Research Article

Green Synthesis, Optimization and Characterization of Silver Nano particles using Aqueous Bark Extract of *Casuarina junghuhniana* and its Bio Efficacy.

Chathurdevi G[1], S. Uma Gowrie[2]*
[1]Research Scholar, Department of Plant Biology and Plant Biotechnology, Ethiraj College for Women, Chennai, Tamil Nadu, India.
[2*]Associate Professor, Department of Plant Biology and Plant Biotechnology, Ethiraj College for Women, Chennai, Tamil Nadu, India.
***Corresponding author's E-mail:** umasezhian@gmail.com

Accepted on: 11-06-2016; **Finalized on:** 31-07-2016.

ABSTRACT

Casuarina junghuhniana Miq. is the most important and widely spread actinorhizal plant due to its uses in soil reclamation, agro-forestry system and as wind breaks. It serves as a rich source of secondary metabolites of potential interest which plays a significant role in regulation of plant communities in growth and resistance to their pests and pathogen. The phytochemicals present in this plant is under explored. Therefore, in our present research work, potential phytochemicals present in aqueous extract of *Casuarina junghuhniana* bark was assessed and the same was used to synthesize silver nano particles. "Green synthesis" of silver nano particles has gained great interest due to eco-friendly and cost effective. Silver nano particles have wider application in various fields such as medicine, environmental sciences and in industries. In this present work, the silver nano particles were synthesized by the reduction of $AgNo_3$ using aqueous extract of *C.junghuhniana* bark. There is a rapid colour change from pale to reddish brown after the addition of bark aqueous extract to silver nitrate solution indicating the synthesis of silver nano particles. The different parameters such as pH, temperature, concentration of the extracts and stability were optimized to attain better synthesis of nano particles. The synthesized nano particles were initially observed using UV-VIS spectrophotometer, which showed the wave length between 415 to 435nm. The presence of functional groups was studied using FT-IR analysis. Further, the synthesized nano particles were characterized using SEM and EDX analysis. Different concentrations of silver nano particles were assayed for *in vitro* antibacterial activity against pathogens. The nano particles were also tested for the degradation of methylene blue dye under photo catalytic activity.

Keywords: Silver nano, *Casuarina,* optimization, characterization, antimicrobial, degradation.

INTRODUCTION

Casuarina junghuhniana Miq., is one of the most important members of nitrogen fixing, tall, fast growing, woody, non-legumes trees in the family of Casuarinaceae. It is an exotic species to India and native to Indonesia.[1] The outstanding ability of *Casuarina* to grow vigorously on nutrient deficient soil is due to their symbiotic association with *Frankia*-an actinomycete that fixes atmospheric nitrogen enabling tolerance to environmental constraints. Initially casuarinas are cultivated extensively for fuel wood purposes. But in recent years, this tree has been recognized as multipurpose tree species useful in area such as in Forestry (agro forestry, planted for crop rotation), Ecorestoration (in land reclamation, soil stabilization), Environmental protection (protective role during tsunami). It is mainly utilized as pulp in paper industry and also used for constructions as poles and pipe due to excellent stem straightness. It has the coppicing ability. However, emphasis is being laid world over on the improvement of yield and quality of these species, admittedly not much work has been documented on secondary metabolites of this plant.

Nanotechnology is one of the emerging fields of science which deals with the synthesis and development of various nano particles with the size less than 100nm. In recent years, green synthesis of silver nano particles has gained great interest. The synthesis of nano particles using plant extracts is the most adopted method due to green, eco-friendly, cost effective, stability and also has an advantage that the plants are widely distributed, easily available, much safer to handle and act as a source of several potent metabolites.[2] Silver nano particles have gained more attention because of their physical, chemical, electronic, catalytic, magnetic, antibacterial and biological activities. It is used as antimicrobials in medicine and agriculture, in pharmaceuticals as drug delivery and in water treatment also. They are also broadly applied in shampoos, soaps, detergents, cosmetics; toothpastes and hence directly encountered by human systems.[3]

The study of the metal bioaccumulation process in plants has revealed that the metals are deposited in the form of nano particles.[4] But the recovery of these nano particles from plant tissue is tedious and expensive. The localization of nano particles are based on the presence of particular enzymes and proteins involved in it and also it needs enzymes to degrade cellulosic materials. To overcome this, the *in vitro* approach of using plant extracts for the bioreduction of metal ions to form nano particles have been developed. The Plant phytochemicals such as primary and secondary metabolites are known as the potent natural resources for the synthesis of metallic nano particles.[5]

According to literature review, the presence of phytochemicals in *Casuarina junghuhniana* bark and its ability to synthesize silver nano particles is not documented so far. Therefore, the present research work was carried out with *Casuarina junghuhniana* bark samples. The objective of the present study is to screen the phytochemicals present in the aqueous bark extract of *Casuarina junghuhniana,* to test the ability of aqueous bark extract to synthesis silver nano particles, to optimize, characterize and to study the bio-efficacy of synthesized nano particles.

