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Green synthesis and characterization of silver nanoparticles from *Ipomoea laxiflora*: A comprehensive evaluation of antimicrobial efficacy

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Abstract
Green nanotechnology is gaining attention in nanoparticle synthesis due to its environmentally friendly approach and the diverse applications of nanotechnologies in fields like drug delivery, sensors, optoelectronics, and magnetic devices. Among nanoparticles, silver nanoparticles (AgNPs) exhibit unique physicochemical and biological properties, making them potent antimicrobial agents. This study focuses on the green synthesis and characterization of AgNPs using *Ipomoea laxiflora* leaf extract, a plant belonging to the Convolvulaceae family, traditionally used to treat fever, headaches, and stomach aches.

To synthesize AgNPs, 10 mL of *I. laxiflora* leaf extract was mixed with 90 mL of 1 mM aqueous silver nitrate solution and heated at 80 °C for three hours, resulting in a color change from yellow to dark brown, indicating nanoparticle formation. The AgNPs were characterized using scanning electron microscopy, Fourier-transform infrared spectroscopy, zeta potential, UV-visible spectroscopy, and X-ray diffraction.

Antimicrobial efficacy was evaluated using the agar well diffusion method. IL-AgNPs exhibited significant antibacterial activity against *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Streptococcus pneumoniae* (*S. pneumoniae*), and *Pseudomonas aeruginosa* (*P. aeruginosa*), with inhibition zones increasing with AgNP concentration (25-100 µL). The antifungal activity was assessed against *Candida albicans* (*C. albicans*) and *Aspergillus niger* (*A. niger*), demonstrating dose-dependent inhibition. *C. albicans* exhibited zones of 12-21 mm, while *A. niger* zones ranged from 9-20 mm.

This study highlights the sustainable synthesis of AgNPs using *I. laxiflora*, emphasizing their potential antibacterial and antifungal properties, which could be further explored for therapeutic and industrial applications.

Keywords: AgNPs, synthesis, characterization, *Ipomoea laxiflora*, convolvulaceae and anti-microbial activity

1. Introduction

The medicinal plants provided the ailments and cure throughout human history. Plants are capable of producing diverse range of chemical compounds that are responsible for various biological actions [1]. Even at the dawn of twenty-first century, about 90% of potential drug molecules have been isolated directly or indirectly [2]. WHO estimated that 80% population of Africo-Asian countries utilize herbal medicine in their primary health care system [3]. The clinical application of herbal drugs faces the same challenges as allopathic medicines like selectivity, drug delivery, solubility, safety, toxicity, efficacy, and frequent dosing [4, 5]. The modern pharmaceutical research could overcome the above-mentioned challenges by developing novel drug distribution systems of herbal medicines, including, micro emulsion, nanoparticles' solid dispersion, liposomes, matrix system as well as solid lipid nanoparticles [6, 7].

About 5000 years ago, many Greeks, Romans, Persians and Egyptians used silver in one form or other to store food products [8]. Use of silver ware during ancient period by various dynasties was common across the globe utensils for drinking and eating and storing various drinkable and eatable items probably due to the knowledge of antimicrobial action [9]. There are records regarding therapeutic application of silver in literature as earlier as 300 BC. In the Hindu religion, till date silver utensils are preferred for the "panchamrit" preparation using curd, *Ocimum sanctum* and other ingredients. The therapeutic potentials of various metals are mentioned in ancient Indian Ayurvedic medicine book medicinal literature named "Charak

Samhita" [10]. Until the discovery of antibiotics by Alexzander Flemming, silver was commonly used as antimicrobial agent.

Silver is extensively used in nano systems and employed in various biomedical purposes. Silver nanoparticles have excellent medical and nonmedical properties and applications when compared with other metal nanoparticles [11]. The green approach of nanoparticles synthesis possesses reduced or no toxicity and number of plants and herbal extracts has been reported to be involved in such synthesis [12]. Plant extracts contain number of secondary metabolite which plays a critical role during the nanoparticle synthesis by acting as reducing or capping agents [13]. Studies have shown that silver nanoparticles are highly stable and toxic to bacteria, fungus, and viruses.

Nano-silver (AgNPs) is a type of metallic nanoparticle with unique features and uses, particularly in the field of nanomedicine [14]. Due to their distinct antibacterial qualities, silver nanoparticles have attracted the most attention among all other nanoparticles. Green synthesis is a new alternative method that was inspired by concerns about the synthesis of these materials, such as the use of harmful solvents and precursor chemicals and the production of toxic byproducts. This environmentally friendly method uses microbial, plant, or biological agents as reducing and capping agents. Green chemistry-produced silver nanoparticles present a novel and potential substitute for chemically-produced nanoparticles [15]. Because of its easy, efficient, eco-friendly, and non-toxic approach, green nanotechnology (Using enzymes, microorganisms, and plant/plant extract) is a potential alternative to pure nanotechnology [16]. Plants are made up of a variety of bioactive substances that can help with the production of metal nanoparticles by acting as reducing and stabilizing agents [17, 18]. Bio-inspired syntheses result in nanoparticles of diverse forms and sizes depending on the synthesis process parameters [19]. The synthesis conditions must be improved by using local plants.

Silver in all its forms has been historically used as an antimicrobial agent by itself or combined with other technologies [20]. This metal has been studied to take advantage of its ability to inhibit bacterial growth by incorporating it as silver nitrate or silver sulfadiazine in creams and dressings to treat burns and ulcers, in food packaging to prevent contamination, in home appliances as refrigerators and washing machines, and several applications in the industrial area [21-26]. Because of the knowledge and evidence existing of the antibacterial activity of silver [27], with the emergence of nanotechnology, the exploration of the antibacterial capacity of AgNPs was an evident path.

AgNPs are defined as a nanomaterial with all its dimensions in the range of 1-100 nm. These have shown greater capacity and higher surface (Area-to-volume ratio) compared to silver in its bulk form. At the nanoscale, this material exhibits unique electrical, optical, and catalytic properties, which has led to the investigation and fabrication of products for targeted drug delivery, diagnosis, detection, and imaging [28, 29]. However, it is the exceptional antibacterial activity exhibited by AgNPs that has focused the attention of researchers and industries on this nanomaterial. AgNPs have shown antimicrobial activity against a variety of infectious and pathogenic microorganisms, including multidrug-resistant bacteria [30, 31].

The enhanced antibacterial activity of Ag at the nanoscale has been most valuable in medical and healthcare areas, where the incorporation of AgNPs into hundreds of products has been studied, including surgical and food handling tools, clothing, cosmetics, dental products, catheters, and dressings [32-,35]. The potential of AgNPs as antibiotics is related to their various mechanisms of action, which attack microorganisms in multiple structures at a time and give them the ability to kill various types of bacteria [36].

Currently, investigations related to the development of new antibiotics are difficult processes; they require years of studying the efficacy and safety of the agents, consuming high amounts of time and resources, while infections caused by multi-resistant microorganisms keep growing and causing deaths worldwide [37]. AgNPs, along with other nanomaterials, have been studied in the defined post-antibiotic era to search for new agents that can help combat pathogenic microorganisms without promoting the appearance of new resistances [37]. As infections caused by antibiotic-resistant microorganisms are a matter of global concern, AgNPs arise as an excellent alternative as they can be applied to prevent infections caused by these microorganisms, decontaminate medical supplies, and even combat infections in course [37, 38]. As an antibiotic alternative, this application has been broadly studied in recent years with the objective of developing new bactericidal products for decontamination or infection treatments taking advantage of the already established knowledge about their efficiency even against multidrug resistant organisms [39].

The attention captured by AgNPs is reflected in the figures of high demand and investment in research related to them. In the last 15 years, the market of AgNPs has been growing steadily, with an estimated production of more than 500 tons of nanoparticles per year to supply the different industries' demands [40]. Due to the growth of the nanoparticle market worldwide and the current offer of products with incorporated nanoparticles, the study of their biological activity and safety has become a matter of issue, along with the elucidation of their exact mechanisms of action in bacterial and mammalian cells [40].

