitrate solution, followed by heating at 80 degrees Celsius for three hours with continuous stirring. The change in color from yellow to dark brown confirmed the formation of silver nanoparticles. The antioxidant activity, determined by DPPH radical scavenging assay, showed 94% scavenging activity for methanolic extract compared to 98% for ascorbic acid. Higher concentrations exhibited increased scavenging activity. Total antioxidant activity ranged from 60% to 89% in hexane and methanol extracts, with methanol showing the highest concentration. Hemolytic activity was observed between concentrations of 10 to 100 µg/ml, with a hemolysis rate of 2.751% at a concentration of 100 µg/mL. The development of green nanoparticles using natural sources like Ipomoea laxiflora holds significant importance for environmental sustainability, health benefits, diverse biomedical applications, resource efficiency, and cost-effectiveness. Embracing such green approaches not only advances nanotechnology but also aligns with broader goals of promoting sustainable development.

Keywords: *Phytochemical, FT-IR, Green synthesis, AgNPs, Ipomoea laxiflora, Antioxidant and Hemolytic activity.*

Introduction

Nanotechnology involves the synthesis of particles with at least one dimension in the range of 1–100 nm, resulting in high surface-to-volume ratios. As particle size decreases, not only does the ratio of surface area to volume increase, but also the physical, chemical, and biological properties of the particles differ compared to their bulk counterparts. The significance of nanomaterials in addressing material science challenges is on the rise. One of the most promising areas of nanotechnology is the production of silver nanoparticles (AgNPs). Various physical, chemical, and biological techniques have been employed to synthesize silver-based nanoparticles in environmentally friendly ways [1-8]. Many research fields are focused on green chemistry to enhance and/or protect our global environment. The applications of silver nanoparticles are extensive, spanning from food processing, cosmetics, home cleaning, catalytic and garment production to medicinal applications. AgNPs have been used medicinally for the treatment of diseases such as cancer, HIV, diabetes, malaria, and tuberculosis. Green chemistry-based strategies have gained attention in recent years for nanoparticle synthesis, offering environmentally friendly approaches among various chemical and physical methods [9-16]. When plant extracts are utilized to reduce and stabilize silver nanoparticles, they do not contain synthetic chemical compounds on their surface, making them non-toxic to humans and the environment. The phytochemicals adhering to the surface of nanoparticles contribute to the scavenging effect of AgNPs against free radicals. Green synthesis stands out as one of the most environmentally friendly and cost-effective approaches, characterized by high reproducibility and yield. In addition to the use of bacteria, fungi, and algae, aqueous extracts of leaves have been found to effectively reduce silver salt and promptly cap the nano-sized silver to prevent agglomeration [17-25].

Medicinal plants have long served as invaluable sources of pharmacologically active substances, offering a diverse array of benefits ranging from medicine to nutrition, flavoring, and beyond. In-fact, herbs are still utilized in 40% of prescription drugs today. The rich diversity of bioactive phytochemicals found in medicinal plants is a remarkable gift of nature, exploited since ancient times in traditional, herbal, and ethnomedicine to combat various ailments and disorders [6]. Despite challenges associated with plant-derived drugs, modern medicine recognizes the value of exploring this phytochemical diversity for discovering new bioactive molecules or templates for drug development. Plant-based therapeutic phytochemicals offer several advantages, including structural diversity, superior quality, and potent curative properties with fewer adverse effects, safety, affordability, and cultural acceptance across diverse populations [26-31].

The use of medicinal plants is increasingly viewed as complementary and alternative therapy, particularly in addressing diseases linked to free radical production. Free radicals, integral to aerobic life and metabolism, can cause cellular damage, but antioxidants found abundantly in plants help protect cells by neutralizing these free radicals. Phytochemicals, often referred to as 'plant-chemicals,' constitute the non-nutritive components of plants with numerous health benefits and disease-prevention properties [7-9].

Plants rich in antioxidants, predominantly containing phenols and flavonoids, exhibit the ability to scavenge free radicals owing to their unique chemical structures. This antioxidant activity contributes to the plant's therapeutic properties and potential to prevent various diseases. The global herbal medicines market has been steadily growing, with a substantial increase projected in the coming years. In 2022, the market was valued at USD 170 billion, and it is anticipated to reach USD 600 billion by 2033, reflecting a compound annual growth rate (CAGR) of 15% from 2023 to 2033. This growth underscores the increasing recognition and utilization of herbal medicines worldwide [32-37].

Ipomoea laxiflora H.J.Chowdhery and Debta, belonging to the Convolvulaceae family, is an annual climbing plant with vines reaching lengths of 5–7 meters. Its distribution spans various regions worldwide, including India, specifically Uttarakhand, Maharashtra (Western Ghats), Gujarat, Uttar Pradesh, and Telangana, with endemism noted in Uttarakhand. Morphologically, I. laxiflora closely resembles I. triloba L., although it can be distinguished by the absence of hairs on the ovary and capsules. Traditionally, *Ipomoea laxiflora* has been utilized for its medicinal properties, particularly in the treatment of fever, headaches, and stomach aches [39-42].

Material and Methods

Collection and Authentication of Plant Material

The leaves of Ipomoea laxiflora were collected from Manneguda Village in Ranga Reddy District of Telangana State, India, during August and September of the year 2022. The plant specimen was authenticated by the Botanical Survey of India, Deccan Regional Centre, Hyderabad, Telangana, with the accession number BSI/DRC/2023-24/Identification/405. A preserved specimen was deposited at the Herbarium Hyderabadensis, Department of Botany, Osmania University, Hyderabad, Telangana, India.