MATERIALS AND METHODS

Collection of Plant Material

The plus trees of *Casuarina junghuhniana* was identified from State Forest Research Institute, Kolapakkam, Chennai. Healthy bark samples were collected from 4 year old plantation site. Identification (authentication) of the plant sample was confirmed at Botanical Survey of India (BSI), Coimbatore, Tamil Nadu. Collected bark samples were washed thoroughly, dried and finely powdered.

Preparation of Aqueous Bark Extract of *Casuarina junghuhniana*

10g of finely powdered bark was extracted with 100 ml of distilled water (percolation process).The extract was filtered and stored for further experiments. Sterility conditions were checked and maintained for the effectiveness and accuracy in results at each and every step of the experiment.

Preliminary Phytochemical Screening

The aqueous bark extract was checked for the presence of following secondary metabolites such as alkaloids, phenols, tannins, flavonoids, terpenoids, proteins and amino acids, carbohydrates, steroids, phlobatanin saponin, Gum and mucilage using standard procedure.[6,7]

Synthesis of Silver Nano Particles

1mM silver nitrate solution was challenged with the aqueous bark extract of *Casuarina junghuhniana* at equal concentration. The reaction mixture was kept in dark (to avoid photo activation of $AgNo_3$) at room temperature for the reduction of silver ions to silver nano particles. Nano particle formation was visually observed by colour change and followed by UV-VIS spectrum analysis (UV 1650PC Shimadzu) at the resolution of 1 nm from 350–600 nm.[3]

Optimization of Nano Particles Synthesis[8]

- **Concentration ratio of bark extract and silver nitrate:** Similarly, the concentration ratio of bark extract and silver nitrate was optimized by using different ratios such as 1:1, 1;2, 1:3 (bark extract and silver nitrate solution 1mM respectively). The absorbance of the resulting solutions was measured spectrophotometrically.
- **pH:** The pH of this reaction was optimized by using different pH such as pH 3, 5, 7, 9, 11. The pH was adjusted using 0.1N HCl and 0.1N NaOH. The absorbance of the resulting mixtures was measured spectrophotometrically.
- **Temperature:** Different temperature such as 20°C, 40°C and 60°C was maintained for the reaction and absorbance of the resulting solutions was measured spectrophotometrically.
- **Stability:** Further, the optimized reaction solution was kept in dark at room temperature and stability of the synthesized silver nano particles was monitored up to 30 days using UV-Vis spectrum.

Characterization of Silver Nano Particles

- **By Colour Change:** The colour change in the reaction mixture was observed visually. The change of colour from yellowish brown to reddish brown indicates the synthesis of silver nano particles.
- **UV-VIS Spectral Analysis:** The synthesized silver nano particles were confirmed by spectrophometrically using double beam UV-Vis spectrophotometer at the range of 300 to 800nm at a resolution of 1 nm (UV 1650PC Shimadzu).
- **FTIR Analysis:** FTIR analysis was recorded using spectrum FT-IR system (Shimadzu, IR Affinity 1, Japan), equipped with a DLATGS detector with a mirror speed of 2.8mm/sec. scan range: from 400-4000cm^{-1} with a resolution of 4cm^{-}1. The synthesized silver nano particles were mixed with a KBr salt and compressed into a thin pellet. Infrared spectra were recorded on KBr pellet on a Shimadzu FTIR spectrometer 4000-500cm^{-1}.[9&3]
- **SEM and EDX Analysis:** The synthesized silver nano particles were centrifuged for 10 min at 10,000g. The pellet thus obtained was washed thrice to remove any residual of silver nitrate. The pellet of silver nano particles thus obtained was air dried and used for the study. The morphology and size of silver nano particles were observed using Scanning Electron Microscope and elemental analysis was carried out using Energy-dispersive X-ray spectroscopy (FEI Quanta FEG 200).[10]

Antibacterial Activity

Different concentrations (50 µg and 100 µg) of silver nano particles were assayed for antibacterial activity using Muller Hinton Agar media against different bacteria such as *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris* and *Staphylococcus aureus.* Assay was carried out by well diffusion method following the standard procedure. Gentamycin (100µg) was used as positive control. The plates were incubated at 37°C. Zone of inhibition around the well was observed after 24hrs for antibacterial assay. Triplicates were maintained.