Nanoparticles have the potential to saturate and stick to the surface of fungal hyphae, preventing harmful fungi from growing [41]. Because of the stabilizing factors that easily allow nanoparticles to connect with other biomolecules and increase their interactions with bacteria, biologically generated nanoparticles have higher antimicrobial efficacy. Biogenic silver nanoparticles (AgNPs), for example, had more antibacterial activity than chemically generated nanoparticles [42]. By interacting with proteins and enzymes, AgNPs can induce persistent cell damage by disrupting the electron transport chain, resulting in membrane permeability barrier disruption [43]. Examples for the biogenic activities of biosynthesized AgNPs from different plants extracts highlighted their antimicrobial, anticancer, and apoptosis inducing ability. For example, a pervious study showed that AgNPs synthesized from the *Lagerstroemia speciosa* (L.) Pers. flower buds had antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Candida glabrata*, besides, its significant anticancer activity against MG-63 cells of Osteosarcoma [44]. Another study used AgNPs bio-fabricated from the aqueous extract of *Ixora brachypoda* leaves and showed antifungal activity against *Bacillus subtilis, Pseudomonas aeruginosa, E. coli, S. aureus, C. albicans, Fusarium oxysporum,* and *A. alternata* [45].

Recent studies have highlighted the effectiveness of AgNPs against a variety of bacterial strains, including clinical isolates and standard strains (ATCCs, NCTCs, PCCs, MTCCs) of various species, such as *Acinetobacter baumannii*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella typhi*, *Enterococcus faecalis*, *Enterobacter* spp., *Bacillus subtilis*, *Micrococcus luteus*, *Proteus vulgari*, among others [46-51]. Furthermore, the synergy between AgNPs and antibiotics, such as *penicillin G*, *amoxicillin*, erythromycin, clindamycin, vancomycin, piperacillin, ciprofloxacin, tetracycline, levofloxacin, cefixime, cefazolin, neomycin, rifampicin, gentamicin, chloramphenicol, imipenem; has been shown to increase the effectiveness of these medications [52-56].

Ipomoea laxiflora H.J. Chowdhery & Debta, it belongs to the Convolvulaceae family. It is an annual climber; 5-7 m long, it is distributed throughout world, in India Uttarakhand, Maharashtra Western Ghats (Gujarat), Uttar Pradesh, Telangana and it is endemic to Uttarakhand. Morphologically, *I. laxiflora* species similar to *I. triloba* L., and is separated by absence of hairs on ovary and capsules [57-63]. It is used to treating fever, headaches, and stomach aches [61].

2. Materials and Methods

2.1 Collection and Authentication of Plant Material

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

2.2 Drying

After cleaning the leaves, they were cut into pieces (0.5─1.5×1×0.2 cm3) with the help of scissors and knives. They were left for shade drying on the newspapers for ten days. After that, the fruit pieces were dried in a hot air oven at 40 °C for an hour just before starting the extraction process to remove the moisture content.

2.3 Successive extraction using Soxhlet apparatus

In order to ensure that the *Ipomoea laxiflora* leaf extract was prepared in the correct manner, fresh leaves were gathered and then passed through water in order to ensure that they were thoroughly cleansed. The purpose of this action was to make sure that any dirt or pollution that might have been there was removed. Additionally, the aqueous solution was utilized in order to accomplish the task of removing the leaves. In order to ensure that the powder had a size that was uniform across the board, the dried leaves were first ground into a powder using a mechanical grinder. After that, the powder was sieved to ensure that it had a consistent size. A Soxhlet apparatus and water were used to extract the leaf powder at a temperature of 100 °C. The extraction process was carried out in stages. In order to obtain an extract that was crystal clear and devoid of any particles, the extract was filtered through Whatman No. 1 filter paper [64]

2.4 Preparation of silver nitrate solution

0.01697 g of $AgNO_3$ was dissolved in 100 mL distilled water to produce 1 mM solution of $AgNO_3$.

2.5 Preparation of 0.1 M NaOH solution

0.4 g of NaOH was dissolved per 100 mL of distilled water to prepare 0.1 M NaOH solution and further used to adjust the pH values in different experiments.

2.6 Green synthesis of silver nanoparticles

2.6.1 Synthesis of silver nanoparticles using leaf extract

The silver nanoparticles were formed using a solution consisting of 90 mL of double-distilled water containing 1 mM silver nitrate, to which 10 mL of *Ipomoea laxiflora* leaf extract was added. The leaf extract and silver nitrate were combined using a ratio of 1:9. The reaction mixture was heated to a temperature of 80 °C using a magnetic stirrer, and then stirred continuously for a duration of three hours at an acceleration of 800 rpm. The color transition from pale yellow to brown indicated the formation of AgNPs. A suspension of AgNPs-IL was obtained and thereafter centrifuged at 15,000 rpm for 45 minutes. For the purpose to eliminate the silver ions and residual leaf extract, the pellet containing the silver nanoparticles underwent a thorough rinsing process with deionized water, which was repeated three to four times. The nanoparticles that had formed were freeze-dried. The lyophilized nanoparticles underwent additional characterization following storage in a cool, dry, and dark environment. The green-synthesized silver nanoparticles, obtained after being tagged, were stored at a temperature of 4 °C until further experimental analysis [65].

2.6.2 Silver nanoparticles synthesis at varying PH

A constant quantity of 15 ml of leaf extract was used to make solutions at a temperature of 80 °C in both acidic and basic environments. This was done in order to promote the production of silver nanoparticles at a variety of pH values, ranging from 3 to 11. Glacial acetic acid was added dropwise to lower pH values, while sodium hydroxide was added dropwise to higher pH levels. This allowed the pH to be adjusted over time.

2.7 Characterization of AgNPs -IL

The synthesized AgNPs of IL extracts were characterized by UV-Vis- spectroscopy (UV-Vis), Fourier-transform infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM), X-ray powder diffraction (XRD) and Zeta Potential (ZT).

2.7.1 UV-visible spectroscopy

This method is primarily used to monitor and assess the stability and synthesis of metallic nanoparticles for the purpose of characterizing them. The predominant technique employed for nanoparticle characterization is UV-visible spectroscopy [66]. Vanden Bout *et al*. [67] (2011) found that the primary absorbance peak in UV-Vis spectra is associated with the surface plasmon resonance (SPR) of silver nanoparticles. The UV-visible spectrum of stable silver nanoparticles and the aqueous extract of fresh IL was measured using a UV-Vis-spectrometer (Shimadzu-Japanese uv-2450) with a resolution of 1 nm. IL extracts were used as a reducing agent. The samples were analyzed at the ambient temperature within the wavelength range of 300-800 nm at a scanning rate of 475 nm per minute, using a 1 cm optical path. UV-vis absorption spectra were obtained 24 hours after incubating the AgNO3 solution with IL extract. One possible cause of yellowing in the backdrop is the use of distilled water [68].

This is the usual wavelength range used to evaluate the optical characteristics of AgNPs and characterize the manufacturing of nanoparticles. A fuller knowledge of the nanoparticle synthesis process was made possible by the analysis of the UV-Vis spectra, which validated the successful synthesis of silver nanoparticles and revealed information on their morphology and size distribution [69].

2.7.2 Fourier Transforms Infrared Spectroscopy (FTIR)
The AgNPs solution was centrifuged for 30 minutes at 10,000 rpm. Three or four thorough washes of the supernatant solution were conducted in order to eliminate any unbound proteins or enzymes that are not capping the AgNPs. A vacuum drier was used to dry the pellet.

A dry leaf powder of IL was obtained and measured at a resolution of 4 cm-1 utilizing the diffuse reflection mode of the potassium bromide (KBr) pellet technique in Fourier transform infrared (FTIR, CFRD, Osmania University, Hyderabad, Telangana) spectroscopy. An infrared light with a wavelength of 500-4000 cm-1 was used to mix the powder with KBr. For the FTIR analyses of AgNPs-IL extract prior to and following bio reduction, a comparable procedure was employed [70, 71]. The force constant and reduced mass are the two factors that determine the frequency of the vibrational peak (v), and these factors can be expressed by the following equation [72, 73].

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

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

2.7.3 Scanning Electron Microscope (SEM)
The structure and size of silver nanoparticles were analyzed using a German-made scanning electron microscope equipped with EDS-Mapping-Line-EBSD capabilities. The evaluation was conducted by subjecting the sample to centrifugation at a rotation speed of 10,000 rpm for 15 minutes. Subsequently, the sample was rinsed with distilled water and then dried at a temperature of 50 °C. The sample was positioned on a platinum mesh that had been coated with palladium. The sample was then subjected to analysis using radiation that passed through it. The resulting image displayed a dispersion spectrum with an energy of 250 INCA [74].

