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Research Article

# Antibacterial Activity of Grandiflora (*Nicotiana alata)* Mediated Green Synthesis and Characterization of Silver Nanoparticles

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**ABSTRACT**

In this study we synthesized Silver nano Particles and screened Phytochemical and antimicrobial activities. Fresh aqueous leaf, Flower, Root extracts of Grandiflora (Nicotiana Alata) were used for synthesis of silver nanoparticles. The biosynthesized AgNPs were characterized by UV-Spectra, FTRI, Transmission Electron Microscope (TEM). Good activity against all the bacteria strains in comparison with Ampicillin. Grandiflora (Nicotiana alata) leaves, flowers, root of the plants were extracted with Hexane Methanol, Pet ether. Methanol, Pet. ether extracts of leaf and flower, Root were tested quantitatively for phytoconstituents. AgNPs bounded protein estimation Lowary's method. The Phytochemical properties of the leaf, flower, Root of Grandiflora (Nicotiana alata) were also done in present investigation. Absorption spectrum at different wavelengths ranging from 400-500 nm revealed a peak of λ max at 430 nm. The TEM images clearly reveals that shape and size of silver nanoparticles was 15-30 nm with spherical shape. TLC Plate of Plant leaf Hexane extract contain not clearly separation componds, Pet. Ether Extract contain only two spots observed (Rf Value 0.35, 0.6cm) and methanol extract of Grandiflora (Nicotiana Alata) plate separation of phytochemical compounds was separation five clear spots observed in methanol extract(Rf Value 0.45, 0.50, 0.7, 0.8, 1.0cm). Results showed the presence of phytoconstituents Alkaloids, tannins, phenolic compounds, proteins, terpenoids and saponins. The Antibacterial activities of the AgNPs were evaluated by silver nano particles. However, the natural products profile and consequently the bioactivity is known to vary with the climate and geographic location of the plants. The present study also correlates with the results of previously reported with little variations. Nanoparticles are important due to their simple experimental procedure and eco-friendliness.

**Key words:** Grandiflora plant leaf, flower and Root, UV-Spectra, FTRI, Transmission Electron Microscope (TEM), antibacterial activity, AgNPs bounded protein estimation.

## INTRODUCTION

Metals Nanoparticles are mostly prepared from gold, silver, platinum, palladium and titanium. Nanoparticles find applications in wide sectors such as Industrial, pharmaceutical, drug delivery, electronics, medicine, environment, textiles etc. Newer methods for synthesis of highly monodisperse AgNPs using microorganisms[1,2] are being explored. Nanoparticles show unusual optical chemical, photo electrochemical. Greener chemistry approaches include synthesis of nanoparticles from medicinal plants. Silver nanoparticles synthesis using leaves of plants such as Azadirachta indica (Neem)[3], Medicago sativa (Alfa alfa)[4,5], Capsicum annuum[6] have been so far investigated. Silver has always been used as an anti-bacterial agent[7]. Biological synthesis can be successfully used for production of small size nanoparticles in large-scale operations when compared to chemical and physical methods[8].

Earlier we are reported synthesis of AgNPs using a greener approach by *Stigmaphyllon littorale* leaves and *Acacia Senegal* characterized with various characterization techniques[9,10]. These medicinal plants can be rich in phenolic compounds, alkaloids, diterpenoid, steroid and other compounds which inhibit gram positive and negative. Phytochemicals in the plant extracts can act as reducing and capping agent in the reduction of metal ions to metal nanoparticles[11] and thus have found widespread use in the biosynthesis of silver metal nanoparticles which can be used in drug delivery[12], tissue/tumor imaging[13], biosensing[14] and catalysis[15]. Biosynthesis of silver metal nanoparticles using plant extracts has received considerable attention in recent days[16,17]. It is also very cost effective[18-21]. Many researchers have reported the biosynthesis of silver metal nanoparticles by using various plant extracts[22-25]. Considering the importance of green synthesis, a systematic investigation was undertaken to screen a local flora, Grandiflora (Nicotiana alata) for the presence of various silver nano particles, phytochemicals study. Further, the Hexane, Methanol, Pet. Ether extracts of Grandiflora (Nicotiana alata) leaf, Root and flower extract was used for the biosynthesis of silver nanoparticles.

