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BIOSYNTHESIS OF SILVER NANOPARTICLES FROM SEED POWDER EXTRACTS OF *LATHYRUS SATIVUS*, MEDICINALLY POTENT LEGUME PLANT

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ABSTRACT

Biological synthesis of nanoparticles has been an exploring research topic in recent days due to their advanced use in biomedical, chemical and related fields. Bioreduction of silver nitrate ($AgNO_3$) with the help of plant extract is a great advantage in green synthesis of nanoparticles. In the present investigation, Silver Nano-particles were rapidly synthesized using seed extract of *Lathyrus sativus, a* member of Leguminaceae family. Seed extract was mixed with 1mM silver nitrate and incubated at 60°C for 15 min. The reddish brown color confirms the formation of *Lathyrus sativus* silver nanoparticles (LsAgNPs). The formed silver nanoparticles were then characterized by UV-Visible spectrum, FTIR-spectrometry. SEM analysis and TEM analysis revealed that the morphology and size of the synthesized silver nanoparticles were in the range of 10-40 nm. These biologically synthesized nanoparticles also showed inhibitory effects on *Pseudomonas putida* and *Staphylococcus aureus.* The plant materials mediated synthesis of silver nanoparticles is comparatively rapid; eco-friendly; less expensive and has wide applications like antibacterial therapy in modern medicine.

KEYWORDS: *Lathyrus sativus, ODAP, Homoarginine, Transmission electron microscope, Bioreduction.*

1. INTRODUCTION

Nanoparticles as the name suggests, are the particles having a size in the nanometer range. A number of recent achievements offer the possibility of generating new types of nanostructure materials with designing surface and structural properties. Nanoparticles can be synthesized by physical, chemical and biological methods. The physical methods for the synthesis of nanoparticles are highly expensive and the chemical processes will involve chemical reactions which may be toxic. Hence, there is every need for the synthesis of nanoparticles in a cost effective and eco-friendly manner. Biological synthesis of silver nanoparticles using bacteria[1-3], fungi[4-6], yeast and plants[7-10] has been reported earlier. Green synthesis of nanoparticles using various plant extracts have been extensively studied[11] and still it is an important area of research due to its diversified applications.

Lathyrus sativus commonly known as grass pea, a member of Leguminaceae (Fabaceae) is an important crop in drought and famine prone areas. It produces high protein seed. In addition, the seeds also contain variable amounts of unusual amino acids like β-N-Oxalyl-L-α, β-diamino propionic acid (ODAP) and Homoarginine[12], was identified by Rao et al and PS Sharma et al., in 1964 seperately. Homoarginine having the property of nitric oxide precursor, which helps in vasodilatations and reduces heart risk.

The present study aims to rapid synthesis of silver nanoparticles using an aqueous extract of *Lathyrus sativus* seed powder and evaluates its antibacterial activity against gram positive and gram negative bacteria respectively. The preparation of uniform nanoparticles with specific requirements in terms of size, shape and physical and chemical properties is of great interest in the formulation of new pharmaceutical products. Due to the increased resistance of bacteria to antibiotics,[14-15] it has become very imperative in finding for new formulations using AgNPs (silver nanoparticles) which can ensure safety and efficacy of treatment.[16]

2. MATERIALS AND METHODS

2.1. Preparation of seed powder extract

The seeds of *Lathyrus sativus* were locally collected from Borptla, Andhra Pradesh, India. The seeds were rinsed twice with water, followed by deionised water in order to remove the fine dust materials and then they are dried under direct sunlight for one week to completely remove the moisture. Then the seeds are ground into fine powder. Then to 5 g of *Lathyrus sativus* seed powder 100 ml of deionised water was added and boiled for 15 min at 60^0C. After cooling, the extract is filtered through Whatman No: 1 filter paper. The filtered extract will serve as the biological extract for the synthesis of silver nanoparticles. The extract can be stored at 4^0C for further use.