2.7.4 X-ray Diffraction
The crystal structure and size of the sample was studied by X-ray Diffraction. About 1 ml of colloidal AgNps was uniformly spread on glass plate and kept to dry in an oven. This process was repeated 2-3 times in order to get a thin film on plates. Before spreading nanoparticles, glass plates were properly washed with acetone and ethyl alcohol in the sonicator. XRD spectra of the resulted AgNps were measured on Rigaku-Ultima IV X-ray Diffractometer (operating at 40 kV voltage and 30 mA current with Cu k (radiations ($\lambda 1 = 1.54056$; $\lambda 2 = 1.54439$). For this analysis, thin film of AgNPs on glass slides was placed in diffraction arm. The diffracted intensities of the sample were recorded at 2 angles ranging from 30 degrees to 80 degrees [75]. The size of the crystalline AgNPs was calculated using Debye-Scherrer equation, i.e. =

$$D = K\lambda / \beta\cos\theta$$

Where, D = Crystalline size of nanoparticles
K = crystalline-shape factor
λ = X-ray wavelength
β = X-ray diffraction broadening, radian
θ = observed peak angle in degree

2.7.5 Zeta potential measurement
The surface electric charge of Ag NPs was established by detecting the particles that are most stable when there is electrostatic repulsion between them. The determination of zeta potential was carried out using the HAS 300 instrument, which is based on photon correlation spectroscopy [76]. The analysis duration was 60 seconds, during which the average zeta potential was measured. The dispersion was determined without any dilution.

2.8 Anti-bacterial activity [77, 78]
2.8.1 Media Preparation for Anti-Bacterial Activity
a) **Nutrient Agar Media:** Nutrient Agar was obtained from a commercial source and measured 28.0 grams of powder. Then dissolved the powder in 1000 milliliters of distilled water and mixed it thoroughly. The dissolved nutrient agar was sterilized in an autoclave at a temperature of 121 ^{0}C for a duration of 15 minutes. The sterilized media was then utilized for plate production in order to investigate its antibacterial properties.
b) **Nutrient Broth:** Nutrient Broth was obtained from a commercial source and measured 1.3 grams of powder. then dissolved in 100 milliliters of distilled water and mixed it thoroughly. The dissolved nutrient broth was sterilized in an autoclave at a temperature of 121 °C for a duration of 15 minutes. The sterilized broth was then utilized for the militance of the inoculum.
c) **Preparation of stock solution:** The stock culture of each organism was prepared by aseptically subculturing each validated test organism onto two nutrient agar slants. One set of slants was maintained as a stock culture, while another set was used as a working culture. The bacterial cultures were kept at a temperature of 4 °C in their respective agar slants and utilized as stock cultures. A single glycerol stock was likewise kept at a temperature of 20 °C.
d) **Inoculum preparation:** The selected bacterial pathogens were inoculated into nutrient broth and incubated at 37 °C for 24 hours and the suspensions were checked to provide approximately 10-5 CFU/ml.

2.8.2 Antibacterial activity of AgNPs- IL
The antibacterial activity of nanoparticles was investigated using the Agar well-diffusion method. Four different concentrations (25, 50, 75, and 100 µl) were evaluated against bacterial pathogens including *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *E. coli*. The plates were placed in an incubator at a temperature of 37 °C for a duration of 18-24 hours. At the conclusion of the experiment, the diameter of the zone where growth was inhibited (measured in millimeters) was recorded, and the activity index was determined. Measurements were obtained in three distinct fixed orientations, and the mean results were documented.

2.8.3 Minimum Inhibitory Concentration (MIC) [79]
Minimum Inhibitory Concentration (MIC), is the lowest concentration of an anti-microbial growth that will inhibit the visible growth of a microorganism after overnight incubation.

Extract Preparation
Extracts were weighed individually 1mg and dissolved in methanol for final stock concentration as 1mg/ml. As same sample, standard ampicillin also prepared.

Culture Preparation

Loop of culture was inoculated in 3 ml of nutrient broth and incubate 37 ^{0}C for overnight in shaking incubator.

Inoculum Preparation:

From overnight grown culture, 20 µl of culture was taken and inoculated in 1.5 ml of nutrient broth and added different concentration of compound and incubated at 37 ^{0} c for overnight in an incubator.

Results

After 24 hrs of compound treatment, tubes were observed, and results were noted.

2.9 Anti-Fungal Activity [80]

2.9.1 Fungal Strains

The fungal strains *Candida albicans* (MTCC 183) and *Aspergillus niger* (MTCC) used in the study was obtained from Microbial type culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh

2.9.2 Sabouraud Dextrose Agar (SDA)

To make a commercially available Sabouraud dextrose agar, 32.5 grams of powder were dissolved and well mixed with 500 milliliters of distilled water. The SDA was used to prepare plates in order to examine its antifungal capabilities after it had been dissolved and autoclaved for 15 minutes at 121 °C.

2.9.3Antifungal activity of AgNPs -IL

The well diffusion method was used to test the antifungal activity. Using the spread plate method, the produced SDA culture plates were inoculated with *Aspergillus niger* and *Candida albicans.* For 48 hours, the plates were incubated for fungal activity at 37+2 ºC. After 48 hours, the zones surrounding the well on the plates were checked for zone formation, and the zone of inhibition (mm) was calculated.

3. Observations and Results

3.1 Morphological description

Annual stem twiners, up to 5 m long; stems turning purplish with age, quadrangular, sparsely hairy at nodes. Leaves simple, variable, usually ovate, entire, cordate at base, simple, entire, 5-10 × 5-8 cm, glabrous; distal leaves trilobed, lobes shallow or sharp; petioles up to 12 cm long, glabrous. Inflorescence cymose, lax, 1-3-5-7 (17)-flowered; peduncles 6-12 cm long, curved up, swollen at apex; pedicels 2.5-3(-5) mm in fruit, quadrangular, glabrous, bulged, cushion-like below the flower but sucked-up when the fruit matures; bracts linear, caducous, 3 mm long. Flowers: Sepals 5, subequal, 8-10 × 2-3 mm, glabrous, faintly veined, purplish at apex, margins white at fruit dehiscence, taller than the capsule (up to 1 cm incl. 1 mm tip), upright, not deflexed; corolla funnel-shaped, pink, 1.5–1.8 cm long; limb 5 lobed, lobes shortly apiculate; stamens 5, filaments subequal, included, 7-8 mm long, hairy at base; ovary 1 × 1.5 mm, glabrous; style 1 cm long; stigma bilobed; capsules ovoid, 5 × 6 mm, apparently 4-partite/4-valved (cf. Chowdhery & Debta, 2009: 121; Plate 1J; Kattee *et al*., 2019: 213) [61, 59] but bilocular with 2 ovules in each loculus, tinged purple when young, glabrous; style-base persistent (Functions like a cap/lock: by abscission it allows the mature capsule to break along the septal suture to release the seeds); the locular partition (Septum) transparent, has a rim and central axis, 6 × 6 mm, slightly ellipsoidal; seeds black, glabrous, 3.5 × 3 mm, with two flat lateral faces and convex back with a shallow ridge at the center (Fig. 1A–E).

Flowering and Fruiting: October-December.

3.1.1 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.

3.1.2 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,

3.2 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 [81], 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 [82, 83, 84]. 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 [85]. The utilization of a variety of analytical methods, such as ultraviolet-visible spectroscopy, scanning electron microscopy, field emission scanning electron microscopy, energy-dispersive X-ray spectroscopy, transmission electron microscopy, X-ray diffraction, and Fourier transform infrared spectroscopy, was also utilized in order to establish the confirmation of the creation of silver nanostructures (AgNPs) (Fig 2).