Photo Catalytic Degradation of Dye

10 mg of methylene blue dye was added to 1000 ml of double distilled water and used as stock solution. About

10 mg of biosynthesized silver nano particles was added to 100 ml of methylene blue dye solution. A control was also maintained without addition of silver nano particles. Before exposing to irradiation, the reaction mixture was kept in shaker for 30 mins and then kept under sunlight and monitored at specific time intervals; aliquots of 2-3 ml suspension were filtered and used to evaluate the photocatalytic degradation of dye. The absorbance spectrum of the supernatant was subsequently measured using UV-Vis spectrophotometer at the different wavelengths. Concentration of dye during degradation was calculated by the absorbance value at 660 nm. Percentage of dye degradation was estimated by the following formula:

$$\%\, decolourization = 100 \times \frac{(C_0 - C)}{C_0}$$

where C_o is the initial concentration of dye solution and C is the concentration of dye solution after photocatalytic degradation.[10]

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The preliminary qualitative phytochemical screening of aqueous bark extract of *Casuarina junghuhniana* showed the presence of alkaloids, phenols, tannins, flavonoids, terpenoids, proteins and amino acids and carbohydrates. This study showed that *Casuarina junghuhniana* bark is a rich source of phyto constituents.

The plant potent metabolites such as polyphenols, terpenoids, sugars, alkaloids and proteins play an important role in bioreduction of metal ions to nano particles. Flavonoids (large group of polyphenolic compounds) can actively chelate and reduce metal ions to nano particles.[4] The phytochemicals present in the plant plays an important role as capping agents which stabilizes the silver nano particles in order to prevent in agglomeration.[11] Plant secondary metabolites have multiple functions and these metabolites serve as mediators in the interaction of the plant with its environment, such as plant–insect, plant–microorganism and plant–plant interactions. The production of secondary metabolites plays an important role in plant's defence system, by the constitutive production of antifeedants and phytoanticipins, and the inducible production of phytoalexins. In addition several plant secondary metabolites are used for the production of medicines, dyes, insecticides, flavours and fragrances.[12&13] Therefore, the presence of rich potent metabolites in the bark keeps *Casuarina junghuhniana* tree to be resistant from pests and pathogens.

Synthesis of Silver Nano Particles

The colour change from yellowish brown to reddish brown was observed after 12hrs of reaction, indicating the formation of silver nano particles by the reduction of $AgNo^3$ to Ag^+. This colour change is due to the Surface Plasmon Resonance (SPR) of the silver nano particles. The reduction of $AgNo^3$ to Ag^+ was due to the presence of rich secondary metabolites in the plant sample. Synthesis of silver nano particles has been reported by various researchers using plant extracts (*Atrocarpus elasticus stem bark)* as reducing agent. Silver nano particles colloidal solution showed intense yellow–brown colour, which occur only in nanoparticles due to strong interaction between light and conduction electron of silver in the solution.[14] Plant mediated synthesis of silver nano particles are broadly accepted method because plants are widely distributed; eco friendly production, safer to handle and plants are rich source of potent metabolites.[15]

Optimization of Nano Particles Synthesis

Concentration Ratio of Bark Extract and Silver Nitrate

Different concentrations of the bark extract and silver nitrate solution was optimized for the better synthesis of silver nano particles. It was observed that maximum synthesis occurred in 1:1 ratio (bark extract: silver nitrate solution). The UV-Vis spectrum for the effect of different concentration ratio in nano particle synthesis showed the λmax 425 nm for 1:1 ratio (Fig-1). As the concentration ratio increases, there was a shift in the peak with variations in intensity. Thus 1:1 ratio was found as optimum concentration and the other parameters were optimized based on this ratio.

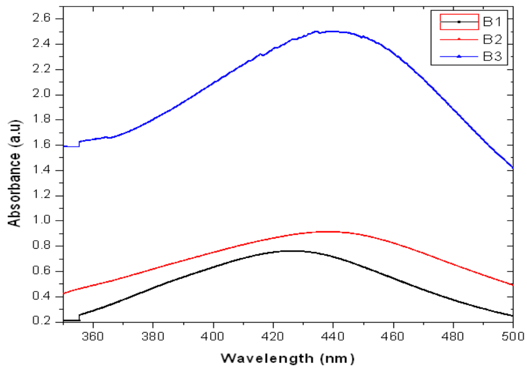

Figure 1: UV spectrum for the effect of different concentration ratio in nano particle synthesis (B1= 1:1, B2= 1:2, B3=1:3)