## MATERIALS AND METHODS:

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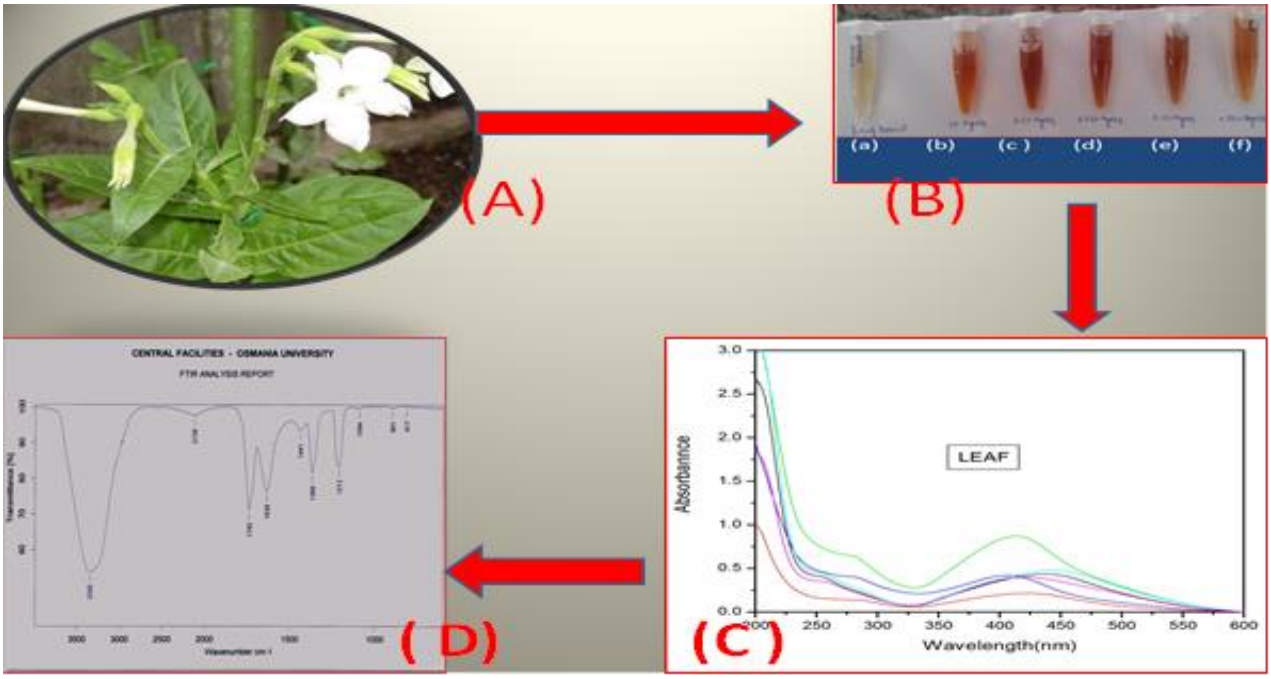


**Figure:1.** Grandiflora (Nicotiana alata) **A).** Plant leaf, Flower and Root **,B).** Grandiflora (Nicotiana alata) leaf Solution **(a)** Grandiflora (Nicotiana alata) plant leaf solution with different Concentrations of silver nitrate (1% to 0.062%) **(b,c,d,e,f)** there was a change in coloure of leaf solution ofter addition of different concentration of $AgNO_3$. **C).** Change in color after addition of Leaf extracts in silver UV-Spectra's. **D)** Change in color after addition of Leaf AgNPs in FTIR Spectra functional group Identification.

