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Green Synthesis of Silver Nanoparticles from *Abutilon Indicum* and Evaluation of their Antibacterial Activity

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Abstract

Recently green synthesis of silver nanoparticles was found to have the potential to overcome the toxicity exhibited by chemical synthesis methods and their antibacterial activity attracts the attention of the research fraternity. This study is a preliminary approach to focus on the synthesis of silver nanoparticles using *Abutilon indicum* (AI-AgNPs) by biological green synthesis method and evaluation of antibacterial properties of *Abutilon indicum* plant extract and AI-AgNP. The aqueous plant extract of *Abutilon indicum* which served as a capping and reducing agent was used to biosynthesize silver nanoparticles. UV Spectrophotometer analysis was used to analyse the biosynthesized *Abutilon indicum* silver nanoparticles (AgNPs) and the SPR peak was observed at the UV region which confirmed the presence of silver nanoparticles. Antibacterial activity of *A.indicum* plant extract and AI-AgNPs were tested by agar well diffusion method using gram-positive bacteria *(Bacillus subtilis, Staphylococcus aureus. sp.*) and gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*). Antimicrobial activity results demonstrated that AI-AgNPs (1mM and 3mM) exhibited significant antibacterial activity with a higher zone of inhibition against test cultures than plain *A.indicum* extract. Thus, the study's findings suggest that *A.indicum* could be an effective agent for synthesizing silver nanoparticles and that the AI-AgNPs could be used as antibacterial agents in therapeutic and biomedical applications.

Keywords
Green synthesis, *A.indicum,* Silver nanoparticles, Antibacterial activity.

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INTRODUCTION:

Since the turn of the century, nanotechnology has been a known research area. Following the introduction of the idea of "nanotechnology" by Nobel laureate Richard P. Feynman in his renowned 1959 presentation "There's Plenty of Room at the Bottom" [1], the area of nanotechnology has seen significant advancements. Materials of all kinds have been developed at the nanoscale with nanotechnology [2]. According to Laurent et al. (2010) [3], nanoparticles (NPs) are a broad material category that includes particulate compounds with at least one dimension smaller than 100 nm. These materials can be 0D, 1D, 2D, or 3D depending on the overall shape [4].

The typical colours of 20-nm gold (Au), platinum (Pt), silver (Ag), and palladium (Pd) NPs are, respectively, wine-red, yellowish gray, black, and dark black [5]. Since ancient times, silver has been known to have powerful antibacterial properties. Infection in burns, open wounds, and persistent ulcers is now commonly treated with topical dressings based on silver [6]. Among the many metallic nanoparticles used in biomedical applications, silver nanoparticles (AgNPs) are one of the most essential and fascinating nanoparticles. AgNPs have a lot of potential, and

they have been used for environmental protection, medication delivery, cosmetics, and nanomedicine [7]. As opposed to their solid silver bulk form, silver nanoparticles have unique biological, chemical, and physical features [8]. Optical behavior, surface-enhanced Raman scattering, electrical conductivity, chemical stability, high thermal stability, catalytic activity, and nonlinearity are all unique properties of AgNPs [9].

Silver nanoparticles were developed with these features for use in electronics and medicine [10]. AgNPs are frequently employed in the realm of antimicrobials to treat infections caused by microorganisms such as fungi, viruses, and bacteria [11]. AgNPs are employed in a variety of applications, including colloidal coating, paints, solid materials like polymer scaffolds, and the textile industry [12].

AgNPs can be produced using a variety of techniques, including physical and chemical ones [13]. It has been reported and is thought to be a well-established technique to chemically reduce silver ions using sodium borohydride [14], hydrazine [15], ascorbic acid, trisodium citrate [16-17], and polyols [18]. Chemical pathways are helpful, but because of the chemicals utilized and the difficulty of getting rid of them, these approaches may be harmful. Also, the chemicals employed in these processes are harmful to the environment [19]. To overcome these issues, green synthesis was developed to mitigate chemical toxicity [20].