Effect of pH

pH is considered to be an important factor for the synthesis of silver nano formation. The reaction was adjusted with 5 different pH. The maximum synthesis was observed at neutral pH (pH7) with the formation of reddish brown colour and the UV-Vis spectrum showed λmax at 422nm. At acidic pH (pH 3 and 5) there was no colour change and at alkaline pH there was a rapid colour change, but a shift in peak was observed (Fig 2). Several researchers have reported that neutral pH was optimum for the synthesis of silver nano particles. An alteration in pH will change the charge in the natural phytochemicals of the plant extract, which affects their binding ability and reduce metal cations and anions in nano particles synthesis. This in turn affects the yield, size and shape of the nano particles.[16] The biomolecules are likely to be inactivated in acidic pH and thus it suppresses the nano particles synthesis. The differences in the colour change could also be due to the presence of various dissociated functional groups present in the plant extracts. At alkaline pH more number of small sized nano particles would be synthesized due to presence of large number of functional groups for silver binding.[10] Alkaline pH influences the formation of spherical shapes nano particles rather than ellipsoidal.[17]

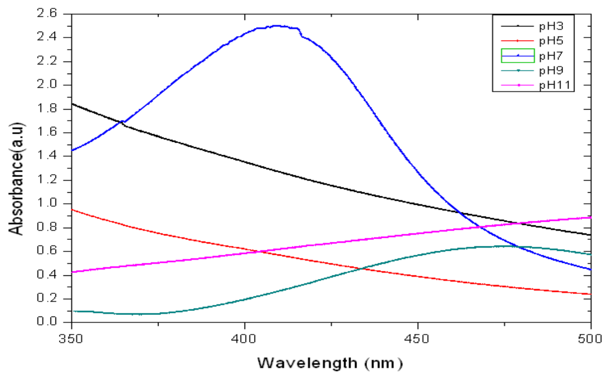

Figure 2: UV spectra for effect of different pH in nano particle synthesis.

Effect of Temperature

Temperature is another major factor which plays an important role in nucleation process in silver nano particles.[18] Three different temperatures were maintained. Maximum synthesis was observed at 40°C which showed a sharp peak at 437.4 nm (Fig 3).

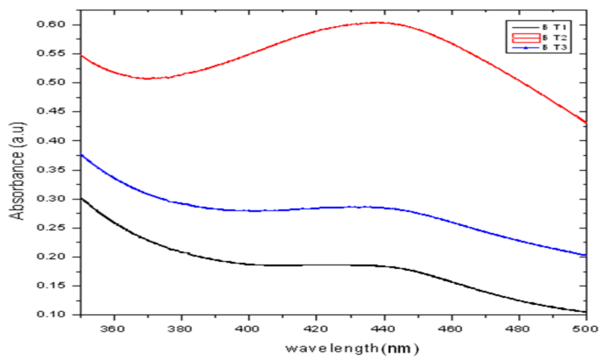

Figure 3: UV spectra for the effect of different temperature in nano particle synthesis. (T1=20^o, T2= 40^o, T3=60^o)

Increase in temperature increased the rate of formation of silver nano particles retarding the secondary reduction process. But further increase in temperature (60^{o}C) aggregation of particles occurs. Triangular silver nano particles was formed only at temperature above 30°C using *Alfalfa* plant.[19] The optimal temperature for synthesis of silver nano particles using *Saraca asoca* bark extract was 45°C which showed 433 nm and a shift in peak to 455nm was observed with further increase in temperature 65°C. The temperature dependent increase in the peak intensity supports temperature of controlled synthesis of silver nano particles.[20]

Stability

Stability is another important factor for synthesized silver nano particles. Fig 4 showed the UV spectrum of the synthesized nano particles at different days (1, 15, 30, 45). The UV spectrum showed λmax at 435nm on 45th day which showed the stability of the synthesized nano particles up to 45th day. The absorbance of silver colloid solution increased with span of time. Nano particles synthesized using plants extracts are surrounded by a thin layer of capping organic material from the plant, thus stabilizes the nano particles in solution up to 4 weeks. This is more advantageous over the chemical synthesis process.[3]

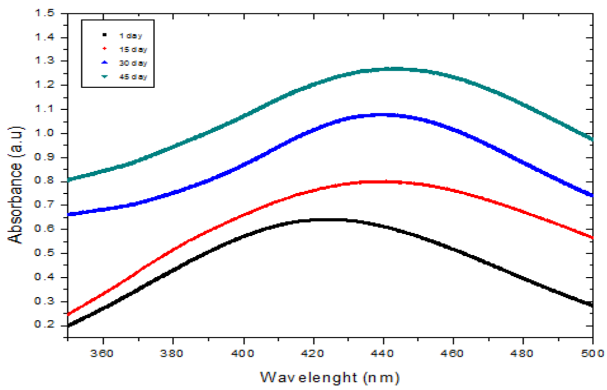

Figure 4: UV spectra for synthesized nano particle synthesis at different days.

