

Synthesis and Characterization of silver nanoparticles using *Lablab purpureus* flowers (Purple colour) and its anti-microbial activities

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Abstract- In recent years, green Bio-synthesis of silver nanoparticles (AgNPs) has gained much interest from chemists and researchers. In this concern, Indian flora has yet to divulge innumerable sources of cost-effective, non-hazardous, reducing and stabilizing compounds utilized in preparing AgNPs. This study investigates an effective and sustainable route of AgNP preparation from 1 mM aqueous AgNO₃ using extracts of *Lablab purpureus* flowers (purple colour) which are well adorned for their wide availability and medicinal property. The AgNPs were characterized by UV-visible (vis) spectrophotometer, Scanning electron microscopy (SEM), Fourier transform infrared spectrometer (FTIR) analysis, and XRD. The AgNPs obtained from extracts significantly higher antimicrobial activities against staphylococcus aureus and E.Coli.in comparison to both AgNO₃ and raw plant extracts. In totality, the AgNPs prepared are safe to be discharged in the environment and possibly utilized in process of pollution remediation. AgNPs may also be efficiently utilized in green research to obtain better health using crop plants as shown by our study.

Keywords: Green synthesis, *Lablab purpureus*, Silver nanoparticles, SEM, Antimicrobial property; etc.,

I. INTRODUCTION

The field of Nanoscience has blossomed over the last twenty years and the need for nanotechnology will only increase, as miniaturization becomes more important in areas such as computing, sensors, and biomedical applications. Advances in this field largely depend on the ability to synthesize nanoparticles of various nanomaterials, based on their sizes, shapes, as well as their efficiency to assemble them into complex architectures. Nanotechnology provides the ability to engineer the properties of materials by controlling their size and this has driven research towards a multitude of potential uses for Nanomaterial. *Phyllanthus acidus* Skeels, an important medicinal plant belonging to the genus *Phyllanthus* (Euphorbiaceae), is widely cultivated in worldwide and its extracts have been used for treating alcoholism. *Phyllanthus acidus* known as the Otaheite gooseberry, Malay gooseberry, Tahitian gooseberry, country gooseberry, star gooseberry, starberry, West India gooseberry, damsel, grosella (in Puerto Rico), jimbilin (in Jamaica), damsel (in Grenada), karamay (in the Northern Philippines), cermai (in Indonesia and Malaysia), Goanbili (in Maldives) or simply gooseberry tree, is one of the trees with edible small yellow berries fruit in the *Phyllanthaceae* family. Despite its name, the plant does not resemble the gooseberry, except for the acidity of its fruits. It is mostly cultivated for ornamentation. The medicinal activities of

Phyllanthus species are antipyretic, analgesic, anti-inflammatory, anti-hepatotoxic and antiviral [1-4]. Fruits of the two well-known species, *P. acidus* L. and *P. emblica* L. contain high contents of vitamin C and have been used for used for improving eyesight and memory. It prevents action against Diabetes and reliefs from cough [5]. Another species of the family, *P. amarum* an important herbal medicine due to its effective antiviral activities especially towards the hepatitis B virus[6-8].Traditionally, *P.acidus* has been used in the treatment of several ailments including inflammatory and oxidative stress-related disorders such as gastric trouble (Jules and Paull,2008) [9].

Green Synthesis and characterization of nanoparticles is under exploration due to its wide medical applications and various research interests in nanotechnology. In the current study, the plant extract of *Persea Americana* (Avocado) (Family-Lauraceae) is used for the synthesis of silver nanoparticles (AgNPs). This study investigates an efficient and sustainable route of AgNPs preparation from 1 mM aqueous AgNO₃ using leaf extracts. The complete reduction of silver ions was observed after 12 hrs of reaction at 40°C under shaking condition. The colour changes in reaction mixture (pale yellow to dark brown colour) was observed during the incubation period, because of the formation of silver nanoparticles (AgNPs) in the reaction mixture enables to produce particular colour due to their specific properties