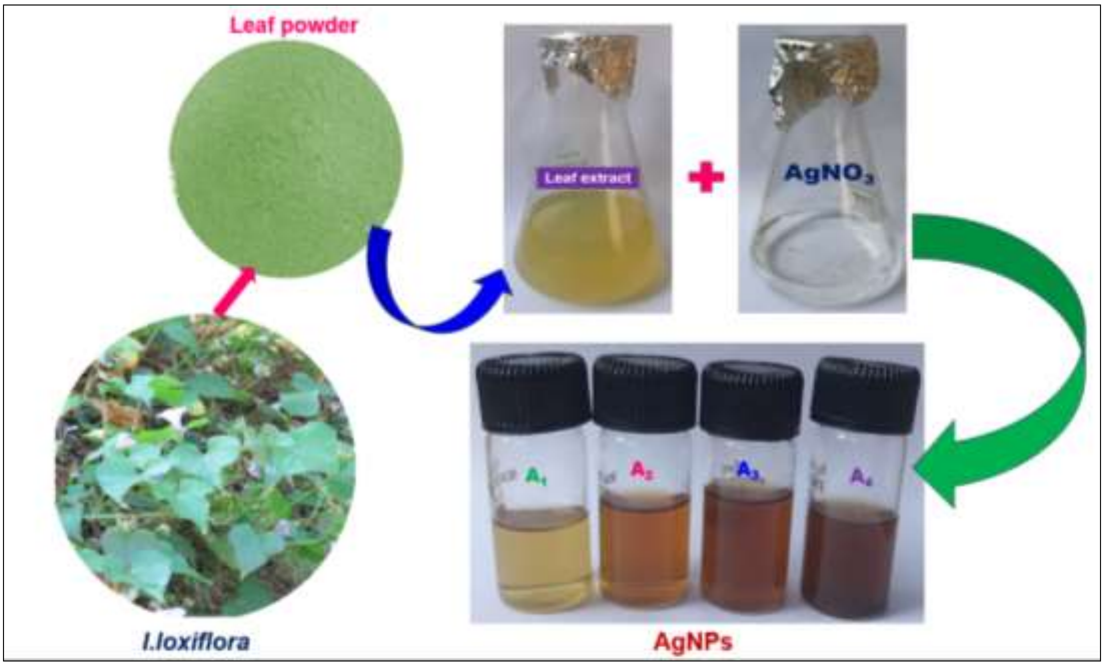

Fig 2: Green synthesis of *I. laxiflora* silver nanoparticles (AgNPs)

3.3 Characterization of *I. laxiflora* silver nanoparticles (AgNPs)
3.3.1 UV-Visible spectroscopy (UV-Vis) study of AgNPs-IL
Characterizing the metal nanoparticles via the application of the surface plasmon resonance (SPR) phenomena is accomplished by the use of ultraviolet-visible spectroscopy [86]. There is no noise in the absorption band. Plasmon resonance that manifests itself at around 400 nanometers for IL AgNPs, and the frequency and breadth of surface plasmon absorption are determined by the size and shape of the produced AgNPs.

3.3.2 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 400-4000 cm^{-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.

3.3.3 Scanning Electron Microscope (SEM) analysis of AgNPS
Scanning electron microscope (SEM), is an electron microscope and studying the surfaces of objects to determine the shape and size. Size and shape of IL synthesized AgNPs studied with Hitachi 500 with 15.0 Kv with 5. 00 µm. IL AgNPs shows the 179-335 nm diameter with rounded aggregated smaller particles (Fig 5).