Drying

After cleaning, the leaves of *Ipomoea laxiflora* were cut into pieces measuring approximately 0.5—1.5×1×0.2 cm^3 using scissors and knives. These leaf pieces were then left for shade drying on newspapers for a period of ten days. Subsequently, the dried leaf pieces were subjected to further drying in a hot air oven at 40°C for one hour to eliminate any remaining moisture content. This preparation step was undertaken just prior to initiating the extraction process.

Hot Continuous Successive Extraction Using Soxhlet Apparatus

To develop extracts from *Ipomoea laxiflora* leaves, fresh leaves were first obtained and thoroughly sanitized with running water to remove all particles and dirt. The leaves were then subjected to extraction using a range of solvents, including hexane, chloroform, ethyl acetate, and methanol. Following extraction, the leaves were once again washed with distilled water to eliminate any residual impurities.

Next, the dried leaf powder was prepared using a mechanical grinder and then sieved to ensure uniform particle size. The leaf powder was then subjected to successive extraction using a Soxhlet apparatus. Hexane extraction was conducted at a temperature of 60°C, chloroform at 61°C, ethyl acetate at 77°C, and methanol at 65°C. The extraction temperatures were adjusted to correspond with the boiling points of the solvents to optimize extraction efficiency. Each solvent was allowed a duration of five hours for continuous and repeated hot extraction.

The resulting extracts were further concentrated and evaporated in an oven at 45°C. The dried extracts obtained were subsequently utilized for phytochemical screening purposes. This comprehensive extraction method allows for the extraction of a wide range of phytochemicals from *Ipomoea laxiflora* leaves, which can then be further analyzed for their potential bioactive properties.

Calculation of percentage yield

The dried extracts obtained from all solvents were weighed, and the final yield was calculated based on the weight of the plant material after complete evaporation in the absence of moisture. This calculation allows for the determination of the concentration and yield of the extracted compounds from *Ipomoea laxiflora* leaves using each solvent.

$$\text{Percentage of yield } (\%) = \left(\frac{\text{Dry weight of extract}}{\text{Dry weight of a plant}} \right) \times 100$$

Qualitative Screening of Phytochemicals

The leaf extract underwent preliminary phytochemical screening to detect the presence of secondary metabolites. Standard procedures were followed for conducting phytochemical tests, which included qualitative analysis for various bioactive compounds. The tests were performed for alkaloids, flavonoids, saponins, steroids & terpenoids, phenolic compounds, tannins, cardiac glycosides, glycosides, coumarins, anthraquinones, quinones, and resins. The results of these tests determined the presence or absence of these bioactive compounds in the leaf extract.

[43,44,45,46,47,48,49,50,51,52,53 54].

The phytochemical screening of the leaf extract of Ipomoea laxiflora was conducted to detect the presence of various secondary metabolites using standard procedures. The following tests were performed qualitatively to determine the presence or absence of specific bioactive compounds:

1. Alkaloids Detection (Mayer's Test): The extract was dissolved in dilute hydrochloric acid and filtered, followed by the addition of Mayer's reagent. Formation of a cream-colored precipitate indicates the presence of alkaloids.

2. Flavonoids Test: The test solution was boiled with magnesium turnings and concentrated hydrochloric acid. Development of a red coloration indicates the presence of flavonoids.

3. Saponins Test: The powdered drug was boiled with water, filtered, and the filtrate was shaken vigorously. Frothing indicates the presence of saponins.

4. Steroids & Terpenoids Detection (Liebermann-Burchardt test): The extract was mixed with chloroform, acetic

anhydride, and concentrated sulfuric acid. The appearance of a dark green color indicates the presence of steroids.

5. Phenolic Compounds Test: The powdered sample was tested with ferric chloride solution and lead acetate solution. A deep bluish-black color with ferric chloride and a white precipitate with lead acetate indicate the presence of phenolic compounds.

6. Tannins Test: Aqueous extract was treated with ferric chloride solution. Formation of a bluish-black color indicates the presence of tannins.

7. Glycosides Detection (Baljet's Test, Legal's Test, Keller-Killiani Test): Various tests were conducted to detect glycosides based on color changes or precipitate formation.

8. Cardio Glycosides Test: The Keller-Killiani test was employed to qualitatively analyze cardiac glycosides in the extracts, based on observed color changes.

9. Coumarins Detection: NaOH was added to the aqueous extract, and the development of a yellow color indicated the presence of coumarins.

10. Phytosterols Test: Concentrated sulfuric acid was added to the extract solution, and a red coloration in the chloroform layer indicated the presence of phytosterols.

11. Quinones Detection: Dilute NaOH was added to the extract, and the presence of blue-green or red coloration indicated the presence of quinones.

12. Resins Test: Acetic anhydride and sulfuric acid were added to the extract, and the appearance of a bright purple color indicated the presence of resins.

13. Leucoanthocyanins Detection: The extract was mixed with isoamyl alcohol, and the upper layer turning red indicated the presence of leucoanthocyanins.

14. Anthraquinone Detection: Chloroform and ammonia solution were added to the powdered plant material, and a bright pink coloration in the upper aqueous layer indicated the presence of anthraquinone.

15. Fixed Oils Test: The extract was pressed between two filter papers, and the presence of an oil stain indicated the presence of fixed oils. These tests provide valuable information about the phytochemical composition of the *Ipomoea laxiflora* leaf extract, aiding in the identification of potential bioactive compounds.

Quantification of phytochemical content from *I. laxiflora*

Total phenolic contents

The total phenolic content of the sample extract was determined spectrophotometrically using the Folin-Ciocalteu method. A mixture containing 0.1 ml of the plant extract, 2.8 ml of deionized water, 2 ml of sodium carbonate solution (20%), and 0.1 ml of 50% Folin-Ciocalteu reagent was incubated for 30 minutes at ambient temperature. After incubation, the absorbance of the reaction mixture was measured at 750 nm using a spectrophotometer. Gallic acid (GA) was utilized as the reference phenol to construct a standard curve.