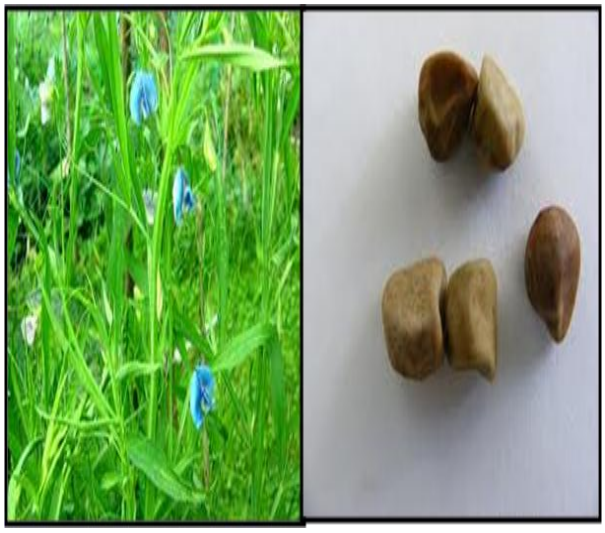

Fig: 1 *Lathyrus sativus* plant and seeds

2.2. Synthesis of silver nanoparticles

1mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 20ml of *Lathyrus sativus* seed powder extract was added to 40ml of 1Mm aqueous silver nitrate and incubated on a hot plate at 60^0C for 30 minutes. A color change from light yellow to reddish brown was observed, indicating the formation of silver nanoparticles as shown in Fig 2b. Color of silver particles is attributed to surface Plasmon resonance (SPR)[17] which arises due to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field. Thus formed silver nanoparticles were centrifuged at 12000 rpm for 30minutes. The pellet was then washed thrice with distilled water and dried in an oven for 48 hours. This stabilized powder form of silver nanoparticles was used for further characterization of the particles by various techniques.

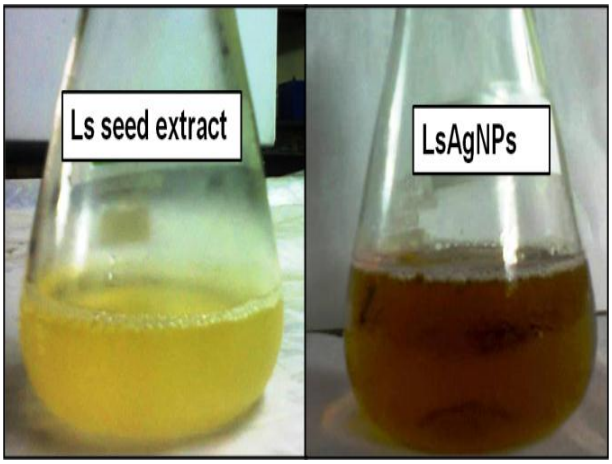

Fig: 2 Lathyrus seed powder extract and silver nanoparticles

3. Characterization of LsAgNPs

The Silver nanoparticles were further characterized by the following methods:

3. 1: UV -visible spectroscopic studies

UV spectroscopic studies were carried out for the confirmation of the formation of silver nanoparticles. An ELICO 159 UV-Vis spectrophotometer was used for the spectrometric analysis of silver nanoparticles. The reduction of silver was measured at 300-700nm. A spectrum of silver nanoparticles was plotted. The seed powder extract served as the blank. The formed silver nanoparticles were then centrifuged at deionised water 20,000rpm for 20mins. Then the pellet was washed three times with deionised water, then the remaining pellet was dried in an oven at 60^0C. This powdered form is used for further analysis.

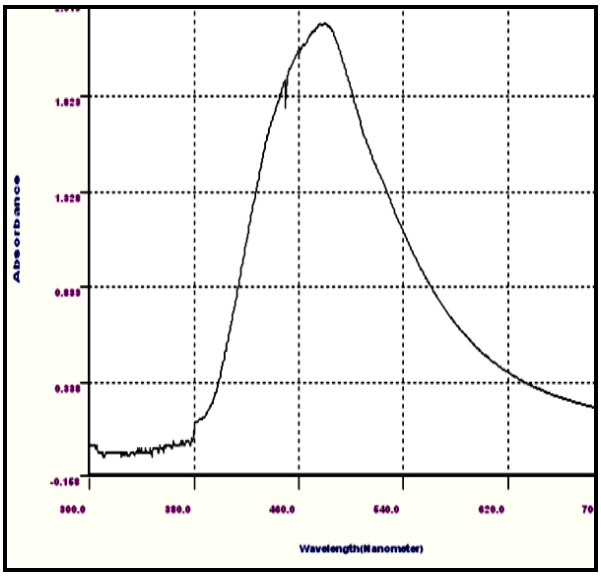

Fig: 3 UV- Visible spectra of silver nanoparticles with the seed extract as the standard