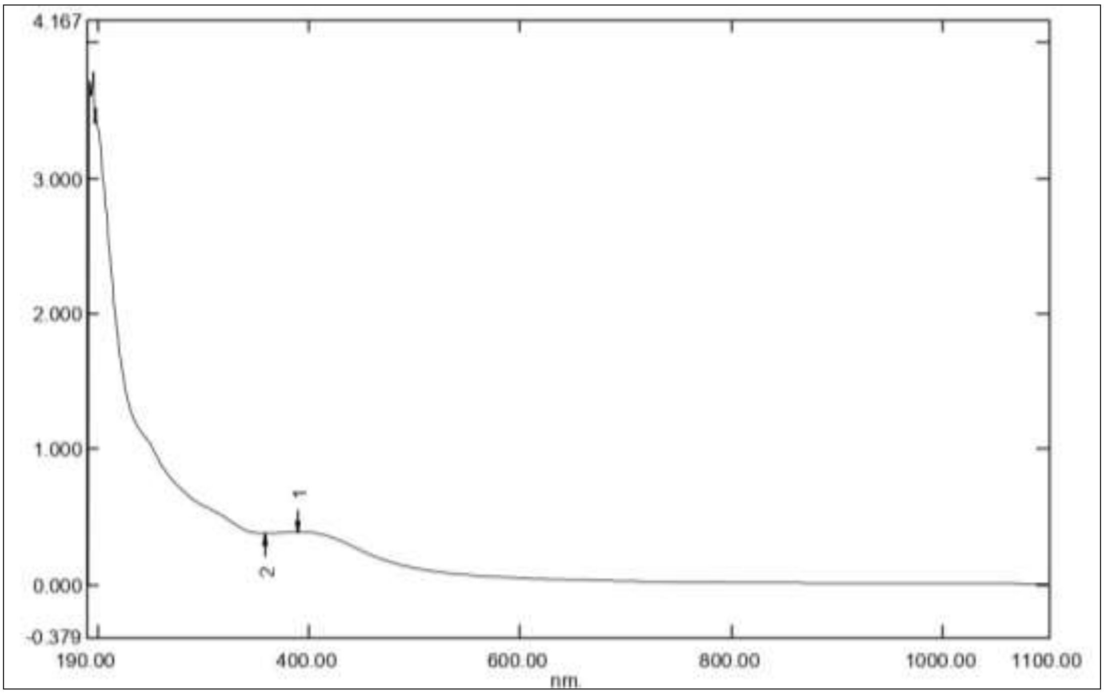

Fig 3: UV-Visible absorption spectra of IL AgNPs

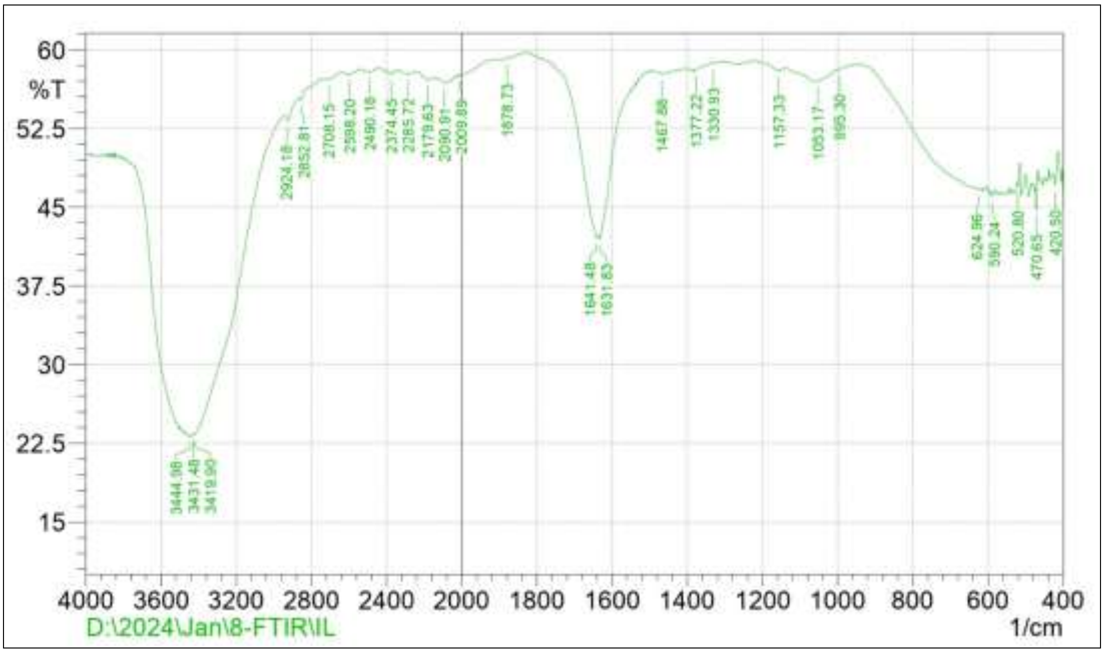

Fig 4: FT-IR spectra of IL AgNPs

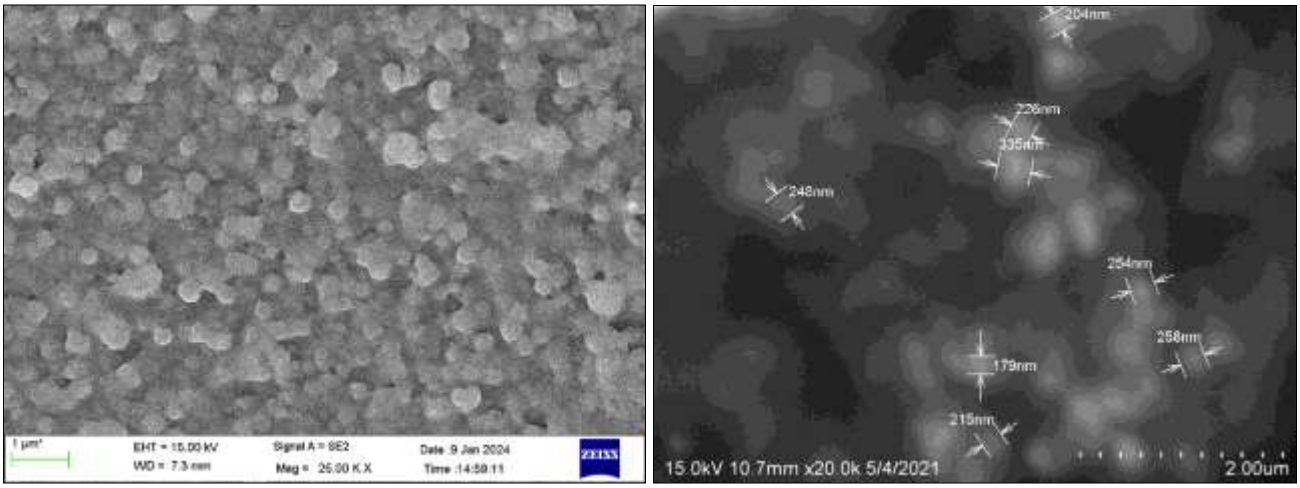

Fig 5: SEM image of IL AgNPs

3.3.4 XRD Spectra of AgNPs-IL
XRD used for characterization of nano particles of any sizes, and the observed changes in positions of diffraction peaks are used to make conclusions on how crystal structure and cell parameters changes with the change in nano particles size and shape and cell dimensions. X-axis indicates Thete-2Theta (deg) and Y-Axis indicates intensity or scan/sec.The X-ray diffraction (XRD) spectrum of IL AgNPs showed the four main characteristic peaks of Bragg diffraction positioned at four different values of 17.9725,32.22157,77.98076 and 82.44159 degrees and these all reflections correspond to (111),(200), (220), (311) facets of the fae centered cubic (fee) structure of AgNPs of IL.These diffraction peaks were consistent with the standard database files of silver(JCPDS card No. 04-0783) and this suggesting that the synthesized AgNPs were pure crystalline in nature (Fig 6).

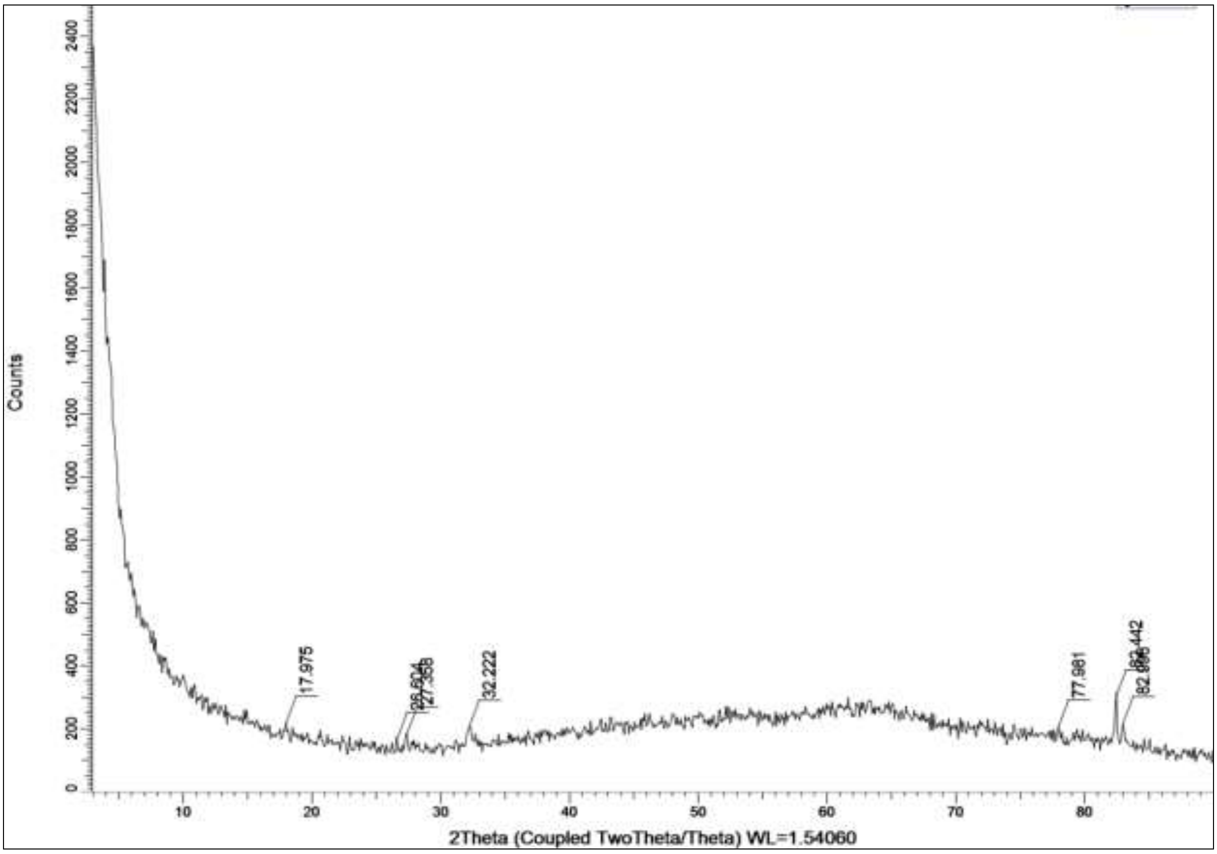

Fig 6: XRD Spectra of IL AgNPs

3.3.5 Characterization of Au-NPs by Zeta Potential
Zeta potential gives the idea about nanoparticles colloidal stability. Nanoparticles with Zeta Potential values greater than +25 mV or less than -25 mV typically have high degrees of stability (Fig 7).

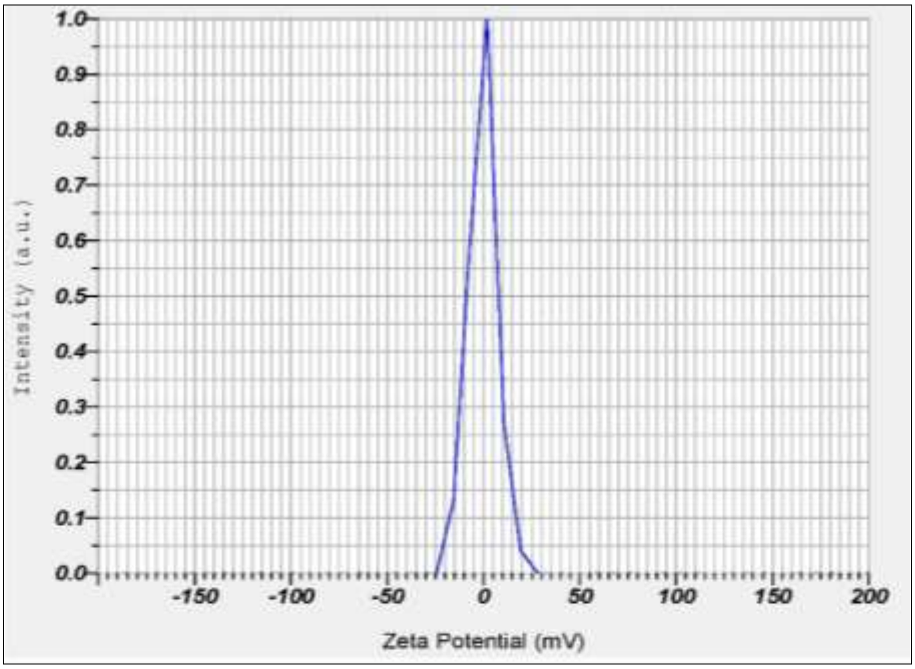

Fig 7: Zeta potential Spectra of IL AgNPs

3.4 Determination of Anti-bacterial activity
The study of the antibacterial activity of *Ipomoea laxiflora* silver nanoparticles using ampicillin as standard were tested against pathogenic bacterial lab cultures that are Gram positive of *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumonia* (ATCC 33400), and Gram negative of *Pseudomonas aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922).