By comparing the absorbance of the sample extract to the standard curve of gallic acid equivalents (GAE), the total phenolic content was determined and expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g d.wt). This method enables the quantitative assessment of the phenolic compounds present in the sample extract, facilitating comparisons with other extracts or standards. [55].

Total flavonoid content

The flavonoid content in the investigated materials was quantified using the aluminum chloride colorimetric method. A mixture was prepared consisting of 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride hexahydrate, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water, from a 0.5 ml extract. The reaction mixture was then incubated at room temperature for 40 minutes, and the absorbance was measured at 415 nm using a spectrophotometer. Quercetin was employed as the reference flavonoid to establish a standard curve. The flavonoid content in the sample was quantified by comparing its absorbance to the standard curve of quercetin equivalents (QE), and the results were expressed as mg quercetin equivalents per 100 grams of dry weight. This method provides a reliable means of assessing the flavonoid content in the investigated materials, allowing for comparisons with other samples or standards [55].

Determination of proanthocyanins content

To generate a calibration curve, test tubes were prepared with volumes of 0.1, 0.2, 0.3, 0.4, and 0.5 ml of catechin, each diluted with ethanol. Subsequently, 0.5 ml of the sample solution was mixed with 1.5 ml of hydrochloric acid and 3 ml of 4% vanillin methanol solution in refluxed conditions. After cooling to room temperature, the absorbance of the mixture was measured at 500 nm. The total proanthocyanin content was then determined in milligrams per gram of catechin equivalent using the calibration curve. This method provides a quantitative assessment of the proanthocyanin content in the sample, allowing for comparisons with other samples or standards [56].

Fourier transform infrared spectroscopy (FTIR)

Dry leaf powder of Ipomoea laxiflora was utilized for Fourier transform infrared (FTIR) spectroscopy analysis, conducted at the Center for Frontier Research and Development (CFRD), Osmania University, Hyderabad, Telangana. The analysis employed the potassium bromide (KBr) pellet technique in diffuse reflection mode, with a resolution of 4 cm^-1. The powdered sample was mixed with KBr and exposed to an infrared source ranging from 500 to 4000 cm^-1. [57,58,59]. Similarly, FTIR studies were conducted on the I. laxiflora extract both before and after bio-reduction using a similar methodology. In FTIR spectroscopy, the frequency of the vibrational peak (ν) is dependent on two factors: the force constant and reduced mass. This relationship is described by an equation that elucidates the underlying principles governing the observed vibrational frequencies [60, 61].

$$\nu = 1/2\pi c \sqrt{(k/\mu)}$$

Here, **c**- is the speed of light,
k- is the force constant
μ- is the reduced mass

Antioxidant activity studies

2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity

Total antioxidant capacity (phosphomolybdenum assay)

The free radical scavenging activity of the extracts was evaluated using the 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) method, as described in [62]. This method relies on the reduction of DPPH in a methanol solution in the presence of hydrogen-donating antioxidants, resulting in the formation of a non-radical species from DPPH-H and a color change from purple to yellow.

For the assay, varying concentrations (50, 75, 100, 150, and 200 µg/ml) of the compound were mixed with 1 ml of methanol solution containing 0.2 mM DPPH. Fresh DPPH solution of 0.2 mM was prepared, and its optical density (OD) was adjusted to 0.8. If the OD was less than 0.8, additional DPPH was added; if it was more than 0.8, methanol was added accordingly. The mixture was thoroughly mixed and incubated for 30 minutes.

After incubation, the optical density of the solution was measured at 517 nm using a spectrophotometer. The percentage inhibition of antioxidant activity was calculated using the following formula, and the readings of the test samples were compared with the positive control of ascorbic acid (Vitamin C): Percentage of inhibition of DPPH = ((Control OD – Test OD) / Control OD) x 100.

This assay provides a quantitative assessment of the antioxidant activity of the extracts by measuring their ability to scavenge DPPH radicals, with higher percentage inhibition indicating stronger antioxidant activity.

To determine the Total Antioxidant Capacity (TAC) of the plant fractions, the phosphomolybdenum method as described by [63] was employed. In summary, 0.3 mL of each solvent fraction and standard drug (at concentrations ranging from 25 µg/mL to 400 µg/mL) were mixed with 3 mL of a reagent solution comprising 0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate in test tubes. These test tubes were then covered and incubated in a water bath at 95°C for 95 minutes.

Following incubation, the samples were allowed to cool to room temperature, and their absorbance was measured at 695 nm. A control mixture containing distilled water instead of the samples was used as a reference. Standard drugs such as ascorbic acid and gallic acid were also included for comparison.

Higher absorbance values indicate a greater total antioxidant potential, providing valuable insights into the antioxidant capacity of the plant fractions being evaluated.

$$\text{The percentage inhibition (\%)} = \left\{ \frac{(\text{absorbance of sample} - \text{absorbance of control})}{(\text{absorbance of sample})} \right\} \times 100$$

Hemolysis activity

The process of hemolysis was employed to assess the toxic effects of the drug on red blood cells (RBCs). A freshly harvested healthy human blood sample was obtained and phosphate-buffered saline (PBS) with a pH of 7.4 was added at a volume ratio of 1:2. Subsequently, the mixture was centrifuged at 10,000 rpm for 15 minutes to isolate the RBCs by removing the supernatant. The isolated RBCs were then washed with sterile PBS five times to remove any remaining erythrocytes. The resulting RBC cells were diluted with 40 mL of PBS [64]. Next, 0.2 mL of RBC cells at different concentrations of synthesized silver nanoparticles (AgNPs) (ranging from 10 to 60 µg/mL) were mixed with the RBC cells using vortexing. These samples were then incubated at room temperature (24°C) for 3 hours.

