Green Synthesis of Silver Nanoparticles Using *Albizia Lebbeck*(L.)*Benth Extract* and Evaluation of Its Antimicrobial Activity

S.Parvathy[1]*, K.Vidhya[1], V. K. Evanjelene[2], B.R.Venkatraman[3]
[1] Department of Chemistry, Government Arts College (Autonomous), Salem- 636 007, TamilNadu, India.
[2] Alpha Omega Hi- Tech Bio Research Centre, Salem – 636 008. TamilNadu. India.
[3] Post Graduate & Research Department of Chemistry, Periyar E.V.R.College (Autonomous)
Trichy-620 023, TamilNadu. India. *Email: parupalani@yahoo.co.in

Abstract—**Green synthesis of silver nanoparticles (AgNp's) is a growing research area because of their potential applications in nanomedicines. Hence, in the present study, we investigated the green synthesis of AgNp's from silver nitrate using methanol and ethyl acetate extracts of leaves of *Albizialebbeck*(L.) *Benth*(AL). The yellowish brown colour of the mixture indicated the formation of AgNp's which monitored with the help of UV-visible spectrophotometer and were characterized by using FTIR spectra and SEM image. The phytochemical investigations were carried out for detection of phytoconstituents by standard procedures. The antibacterial activity of methanolic extract of AgNp's against two pathogenic bacteria is evaluated using disc diffusion method in two different concentrations. The bacterial property of AgNp's was analyzed by measuring their inhibitory zone.**

Keywords; silver nanoparticles, green synthesis, *Albizialebbeck, phytochemical screening, antimicrobial activity.*

I. INTRODUCTION

Medicinal plants are of great importance to the health of individual and communities. The medicinal value of the plant lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. The use of plants to cure diseases and relieve physical sufferings had started from the earliest times of humankind's history [1]. Today, medicinal plants play a great role in human health services worldwide. Many people in the modern world are turning to herbal medicine. For example, in USA about 25% of all prescriptions dispensed in public pharmacies in 1973 contained drugs extracted from higher plants and about 64% of the total global populations remain dependent on traditional medicine for their healthcare needs [2], [3].

Nano sized particles are quite unique in nature because nano size increase surface to volume ratio and also its physical, chemical and biological properties are different from bulk material. So the main aim to study its minute size is to trigger chemical activity with distinct crystallography that increases the surface area [4], [5],-[6]. Thus in recent years much research is going on metallic nanoparticles and their properties like catalyst, sensing to optics, antibacterial activity and data storage capacity [7].

Phytochemicals are bioactive compounds found in plants that work with nutrients and dietary fiber to protect against diseases. They are non-nutritive compounds. These phytochemicals often secondary metabolites in smaller quantities in higher plants include the alkaloids, Steroids, flavonoids, terpenoids, tannins and many others [8]. Many phytochemicals have antioxidant activity and reduce the risk many diseases. It is crucial to know the type of phytochemical constituent, thus knowing the type of biological activity, which might be exhibited by the plant [9]. The importance of medicinal plants and the contribution of phytomedicine to the well-being of a significant member of the world's population have attracted interest from diverse disciplines.

Green synthesis provides enhancement over chemical and physical method it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. The use of environmentally benign materials like plant extract for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and biocompatibility for pharmaceutical and other biomedical applications. Silver nanoparticles can be produced either intra or extra-cellularly by using living organisms [10], [11], [12], [13].

The need of the hour is to screen a medicinal plant for promising biological activity. Considering the aforesaid, *Albizia lebbeck* (L.)*Benth*, a medicinal plant and a member of Mimosaceae family, found in India, Bangladesh, tropical and subtropical Asia and Africa [14].The common name of the plant is Siris tree and in Tamil, it is called as vaagei. It is a deciduous tree with compound leaves, flat oblong fruits, round cream-colored seeds, grow wild.

II. MATERIALS AND METHODS

A. Procurement of plant Material

The fresh plant leaves of *Albizia lebbeck* (L.) *Benth* (AL) were collected from Yercard main road, Salem District, Tamil Nadu. Plant materials were washed under running tap water, air-dried and then homogenized to fine powder and stored in airtight bottles in refrigerator.