3.4.1 Antibacterial activity of *IL silver nanoparticles*
The results presented in Table 1, which investigate the antimicrobial activity of silver nanoparticles synthesized using leaf of *Ipomoea laxiflora (IL)* extract against four pathogenic bacteria strains: *Staphylococcus aureus, Escherichia coli (E. coli)*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*. The study employed the disc diffusion plate method of antibiotic sensitivity testing, with ampicillin serving as the standard antibiotic for comparison.

Starting with the results for ampicillin, we observe varying degrees of effectiveness against the different bacterial strains at concentrations ranging from 25 µg to 100 µg. For instance, against *Staphylococcus aureus*, ampicillin exhibited a zone of inhibition (ZOI) of 13 mm at a concentration of 25 µg, which increased to 20 mm at 100 µg. Similarly, for *E. coli*, the ZOI ranged from 14 mm to 19 mm across the same concentration range. These results suggest a concentration-dependent antibacterial effect of ampicillin against the tested strains, consistent with its known antimicrobial properties. The test plates are shown in Figure 8 and Figure 9 for reference.

The silver nanoparticles (AgNPs) synthesized from the *Ipomoea laxiflora* leaf extract demonstrated notable antimicrobial efficacy, as evident from their ability to inhibit bacterial growth at varying concentrations. Against *Staphylococcus aureus*, AgNPs showed a progressive increase in the zone of inhibition, with measured diameters of 0.8 mm at 25 µg, 2.5 mm at 50 µg, 3.6 mm at 75 µg, and reaching a maximum of 5.5 mm at 100 µg. This demonstrates a clear dose-dependent inhibitory effect, with higher nanoparticle concentrations yielding greater antibacterial action.

Similarly, the AgNPs inhibited *Escherichia coli* with inhibition zones of 0.5 mm at 25 µg, 1.2 mm at 50 µg, 2.0 mm at 75 µg, and a substantial 3.5 mm at the 100 µg concentration. *Streptococcus pneumoniae* displayed zones of inhibition that included 0.8 mm at 25 µg, 1.6 mm at 50 µg, 2.4 mm at 75 µg, and 3.6 mm at 100 µg, indicating the nanoparticles' broad-spectrum efficacy against Gram-positive bacteria.

Against *Pseudomonas aeruginosa*, an increasing inhibition trend was also observed, with inhibition zones of 0.6 mm at 25 µg, 1.8 mm at 50 µg, 2.3 mm at 75 µg, and 3.8 mm at 100 µg. This highlights the AgNPs' effectiveness against Gram-negative bacteria as well. The increasing zones of inhibition at higher nanoparticle concentrations (up to 100 µg) underscore the potency of *Ipomoea laxiflora*-derived AgNPs, showing potential as a natural, bio-synthesized antimicrobial agent with broad-spectrum capabilities.

In summary, silver nanoparticles synthesized from *Ipomoea laxiflora* leaf extract showed strong, dose-dependent antibacterial effects against both Gram-positive and Gram-negative bacteria. The increasing inhibition zones with higher concentrations highlight the potential of *Ipomoea laxiflora*-derived AgNPs as a natural, eco-friendly antimicrobial agent.

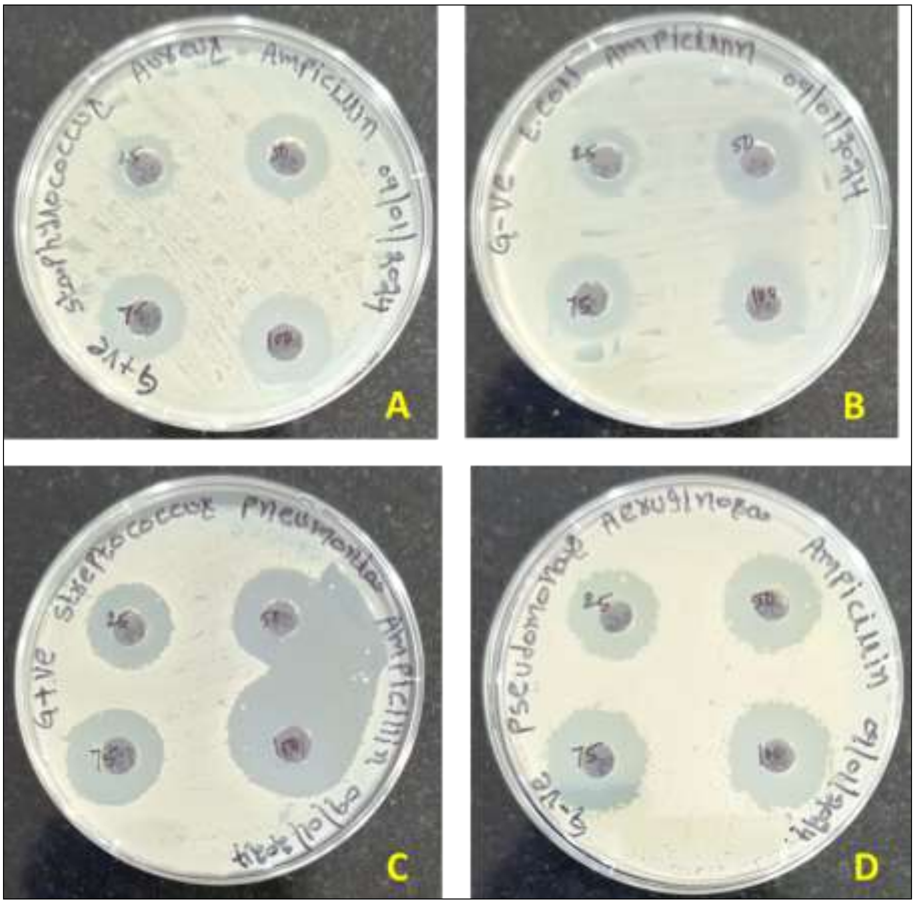

Fig 8: Anti-bacterial activity against various pathogenic bacteria. (A, B, C& D represents zone of inhibition with standard drug ampicillin against *Staphylococcus aureus*, *E. coli*, *Streptococcus pneumonia* and *Pseudomonas aeruginosa* respectively)

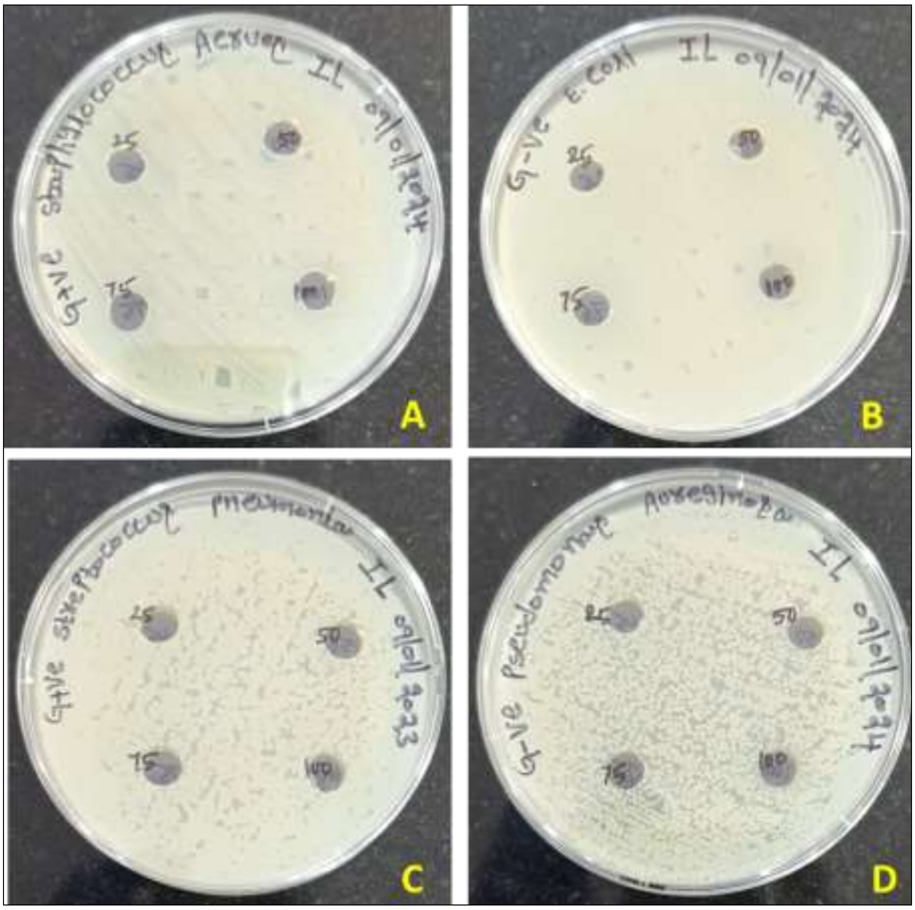

Fig 9: Anti-bacterial activity against various pathogenic bacteria. (A, B, C& D represents zone of inhibition with *IL* Silver nanoparticles against *S. aureus*, *E. coli*, *S. pneumonia* and *P. aeruginosa* respectively)