After incubation, the samples were centrifuged at 10,000 rpm for 2 minutes, and the supernatant was collected for optical absorbance analysis at a wavelength of 541 nm. The percentage of hemolysis was estimated using the following relation:

$$Hemolysis = \frac{Sample\ \ Abs - Negative\ Control}{Postive\ Control - Negative\ Control} \times 100$$

Note that for positive and negative controls, RBC cells were separately incubated with water and phosphate-buffered saline (PBS) instead of the synthesized silver nanoparticles. These controls were included to provide a baseline for comparison with the samples incubated with AgNPs, allowing for the assessment of the extent of hemolysis induced by the nanoparticles.

Observations and Results

4.1 Plant profile

Table: 1 Classification of I. laxiflora H.J. Chowdhery & Debta

Kingdom	Plantae
Subkingdom	Tracheobionta
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Solanales
Family	Convolvulaceae
Genus	*Ipomoea*
Species	*laxiflora*

Morphological description

The annual stem twiners of Ipomoea laxiflora can reach up to 5 meters in length, with stems that turn purplish as they age. The stems are quadrangular in shape and sparsely hairy at the nodes. The leaves are simple, typically ovate, and range from 5 to 10 cm in length and 5 to 8 cm in width, with a cordate base. They are usually glabrous, though distal leaves may be trilobed with shallow or sharp lobes. The petioles can grow up to 12 cm in length and are also glabrous. The inflorescence is cymose and lax, typically bearing 1 to 3 to 5 to 7 flowers, occasionally up to 17. The peduncles are 6 to 12 cm long, curved upward, and swollen at the apex. Pedicels, which measure 2.5 to 3 to 5 mm in fruit, are quadrangular, glabrous, and bulged, with a cushion-like appearance below the flower that becomes sucked up when the fruit matures. Bracts are linear, caducous, and about 3 mm long. The flowers themselves consist of 5 subequal sepals, measuring 8 to 10 mm in length and 2 to 3 mm in width. They are glabrous, faintly veined, and purplish at the apex, with white margins at fruit dehiscence. The corolla is funnel-shaped and pink, typically 1.5 to 1.8 cm long, with a 5-lobed limb. Stamens number 5, with subequal filaments that are included and measure 7 to 8 mm in length, hairy at the base. The ovary is glabrous, measuring 1 × 1.5 mm, with a 1 cm long style and a bilobed stigma. The capsules are ovoid, measuring 5 × 6 mm, and apparently 4-partite or 4-valved, though they are bilocular with 2 ovules in each loculus. When young, they are tinged purple and glabrous. The persistent style-base functions as a cap or lock, allowing the mature capsule to break along the septal suture to release the seeds. The locular partition (septum) is transparent, with a rim and central axis, measuring 6 × 6 mm and slightly ellipsoidal. Seeds are black, glabrous, measuring 3.5 × 3 mm, with two flat lateral faces and a convex back bearing a shallow ridge at the center.

Flowering and Fruiting: October–December.

Taxonomic Note: Morphologically *I. laxiflora* is similar to *I. triloba*, but its ovate, obtuse calyx, sepal shape, the range of corolla sizes, infundibuliform tube and indumentum that bridges the two species with black seeds may be used to identify and discriminate between both of them[36].

Variations: There is another form of *I. laxiflora*, which shows an entire green stem, green veins of leaves, flowers white with yellow throat; seeds white with dark brown ting on the dorsal surface

Fig. 1. A-E: I. laxiflora – A. Habit; B. Flower, C. Sepals; D. Capsules mature, E. Capsules immature,

Preliminary screening for phytochemicals

The leaf extracts of *I. laxiflora* were subjected to phytochemical screening in order to ascertain the presence of secondary metabolites. A qualitative examination revealed the presence of several phytochemicals in the leaf of *I. laxiflora*. The analysis's results were presented in Table and Figures to facilitate a comparison of the extraction solvents.

The phytochemical screening was carried out with the help of the Soxhlet apparatus and a hot continuous extraction method, which was utilized in the current experiment. Based on the findings, it was determined that the chloroform and methanol extracts contained alkaloids, glycosides, and cardiac glycosides. The pet. ether extract was the only one that did not contain any flavonoids, steroids, or terpenoids. All of the other extracts, however, did contain these compounds. The methanol extract was the only one that produced yields that were positive for saponins and coumarins. The phenols, tannins, and leuco anthocyanins that are present in ethyl acetate and methanol extracts are the primary factors that are investigated in this inquiry. All of the extracts, with the exception of the chloroform extract, were found to contain anthraquinones, in contrast. All of the extracts, with the exception of the ethyl acetate extract, were found to have an abundance of quinones and resins. Extracts of low polarity solvents, notably ether and chloroform, were the only ones in which the fixed oils were found to be present. At the end of the day, none of the extracts revealed any signs of gums or mucilage being present. In comparison to all of the other solvent extracts, methanolic leaf extracts displayed a higher level of precipitation (+++) and a greater number of secondary metabolites to be found in the leaves. Pet ether, on the other hand, was shown to contain a less quantity of phytoconstituents than other components. It has been determined that the yield percentages of the crude extracts of pet. ether, chloroform, ethyl acetate, and methanol are as follows: 5.8%, 36.18%, 25.8%, and 46.25%, respectively (Fig:2& Table 2).