(Surface Plasmon Resonance). The formation of silver nanoparticles was confirmed by UV-Visible spectroscopy, Fourier Transform Infra-Red (FT-IR) spectroscopy analysis, X-Ray Diffraction (XRD) pattern, Transmission electron microscopy (TEM). The results showed that the leaf extract is optimum for the synthesis of silver nanoparticles and it is also known to have the ability to inhibit the growth of various pathogenic microorganisms. The average size of synthesized silver nanoparticles is found to be 27.42 nm using XRD data by Scherrer's formula, which is approximately similar as the size obtained in TEM Analysis (27.58nm). In total, the AgNPs prepared are safe to be discharged in the environment and possibly utilized in processes of pollution remediation. AgNPs may also be efficiently utilized in anti-inflammatory activity of Pharmaceutical research to obtain better result of plant as shown by our study [10].

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different sizes, shape and controlled dispersity. With the development of new chemical or physical methods, the concern for environmental contaminations are also heightened as the chemical procedures involved in the synthesis of nanoparticles generates a large amount of hazardous byproducts. Thus, there is a need for green method that includes a clean, non-toxic and environment friendly method of nanoparticles synthesis [11]. As a result, researchers in the field of nanoparticles synthesis and assembly have turned to biological system of inspiration. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extra cellularly using plants or their extracts in a controlled manner according to their size, shape and dispersity [12]. The aqueous silver nitrate solution, after reacting with geranium leaf extract, led to rapid formation of highly stable, crystalline silver nanoparticles (16 to 40 nm) [13]. Various approaches available for the synthesis of silver NPs include chemical [14], electrochemical [14], radiation[15], photochemical methods [16] and Langmuir-Blodget [17] and biological techniques [18].

The uses of engineered nanomaterials have increased as a result of their positive impact on many sectors of the economy including agriculture. In the current study, the plant extract of *Citrullus Lanatus* is used for the synthesis of silver nanoparticles. The plant extract is mixed with AgNO₃, and then it is incubated. The extract is kept in microwave oven for exposure of heat, then it is dried and powdered. The synthesized dried powder is confirmed as nanoparticles by colour transformation. The characterization of silver nanoparticles was studied by UV-Vis spectroscopy,

FTIR, XRD& TEM. The silver nanoparticles synthesized were generally found to be in the size ranging from 1-100 nm. The average size of synthesized silver nanoparticles is found to be 15.98 nm using XRD data by Scherrer's formula, which is approximately similar as the size obtained in TEM Analysis 16.32 nm. In totality, the AgNPs prepared are safe to be discharged in the environment and possibly utilized in processes of pollution remediation. AgNPs may also be efficiently utilized in Anti-inflammatory activity of Pharmaceutical research to obtain better result of plant as shown by our study. The Anti-inflammatory activity of silver nanoparticles was tested on human blood cells which confirms that the plant mediated synthesis of silver nanoparticles have a significant Anti-inflammatory effect on human blood cells [19]. In the present work, the synthesis of silver nanoparticles from aqueous solution of silver nitrate using *Lablab purpureus* flowers (purple colour). Further silver nanoparticles were characterized by using UV-visible (vis) spectrophotometer, Scanning electron microscopy (SEM), Fourier transform infrared spectrometer (FTIR) and anti-microbial activities.

II. MATERIALS AND METHODS

PREPARATION OF FLOWER EXTRACT

The fresh flowers of *Lablab purpureus* flowers (purple colour) was collected and washed thoroughly with sterile double distilled water (DDW). Twenty gram of sterilized flower samples were taken and cut into small pieces. Finely cut leaves were placed in a 500 ml Erlenmeyer flask containing 100 ml of sterile DDW After that, the mixture was boiled for 5 minutes and then filtered. The extract was stored in 4⁰C.