Table 1: Anti-bacterial activity of *IL* Silver nanoparticles against pathogenic bacteria

Sr. No	Bacterial Strain	Ampicillin				Silver nanoparticle using *IL* extract			
		Concentration (µg)/ Zone of Inhibition (mm)							
		25	50	75	100	25	50	75	100
1	*Staphylococcus aureus*	13	17	18	20	0.8	2.5	3.6	5.5
2	*E coli*	14	17	18	19	0.5	1.2	2	3.5
3	*Streptococcus pneumonia*	17	18	21	27	0.8	1.6	2.4	3.6
4	*Pseudomonas aeruginosa*	18	20	21	24	0.6	1.8	2.3	3.8

3.4.2 MIC Studies

The Minimum Inhibitory Concentration (MIC) determination results presented in the Table 2 and Figure 10 for both ampicillin and silver nanoparticles synthesized using *Ipomoea laxiflora (IL)* extract against two bacterial strains: *Staphylococcus aureus* and *Escherichia coli (E. coli)*. MIC is defined as the lowest concentration of an antimicrobial agent that inhibits visible growth of a microorganism after overnight incubation. It serves as a crucial parameter for assessing the effectiveness of antimicrobial agents.

Looking at the results for ampicillin, we observe a concentration-dependent inhibition of bacterial growth for both *Staphylococcus aureus* and *E. coli*. For instance, against *Staphylococcus aureus*, the MIC values ranged from 0.337 µg/ml at the lowest concentration to 0.095 µg/ml at the highest concentration tested (200 µg/ml). Similarly, for *E. coli*, the MIC values ranged from 0.295 µg/ml to 0.113 µg/ml across the same concentration range.

On the other hand, the MIC determination for *IL* silver nanoparticles reveals a different pattern. Against both *Staphylococcus aureus* and *E. coli*, the silver nanoparticles did not exhibit significant inhibitory effects at any of the tested concentrations (5 µg/ml to 200 µg/ml). Even at the highest concentration tested (200 µg/ml), the growth of both bacterial strains was not visibly inhibited by the silver nanoparticles.

These results suggest that while ampicillin demonstrated concentration-dependent inhibitory effects against both *Staphylococcus aureus* and *E. coli*, the *IL* silver nanoparticles did not exert significant antimicrobial activity at any of the tested concentrations.

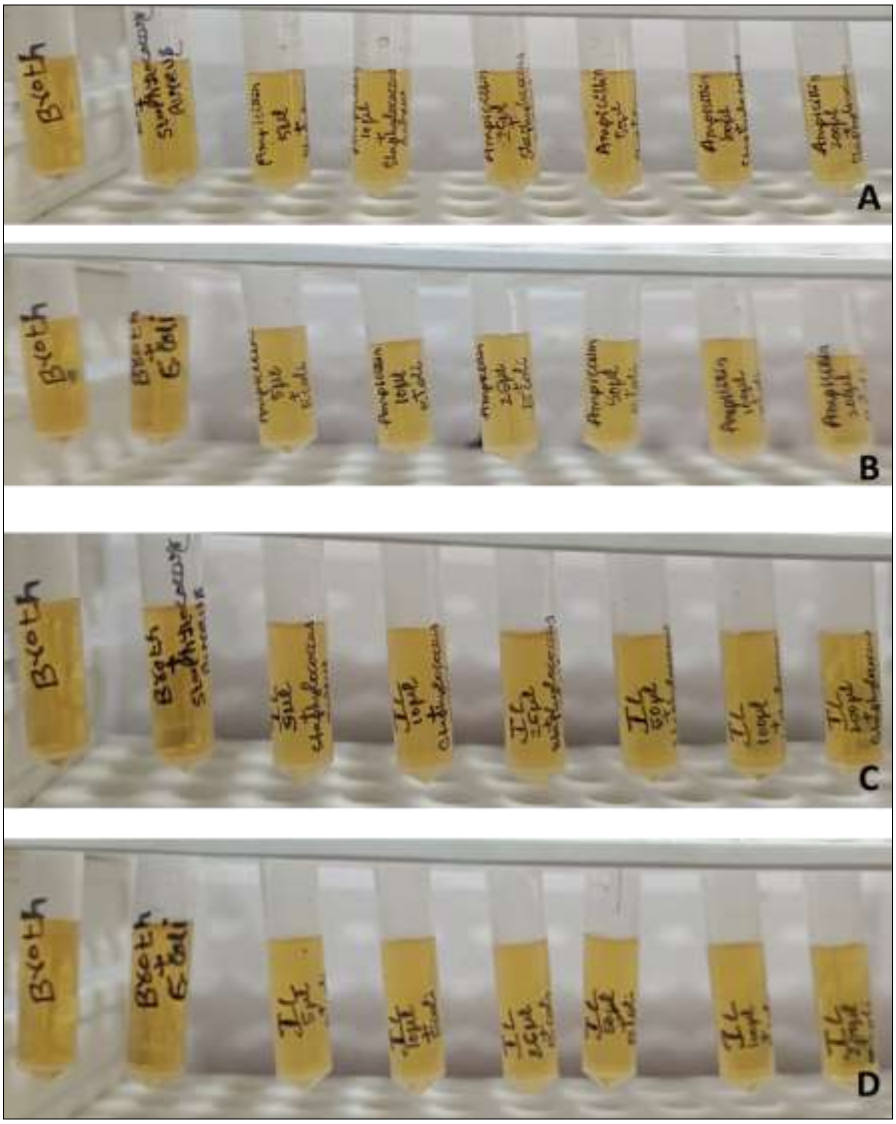

Fig 10: A: MIC activity of Ampicillin on *S. aureus*, B: MIC activity of Ampicillin on *E. coli*, C: MIC activity of IL silver nanoparticles on *S. aureus*, D: MIC activity of IL silver nanoparticles of plant on *E. coli*.

Table 2: Minimum Inhibitory concentration determination for *IL* silver nanoparticles in comparison with Ampicillin.

S. No	Bacteria	Concentration (µg/ml) Ampicillin						Concentration (µg/ml) IL silver nanoparticles					
		5	10	25	50	100	200	5	10	25	50	100	200
1	*Staphylococcus aureus*	0.337	0.300	0.257	0.253	0.192	0.095	0.315	0.326	0.336	.282	0.216	0.118
2	*E. coli*	0.295	0.282	0.278	0.283	0.203	0.113	0.302	0.293	0.286	0.253	0.196	0.116

3.5 Anti-fungal activity

Anti-fungal activity study of silver nanoparticles synthesized using leaf of *Ipomoea laxiflora* (IL) extract against two pathogenic fungal strains: *Candida albicans* and *Aspergillus niger* are presented in Table 3 and Figure 11. The comparison is made with fluconazole, a commonly used antifungal medication.

Starting with fluconazole, we observe a concentration-dependent inhibition of fungal growth for both *Candida albicans* and *Aspergillus niger*. For instance, against *Candida albicans*, the inhibition zone increases from 12 mm at 25 µg/ml to 21 mm at 100 µg/ml of fluconazole. Similarly, for *Aspergillus niger*, the inhibition zone ranges from 9 mm to 20 mm across the same concentration range.

Now, turning to the results for *IL* silver nanoparticles, we notice differences in their antifungal activity compared to fluconazole. Against *Candida albicans*, the silver nanoparticles showed 0.5mm, 1.8mm, 9mm, and 10mm, However, at higher concentrations (75 µg/ml and 100 µg/ml), some antifungal activity is observed, with inhibition zones of 9 mm and 10 mm, respectively.