Fig:2 Phytochemical leaf extractions with different solvents

Table 2: Preliminary phytochemical constituents of I. laxiflora

Sr. No.	Name	Pet.ether	Chloroform	Ethyl acetate	Methanol
1.	**Alkaloids** Mayer's Test	-	+++	-	+++
2.	**Flavonoids** Zinc-hydrochloride reduction test	-	+++	+++	+++
3.	**Saponins** Foam test	-	-	-	+++
4.	**Steroids& Terpenoids** Salkowki's test	-	++	+++	+++
5.	**Phenols** Ferric chloride test	-	-	++	+++
6.	**Tannins** Precipitate test	-	-	++	+++

7.	**Glycosides** Borntrager's Test (Modified)	-	++	-	+++
8.	**Cardio glycosides** Kellar - Kiliani	-	++	-	++
9.	**Coumarins** Ferric chloride test	-	-	-	+++
10.	**Anthraquinones** Borntrager's	+	-	++	+++
11.	**Quinones** Precipitate test	+	+	-	+++
12.	**Resins** Acetic anhydride test	++	+++	-	+++
13.	**Leuco anthocyanins** Isoamyl alcohol test	-	-	++	+++
14.	**Fixed oils** Spot test	+++	+++	-	-
15.	**Gums & Mucilage's** Alcohol test	-	-	-	-

Note: (+) mildly positive, (++) moderately Positive, and (+++) highly positive (significantly visible color change).

Quantification of phytochemical content from *I. laxiflora*

The amounts of phenolic compounds, flavonoids, and proanthocyanins were determined using the Folin-Ciocalteu test, the vanillin methanol-HCL test, and the aluminum chloride colorimetric test, respectively.

Total phenolic contents

In accordance with the findings of particularly strongly current quantitative analysis, pet. ether (160 mg/g), ethyl acetate (190 mg/g), chloroform (210 mg/g), and methanol (230 mg/g) are all significant concentrations. The methanol exhibited the highest concentration of phenol among all of the tests (Fig3).

Total Flavonoid content

Based on the results of the most recent quantitative analysis, methanol, with a flavonoid concentration of 640 mg/g, is the solvent type with the highest total flavonoid content. The remaining three substances are, according to that sequence, from pet ether (320 mg/g), chloroform (450 mg/g), and ethyl acetate (540 mg/g) (Fig.3).

Determination of proanthocyanins content

According to the results of most recent quantitative studies, methanol encompasses the highest concentration of proanthocyanidin among organic solvents, with a concentration of 520 mg/g. This is followed by ethyl acetate at 540 mg/g, chloroform at 450 mg/g, and pet. ether at 390 mg/g. When compared to other commonly used organic solvents, methanol has the highest concentrations of proanthocyanidins among all substances (Fig:3).

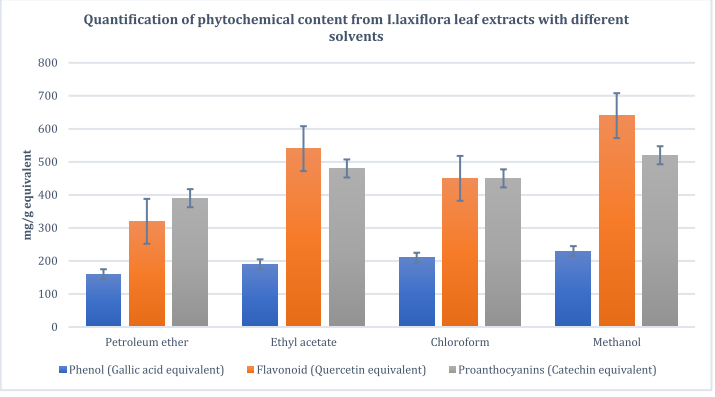

Fig 3 An illustration showing the phytoconstituents' quantification

Fourier Transforms Infrared Spectroscopy (FTIR)

The bio-reduction compounds that are responsible for the synthesis of AgNPs were determined using Fourier Transform Infrared spectroscopy (FT-IR). The FT-IR spectra were collected from 10 scans per sample at a resolution of 4 cm^{-1} at a range of 400-4000cm^{-1}. The FT-IR spectrum of IL AgNPs showed the absorption peaks at 1053.17, 1157.33, 1631.83, 1641.48, 2374.45, 2724.18, 3431.48 and 3444.98cm^{-1}. Peak at 1053.17 and 1157.33 could be due to C-H stretch. The absorption peak at 1631.83 and 1641.48 may due to C=C stretch. The presence of peak at 2374.45 & 2724.18 may be assigned to C=H stretch. The absorption bands at 3431.48 and 3444.98 are assigned to amine N-H stretch. These bonds are derived from water soluble compounds like Fflavonoids, terpenoids, proteins and carbohydrates have been reported in *I.laxiflora* extract (Fig 4).

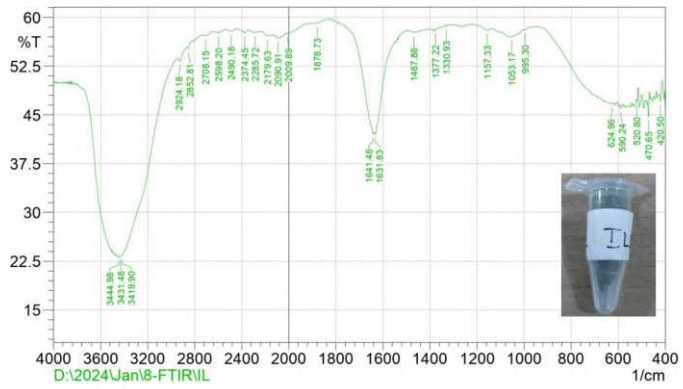

Fig 4: Fourier Transforms Infrared Spectroscopy (FTIR) analysis of I. laxiflora

Green synthesis of *I. laxiflora* silver nanoparticles (AgNPs)