Characterization of Silver Nano Particles

Synthesis of silver nano particles was confirmed by the characteristic Surface Plasmon absorption band at 421nm in uv vis spectrophotometer (Fig 5).

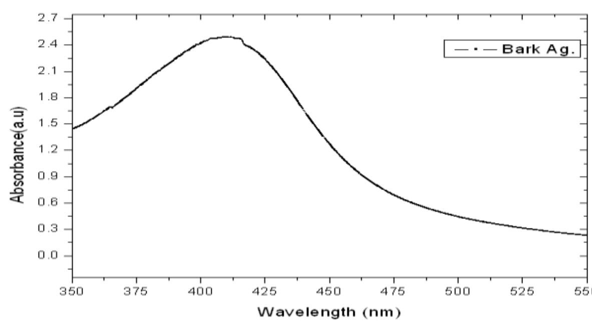

Figure 5: UV-Vis spectra recorded after 24hrs of reaction, showed peak at 421nm.

In this study, a single spectrum band was observed which indicates the spherical shape of silver nano particles. The UV visible spectroscopy is commonly used preliminary method for characterization of synthesized silver nano particles based on optical properties called Surface Plasmon Resonance (SPR). Light wavelengths in 300-800 nm are generally used for characterizing various metal nano particles in size range of 2 to 100nm. A single

Surface Plasmon Resonance (SPR) band corresponds to the spherical nano particles. A characteristic single peak at 420nm for synthesized silver nano particles was reported using *Annona reticulata* leaf extract.[21] Two or three SPR bands correspond to the anisotropic molecules.[22]

FTIR spectroscopy is useful in characterizing the surface chemistry. This FTIR study reveals the organic functional groups attached to the surface of nano particles and other surface chemical residues. FTIR spectrum for the synthesized silver nano particles was shown in (Fig 6). The intense band was observed at 3424.76 due to O-H stretching vibrations of phenolic compounds and other bands were 2927.01 -- C-H stretching of alkanes amides I band of proteins, 1643.20 – N-H bands of primary amines, 1380.13 – C-N stretching of aliphatic amines. The functional biomolecules such as hydroxyl, carboxylic, phenolic and amino group in *Morinda tintoria* aqueous leaf extract was involved in the reduction of silver ions to silver nano particles.[10] The carbonyl group from amino acid residues and proteins has the stronger ability to bind metal surfaces. Proteins could protect the nano particles and also capping the silver nano particles to prevent agglomeration and thereby stabilizes the particle size. Thus, biological molecules could perform both functions such as formation and also stabilization of silver nano particles.[20]

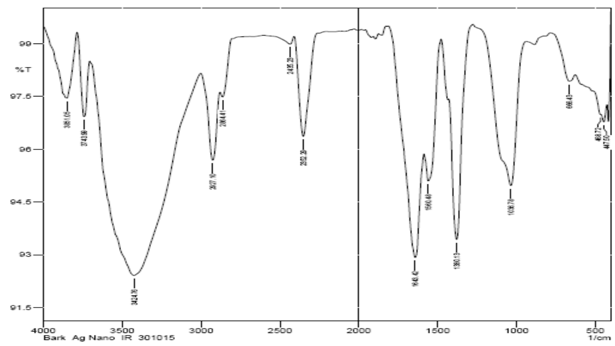

Figure 6: FTIR spectrum of synthesized silver nano particles

Scanning Electron microscope (SEM) image shows the shape and size of the synthesized silver nano particles. Size of the nano particles was observed at different magnifications. Spherical nano particles were observed with the size range from 39 to 42 nm (Fig 7).