Similarly, against *Aspergillus niger*, the silver nanoparticles showed limited antifungal activity at lower concentrations (25 µg/ml and 50 µg/ml), with inhibition zones of 1.4 mm and 6 mm, respectively. However, at higher concentrations (75 µg/ml and 100 µg/ml), the antifungal activity increased, resulting in inhibition zones of 12 mm and 14 mm, respectively.

These results suggest that while fluconazole demonstrated concentration-dependent antifungal effects against both *Candida albicans* and *Aspergillus niger*, the *IL* silver nanoparticles exhibited moderate antifungal activity, particularly at lower concentrations. However, at higher concentrations, some antifungal activity against both fungal strains were observed, albeit to a lesser extent compared to fluconazole.

This highlights the potential of IL silver nanoparticles as antifungal agents, but also indicates the need for further optimization to enhance their efficacy. Additionally, the mechanisms underlying the antifungal activity of these nanoparticles warrant further investigation to better understand their mode of action and potential applications in combating fungal infections. The study compared silver nanoparticles synthesized from *Ipomoea laxiflora* leaf extract against pathogenic fungal strains, *Candida albicans* and *Aspergillus niger*. Fluconazole showed concentration-dependent inhibition of fungal growth against both strains. However, IL silver nanoparticles exhibited moderate antifungal activity, particularly at lower concentrations. At higher concentrations, some antifungal activity was observed, albeit to a lesser extent compared to fluconazole. This suggests the potential of IL silver nanoparticles as antifungal agents, but also suggests the need for further optimization to enhance their efficacy. Further investigation is needed to better understand their mechanism of action and potential applications in combating fungal infections.

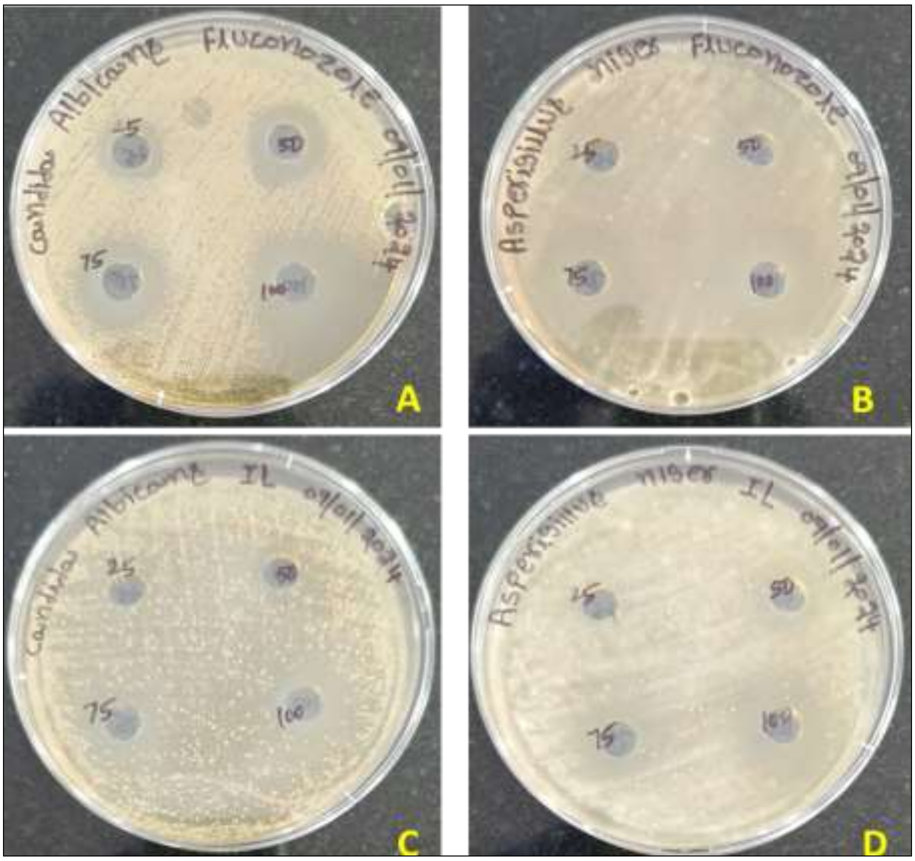

Fig 11: Anti-fungal activity, A & B represents for fluconazole against *Candida albicans* and *Aspergillus niger* respectively whereas C and D represents for IL silver nanoparticles activity against *Candida albicans* and *Aspergillus niger* respectively.

Table 3: Anti-fungal activity of IL nanoparticles against pathogenic fungal strains

S. No	Fungal Strain	Concentration (µg/ml) Fluconazole				Concentration (µg/ml) IL silver nanoparticles			
		25	50	75	100	25	50	75	100
1	*Candida albicans*	12	15	18	21	0.5	1.8	9	10
2	*Aspergillus niger*	9	12	18	20	1.4	6	12	14

4. Discussions

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 world, in India). It is used to treating fever, headaches, and stomach aches (Chowdhery and Debta, 2009) [61]. 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 reduction of Ag+ ions cause surface plasmon resonance vibration, which is necessary for the formation of AgNP. Measurements of the absorbance peak at 428 nm indicated AgNP biogenesis using *I. laxiflora* leaf extract. The XRD pattern of AgNP shows strong peaks at 2^{0}-38.31, 43.54, 64.62, and 77.46, corresponding to the (111), (2 0 0), (2 2 0), (3 1 1), and (2 2 2) Bragg's reflections of the face-centered cubic structure of silver nano particles. The SEM analysis of IL synthesized AgNPs using Hitachi 500 reveals a diameter of 179-335nm, with rounded aggregated particles. Zeta potential indicates nanoparticles' colloidal stability, with values greater than +25 mV or less than -25 mV indicating high degrees of stability. The antimicrobial activity of silver nanoparticles (AgNPs) synthesized using *Ipomoea laxiflora* leaf extract was assessed against four bacterial pathogens: *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*. Ampicillin served as the standard antibiotic for comparison.

Ampicillin exhibited concentration-dependent antibacterial activity, with inhibition zones ranging from 13-20 mm for *S. aureus* and 14-19 mm for *E. coli* across 25-100 µg concentrations. Similarly, significant inhibition was observed against other bacterial strains, confirming its broad-spectrum efficacy. The study analyzed the Minimum Inhibitory Concentration (MIC) of ampicillin and silver nanoparticles synthesized from *Ipomoea laxiflora* extract against *Staphylococcus aureus* and *Escherichia coli*. The results showed a concentration-dependent inhibition of bacterial growth for both strains. However, the MIC for silver nanoparticles did not show significant inhibitory effects against both strains. The findings suggest that ampicillin showed concentration-dependent inhibitory effects against both strains, but silver nanoparticles did not exert significant antimicrobial activity.

The synthesized AgNPs demonstrated notable antimicrobial potency in a dose-dependent manner. Against *S. aureus*, inhibition zones increased from 0.8 mm at 25 µg to 5.5 mm at 100 µg. For *E. coli*, the zones ranged from 0.5 mm to 3.5 mm. *S. pneumoniae* and *P. aeruginosa* displayed similar trends, with inhibition zones of 0.8-3.6 mm and 0.6-3.8 mm, respectively, highlighting the AgNPs' effectiveness against both Gram-positive and Gram-negative bacteria.

These results underscore the potential of *I. laxiflora*-derived AgNPs as natural, eco-friendly antimicrobial agents, demonstrating broad-spectrum activity and concentration-dependent effectiveness.

5. Conclusion

The study successfully synthesized silver nanoparticles (AgNPs) from *Ipomoea laxiflora* leaf extract, confirmed by characteristic color changes, UV-vis spectroscopy, XRD analysis, and SEM imaging, revealing stable nanoparticles with diameters of 179-335 nm. While *I. laxiflora*-derived AgNPs demonstrated dose-dependent antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*, their efficacy was lower compared to the standard antibiotic ampicillin. These findings highlight the potential of *I. laxiflora* as a natural, sustainable source for AgNP synthesis, offering moderate antimicrobial capabilities and scope for further optimization.

6. Conflicts of Interest

The authors declare that they have no conflicts of interest.

7. Acknowledgements

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