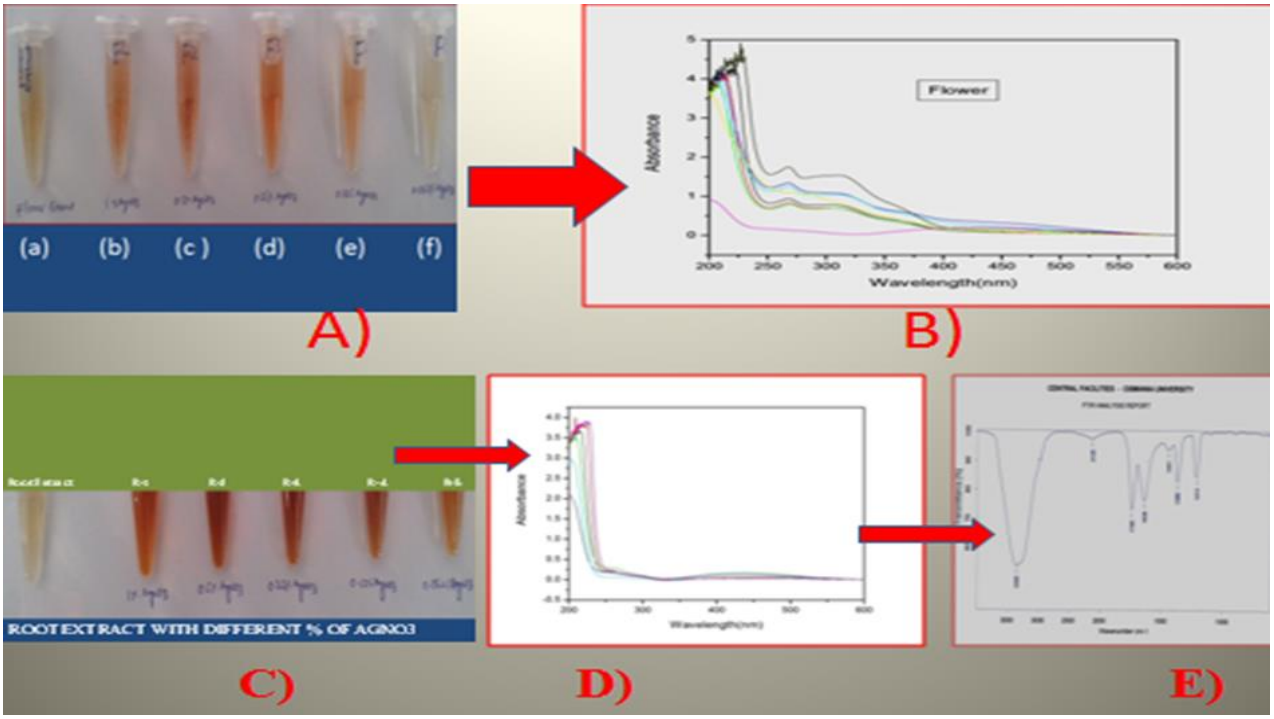


**Figure:2.** Grandiflora (Nicotiana alata) flower Solution **(a)** Grandiflora (Nicotiana alata) plant flower solution with different Con,c of silver nitrate (1% to 0.062%) **(b,c,d,e,f)** there was a change in coloure of flower solution ofter addition of different concentration of $AgNO_3$. **B).** Change in color of extract (pink line) after addition of silver Nitrate (UV-Spectra's). **C).** Grandiflora (Nicotiana alata) Root Solution **(a)** Grandiflora (Nicotiana alata) plant Root solution with different Con,c of silver nitrate (1% to 0.062%) **(b,c,d,e,f)** there was a change in coloure of Root solution ofter addition of different concentration of $AgNO_3$. **D).** Change in color after addition of Root extracts in AgNPs (UV-Spectra's). **E).**change in color after addition of Root AgNPs FTIR Spectra Identification of functional group

All reagents used were of analytical grade. Double distilled Millipore water was used in study. The leaves,flower,root from Grandiflora (Nicotiana) were procured from a local plant in botanical garden,Osmania University.,Hyderbad. Silver nitrate solution (1mM) was used throughout the study Chemicals all reagents used in the study were of analytical grade. Silver nitrate (AgNO3) was obtained from Sigma Aldrich.

*Synthesis Of Silver Nanoparticles*

Silver nanoparticles were synthesised from silver nitrate using aqueous leaf,flower,Root extract of Grandiflora (Nicotiana) as reducing agent. Different conditions and different methods were employed for the synthesis of silver nanoparticles for optimising the time of synthesis and yield of silver nanoparticles. The following parameters were varied for optimising the process of synthesis of silver nanoparticles.

*Room temperature*

*Variation of concentration of silver nitrate*

*Variation of pH*

Silver nanoparticles were synthesized by the addition of 5ml of silver nitrate solution to different volumes (1ml, 2ml, 3ml, 4ml, 5ml) of plant extract. The colour changed to reddish brown after 10 minutes at different intervals of time under room temperature at constant pH (6).The formation of silver nanoparticles was monitored by UVstudies. The study was conducted in a similar manner at 75˚C at constant pH 6 to find the effect of temperature on the ease of formation of silver nanoparticles. The effect of variation of volume of silver nitrate was with varying volumes (1ml, 2ml, 3ml, 4ml, 5ml) of silver nitrate solution at room temperature and constant pH(6). In the variation of pH studies, 1ml of aqueous extract is treated with 5ml of silver nitrate solution at room temperature and different pH (7, 8, 9, 10 ,11 and 12). In the sonication method water bath was used. Eight ml of 1mM of silver nitrate solution was added to different volumes of aqueous plant extract such as (1ml, 2ml, 3ml, 4ml, 5ml ) and water till the formation of nanoparticles as evidenced by the colour change to reddish brown. Further confirmation of the formation of silver nanoparticles was made using analytical and spectroscopic techniques mentioned below.

*Bio synthesis of silver nanoparticles from the leaf, flower, root extract of grandiflora (nicotiana alata)*

Plant Leaf, Flower, Root of Grandiflora (Nicotiana) was pulverized into fine powder. 5 grams of the leaf flower, root powder was taken in a 250 ml Erlenmeyer flask and to it 100ml of milliQ water was added. The mixture was kept on a sand bath at $60^0$C for 15 minutes. Then, the extract was filtered by Whatmann-no 1 filter paper. The liquid obtained was used for the synthesis of silver nanoparticles. For the synthesis of silver nanoparticles, seed powder extract was added to the silver nitrate solution in the ratio 1:10 and kept in a domestic microwave for 15 mins to synthesize the nanoparticles.

UV-Vis Spectra Analysis

The reduction of pure Ag+ion was monitored by measuring the UV-Visible spectrum of the reaction mixture after diluting a small aliquot of the sample with distilled water after regular interval of time. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-1700 (Shimadzu).

*FTIR analysis of dried biomass after bioreduction*

To remove any free biomass residue, the residual solution was centrifuged at 5000 rpm for 15 min and the resulting suspension was redispersed in 10 ml sterile distilled water. The centrifuging and redispersing process was repeated three times. Thereafter, the purified

Table:1. Phytoconstituents present in different extracts of Grandiflora (Nicotiana) plant Leaf, Flower Extract detected by phytochemical analysis

| S.No | Phytoconstituents | Petroleum ether Leaf extract | Methanol Root extract | Methanol Leaf extract | Ethanol Leaf extract | Methanol flower extract |
|---|---|---|---|---|---|---|
| 1 | Tannins | -Ve | -Ve | -Ve | +Ve | -Ve |
| 2 | Saponins | -Ve | -Ve | -Ve | -Ve | -Ve |
| 3 | Alkaloids | +Ve | -Ve | -Ve | -Ve | -Ve |
| 4 | Flavonoids | -Ve | -Ve | -Ve | -Ve | -Ve |
| 5 | Terpenoids | -Ve | -Ve | -Ve | -Ve | -Ve |
| 6 | Glycosides | -Ve | -Ve | +Ve | +Ve | +Ve |
| 7 | Steroids | -Ve | -Ve | +Ve | +Ve | -Ve |

(+ve) - indicates the presence of phytoconstituent; (-ve) - indicates the absence of phytoconstituent.