This biosynthetic process of metal nanoparticles has been recommended as a cost- effective and eco-friendly approach to producing these materials. Synthesis of AgNPs using microorganisms or plant extracts has become an attractive alternative. These biosynthetic approaches offer several advantages. They are simple, low-cost, provide high yields, and are environmentally benign [21]. Plant extracts are said to have been successfully employed in the synthesis of AgNPs [22].

A plant extract-mediated bio reduction generally includes combining the aqueous extract with an aqueous solution of the suitable metal salt. The fabrication of nanoparticles takes place at room temperature and is completed in a matter of minutes. Several plants have therapeutic characteristics in addition to being employed as reducing agents in the manufacture of silver nanoparticles [23]. For thousands of years, plants have been utilized as remedies. They have always been employed as a rich source of physiologically active medications and have a variety of traditional uses that have served humanity for thousands of years [24-30]. According to the World Health Organization (WHO) assessment, around 80% of the population, primarily in underdeveloped nations, continues to rely on traditional medical systems for basic health care.

Medicinal plants provide a variety of biologically active substances that aid in the management of different illnesses and the overall quality of life. The existence of diverse life-sustaining elements in plants prompted scientists to examine their potential applications in treating infectious disorders and managing chronic wounds [31-34]. The *Abutilon indicum* is one such plant that grows as a shrub and belongs to the *Malvaceae* family. It is found all across the tropical and subtropical zones but is most common in India, Asia, and Africa. Some of the plants in the genus are well-known Ayurvedic herbs, and there has recently been renewed scientific interest in studying the species. Terpenoids, Tannins, Alkaloids, Flavonoids, Saponins, hexoses, n-alkane mixtures (C_{22-34}), alkanols, -sitosterol, vanillic, p-coumaric, caffeic acid, fumaric acid, sesquiterpene lactones (Alantolactone and isoalantolactone), amino acids, and Anthraquinones, which have been found in vitro to have antimicrobial properties against bacteria and fungi [35-36]. *A.indicum* essential oil comprises 0.15% -pinene, caryophyllene, caryophyllene oxide, endesmol, farnesol, borenol, geraniol, geranyl acetate, elements and 1:8-cineole, among other minor compounds [37-42]. *Abutilon indicum* also has hepatoprotective, wound healing, immunomodulatory, analgesic, antimalarial, antibacterial and hypoglycemic action [43].

It is found from several studies that, *A. indicum* has been acting as an effective reducing agent and used in the synthesis of AI-AgNPs [44-46]. So far the antibacterial properties of silver nanoparticles prepared from *A.indicum* leaf extract have not been much explored. Hence, this study is focused on the preparation of silver nanoparticles using the *Abutilon indicum* leaf extract, and assessment of their antibacterial properties.

MATERIALS AND METHODS:

Collection of plant samples:

The plant sample (Figure 1) was collected from local fields in Nizampet, JNTU, Hyderabad. Here we have chosen the plant grown in a natural environment, i.e., wild conditions, because of its higher antimicrobial activity compared to the plants grown in laboratory conditions or botanical gardens.

Fig 1: *Abutilon indicum* plant

Preparation of plant extract

Fresh leaves were collected, surface cleaned with running tap water to remove any dust particles and other contaminated organic materials, and then cleaned with double-distilled water, and finally air dried at room temperature. About 20 grams of finely cut leaves were kept in a beaker containing 200 mL of double-distilled water and boiled for 30 minutes. Then the extract was allowed to cool and filtered using Whatman No. 1 filter paper. Finally, the extract was stored at 4°C for further use [23]. The plant extract was also prepared by homogenizing 20 grams of plant leaves [47] using a mortar and pestle (Figure 2). The crushed homogenate was then filtered with the help of a sterile gauze cloth, and the extract was collected in a sterile container. The extract was stored at 4°C for further use.