The current investigation involved the synthesis of silver nanoparticles from *I. laxiflora*. In this particular investigation work as well as the reduction of AgNO3 was accomplished by utilizing the various components of the methanolic extract of *I. laxiflora* leaf. During the reduction process, which lasted for a total of 48 hours, the temperature range was between 68 °C, and the stirring was continuous. The color changed from a bright yellow hue to a brown shade, which allowed for the detection of the presence of silver nanoparticles (AgNPs). The observed shift in hue is the most important indicator of the presence of silver nanoparticles (AgNPs). This conclusion is further supported by the presence of a unique surface plasmon resonance (SPR) band at a wavelength of 445 nm. Studies conducted by other researchers who used plant extracts as a reducing agent [65], have reported findings that are equivalent to those described here. According to the findings of the study that was carried out by [66], the solution was maintained at a temperature that was higher than sixty degrees Celsius in order to successfully attain the ideal particle size of *I. laxiflora*. The various research has shown that the creation of smaller AgNPs using extracts from various plant leaves takes occur at elevated temperatures [67,68,69]. These findings have been supported by the findings of the aforementioned investigations. The production of silver nanoparticles (AgNPs) with a particle size of 51 nanometers was accomplished by the utilization of a green strategy that included the utilization of silver nitrate (AgNO3) and *Rivina humilis* leaf extract in the sunshine [70]. A diverse range of analytical methods was employed to confirm the synthesis of silver nanostructures (AgNPs). These methods included ultraviolet-visible spectroscopy, scanning electron microscopy (SEM), field emission scanning electron microscopy (FE-SEM), energy-dispersive X-ray spectroscopy (EDX), transmission electron microscopy (TEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR). Ultraviolet-visible spectroscopy was used to analyze the optical properties of the synthesized AgNPs. SEM and FE-SEM were employed to examine the surface morphology and structure of the nanoparticles at high resolution. EDX provided information on the elemental composition of the AgNPs. TEM allowed for direct visualization of the size, shape, and distribution of the nanoparticles at the nanoscale level. XRD analysis was utilized to determine the crystalline structure of the synthesized AgNPs. FTIR spectroscopy provided insight into the functional groups present on the surface of the nanoparticles. By employing this array of analytical techniques, comprehensive characterization of the synthesized AgNPs was achieved, confirming their successful formation and providing valuable information about their physical and chemical properties (Fig5).

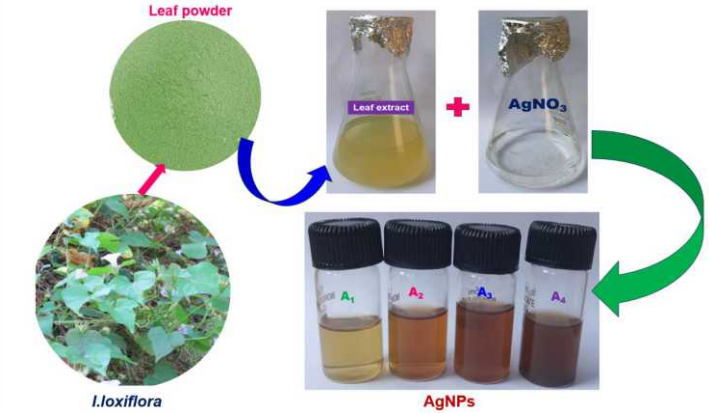

Fig:5 Green synthesis of I. laxiflora silver nanoparticles (AgNPs)

Antioxidant activity of *I. laxiflora* -AgNPs

DPPH free radical scavenging tests were carried out in order to ascertain the level of antioxidant activity possessed by *I. laxiflora* -AgNPs. In *I. laxiflora* -AgNPs, the efficiency of the scavenging effect against DPPH was depending on the concentration. The DPPH scavenging activity increased from 6% to 94% when the concentration *of I. laxiflora* -AgNPs was increased from 5, 10, 20, 40, and 80 µg/mL in a variety of solvents including pet ether, ethyl acetate chloroform, and methanol. Vitamin C and BTH were used as standards in this experiment (Fig.6). As the concentration of *I. laxiflora* -AgNPs increased from 5 to 80 µg/mL, it was discovered that the radicals in pet. ether leaf extract was suppressed by a range of 8 to 70%. In a similar manner, the scavenging activity of vitamin C went from 5 to 98%, and the activity of BTH increased from 16 to 65%. In addition, a mixture consisting of 10–78% ethyl acetate, 6–55% chloroform, and 13–94% methanol, in that particular order. It was established through the methanol leaf extract inhibition percentages that the *I. laxiflora* -AgNPs exhibited the most powerful antioxidant activity when compared to vitamin C and BTH. The antioxidant activity observed in the AgNPs synthesized from Ipomoea laxiflora can be attributed to the presence of flavonoids, polyphenols, and proteins involved in the bio-reduction and stabilization process of these nanoparticles. These compounds possess inherent antioxidant properties that help in neutralizing harmful free radicals and reducing oxidative stress.

Oxidative stress, resulting from an imbalance between antioxidants and the oxidative system, is implicated in various disorders such as aging, cancer, diabetes, hypertension, atherosclerosis, cardiovascular, and neurodegenerative disorders. Therefore, there is a critical need to counteract oxidative stress by augmenting the antioxidant defense mechanisms in the body. Given the potential side effects associated with synthetic antioxidants, researchers are increasingly turning to natural sources for effective antioxidants. Phytochemicals like polyphenols and flavonoids found in plants have been demonstrated to exhibit potent antioxidant activity. Incorporating these natural antioxidants, either directly from plant extracts or via green-synthesized nanoparticles like I. laxiflora-AgNPs, could offer a safer and more sustainable approach to combating oxidative stress and its associated health implications [72]. (Fig: 6).