B. Preparation of extract

Crude plant extract was prepared by Soxhlet extraction method. About 20g of powdered plant material was uniformly packed into a thimble, extracted with 250mL of methanol, and aqueous separately. The process of extraction has to be continued for 24 hours or until the solvent in siphon tube of extractor become colourless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40ºCtill all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC till future use.

C. Synthesis of AgNp's

One mM $AgNO_3$ was added to the plant extracts separately to make up a final solution 200mL and centrifuged at 18,000rpm for 25 min. The collected pellets were stored at 4^0C.The supernatants were heated at 50^0C to 95^0C. A change in the colour of solutions was observed during the heating process.

D. Characterization of AgNp's

The optical property of AgNp's was determined by UV-Vis spectrophotometer (Perkin-Elmer, Lamda 35, Germany). After the addition of $AgNO_3$ to the plant extract, the spectra's were taken in 24 hr. between 350nm to 500nm. Then the spectra were taken after 24 hr. of $AgNO_3$additions. The chemical composition of the synthesized AgNp's from AL plant extract was studied by using FTIR spectrometer (Perkin-Elmer LS-55-Luminescence spectrometer). The solutions were dried at 75^o C and the dried powders were characterized in the range 4000–400cm^{-1}using KBr pellet method. The morphological features of synthesized AgNp's from AL plant extract were studied by SEM (JSM-6480 LV). After 24 hrs. of the addition of $AgNO_3$, the SEM image is prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the sample is characterized in the SEM at an accelerating voltage of 20KV.

E. Phytochemical Screening

Preliminary phytochemical analysis was carried out for both methanol ethyl acetate extracts of AL as per standard methods [15], [16]. Natural chemical groups such as alkaloids, flavonoids, steroids, terpenoids, anthraquinones, phenols, saponins, tannins, carbohydrates, oils and resins were probed.

F. Antibacterial activity study

Bacterial cultures *Escherichia coli* and *Corneybacterium* were taken and disc diffusion method [17] was used to screen the antibacterial activity. *In-vitro* antibacterial activity was screened by using Muller Hinton Agar (MHA) obtained from Hi-media (Mumbai). The MHA plates were prepared by pouring 15mL of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes, 0.1% inoculums suspension was swabbed uniformly, and the inoculums were allowed to dry for 5 minutes. The concentration of extract 40mg/disc was loaded on 6mm sterile disc. The loaded disc was placed on the surface of medium, the extract was allowed to diffuse for 5 minutes, and the plates were kept for incubation at 37^oC for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

III. RESULTS AND DISCUSSION

The AgNp's is synthesized in both the extracts *i.e.,* methanol and ethyl acetate extract of AL. The results reddish brown colour formation is obtained only in the methanol extract and there is absence reddish brown colour formation in the ethyl acetate extracts. The AgNp's solution has dark brown or dark reddish in colour. In *Albizia lebbeck* before addition of $AgNO_3$ its colour was dark green but after its treatment with $AgNO_3$ its colour changes to dark brown [18] which indicated the formation of AgNp's(Fig. 1). This colour change is due to the property of quantum confinement, which is a size dependent property of nanoparticles and affects the optical property of the nanoparticles. The highest yield (4.89 g) was obtained in methanol extract and least yield was found in ethyl acetate extract (3.65 g).

Figure 1.AgNp's using methanolic AL plant extract before synthesis (control) and after synthesis (synthesized AgNp's)

The synthesis of AgNp's had been confirmed by measuring the UV-Visible spectrum of the reaction media (Fig. 2). Longitudinal vibrations corresponding to silver nanoparticles were convincing with UV spectral peak at 407nm and with no considerable peak in the figure (Fig. 2). This clearly indicates the interaction between Ag^{+} ions and phytochemicals present in the methanol extract [19]. Intensity of band increased upon varying time without any shift in peak position.