3. 2: FTIR Analysis

The stabilized forms of the nanoparticles were then analyzed by FTIR to find out the constituents of the powder (Paragon 500, Perkin Elmer-RX1 spectrophotometer).

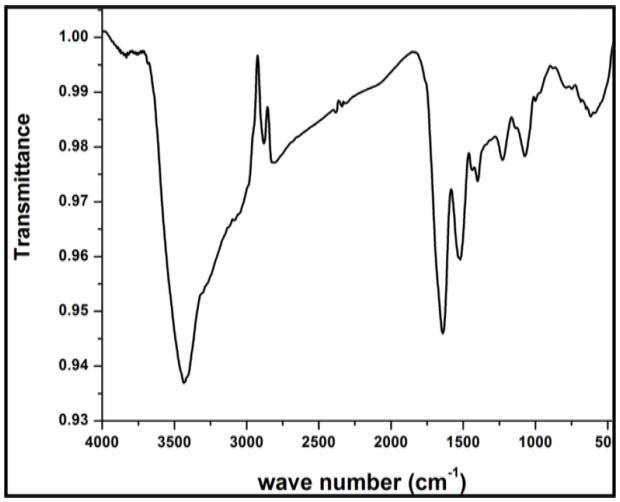

Fig: 4 FTIR analysis of silver nano particles

3. 3: XRD Analysis

The crystallite sizes of Ag nanoparticles were calculated from the line width of intense line (111) using the Scherer's formula [Cullity BD, Elements of X-ray Diffraction, Addison-Wesley publishing Co; Reading, MA. 1997].

$$t = \frac{0.9\lambda}{\beta \cos\theta}$$

Where it is the thickness in Angstroms (A^o) and corresponds to the crystallite diameter, assuming a spherical shape, λ is the wave length of the X-ray used, θ is the Bragg angle and β is the full width at half maximum measured in radians of (111) line in the powder XRD pattern.

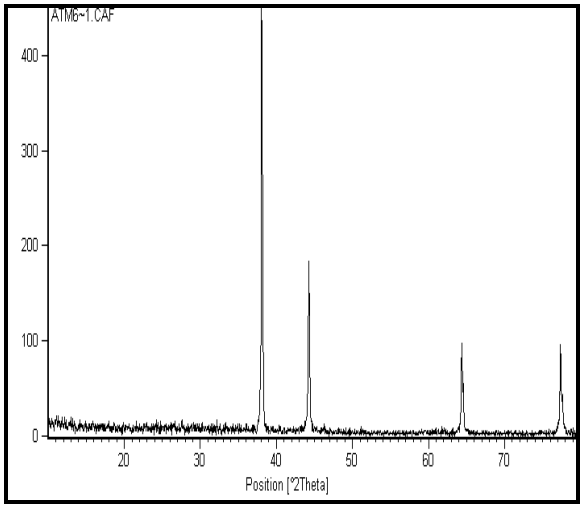

Fig: 5 XRD spectrum of silver nanoparticles

3.4. Electron microscopy studies

In order to study the morphology and size of the synthesized silver nanoparticles, the formed silver nanoparticles were subjected to SEM (Scanning electron microscopy) and TEM (Transmission Electron Microscopy).

3.4.1. SEM Analysis

SEM analysis was done using Zeiss 700 Scanning electron microscope. The powdered sample of 10mg was prepared in thin films of carbon coated cooper grid and the sample was analyzed for size determination of the nanoparticles.