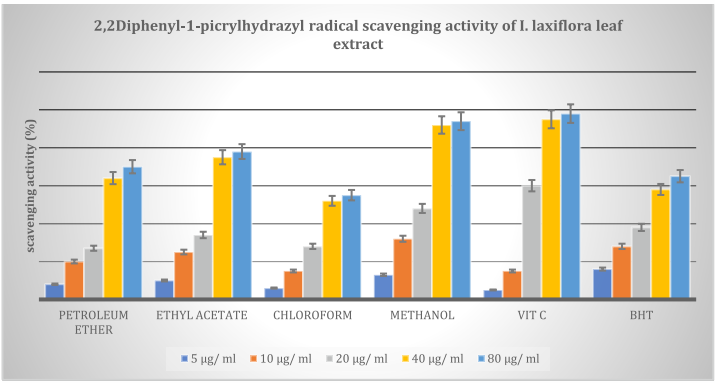

Fig 6: A pictorial representation showing the antioxidant activity of I. laxiflora

Total antioxidant activity of *I. laxiflora* -AgNPs

Antioxidant capacity total (TAC) Figure 3 shows that all of the extracts showed varying degrees of activity in the phosphomolybdenum assay, a quantitative approach for evaluating overall antioxidant capacity [73]. At concentrations of 25, 50, 100, 200, and 400 µg/ml, the antioxidant activity was observed to be dosage dependent. The antioxidant activity of all of the AgNPs leaf extracts increased as the concentration increased. However, at a greater dosage (400 µg/mL), the methanolic extract of the leaves had a significantly higher total antioxidant capacity (89%), followed by the ethyl acetate extract (70%), and finally the ascorbic acid (85%). Using gallic acid as a positive control, we discovered that at the same concentration, it had an efficiency of 88% (85% ascorbic acid equivalents). In addition, The pet. ether extract had a concentration of 68%, whereas the chloroform extract had 58% at a higher concentration of 400 µg/mL (Fig 7).

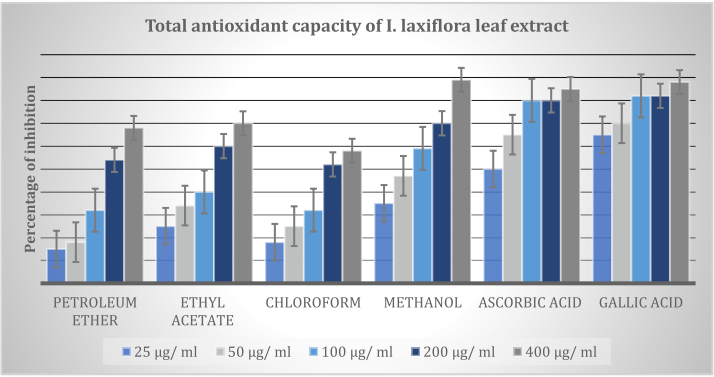

Fig 7: A depiction showing the total antioxidant activity of I. laxiflora

Haemolysis Activity of *I. laxiflora* -AgNPs

According to Shim *et al.,* (1996), hemolysis of the blood is a serious problem that arises when foreign objects, such as implants, enter the human body. Water will cause hemolysis of the red blood cells (RBC). Hemolytic activity in RBC cells is therefore used to examine the biocompatibility of AgNPs produced from plant extracts.

The circulatory system eventually comes into contact with the nanomaterials after they reach the body. The blood cells must be handled carefully while dealing with foreign particles because they are an essential aspect of our body. Water was added to the RBC cells for this assay, and an optical spectrophotometer was used to measure the amount of hemoglobin that was released (100% hemolysis). A range of concentrations (10, 25, 50, 75, and 100 µg/ml) of *I. laxiflora*-AgNPs were tested against human RBCs. At the maximal concentration of *I. laxiflora*-AgNPs, 2.751% and 2.382%, respectively, were shown to represent the highest percentages of hemolysis in Figure 7. Haemolysis percentage dropped to 0.862 % at the very least concentration of *I. laxiflora* (5 µg/ml)-(Table 3& Fig). Studies have analyzed the hemolytic activity of polymeric nanoparticles, indicating that at concentrations of 40 µg/ml, these nanoparticles can cause hemolysis of greater than 10%. However, in contrast, the hemolytic activity observed for silver nanoparticles synthesized using fenugreek and papaya leaves was found to be even less, at less than 5%. This suggests that the hemolytic potential varies among different types of nanoparticles, with polymeric nanoparticles exhibiting higher hemolytic activity compared to certain biogenic silver nanoparticles. Such findings underscore the importance of understanding and evaluating the potential toxicity of nanoparticles, particularly in biomedical applications where they may come into contact with blood components, emphasizing the need for thorough biocompatibility assessments prior to their utilization [74-75].

Table 3: Haemolysis Activity of I.laxifolra -AgNPs

S.No	IL Nanoparticles (µg/ml)	OD at 545nm	% of Haemolysis
1	10	0.042	0.862
2	25	0.068	1.396
3	50	0.084	1.724
4	75	0.116	2.382
5	100	0.134	2.751
6	Water (Positive Control)	4.87	100
7	Saline (Negative Control)	0.019	0

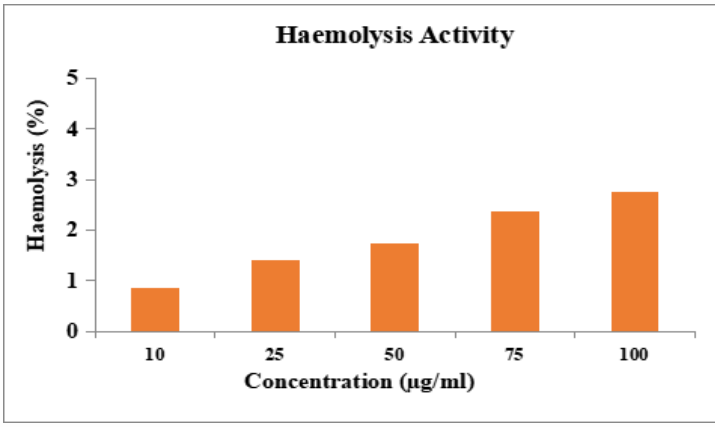

Fig 8: A graphical illustrating the haemolysis Activity of I.laxifolra -AgNPs.