Fig 2: Preparation of *A.indicum* leaf extract by A) Boiling and B) Crushing

Green Synthesis of AI-AgNP and Characterization by UV-Vis Spectroscopy:

Silver nitrate was used as such (purchased from Merck, India). Using deionized water, 1 mM and 3 mM solutions of silver nitrate was prepared in an Erlenmeyer flask. Then 1 mL of plant extract was added separately to 10 mL of 1 mM and 3 mM silver nitrate solutions and incubated in a dark chamber to minimize photo-activation of silver nitrate at room temperature [46]. Then the tubes were observed for the reduction of Ag^{+} to Ag^{0} by the colour change of the solution from colourless to brown. Then, prepared silver nanoparticles were characterized using a UV-visible spectrophotometer at a wavelength ranging from 350 – 600nm [48].

Screening for the antimicrobial activity:

The antimicrobial activity of a plain extract of *A.indicum* and silver nanoparticles prepared using *A.indicum* was tested using the agar-well diffusion assay method as previously described by the Clinical and Laboratory Standards Institute [49]. The test bacterial cultures used for the assay were *Bacillus subitilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa.* The test cultures were obtained from the Department of Microbiology, Osmania University, Hyderabad. They were maintained on Nutrient agar plates (Hi-Media Laboratories Pvt. Limited, Mumbai, India) at 4°C and subcultured onto Nutrient broth for 24 h before testing [50].

Agar well diffusion method

The following procedure [49] was adopted for the boiled and crushed leaf extracts separately. The test organisms were subcultured into prepared normal saline and incubated at 37°C for 30 min. The concentration of each organism was increased to form turbidity that matched 0.5 McFarland's standard by visual comparison, at which it was assumed that the number of cells was 1.5×10^{8} CFU/ml. Inoculum containing 1.5×10^{8} CFU/ml of bacterial suspension to be tested was spread on Mueller-Hinton agar plates with a sterile swab moistened with the test culture suspensions. Subsequently, wells were then made using a sterile cork borer of 6 mm in diameter on the surface of pre-seeded agar plates and filled with 100 µl of each extract (i.e., 1 mM $AgNO_3$, 3 mM $AgNO_3$, and plain *A. indicum* extract) and allowed to diffuse at room temperature for 2 h for proper diffusion. All the plates were incubated at 37°C for 24 h. The presence of a zone of inhibition was regarded as evidence of antimicrobial action. From the inhibition zones seen, antimicrobial activity was expressed in terms of the diameter (in millimeters) of the zones of inhibition measured.

RESULTS AND DISCUSSION:

Green synthesis of AI-AgNP:

The addition of plant extract of *Abutilon indicum* into the aqueous solution of silver nitrate led to the change in the colour of the solution from yellow to dark brown (Figure 3). The visual colour change to dark brown with time is thought to be evidence of silver ion reduction to AgNP. The color change of biosynthesized AgNP is caused by the excitation of surface plasmon resonance (SPR). Metal nanoparticles have unbound electrons, which provide the SPR absorption band due to the coupled vibrations of metal nanoparticle electrons in resonance with light waves [51]. Silver nanoparticles were synthesized at different concentrations (1mM

and 3mM) by keeping the plant extract (1 mL) constant. It was also witnessed in Figure 3 that the intensity of the brown colour increased as the concentration increased [52].

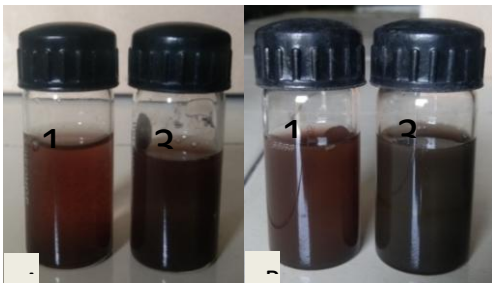


Fig 3: A) Silver nanoparticles (1mM & 3 mM) prepared from *A.indicum* (boiled) leaf extract; B) Silver nanoparticles (1mM & 3 mM) prepared from *A.indicum* (crushed) leaf extract

Characterization of AI-AgNPs by UV-Vis Spectroscopy:

The prepared AgNP solution was analysed with a UV spectrophotometer at wavelengths ranging from 350 to 600nm. Silver nanoparticles demonstrated an SPR peak at 400nm (Figure 4 & Figure 5). It confirmed that *Abutilon indicum* leaf extract has a higher capacity to reduce Ag ions into silver nanoparticles, leading us to do additional research on the fabrication of silver nanoparticles from *Abutilon indicum* leaf extracts [53].