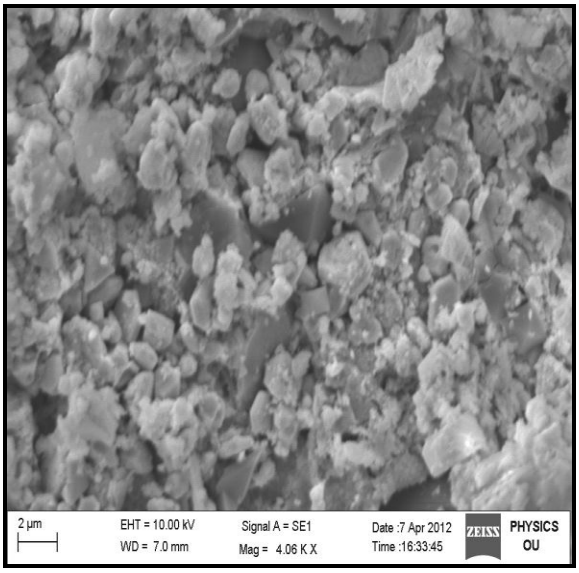

Fig: 6 SEM analysis of silver nanoparticles

3.4.2: TEM Analysis

TEM analysis was done using Philips Tacna G2 FEI F12, operating at 80–100 KV. TEM specimens were prepared by the drop casting method. One or two drops of LsAgNPs aqueous solution onto carbon coated copper grids, which were allowed to dry at room temperature overnight. The powdered form was then used.[14-15]

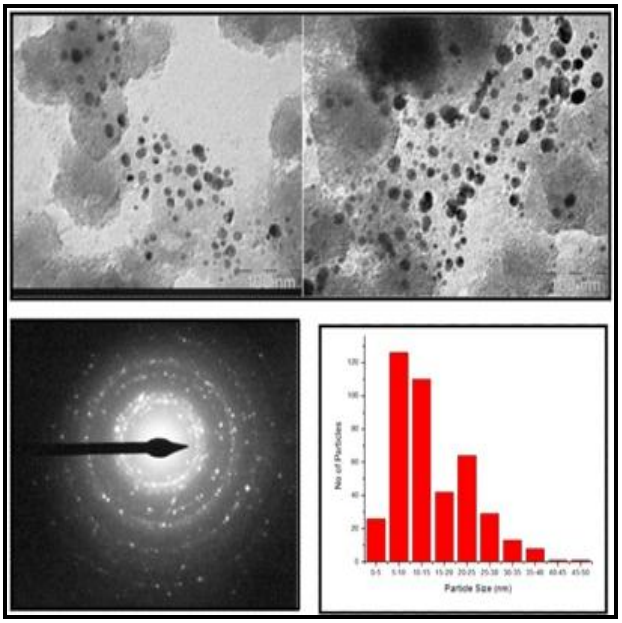

Fig: 7 TEM images of silver nanoparticles, silver (Selected area electron diffraction) SAED image and particle size demo gram of silver nanoparticles

3.7: Antibacterial activity of silver nanoparticles

The test strains, *Pseudomonas putida* and S*taphylococcus areas* were purchased from IMTECH, Chandigarh. Yeast extract, Tryptophan and Bacterial grade Agar-Agar purchased from Himedia laboratories, Mumbai, India.

The antimicrobial activity of synthesized silver nanoparticles was determined using the disc diffusion assay. LB media were prepared and poured into sterilized petri plates and then the plates were inoculated with overnight lag phase cultures of *Pseudomonas putida* and *Staphylococcus aureus* separately, then the samples were added at different concentrations to the discs. The plates were then incubated overnight at 37°C.