In SEM image some of the nano particles showed large size due to aggregation of small size of silver nano particles. Similarly aggregation of nano particles was reported.[23,10] Control over size and morphology of nano structures may be related to the interactions of biomolecules present in the plant with the metal ions. Variations in the concentration and composition of potent metabolites in the plant may be also responsible for the morphological diversity of the nano particles.[4]

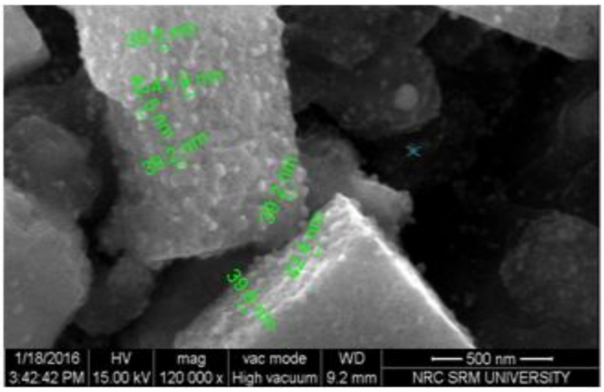

Figure 7: SEM image of synthesized silver nano particles

Elemental analysis of silver nano particles was measured by Energy-dispersive X-ray spectroscopy (EDX). Edx spectrum showed strong signals in silver region at 3 KeV and confirmed the formation of silver nano particles and its elemental nature (Fig 8). The signal was formed due to the excitation of Surface Palsmon Resonance of silver nano particles. EdX profile shows strong silver along with weak oxygen and carbon which may have originated from biomolecules bound to surface of silver nano particles. Strong signal in silver region at 3KeV was observed in synthesized silver nano particles using *M.tinctoria* leaf extracts. Some weak signals of Cl were also observed due to impurity in leaf extracts.[4]

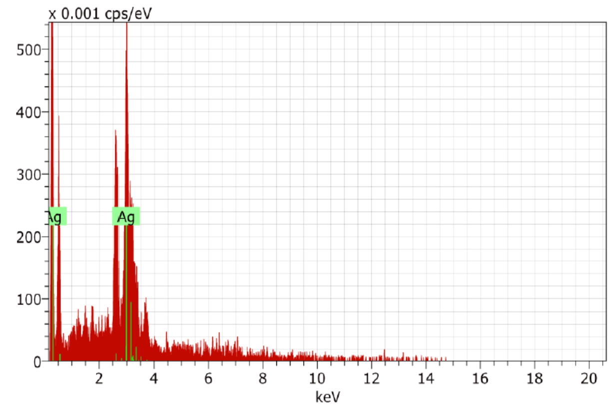

Figure 8: EDx spectrum of synthesized silver nano particles

Antibacterial Activity

Silver nano particles are reported for its wide range of antibacterial activity**.** In this study, synthesized silver nano particles at 100µg concentration showed maximum zone of inhibition against *Bacillus subtilis* (22±0.4mm), followed by *Pseudomonas aeruginosa* (18±0.2mm) and *Staphylococcus aureus* (16±0.3mm). At 50 µg concentration inhibition was found only against *Staphylococcus aureus* only (Fig 9). Different studies have proved that silver nano has range of antibacterial activity. AgNps synthesized using *Skimmia laureola* leaf extract showed maximum inhibition against *Staphylococcus aureus.*[24] AgNps utilizes multivalent or polyvalent mechanisms to exert their antibacterial activities.[25] AgNps, which are filled with polyphenolic compounds, disrupt the cell walls of bacteria. Polyphenolic compounds generate free radicals and other reactive oxygen species which can induce damage and toxicity to the cells.[26] Silver nano particles can affect the membrane permeability and respiratory function by attaching to the cell surface of the microorganisms.[2] Variations in the thickness and molecular composition of the membrane structures of gram positive and gram negative bacteria account for differences in their sensitivity to AgNps.[27]

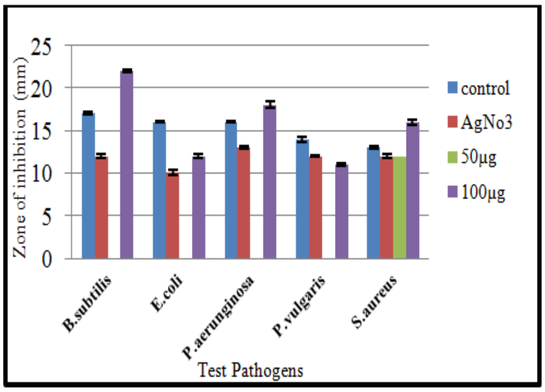

Figure 9: Antibacterial activity of silver nano particles against different bacterial pathogens

Photocatalytic Degradation of Dye

Synthesized nano particles were used for the degradation of methylene blue dye using photocatalytic activity. Dye degradation was initially noted by colour change from deep blue to light blue after 12 hrs of reaction time. The intensity in the absorbance value in absorption spectrum at 660nm for methylene blue was decreased gradually with increase in time and thus showed the photocatylic activity of methylene blue dye (Fig 10). The percentage of dye degradation was increased with increase in the exposure time. The percentage of degradation of dye was found to be 33.5% (at 36 hrs).