It was also observed that the intensity of absorption peaks increases with an increase in the concentration of the silver nitrate salt. These results were found to be consistent with several literature such as the absorbance at 420 nm noticed for the AI-AgNPs [46]. In another study, an SPR peak was observed around 440nm for the silver nanoparticles synthesized by *Cochlospermum religiosum* extract [54] and *Pithophoraoe dogonia* extract [55].

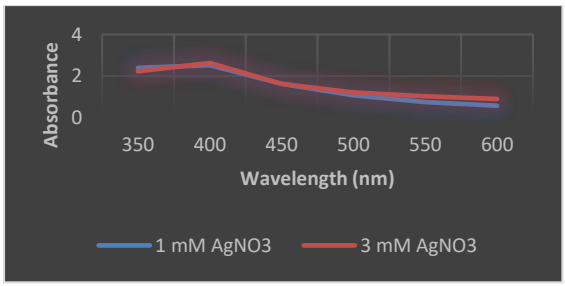


Fig 4: UV spectra of green synthesized AI-AgNPs (crushed)

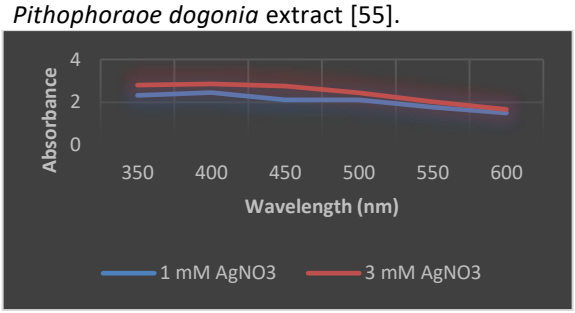
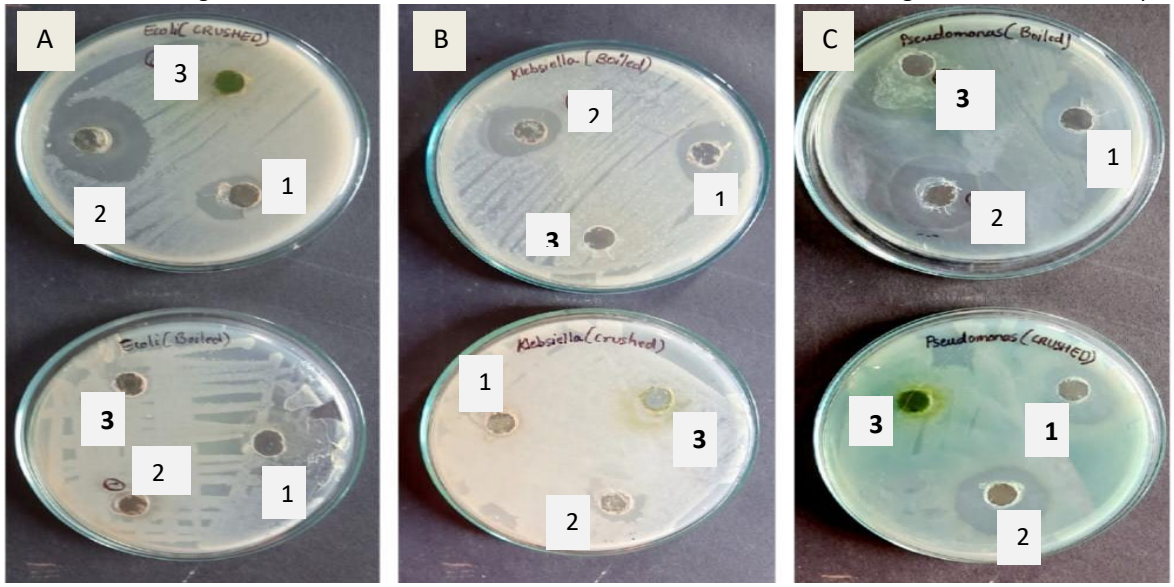