Figure: 1 *Lablab purpureus* flowers (purple colour)

SYNTHESIS OF SILVER NANOPARTICLES

Silver nitrate was used as precursor in the synthesis of silver nanoparticles. 100 ml flower extract was added to 100 ml of 0.1N AgNO₃ aqueous solution in conical flask of 250 ml content at room temperature. The flask was thereafter put into shaker (100 rpm) at 500 °C and reaction was carried out for a period of 12 hrs. Then the mixture (Flower extracts & Silver nitrate solution) is kept in microwave oven for exposure of heat. Finally, the mixture was completely dried after a period of 20 minutes and hence Nanoparticles in form of powders were obtained.

UV VISIBLE SPECTROSCOPY ANALYSIS

The colour change in reaction mixture (metal ion solution + flower extract) was recorded through visual observation. The bio reduction of silver ions in aqueous solution was monitored by periodic sampling of solid and subsequently measuring UV visible spectra of the solid sample. UV-visible spectra of sample were monitored as a function of time of reaction on the UV-visible spectroscopy and the investigation was carried out using PERKIN ELMER (Lambda 35 model) spectrometer in the range of 190 nm to 1100 nm.

FT-IR MEASUREMENT

The Fourier transform infrared (FTIR) investigation is carried out using PERKIN ELMER (Spectrum RXI) spectrometer in the range of 400 cm⁻¹ to 4000 cm⁻¹. The functional groups were identified using the peak assignments.

XRD MEASUREMENT

The sample was drop-coated onto Nickel plate by just dropping a small amount of sample on the plate frequently, allowed to dry and finally thick coat of sample was prepared. The particle size and nature of the silver nanoparticle was determined using X-ray diffraction (XRD). This was carried out using Rigaku miniflex-3 model with 30kv, 30mA with CuK α radiations at 2 θ angle.

SEM ANALYSIS

Sample is dispersed with acetone and exposed in ultrasonics for 5 minutes. Take a drop of a solution from the sample and drop it on the grid, leave until it dries. After drying the sample is inserted into SEM instruments using model is Tecnai T20 Making in FEI, Netherlands operating at 200KeV Tungsten Filament.

ANTIBACTERIAL ACTIVITY

Micro - organisms and culture media

Bacterial cultures such as, Staphylococcus aureus, E.coli, were obtained from Eumic analytical Lab and Research Institute, Tiruchirappalli. Bacterial strains were maintained on Nutrient agar slants (Hi media) at 4°C.

Inoculum preparation

Bacterial cultures were subcultured in liquid medium (Nutrient broth) at 37°C for 8hrs and further used for the test (10⁵-10⁶CFU/ml). These suspensions were prepared immediately before the test was carried out.

Preparation of culture media

Nutrient agar medium

Nutrient agar medium is one of the most commonly used medium for several routine bacteriological purposes:

Ingredients	
Grams/Litre	
Peptone	: 5gm
Beef extract	: 3gm
Agar	: 15gm
Sodium chloride	: 5gm
Yeast extract	:
1.5gm	
p ^H	: 7.0

After adding all the ingredients into the distilled water it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 Ib psi pressure (121°C) for 15 minutes.

Nutrient broth

The nutrient broth was prepared by the same composition without agar. After adding all the ingredients into the distilled water it is boiled to dissolve in the medium completely and it is sterilized by autoclaving at 15 ib psi pressure(121°C) for 15 minutes.

Preparation of plant material

Flowers of the plant materials taken for this study were shade dried individually at room temperature and then powdered by using electric blender. About 10gm of fresh plant materials (flower) were extracted with 100ml of distilled water 91:10. They were kept for seven days at room temperature (31°C) for complete extraction. After seven days. The extracts were filtered through what man no.1 filter paper. This extract was collected in beaker and kept in refrigerator.

Continuous hot extraction using soxhlet apparatus

In concentrated preparation, there is first extraction followed by evaporation. In continuous the operations i.e., extraction and evaporation are combined in the apparatus were used for this purpose. To execute continuous extraction a soxhlet apparatus is uses soxhlet continuous extractor. The apparatus is used for the extraction on coarse drug powder placed in a thimble made of filter paper is inserted into the wide tube of the extractor. The solvent which is taken in the flask is heated, the vapours arise from the solvent get into the condenser through a side tube and the liquid condensed from the vapours drips into the thimble. The solvent liquid level slowly rises and during

this period the dried flower materials gets extracted of its soluble constituents. When the level of the liquid reaches the top of the siphon it gets siphoned into flask. The suction effect of the siphoning assists permeation of the solvent through the drug.