Table:2. Zone of Inhibition Study on Gram Positive and Negative Bacteria by Synthesis of Grandiflora (Nicotiana alata) Leaf, Flower, Root AgNPs

| S.No | Microorganism | Diameter of zone of inhibition (in mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Concentration of AgNPs and Drug | Leaf AgNPs | | Flower AgNPs | | Root AgNPs | | Ampicillin (10µg/µl/disc) |
| | | (10µg /µl/disc) | (20µg /µl/disc) | (10µg /µl/disc) | 20µg /µl/disc) | (10µg /µl/disc) | 20µg /µl/disc) | |
| 1 | Staphylococcus aureus | 6mm | 7mm | 5mm | 6.5mm | 8mm | 8.5mm | 18mm |
| 5 | E.coli | 7mm | 8.5mm | 4mm | 5mm | 8mm | 9.5mm | 21mm |

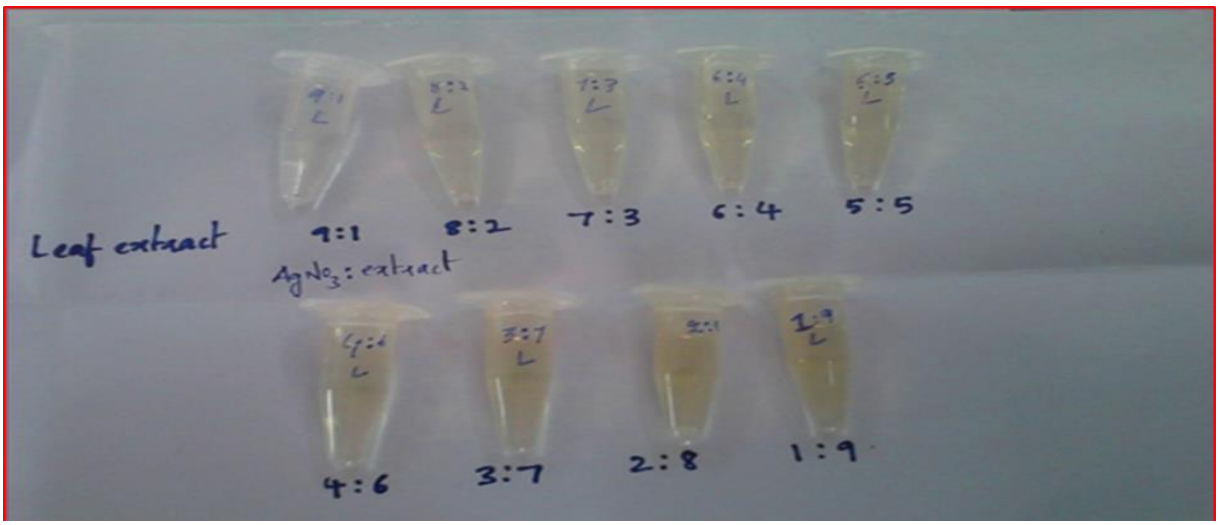


**Figure:3.** Different Concentration of plant leaf extract and silver nitrate (1mM) there was a change in color of extract after addition of $AgNO_3$