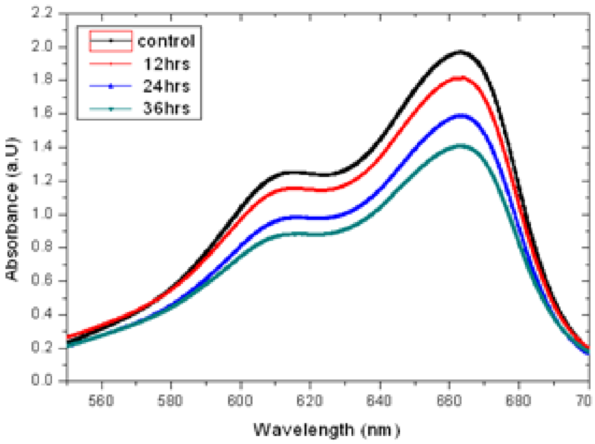

Figure 10: The absorption spectra of aqueous solution of methylene blue treated with synthesized silver nano particles.

This photocatalytic study of methylene blue dye by synthesized silver nano particles can be applied in degradation of the organic dye released from many industries. In the textile industry, many dyes specifically such as methylene blue or methyl red, are released into ecosystems through water waste. Many of these dyes are carcinogenic and cause deteriorating effects in humans. In photocatalytic reactions, surface area and number of active places used by the catalyst for absorbing pollutants, play an important role in degradation level.[20] AgNps synthesized using *M.tinctoria* leaf extract showed 95.3% of methylene blue dye degradation at 72hrs[10] and 41.3% degradation of dye was reported at 72hrs by AgNps synthesized using *Kalanchoe pinnata* leaf extracts.[11]

CONCLUSION

The present study revealed the potent phytoconstituents in aqueous bark extract of *Casuarina junghuhniana.* Effective green synthesis of silver nano particles using aqueous bark extract was observed. Optimization parameters for better synthesis of silver nano particles was found at concentration ratio 1:1, neutral pH (7), temperature 40°C and stability up to 30days.

Characterization of silver nano particles confirms the formation (UV spectrum), possible biomolecules (FT-IR), shape and size of particle (SEM & EDX). Synthesized silver nano particles are found to be effective in antimicrobial activity and dye degradation. Thus, *Casuarina junghuhniana* bark is found to be the source of potent secondary metabolites and able to synthesize silver nano particles.

The synthesized nano particles can be effectively used in pharmaceutical industries, for environmental protection to biodegrade organic dyes and as bioprotectant in agriculture.

Acknowledgement: The author thanks Mrs. Prema Sampathkumar, Associate professor and Head, the Faculty members and non teaching staff of the Department of Plant Biology and Plant Biotechnology, Dr. Mrs A. Nirmala Principal Ethiraj College for Women, (autonomous) Chennai 600008, for their valuable support and encouragement throughout the entire period of research. We would also like to express our thanks for the facilities extended by the Central Instrumentation centre of Ethiraj College for Women. Sincere Thanks to State Forest Research Institute, kolapakkam, Chennai-48 and SRM University for extension of facilities.

REFERENCES

1. Pinyopusarerk K & E. R. Williams. Variations in growth and morphological Characteristics of *Casuarina junghuhniana* Provenances grown in Thailand. *Journal of Tropical Forest Science*, 17(4), 2005, 574—587.

2. Geoprincy G, B.N. Vidhya Sri, U. Poonguzhali, N. Nagendra Gandhi and S. Renganathan. A Review on green synthesis of silver nano particles. Asian Journal of Pharmaceutical and Clinical Research, 6(1), 2013, 8-12.

3. Priya Banerjee, Mantosh Satapathy, Aniruddha Mukhopahayay and Papita Das. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization,

antimicrobial property and toxicity analysis. Bioresources and Bioprocessing, 1, 2014, 3.

4. Makarov V.V, A. J. Love, O. V. Sinitsyna, S. S. Makarova, I. V. Yaminsky, M. E. Taliansky and N. O. Kalinina. Green Nanotechnologies: Synthesis of metal nano particles using plants. Acta naturae, 6, 1(20), 2014, 35-44.

5. Marshall A.T, Richard G. Haverkamp, Clive E. Davies, Jason G. Parsons, Jorge L. Gardea-Torresdey, Dimitri van Agterveld. Accumulation of gold nanoparticles in *Brassica juncea*. International Journal of Phytoremediation, 9, 2007, 197–206.

6. Harborne A. J. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. 3, Springer Netherlands, 1998, 1-8.

7. Raaman N. Phytochmeical Techniques. New India Publishing Agency, 5, 2006, 19-25.

8. Krishnaraj C, R. Ramachandran, K. Mohan and P.T. Kalaichelvan. Optimization for rapid synthesis of silver nano particles and its effect on phytopathogenic fungi. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 93, 2012, 95–99.