Fig 5: UV spectra of green synthesized AI-AgNPs (boiled)

Screening of Antimicrobial Activity:

The antimicrobial activity of *A.indicum* leaf extract and AI-AgNP solution (1 mM & 3 mM) was evaluated using the agar well diffusion method against test cultures *Bacillus subitilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa*. The diameter of the zone of inhibition was measured in millimeters (Figure 6) and the results were tabulated (Table 1) and interpreted in the graph (Figure 7). The results showed that silver nanoparticles prepared from both crushed and boiled leaf extracts developed zones of inhibition against all the test organisms except *E. coli* which showed resistance against all the test samples.



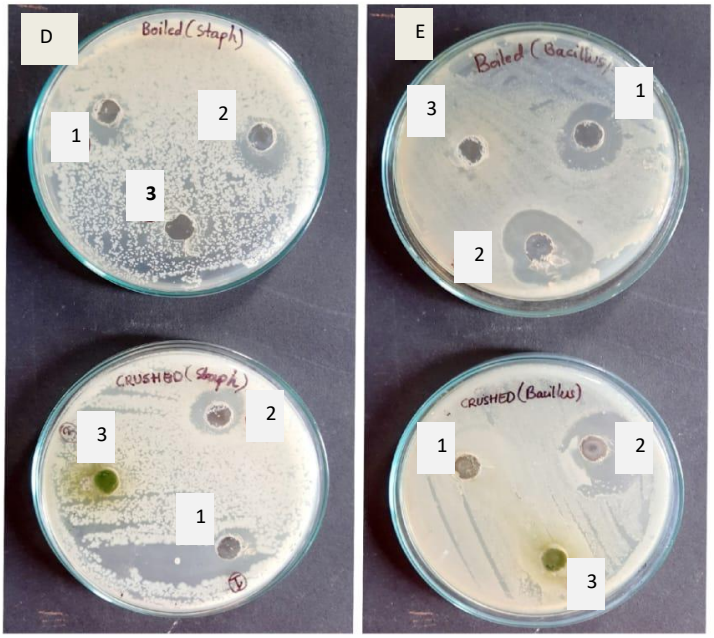
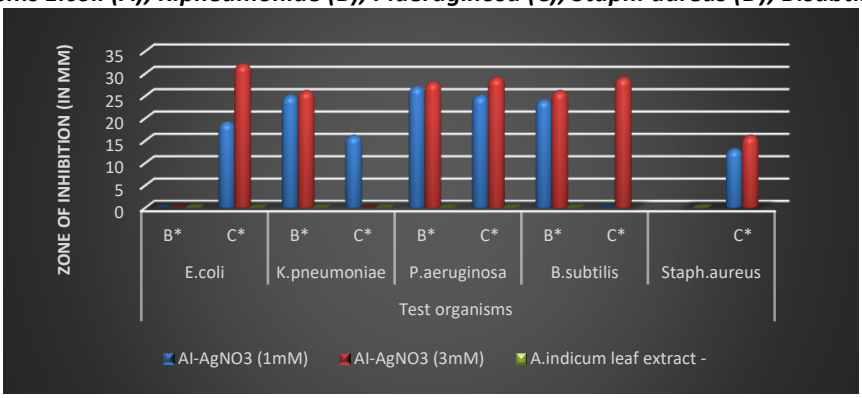