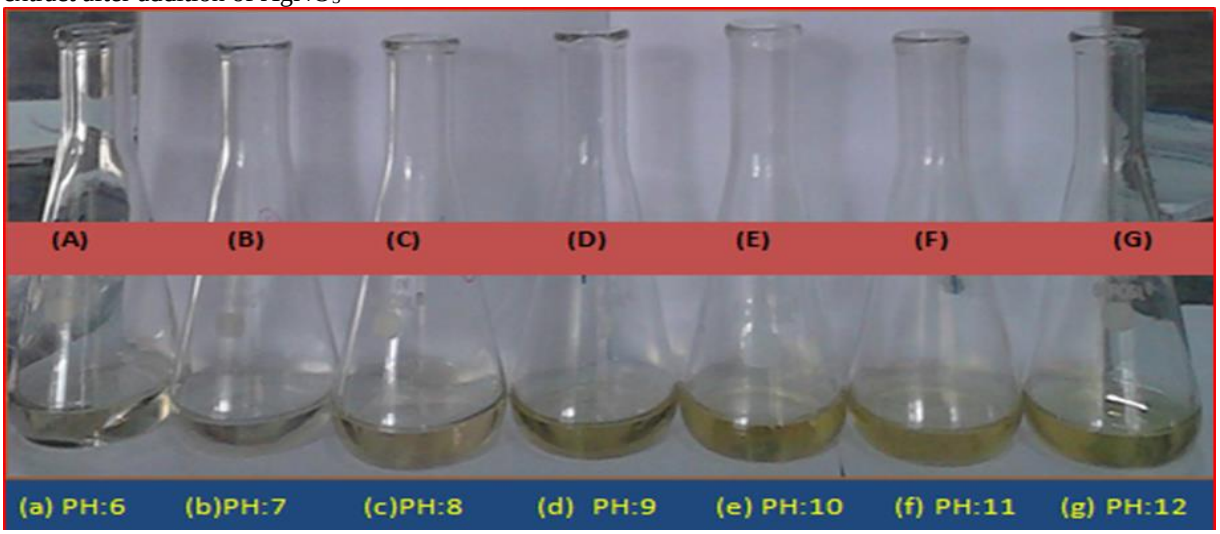


**Figure:4** Grandiflora (Nicotiana) plant Leaf Extract with silver nitrate (1mM) at different pH

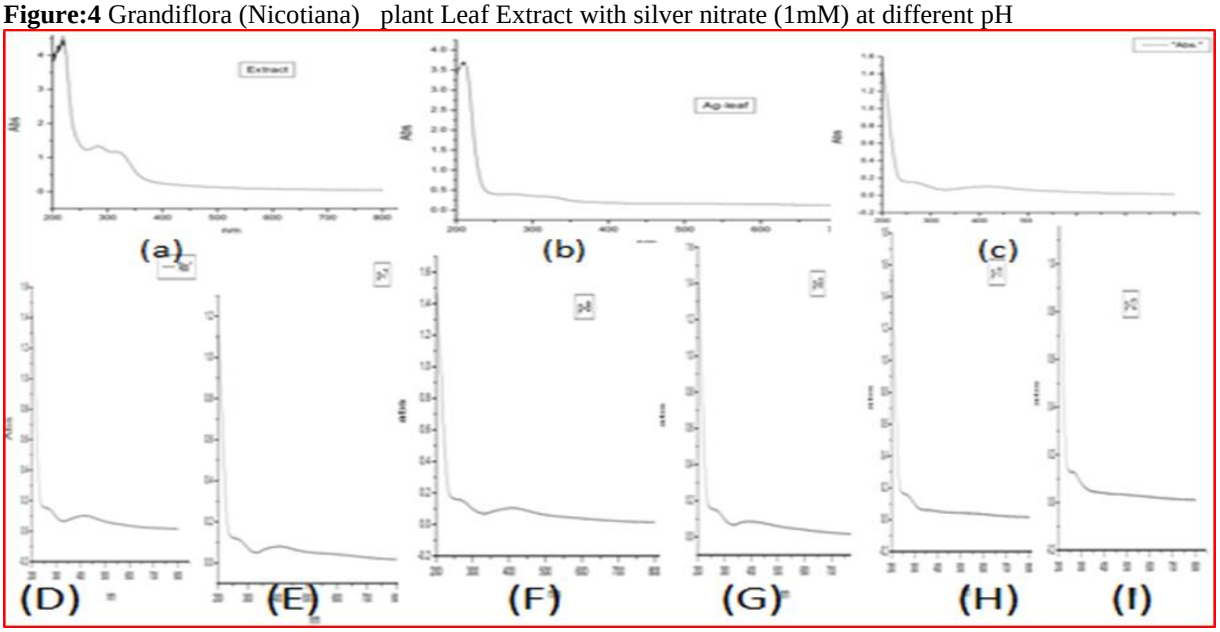


**Figure:5.** UV-spectra of Grandiflora plant leaf extract, with silver nitrate at different pH levels.
a) leaf extract b) AgNPs c ) pH-6 d) pH-7 e) pH-8 f) pH-9 g) pH-10 h) pH-11 i) pH-12

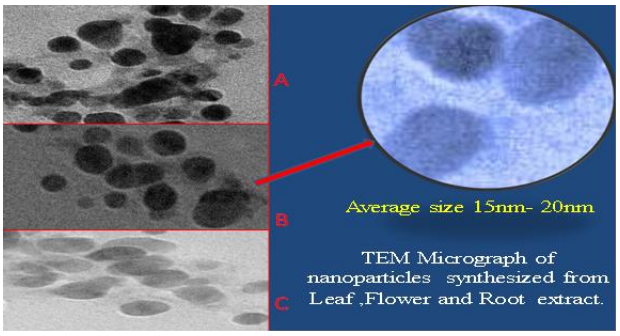


**Figure: 6.** TEM micrograph of size of AgNPs synthesized from A) leaf B) Flower and C) Root Extract from Grandiflora (Nicotiana alata).


**Figure: 7.** Methanol and Pet. ether extract from Grandiflora (Nicotiana) Plant leaf, flower and Root synthesis of silver nano particles.