Discussion

The primary objective of this study was to explore the potential of Ipomoea laxiflora in the synthesis of silver nanoparticles (AgNPs) for the first time, alongside investigating its phytochemical properties and assessing its hemolytic and antioxidant capabilities. Nanotechnology, by synthesizing particles within the range of 1 - 100 nm, creates materials with high surface-to-volume ratios. As particle size decreases, this ratio increases, leading to divergent physicochemical, chemical, and biological properties compared to bulk particles. The field of nanomaterials holds increasing significance in material science, with silver nanoparticle production being particularly promising. These nanoparticles can be synthesized using green methods, encompassing physical, chemical, and biological approaches, which offer environmentally friendly alternatives for various applications [3,4].

The *I. laxiflora* leaves were taken from Manneguda Village in Ranga Reddy District, Telangana, India. The plant was

authenticated by Botanical Survey of India, Deccan Regional Centre, Hyderabad, Telangana (Accession number-BSI/DRC/2023-24/Identification/405). It belongs to the Convolvulaceae family. It is an annual climber; 5–7 m long, it is distributed throughout the world, in India). It is used to treating fever, headaches, and stomach aches [35].

The phytochemical screening was conducted using the Soxhlet apparatus and a hot continuous extraction method. Results showed that chloroform and methanol extracts contained alkaloids, glycosides, and cardiac glycosides. Pet ether extract was the only extract without flavonoids, steroids, or terpenoids. The methanol extract produced positive yields for saponins and coumarins. The primary factors investigated were phenols, tannins, and leuco anthocyanins in ethyl acetate and methanol extracts. All extracts, except chloroform, contained anthraquinones and resins. Low polarity solvent extracts, notably ether and chloroform, contained fixed oils. No gums or mucilage were found in any extracts. Methanolic leaf extracts had a higher precipitation level and more secondary metabolites. The crude methanol extracts had a high yield percentage of 46.25%, while the pet ether extracts had the lowest yield percentage of 5.8%. The amounts of phenolic compounds, flavonoids, and proanthocyanins were determined, the total phenolics compounds quantitative analysis revealed the methanol exhibited the highest concentration(230mg/g) of phenol among all of the tests. Similarly, the total flavonoid (640 mg/g,) and proanthocyanin (530 mg/g) content analysis showed in methanol extracts. The FT-IR spectra of *I. laxiflora*-AgNPs revealed absorption peaks at 1053.17, 1157.33, 1631.83, 1641.48, 2374.45, 2724.18, 3431.48, and 3444.98cm-1, indicating C-H, C=C, C=H, and amine N-H stretching. These peaks are derived from water-soluble compounds in *I. laxiflora* extract, including flavonoids, terpenoids, proteins, and carbohydrates. The silver nanoparticles (AgNPs) are synthesized from *I. loxiflora* leaf extract using a reduction method, confirmed by a color change from pale yellow to brown and a sharp surface plasmon resonance band at 445 nm. The *I. laxiflora*-AgNPs were tested for DPPH antioxidant activity. As concentration increased, DPPH scavenging activity increased from 6% to 94%. A mixture of ethyl acetate, chloroform, and methanol had the highest antioxidant activity. Flavonoids, polyphenols, and proteins involved in *I. laxiflora*-AgNP bio-reduction and stabilization provided antioxidant action and also the total antioxidant activity was performed of AgNPs leaf extracts increased with concentration, with methanolic leaf extract showing superior activity (89%), followed by ethyl acetate (70%), ascorbic acid (85%), pet. ether (68%), and chloroform (58%). The circulatory system contacts nanomaterials, affecting blood cells. A study tested different concentrations of *I. laxiflora*-AgNPs on human red blood cells. The highest hemolysis percentages were observed at the highest concentrations, 2.751% and 2.382%, while the lowest concentration was 0.862%.

Conclusions

In conclusion, the study highlights the successful production of eco-friendly silver nanoparticles using *Ipomoea laxiflora* leaf extract and explores their antioxidant and hemolytic effects. This research represents a pioneering effort in understanding the potential of this plant species for green synthesis. The use of natural sources like *Ipomoea laxiflora* for nanoparticle synthesis aligns with the growing emphasis on environmentally friendly technologies and sustainable practices.

The findings underscore the importance of green nanoparticles in various fields, including medicine and environmental protection. By utilizing less hazardous chemicals and tapping into the reducing and capping agents present in plants, we can create nanoparticles with minimal environmental impact. The phytochemical analysis and characterization of nanoparticles provide valuable insights into their composition and properties. Furthermore, the study demonstrates the antioxidant properties of the synthesized nanoparticles, showcasing their potential for biomedical applications. The hemolytic activity evaluation also sheds light on their safety profile, which is crucial for their use in medical settings.

Overall, the development of green nanoparticles through methodologies like green synthesis offers a promising avenue for promoting environmental sustainability, improving human health, and advancing technological innovation. Embracing these green approaches not only contributes to the field of nanotechnology but also supports broader goals of sustainable and equitable development.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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