Again a portion of the solvent from the solution vapourised leaving the constituents in the flask itself and the process mentioned above is repeated. The same process is repeated again and again until all the solutes are extracted. This kind of continuous not percolation (soxhlation) is undertaken when the active constituents are not readily soluble in the cold and are thermo labile e.g., grainder Oleoresin is extracted with ethanol.

Assay of antimicrobial activity
Microbial inoculum preparation

The nutrient broth were prepared, then identified bacterial colonies were inoculated into the broth culture were used for antimicrobial activity.

Kirby bauer agar well diffusion assay

The nutrient agar medium was prepared and sterilized by autoclaving at 121°C 15 lbs pressure for 15 minutes then aseptically poured the medium into the sterile petriplates and allowed to solidify the Bacterial broth culture was swabbed on each petriplates using a sterile buds. Then wells were made by well cutter. The organic solvent extracts of flower were added to each well aseptically.

This procedure was repeated for each Petri plates then the petriplates were incubated at 37°C for 24 hrs. After incubation the plates were observed for the zone of inhibition.

III. RESULT AND DISCUSSION

UV-Visible Spectroscopy Analysis

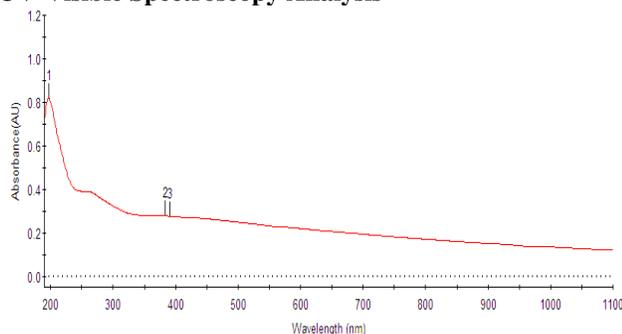


Figure: 2 UV-Visible spectrum of synthesized silver nanoparticles using flower extract of *Lablab purpureus* (purple colour)

UV-Visible spectroscopy analysis showed the absorbance band of silver nanoparticles synthesized using *Lablab purpureus* (purple colour) flower extract at 197.10nm,382.60nm,and 391.30nm which confirms the

presence of poly-unsaturated and aromatic compound (Isoguinoline) (Advanced strategies in food analysis, UV-VIS spectrometry International Journal of medicine and Pharmaceutical Research by Richart koplík.

FT-IR measurement

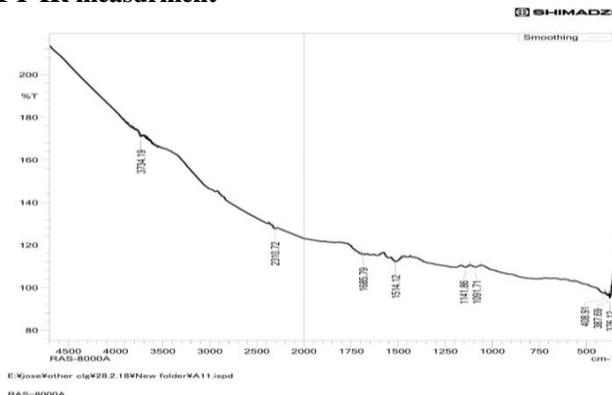


Figure : 3 FT-IR Spectrum of synthesized silver nanoparticles using flower extract of *Lablab purpureus* (purple colour)

The *lablab purpureous* (purple colour) related functional groups were identified using the peak assignments. A medium peak at 3734.19cm⁻¹ was assigned to the N-H stretching in amide group, multible brode peak at 2310.72 cm⁻¹ was assigned to N-H stretching primary ammonium ions group, similar conjugated effets at 1685.79cm⁻¹ was assigned to C=O Stretching anhydride group,a strong peak at 1514.12cm⁻¹ was assigned to N-O stretching Nitro compound group, a strong peak at 1141.86cm⁻¹ was assigned to P-O stretching Phosphorus oxide, strong peck at 1091.71cm⁻¹ was assigned to C-O stretching Alkyl aryl ether medium are observed.