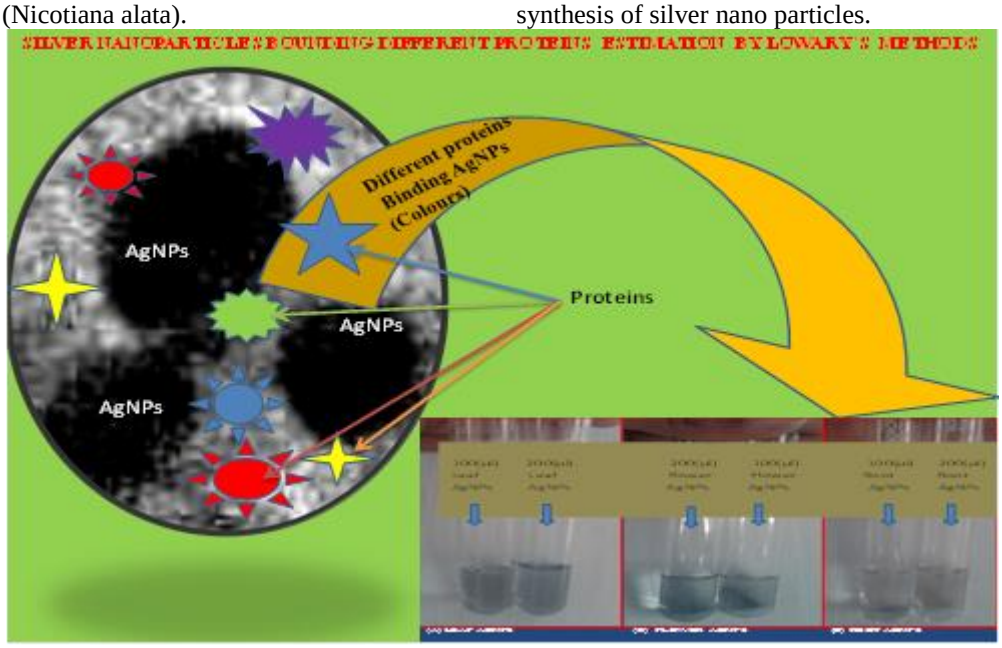


**Figure: 8.** Diagrammatic representation of AgNPs bounded protein (Estimation by Lowry's method)

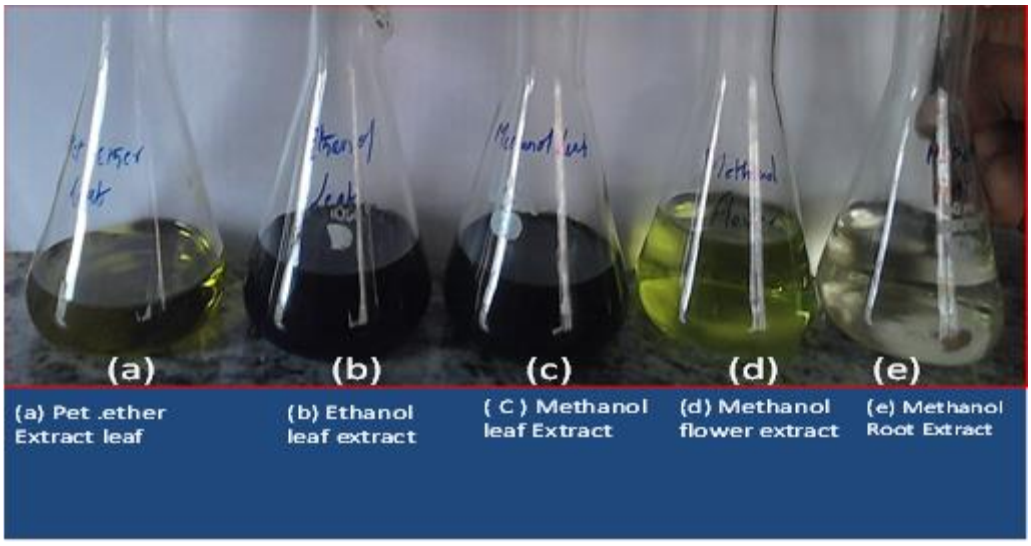


**Figure:9.** Grandiflora (Nicotiana) plant Leaf, Flower, Root Extract in different solvents (Polar, Non-Polar solvents).

suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR.

TEM analysis

Sample for TEM was of prepared by placing drops of silver nanoparticles suspension over carbon coated grid and allowing the solvent to evaporate the solvent. TEM

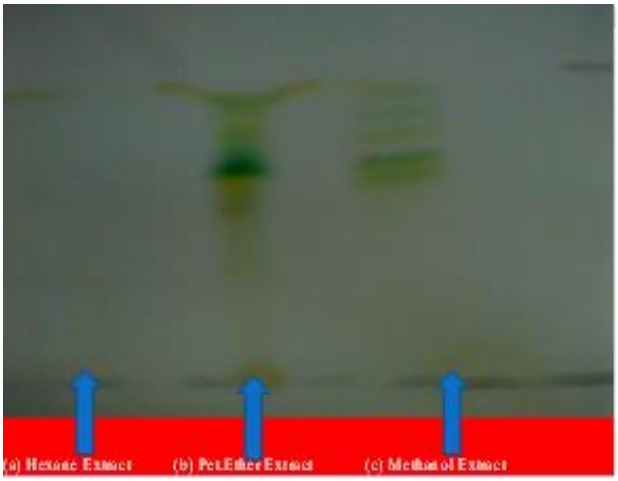


**Figure: 10.** TLC Plate of Plant leaf **a)** Hexane **b)** Pet. Ether **c)** methanol Extract of Grandi flora (Nicotiana Alata)