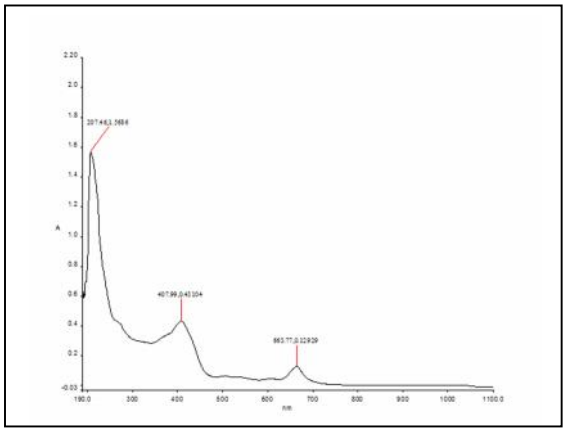

Figure 2. UV-Vis absorption spectra of AgNp's synthesized by AL plant extract

FTIR spectrum of AgNp's synthesized from AL plant extract is shown in the figure (Fig.3). FTIR measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the AgNp's synthesized by leaf broth [20]. The Peaks at 3472cm^{-1} and 2924cm^{-1} assigned to OH stretching and aldehydic C-H stretching respectively. Peak at 1034cm^{-1} corresponds to C-N stretching vibration of the aliphatic amine. The peak near 825cm^{-1} assigned to $–C=CH_2$.A peak at 1132cm^{-1} may be due to C-O-C stretching of carbohydrate as polysaccharides. A peak at 1577cm^{-1} amide-II bonds involving carbonyl and N-H stretching of the proteins and absorption peak at 1403cm^{-1} CH_2 and CH_3 groups of proteins. These findings indicate the presence and binding of proteins with AgNp's which can lead to their possible stabilization [21].

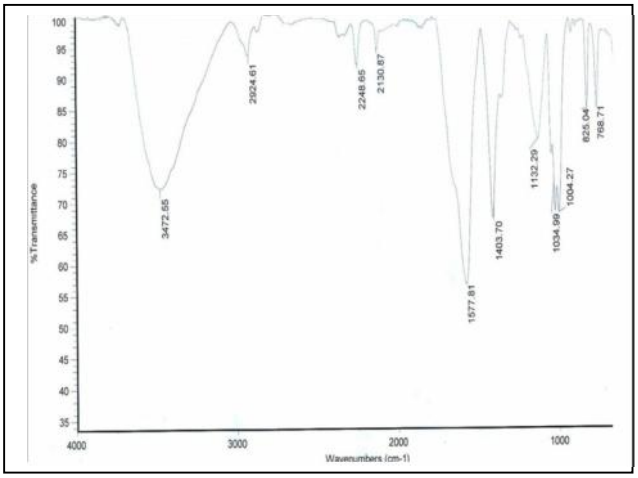

Figure 3. FTIR spectra of AgNp's synthesized by AL plant extract

A scanning electron microscope was employed to analyze the shape of the synthesized AgNp's. It is well known that when liquids that contain fine particles were evaporated on a flat surface, the particles accumulate along the outer edge and form typical structures [22]. SEM analysis shows that the plant, AL have tremendous capability to synthesizing AgNp's which are roughly spherical in shape (Fig. 4).

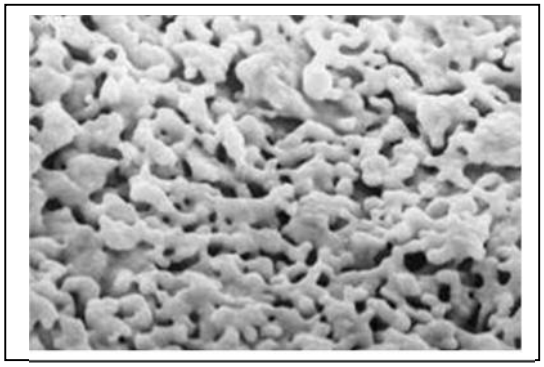

Figure 4.SEM micrograph of AgNp's synthesized by AL plant extract

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents, which are known to exhibit medicinal as well as physiological activities [23].The phytochemical investigation of AL extract (methanol and ethyl acetate) revealed differences in their phytoconstituents (Table 1). Steroids, tannins and carbohydrates were present in both the methanolic and ethyl extracts of AL Plant where as flavonoids, anthraquinone and terpenoids were absent in both of extracts. While comparing the phytochemical activity of the extracts of AL, more number of phytochemical constituents was present in methanol extract. Tannins bind to proline rich protein and interfere with protein synthesis. Steroids have been reported tohave antibacterial properties [24].