Fig 6: Antibacterial activity of 1mM Al-AgNP (1), 3mM Al-AgNP (2), and *A. indicum* plant extract (3) against the test organisms *E.coli (A), K.pneumoniae (B), P.aeruginosa (C), Staph. aureus (D), B.subtilis (E).*



Note: B* - Boiled *A.indicum* leaf extract; C* - Crushed *A.indicum* leaf extract

Fig 7: Antibacterial activity of *A. indicum* plant extract and green synthesized Al-AgNP against the test organisms

Table 1: Antibacterial activity of *A. indicum* plant extract and green synthesized Al-AgNP against the test organisms

Test Samples	Test organisms (Diameter of Zone of inhibition in mm)									
	E.coli		***K.pneumoniae***		***P.aeruginosa***		***B.subtilis***		***Staph.aureus***	
	B*	**C***	**B***	**C***	**B***	**C***	**B***	**C***	**B***	**C***
Al-AgNO$_3$ (1mM)	-	19	25	16	27	25	24	-	15	13
Al-AgNO$_3$ (3mM)	-	32	26	-	28	29	26	29	18	16
***A.indicum* leaf extract**	-	-	-	-	-	-	-	-	-	-

Note: B* - Boiled *A.indicum* leaf extract; C* - Crushed *A.indicum* leaf extract

These results were in accordance with Pratap et al, (2014) who revealed that silver nanoparticles prepared using *A.indicum* showed potent antibacterial activity against four strains of laboratory pathogens such as *Bacillus subtilis, Klebsiella pneumoniae, Salmonella typhi, and Proteus vulgaris.* Our findings were also consistent with the results obtained from the previous study on *Abutilon indicum* extracts, which exhibited potent antibacterial activities against *Staph. aureus, E. coli, and Klebsiella sp* [56]. However, the diameter of the zone of inhibition developed by 3mM Al-AgNO3 solutions was greater than that of 1mM Al-AgNO3 solutions. Concurrently, no inhibition zone was seen when a plain *A.indicum* leaf extract was tested against the test cultures. They might have shown

activity if prepared using the solvent extraction method.

The antibacterial activity of silver nanoparticles (AgNPs) is attributed to several mechanisms and can vary depending on factors such as nanoparticle properties (size, shape, surface chemistry), bacterial characteristics, and environmental conditions [2]. Frequently they attack the bacterial cells through membrane disruption, reactive oxygen species generation, inhibition of enzyme activity, DNA binding and disruption, protein interaction, etc., [44].

CONCLUSION:

In this study, we have prepared AI-AgNPs *Abutilon indicum* leaf extract (crushed and boiled) using the economic and eco-friendly approach. The prepared AI-AgNPs were characterized by UV spectrophotometer analysis. This method demonstrated that the silver nanoparticles displayed the absorption maxima in the UV region (around 400nm) which supported that the leaf extract is a rich source of polyphenols and flavonoids as these biological phytomolecules absorb UV light due to the presence of hydroxyl (OH) moieties [57-58]. They have developed an SPR peak at 400nm which confirmed the presence of AgNPs in the solution. Antibacterial activity of the synthesized AI-AgNPs was evaluated on bacterial species, which include *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis* by using the agar well diffusion method. The results confirmed that the AI-AgNPs have remarkable antibacterial activity and it is a preliminary approach to screen the AI-AgNPs for their antibacterial properties. They could be further studied with detailed statistical analysis for their significance against various pathogens and their potential for use in biomedical applications.

Studies suggested that the green-synthesized AI-AgNPs have enormous potential for applications in cosmetics, wastewater treatment, and pharmacological and nutraceutical industries [13, 48, 44]. In conclusion, the *A. indicum* based green synthesis of silver nanoparticles is economical and beneficial for the fabrication of plant-mediated, low-cost, less toxic, and more biocompatible nanomaterials. They have to be further characterized and evaluated for their successful application in various fields which will develop a novel platform for the green fabrication of nanoparticles for their biomedical applications.

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CONFLICT OF INTEREST:

The authors have not declared any conflict of interest.

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