9. John Coates. Interpretation of infrared spectra; A practical approach in encyclopedia of analytical chemistry. Meyers RA (ED). John Wiley and Sons Ltd, Chinchester., 2000, 10815-10837.

10. Vanaja M, K. Paulkumar, M. Baburaja, S. Rajeshkumar, G. Gnanajobitha, C. Malarkodi, M. Sivakavinesan, and G. Annadurai. Degradation of Methylene Blue Using Biologically Synthesized Silver Nanoparticles. Bioinorganic Chemistry and Applications, 2014.

11. Rohan Sharadanand Phatak and Anup Subhash Hendre. Sunlight induced green synthesis of silver nanoparticles using sundried leaves extract of *Kalanchoe pinnata* and evaluation of its photocatalytic potential. Scholars Research Library, 7(5), 2015, 313-324.

12. Harborne JB: Twenty-five years of chemical ecology. Natural Product Reports. 18, 2001, 361-379.

13. Dixon RA: Natural products and plant disease resistance. Nature, 411, 2001, 843-847.

14. Nur Iffah Shafiqah Binti Abdullah, Mansor B.Ahmad* and Kamyar Shameli. Biosynthesis of silver nanoparticles using *Artocarpus elasticus* stem bark extract. Chemistry Central Journal, 9, 61, 2015.

15. Ankamwar B, Damle C, Ahmad A, Sastry M. Biosynthesis of gold and silver nanoparticles using *Emblics officinalis* Fruit extract and their Phase Transfer and Transmetallation in an Organic Solution. Journal of nanoscience nanotechnology, 5(10), 2005, 1665-1671.

16. Pei Pei Gan and Sam Fong Yau Li. Potential of plant as a biological factory to synthesize gold and silver nanoparticles and their applications. Reviews in Environmental Science and Biotechnology, 11, 2012, 169–206.

17. Sathish Kumar.M, K. Sneha, S. W. Won, C. W. Cho, S. Kim and Y. S. Yun, "*Cinnamon zeylanicum* bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity," Colloids and Surfaces B, 73, 2, 2009, 332–338.

18. Neran Ali Thamer A and Lamia A. Almashhedy. Green synthesis optimization and characterization of silver nano particles using aqueous extract of *Crocus sativus* L. International Journal of Pharma and Bio Sciences, 5(4), 2014, 759–770.

19. Audra I. Lukman, Bin Gongb, Christopher E. Marjo, Ute Roessner and Andrew T. Harris. Facile synthesis, stabilization, and anti-bacterial performance of discrete Ag nanoparticles using *Medicago sativa* seed exudates. Journal of Colloid and Interface Science, 353, 2, 2011, 433–444.

20. Pratyusha Banerjee and Debjani Nath. A Phytochemical approach to synthesize silver nanoparticles for non-toxic biomedical application and study on their antibacterial efficacy. Nanoscience & Technology, 2015.

21. Shivakumar Singh P, Vidyasagar GM. Biosynthesis, characterization, and antidermatophytic activity of silver nanoparticles using Raamphal Plant (*Annona reticulata*). Aqueous Leaves Extract. Indian Journal of Material Science, 5, 2014.

22. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids and Surfaces B: Biointerfaces. 76, 1, 2010, 50-56.

23. Ramgopal M, C. H. Saisushma, I. H. Attitalla, and A. M. Alhasin, "A facile green synthesis of silver nanoparticles using soap nuts," Research Journal of Microbiology, 6, 5, 2011, 432–438.

24. Ahmed KBA, Subramanian S, Sivasubramanian A, Veerappan G, Veerappan A. Preparation of gold nanoparticles using *Salicornia brachiata* plant extract and evaluation of catalytic and antibacterial activity. Spectrochimica Acta Part A Molecul Biomol Spectros 130, 2014, 54–58.

25. Youmie Park. A New Paradigm Shift for the Green Synthesis of Antibacterial Silver Nanoparticles Utilizing Plant Extracts. Toxicology Research, 30, 3, 2014, 169-178.

26. Monteiro DR, Gorup LF, Takamiya AS, Ruvollo-Filho AC, de Camargo ER, Barbosa DB. The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. Int J Antimicrob Agents, 34, 2, 2009, 103–110.

27. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ. Antimicrobial effects of silver nanoparticles. Nanomed Nanotechnol Biol Med, 3, 2007, 95–101.

Source of Support: Nil, **Conflict of Interest:** None.