TABLE 1.PHYTOCHEMICAL STUDY OF AL EXTRACTS

Phytochemical	**Methanolic extract**	**Ethyl acetate extract**
Alkaloids	+	-
Flavonoids	-	-
Steroids	+	+
Terpenoids	-	-
Anthroquinone	-	-
Phenols	+	-
Saponins	-	-
Tannins	+	+
Carbohydrates	+	+
Oils and Resins	-	+

(+) sign: presence of constituent; (-) sign: absence of constituent

The AgNp's of AL plant extract showed inhibition against *E.coli* in both concentrations (30 and 40µg/mL), whereas against *Corneybacterium*, it shows inhibition only in higher concentration (40µg/mL).

TABLE 3. ANTIBACTERIAL ACTIVITY IN METHANOLIC EXTRACT OF AgNp's

S. No.	Name of bacteria	Diameter of zone of inhibition (mm)				
		Control*	*AL Plant Extract*		*AgNp's of AL Plant extract*	
			Concentration (µg/mL)			
			30	**40**	**30**	**40**
1.	*Escherichia coli*	20	11	16	15	13
2.	*Corneybacterium*	20	04	10	-	06

***Control: Chloramphenicol**

IV. CONCLUSION

In the present study, it has been observed that the methanolic extract of *Albizia lebbeck* is a good source for the synthesis of AgNp's by a green approach. AgNp's synthesized by the present method are of spherical shape.FT-IR provided information about functional groups, which participates in the synthesis of silver nanoparticles. The AgNp's were characterized by UV-visible spectra, FTIR spectra and SEM image. The phytochemical investigation revealed that more number of phytochemicals present in methanolic extract of *Albizia lebbeck.* When evaluated for their antibacterial activity against *Escherichia coli* and *Corneybacterium*, it shows significant inhibition zone.

REFERENCES

[1] A.F.Hill, "Economic Botany: a textbook of useful plants and plant products", 2nd edn., McGraw-Hill Book Company Inc., New York, p.560, 1989.

[2] N.R.Farnsworth, R.W.Morris, "Higher plants: the sleeping giant of drug development", Am. J. Pharm. Educ., vol.148, pp.46-52, 1976.

[3] A.Sofowora, "Medicinal plants and traditional medicinal in Africa", John Willey & Sons Ltd., New York, p.256, 1982.

[4] T.Osaka, T.Matsunaga, T.Nakanishi, A.Arakaki, D.Niwa, H.Iida, "Synthesis of Magnetic nanoparticles and their application to bioassays", Anal. Bioanal.Chem., vol.384, pp.593-600, 2006.

[5] C.Singh, V.Sharma, P.K.Naik, V. Khandelwal, H.Singh, "Green biogenic approach for synthesis of gold and silver nanoparticles using *Zingiberofficinale"*. Dig. J. Nanomater. Bios.,vol. 6, pp.535- 542, 2011.

[6] S.Sinha, I.Pan, P.Chanda, S.K.Sen,"Nanoparticles fabrication using ambient biological resources", J. Appl. Biosci., vol.19, pp.1113 – 1130, 2009.

[7] K.V.Sharma, A.R.Yngard, Y.Lin Y, "Silver nanoparticle: Green synthesis and their antimicrobial activities", Adv. Colloid Interface Sci., vol.145, pp.83–96, 2009.

[8] N.P.Peteros, "VYMM.Antioxidant & cytotoxic activities & phytochemical screening of four Phillppine medicinal plants". J. Med. Plant. Res., vol. 4, 407-414, 2010.