XRD measuremt

Gauss value:

According to Gauss formula,

$$\varnothing = Q / \epsilon$$

Partical size D=14.9143nm

Surface area S=59.2920m²/g

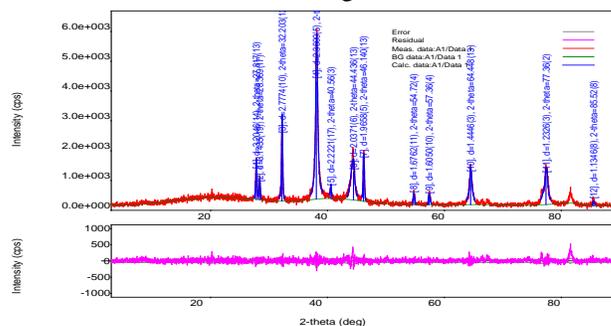


Figure: 4 XRD Spectrum of synthesized silver nanoparticles using flower extract of *Lablab purpureus* (purple colour)

SEM ANALYSIS

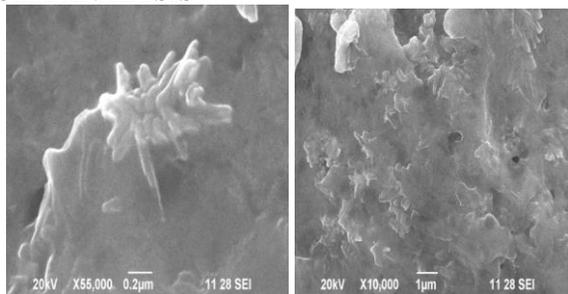


Figure: 5 SEM image of synthesized silver nanoparticles using flower extract of *Lablab purpureus* (purple colour)

SEM analysis shows uniformly distributed silver nanoparticles on the surfaces of the cells. SEM analysis reveals individual spherical polydispersed AgNPs as well as number of aggregates, which wear irregular in shape. The size of the silver nanoparticles was found to be 5-50nm, with an average size 14.91nm. The larger silver particles may be due to the aggregation of the smaller ones.

Anti-microbial activity

In the present study, flowers of *Lablab purpureus* (purple colour) exhibited significant anti- microbial activity

when compared with standard drug. It is evident from the data presented in table 1 that the sample possesses antibacterial activity. The disc diffusion method result showed the zone of inhibition for 25mg/ml as 15mm, and 15mm, for 50mg/ml showing 18mm and 17mm 75mg/ml showed 20mm and 19mm for 100 mg/ml as 24mm, and 22mm, against staphylococcus aureus ,E.coli respectively when compared with standard drug Gentamicin showing 22mm, and 23mm zone of inhibition respectively . Then it is evident from the data presented in table 1 that the sample possesses antibacterial activity. The above result shows that the activity of the compound of *Lablab purpureus* flower (purple colour) shows significant antibacterial activities.

Table1: Anti-bacterial activity of synthesized silver nanoparticles using flower extract of *Lablab purpureus* (purple colour)

SAMPLE	Extract 100 µl added and Zone of inhibition (mm/ml)				
	25 µl	50 µl	75 µl	100 µl	Control
Staphylococcus aureus	15	18	20	24	22
E.coli	15	17	19	22	23