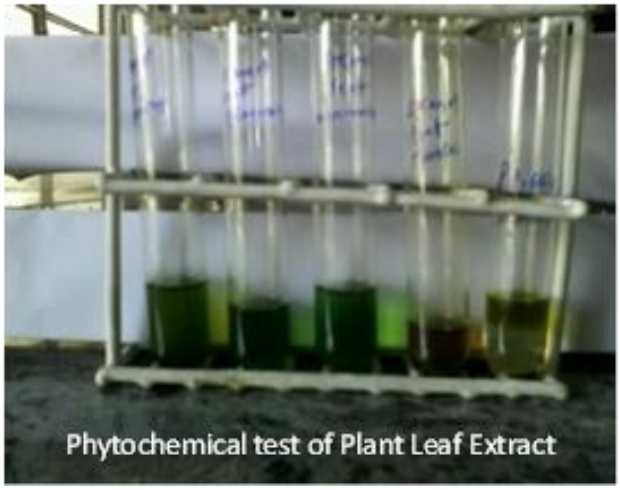


**Figure: 11**. Phytochemical analysis of Grandiflora (Nicotiana) plant Leaf Extract

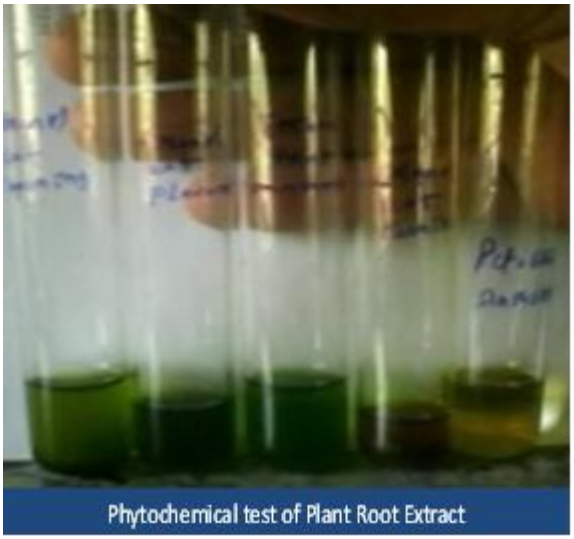


**Figure:12.** Phytochemical analysis of Grandiflora (Nicotiana) plant Root Extract

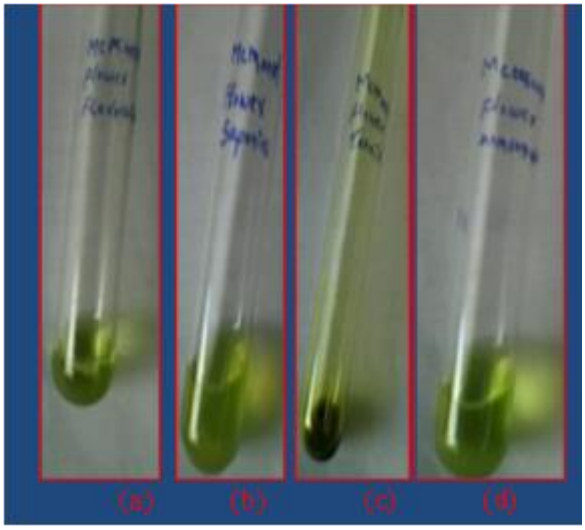


**Figure:13.** Phytochemical analysis of Grandiflora (Nicotiana) plant Flower Extract

was performed by JOEL Model 1200 FX instrument operating at 80 K voltage for the characterization of size and shape of synthesized silver nanoparticles.

*Anti microbial studies*

The agar disc diffusion method was employed for the determination of antimicrobial activities of the synthesized silver nanoparticles. 0.1 ml from 108 cfu/ml of different pathogenic bacteria suspension was spread on different plates nourished with LB (Luria-Bertani) media. Filter paper discs (5 mm in diameter) were placed on the plates and then onto the discs synthesized nanoparticles were impregnated in different concentrations. Ampicillin (10µg/µl concentration) served as the standard for measuring the antibacterial activity. The plates were then incubated at $37^0$C for 24h. The Zones of inhibition were measured in mm.

*Preliminary investigation of phytochemicals*

*Collection and processing of plant samples*

Fresh leaves of Grandiflora (Nicotiana) were collected in the month of March - 2014 from botanical garden, Osmania University, Hyderabad, India.

*Phytochemical screening*

The samples were crushed into fine powder and dissolved separately in 100ml of solvent. The solution was kept at room temperature for seven days to allow the extraction of compounds from seeds. The solution of each sample was stirred after every 24 hrs using sterile glass rod. After 7 days the Solution was filtered through what man filter paper No-1 and a greenish filtrate was obtained. The solvent is allowed to evaporate and stidey substances obtained One stired on rifrigerator.

*Thin layer chromatography*

For the study analysis of TLC plate, the Hexane,Pet Ether and methanolic extract were subjected to thin layer chromatographic analysis, to find the presence of number of chemical constituents to support the chemical test. Analytical TLC plates were prepared by pouring the silica gel G slurry on the glass plates. Drying the thin layer

plates, for 30 min in air for qualitative work, spot was applied in a row along one side of plate, about 2 cm from edge by using capillary tubes. The range of sample volume was controlled, spreading not more than 0.5 cm. The plate was placed in previously saturated TLC chamber with mobile phase. Pet. Ether, methanol and water (5:4:1 ratio) were used as mobile phase.

*Preliminary investigation for the presence of phytochemicals*

Chemical tests were carried out both on the methonal extract and on the powdered specimen using standard procedures to identify the constituents as described by. The specific procedure involved for the evaluation of a particular group of chemical is mentiomed below. Methanol, Pet ether, ethanol extracts of the leaves of Grandiflora (Nicotiana) and methanol flower extract and methanol root extract were investigated for the presence of phytochemicals viz. Tannins, saponins, polyphenols, alkaloids, terpenoids, flavonoids, Steroids and Anthraquinone by following standard biochemical methods.