[9] K.N. Agbafor, N. Nwachukwu, "Phytochemical analysis and antioxidant property ofleaf extract of *vitexdoniana* and *mucunapruriens*", Biochem.Res. Int., pp.1-4, 2011.

[10] P.Mohanpuria, N.K.Rana, S.K. Yadav, "Biosynthesis of nanoparticles: technological concepts and future applications", J. Nanopart. Res., vol.10, pp.507-517, 2008.

[11] Y.S.Jae, S.K.Beom, "Rapid biological synthesis of silver nanoparticles using plant leaf extracts", Bioprocess. Biosyst.Eng., vol.32, pp.79-84, 2009.

[12] V.Parashar, R.Parashar, B.Sharma, A.C.Pandey, "Parthenium leaf extract mediated synthesis silver nanoparticles: A novel approach towards weed utilization", Digest J. Nanomater. Biostruct., vol.4, pp.45-50, 2009.

[13] R.Geethalaksmi, D.V.L.Sarada, "Synthesis of plant-mediated silver nanoparticles using *Trianthemadecandra* extract and evaluation of their anti microbial activities",Int. J. Engg. Sci. Tech., vol.2, pp.970-975, 2010.

[14] K.R.Kirtikar, B.D.Basu, "In: Indian Medicinal Plants", India, Part II, p.937, 1980.

[15] K.R.Brain, T.D.Turner, "Practical Evaluation of phytopharmaceuticals", Wright– Scientechnica, Bristol, 1st edn., p.144, 1975.

[16] W.C.Evans, "Trease and Evans' Pharmacognosy", 14th edn., London, England: W.B. Sounders Company Ltd., pp.545-46, 1996.

[17] A.W.Bauer, W.M.Kirby, J.C.Sherris, M.Turck, "Antimicrobialsusceptibilitytesting by a standardized single disc method", Am. J. Clin. Pathol., vol. 45, pp.493- 496, 1966.

[18] A.Ahmad, P.Mukherjee, S.Senapati, D.Mandal, K.M.Islam, R.Kumar,"Extracellular biosynthesis of silver nanoparticles using the fungus *Fusariumoxysporum"*, *Colloids Surf B Biointerfaces*, vol. 28, pp.313–318, 2003.

[19] P.Kumar, S.SenthamilSelvi, A.LakshmiPrabha, K.Prem Kumar, R.S.Ganesh Kumar, M.Govindaraju,"Synthesis of silver nanoparticles from *Sargassumtenerrimum* and screening phytochemicals for its antibacterial activity", Nano Biomed. Eng., vol.4, pp.12-16, 2012.

[20]J. Saraniya Devi, B. Valentin Bhimba, "Silver nanoparticles: Antibacterial activity against wound isolates &*in vitro* cytotoxic activity on Human Caucasian colon adenocarcinoma", Asian Pacific Journal of Tropical Disease, pp.S87-S93, 2012.

[21] J.Jayashree, P.Nilotpala, D.Bisnu Prasad, S.LalaBehari, P.Prasannakumar, "Biosynthesis and characterization of silver nanoparticles using microalga *chlorococcumhumicola* and its antibacterial activity", International Journal of Nanomaterials and Biostructures vol.3, pp.1-8, 2013.

[22] C.M.Samidoss, K.Murugan, R.Mathath, S.Sivapriyajothi,N.A.Suganya, Larvicidal potential of silver nanoparticles synthesized using Adiantumcapillusverenis against anopheles stephensi [diptera:culicidae], International Journal of Current Tropical Medicine and Health Research, vol.1, pp. 9-18, 2013.

[23] A.Sofowra, "Medicinal Plants and traditional Medicine in Africa", Spectrum Books Ltd., Nigeria, pp.191-289, 1993.

[24] K.Vindhya, K.K.Sampath Kumara, H.S.Neelambika, S.Leelavathi, "Preliminary phytochemical screening of *Gardenia latifolia* Ait. and *Gardenia gummifera* Linn.", Res. J. Pharm. Biol.Chem.Sci., vol.5, pp.527-532, 2014.