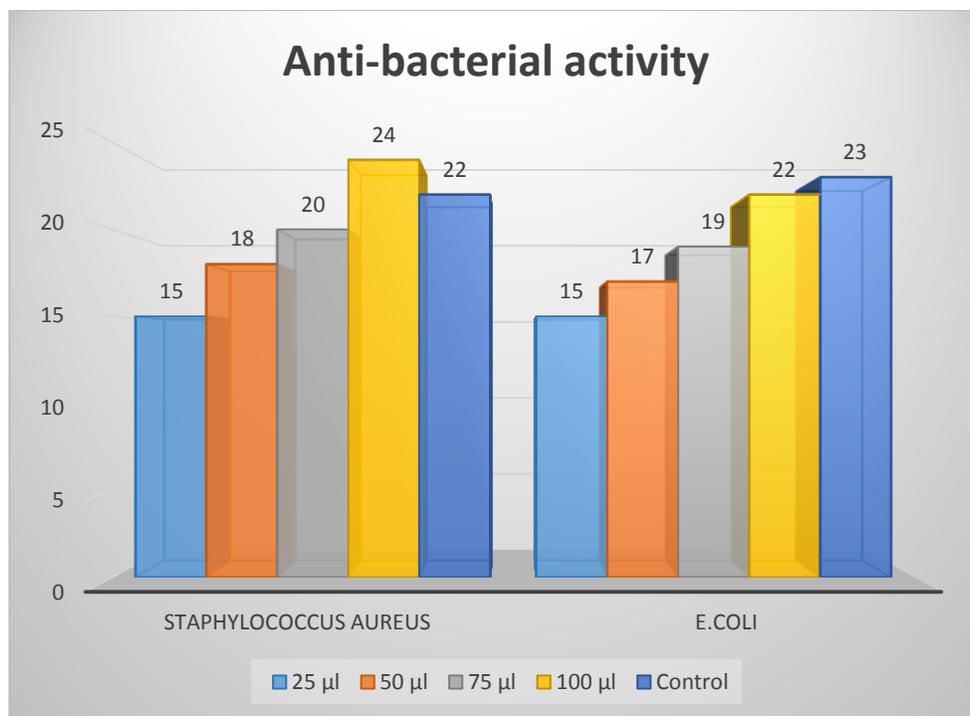
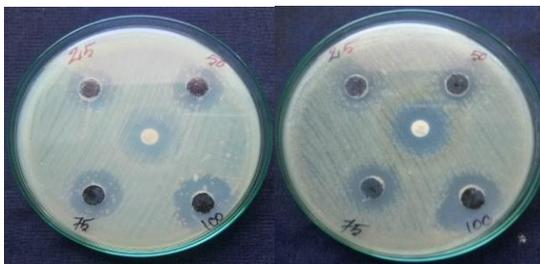


Figure: 6 Graphical representation Anti-bacterial activity of synthesized silver nanoparticles using flower extract of *Lablab purpureus* (purple colour) (standard: Gentamicin, concentration 1mg/ml)



Staphylococcus E.Coli

Figure: 7 Image of Anti-bacterial activities of synthesized silver nanoparticles using flower extract of *Lablab purpureus* (purple colour) (standard: Gentamicin, concentration 1mg/ml)

IV. CONCLUSION

The present study reports the facile approach of biosynthesizing AgNPs from AgNO₃ using the aqueous extract of *Lablab purpureus* (purple colour) flower. The adopted method is well suited with green chemistry principles as the plant extract serves as a dual functional molecule as reductant and a stabilizing agent for the synthesis of AgNPs. The efficiency and the influence of various process variables in the biosynthesis of AgNPs analysed include reductant concentration, temperature and time. The interesting conclusion arrived from the study is that the shape and size of the nanoparticles synthesized have the direct and strong influence and dependent on process variables used in the experiment. The UV-visible spectra confirmed the reduction of silver ions at 391.30 nm. XRD analysis confirmed the crystalline structure of AgNPs. From FTIR and XRD analyses it was observed that *Lablab purpureus* (purple colour) flower extract acted as apparent stabilizer for the synthesis of AgNPs. The size and morphology of particles were characterized using SEM and the images showed the AgNPs in the average range from 14-50 nm and Surface area S=59.2920m²/g. Further, the biosynthesized AgNPs showed significant antibacterial action on tested pathogenic microorganisms. As a result it is observed that a fine tuning of process variables may give the end product with typical physical characteristics.

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