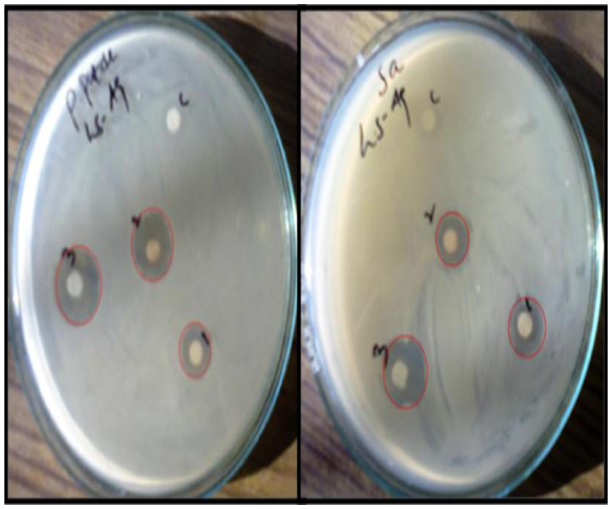

Fig: 8 Antimicrobial activity *Lathyrus sativus* silver nano particles

4. RESULTS AND DISCUSSION

Several approaches have been employed to obtain a better synthesis of silver nanoparticles such as chemical and biological methods. Recently, synthesis of silver nanoparticles using plant extracts getting more popular. Synthesis of silver nanoparticles by using plant extracts is a more advanced study in modern bio nanotechnology.

Silver nanoparticles were synthesized using the Aloe Vera extract at 24 h of incubation[18]. Similarly, in the present study silver nanoparticles were synthesized using seed powder extract of Lathyrus sativus, a Leguminaceae plant which is a more pretentious pulse. Interestingly, silver nanoparticles were synthesized rapidly within 30 min of incubation period at 60^0C. The colorless aqueous silver nitrate solution was turned to orange brown color within 30 min, with the addition of powder extract (Fig. 2a and 2b). Intensity of brown color increased in direct proportion to the incubation period. It may be due to the excitation of Surface Plasmon Resonance (SPR) effect and reduction of $AgNO_3$. The control $AgNO_3$ solution (without powder extract) showed no change in color. SPR patterns, characteristics of metal nanoparticles strongly depend on particle size, stabilizing molecules or the surface adsorbed particles and the dielectric constant of the medium.

4.1. Spectrophotometric analysis of silver nanoparticles

The characteristic absorption peak at 480 nm in UV–vis spectrum (Fig. 3) Confirmed the formation of silver nanoparticles. The single SPR band in the early stages of synthesis corresponds to the absorption spectra of spherical nanoparticles.

4.2. FTIR analysis of silver nanoparticles

A typical infrared spectrum can be visually divided into two regions. The left half, above 2000 cm^{-1}, usually contains relatively few peaks, but some very diagnostic information can be found here. Alkane C-H stretching absorptions just below 3000 cm^{-1} demonstrate the presence of saturated carbons, and signals just above 3000 cm^{-1} demonstrate unsaturation. A very broad peak in the region between 3100 and 3600 cm^{-1} indicates the presence of exchangeable protons, typically from alcohol, amine, amide or carboxylic acid groups (see further discussion of this below). The frequencies from 2800 to 2000 cm^{-1} are normally void of other absorptions, so the presence of alkynes or nitrile groups can be easily seen here.

In contrast, the right half of the spectrum, below 2000 cm^{-1}, normally contains many peaks of varying intensities, many of which are not readily identifiable. Two signals which can be seen clearly in this area is the carbonyl group, which is a very strong peak around 1700 cm^{-1} and the C-O bond[19] with can be one or two strong peaks around 1200 cm^{-1}. This complex lower region is also known as the "fingerprint region" because almost every organic compound produces a unique pattern in this area. Therefore identity can often be confirmed by comparison of this region to a known spectrum. FTIR analysis was used for the characterization of the resulting nanoparticles (Figure 3). FTIR absorption spectra of water soluble extract after reduction of Ag ions are shown in Fig. 3., whereby they themselves get oxidized to unsaturated carbonyl groups leading to a broad peak at *1612* cm-1 (for reduction of Ag).

4.3: XRD Results

Fig: 8 shows a representative XRD analysis of *Lathyrus sativus* silver nanoparticles, which showed three distinct diffraction peaks at 38.1^0, 44.1^0 and 64.1^0, which indexed the planes 111, 200 and 220 of the cubic faces-centered silver. The lattice constant calculated from this pattern was a = 4.086Å and the data obtained was matched with the database of the Joint Committee on Powder Diffraction Standards (JCPDS) file No. 04-0783. The average grain size of the silver nanoparticles formed in the bioreduction process was determined using Scherer's formula, $d = (0.9\_\times 180^0)/\cos^{\varphi}$ and was estimated as 35 nm (Fig. 6).