*Tannins*

1ml of water and 1-2 drops of ferric chloride solution were added in 0.5ml of extracted solution, blue colour was observed for Gallic tannins and green black for catecholic tannins.

*Saponins*

*Foam test*

small amount of extract was shaken with little quantity of water if foam is produced,which is Persistent for ten minutes it indicates the presence of saponins .

*Flavanoids (alkaline reagents test)*

4 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids .

*Terpenoids (Salkowski test)*

Five ml of each extract was mixed in 2ml of chloroform and concentrated $H_2SO_4$ (3ml) was carefully added to form a layer. A reddish brown colouration of the interface shows the presence of terpenoids.

*Glycoside*

1.0 g of extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayered with 1 ml of concentrated sulphuric acid ($H SO_2$).

*Alkaloids*

The extract of Nicotiana alata was evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

*Steroids*

1ml of test solution 5ml of chloroform was added and then few drops of Conc.$H_2SO_4$ was added to the above mixture and mix well .Allow the mixture to stand for some time ,reddish precipite in the lower layer indicates the presence of steroids.

*Biosynthesis of silver nanoparticles from the leaf extract of grandiflora (nicotiana)*

1 ml of the ethanol extract was added into 15 ml of aqueous solution of 1 mM silver nitrate for reduction of Ag+ions and stirred at room temperature for 6 hours.

*UV-Vis spectra analysis*

The reduction of pure Ag+ion was monitored by measuring the UV-Visible spectrum of the reaction mixture after diluting a small aliquot of the sample with distilled water after regular interval of time. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-1700 (Shimadzu).

*Estimation of protein by lowry method*

The reduction of pure Ag+ion Causes binding of some protein. This protein was estimated by Lowry method.

*Thin layer chromatography*

The separation and purification of phytoconstituents of extract were mainly carried out using a combination of chromatographic techniques. The choice of techniques depends upon the solubility properties and volatilities of compound to be separated. Thin layer chromatography is a method of choice for separating all soluble components, i.e. the lipids, steroids, carotenoids, flavonoids, glycosides, tannins, simple quinines and chlorophyll. TLC of Methanol and Pet. ether, Hexane extracts resulted.

## RESULTS AND DISCUSSION

The colour change was noted by virtual observation in Grandiflora (Nicotiana alata) leaf, flower and root extract incubated with aqueous solution of $AgNO_3$. It started to change from watery to yellowish brown due to the reduction of silver ions, confirming the formation of silver nanoparticles (Figure:1). Absorption spectrum at different wavelengths ranging from 400-500 nm revealed a peak of λ max at 430 nm (Figure 2 and 3). The Grandiflora (Nicotiana alata) Leaf, Flower, Root extract without silver nitrate solution did not show any change in colour.

The FTIR spectra study of functional group and shifted electron regions of leaf AgNPs are shown in Figure 2 and 3. The TEM images clearly reveals that shape and size of silver nanoparticles was 15-30 nm with spherical shape. It was noticeable that edges of particles were lighter than the centre (Figure: 6). The shape diffraction spots clearly suggest that the particle is of single crystal quality. Change in color after addition of Root AgNPs and Spectral Identification of functional group is seen in Figure 4. Grandiflora (Nicotiana alata) flower, root Solution (a) Grandiflora (Nicotiana alata) plant flower solution with different Concentrations of silver nitrate (1% to 0.062%) (b,c,d,e,f) change in colour of flower solution after addition of different concentrations of $AgNO_3$.(figure: 3 ). The change in color after addition of root extracts in AgNPs is seen in UV-Spectra's (figure: 4). UV-spectra of Grandiflora plant leaf extract, with silver nitrate at different pH levels is shown in Figure 5. In the disc diffusion method 10µl to 20 µl nanoparticles were placed on disc and tested against pathogen. The zone around the disc was compared with inhibition zone of standard antibiotic Ampicillin (Table 2). The present work demonstrated that extract was capable of producing Ag

from aqueous solution of Ag+. One ml of the methanol, pet ether extract was added into 15 ml of aqueous solution of 1 mM silver nitrate for reduction of Ag+ ions. The colour change was noted by virtual observation in Grandiflora (Nicotiana alata) leaf, root and flower extract incubated with aqueous solution of AgNO3 (Figure 9). AgNPs bounded protein in leaf are shown in (Figure 8). Application of such ecofriendly nanoparticles is bactericidal against highly pathogens and has therapeutic use and electronic applications. TLC Plate of Plant leaf Hexane extract contain not clearly separation componds, Pet. Ether Extract contain only two spots observed (Rf Value 0.35, 0.6cm) and methanol extract of Grandiflora (Nicotiana Alata) plate separation of phytochemical compounds was separation five clear spots observed in methanol extract(Rf Value 0.45, 0.50, 0.7, 0.8, 1.0cm) (Figure:10). The phytochemical screening of the plants revealed some differences in the constituents of the Leaf, Flower and Root of plants tested. The results of phytochemical screening are shown in positive tests (Figure 11, 12, 13). The photochemical results are shown in Table 1. However, the natural products profile and consequently the bioactivity is known to vary with the climate and geographic location of the plants. The present study also correlates with the results of previously reported with little variations. Nanoparticles are important due to their simple experimental procedure and eco-friendliness.

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