4.4: Electron microscopy studies: SEM and TEM analysis of silver nanoparticles

The SEM image showed relatively spherical shape nanoparticles formed with diameter range of 40 mm which were observed at a magnification of X 4.06 K (Fig.3). The transmission electron microscopy gave a detailed descriptive image of the silver nanoparticles synthesized with their structural details and their size. The silver particles are crystalline, as can be seen from

the selected area diffraction pattern recorded from one of the nanoparticles in the aggregates. Transmission electron microscope concluded that the average mean sizes of silver nanoparticles were in between 5- 40 nm and seems to be spherical in morphology as shown in Fig: 4. (Fig: 7a). SAED pattern (Selected Area Electron Diffraction Pattern) (Fig: 7b) shows the selected area of silver nanoparticles in the sample. (Fig: 7c) shows the average particle size ranges 35 nm.

4.5: Anti bacterial studies

The antimicrobial activity of LsAgNPs was tested against *Pseudomonas putida* and *Staphylococcus areas* which are Gram positive and Gram negative bacteria respectively. The inhibition zone appeared around the disc was measured and recorded as the antibacterial effect of LsAgNPs. It was found that zone of inhibition in *Pseudomonas putida* was 7 mm and *Staphylococcus areas* were 6 mm for 10 µl of nano particles added to the disc having 10 micro grams of LsAgNPs.

CONCLUSION

The biosynthesis of silver nanoparticles using *Lathyrus sativus* seed extract proved to be an eco-friendly approach besides other chemical and biological processes available. We conclude that it is very imperative for the formulation of new pharmaceutical products using AgNPs, which in the nearby future and may address strategies against increasing drug resistance by superbugs. The possible mechanism of biosynthesis of nanoparticles by biological system was reductases and any other equivalent. The plant extract after addition of aqueous 1mM silver nitrate turned in to reddish brown color in the reaction vessel which suggested the formation of silver nanoparticles, which was then subsequently subjected to optical measurements by UV–vis spectrophotometer. Absorbance peak was noted at 445 nm which was specifically for the synthesized Ag nanoparticles. The present study established that use of a natural, low-cost biological reducing agent; *Lathyrus sativus* seed powder extracts (aqueous) can produce metal nanostructures, through efficient green nanochemistry methodology, avoiding the presence of hazardous and toxic solvents and waste; furthermore, the nanostructures LsAgNPs showed excellent antimicrobial activity could be used as antimicrobial agent to sewage and stream water plants, further analysis of specified human cell lines to check the protein folding and refolding affect as LS plant contains β-ODAP having chaperon activity as it high accumulation in stress conitions.

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REFERENCES

1. Malarkodi C, Rajeshkumar S, Paulkumar K, Gnanajobitha G, Vanaja M, Annadurai G. Bacterial Synthesis of Silver Nanoparticles by Using Optimized Biomass Growth of *Bacillus Sp.* Nanoscience and Nanotechnology: An International Journal. 2013; 3(2): 26-32.
2. Reena P, K Rama and A Adith Rena Extracellular Biosynthesis of Silver Nanoparticles by Culture Supernatant of *Pseudomonas aerusinosa.*. International Journal of Biotechnology, 2012; 11(1): 72-76.
3. Ritika Chauhan, Abhishek Kumar, Jayanthi Abraham. A Biological Approach to the Synthesis of Silver Nanoparticles With *Streptomyces Sp JAR1* And Its Antimicrobial Activity. SCI Pharm. 2013; 81: 607–621.
4. Prashant Singh and R.Balaji Raja.Biological Synthesis and Characterization of Silver Nanoparticles Using the Fungus *Trichoderma harzianum*. Asian J. Exp. Biol. SCI. 2011; 2(4).
5. Karthick Raja S., Namasivayam, S. Ganesh and Avimanyu. Evaluation of Antibacterial Activity of Silver Nanoparticles Synthesized from *Candida glabrata and Fusarium oxysporum*. Int J Med Res. 2011; 1(3): 130-136.
6. Vahabi K., Mansoori G. A. and Karimi S. Biosynthesis of Silver Nanoparticles by Fungus *Trichoderma reesei*. Insciences J. 2011; 1(1): 65-79.
7. Karunakar Rao Kudle, Manisha R Donda, Jahnavi Alwala, Rama Koyyati, Veerababu Nagati, Ramchander Merugu, Y.Prashanthi, M.P.Pratap Rudra. Biofabrication of Silver Nanoparticles Using *Cuminum cyminum* through Microwave Irradiation. International Journal Of Nanomaterials And Biostructures, 2012; 2(4): 65-69.
8. Karunakar Rao Kudle, Manisha R Donda, Ramchander Merugu, Y.Prashanthi, M.P.Pratap Rudra Microwave Assisted Green Synthesis of Silver Nanoparticles Using *Stigmaphyllon littorale* Leaves, Their Characterization and Antimicrobial Activity. International Journal Of Nanomaterials And Biostructures, 2013; 3(1): 13-16.
9. Madhukar Rao Kudle, Karunakar Rao Kudle, Manisha. R. Donda, M. P. Pratap Rudra Synthesis, Characterization And Antimicrobial Activity of Silver Nanoparticles Formed Using *Moringa oleifera Lam Gums.*. Nanoscience And Nanotechnology: An International Journal, 2013; 3(3): 45-48.
10. Veera Babu Nagati, Raju Nalvothula, Rama Koyyati, Ramchander Merugu, Pratap Rudra Manthur Padigya, Green synthesized of silver nanoparticles using Canna indica leaf extract and its characterization International Journal of Chem Tech Research, 2014; 6(4): 2271-2276.
11. Geoprincy G., Vidhya Sri B.N., Poonguzhali U., Nagendra Gandhi N., Renganathan S. A Review on Green Synthesis of Silver Nanoparticles. Asian J Pharm Clin Res, 2013; 6(Suppl 1): 8-12.
12. Bell, E.A, Harbroune JB., Boulten D., Turner BL Comparative biochemistry of nonprotein amino

acids, in chemotaxonomy of leguminosae., (Eds) Academic Press, 1971; 179-206.
13. Prashant Singh and Balaji Raja R. Biological Synthesis and Characterization Of Silver Nanoparticles Using the Fungus *Trichoderma harzianum*. Asian J. Exp. Biol. Sci. 2011; 2(4).
14. Asmita J. Gavhane P. Padmanabhan, Suresh P. Kamble and Suresh, N. Jangle, Synthesis of Silver Nanoparticles Using Extract of Neem Leaf and Triphala and Evaluation of Their Antimicrobial Activities, Int J Pharm Bio Sci. 2012; 3(3): 88-100.
15. Jaidev LR, Narasimha G. Fungal mediated biosynthesis of silver nanoparticles, characterization and antimicrobial activity, Colloids Surf B: Biointerfaces, 2010; 81(2): 430–433.
16. Sathyavathi R, Balamurali Krishna M, Venugopal Rao S, Saritha R and Narayana Rao D. Biosynthesis of Silver Nanoparticles Using Coriandrum Sativum Leaf Extract and Their Application in Nonlinear Optics. Advanced Science Letters. 2010; 3: 1-6.
17. Laura Christensen, Singaravelu Vivekanandhan, Manjusri Misra, Amar Kumar Mohanty, Biosynthesis of silver nanoparticles using murraya koenigii (curry leaf): An investigation on the effect of broth concentration in reduction mechanism and particle size. Adv. Mat. Lett, 2011; 2(6): 429-434.
18. Chandran, S.P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad, M. Sastry Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract Biotechnol. Prog., 2006; 22: 577–583
19. H.A. Szymanski, R.E. Erickson, Infrared Band Handbook, Plenum Press, New York, 2 Volumes, 1970. 2. P. Gans, Vibrating Molecules: an Introduction to the Interpretation of Infrared and Raman Spectra, Chapman & Hall, London, 1975.