Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(18):14-19
(http://derpharmachemica.com/archive.html)

Green synthesis, characterization and biological studies on silver nanoparticles from *Caesalpinia bonduc* stem bark extract

N. Srinivasa Rao[a,b] and Mandava V. Basaveswara Rao[a*]

[a]*Department of Chemistry, Krishna University, Krishna Dist., Andhra Pradesh, India*
[b]*Department of Chemistry, SKBR Govt. Degree College, Macherla, Guntur Dist., Andhra Pradesh, India*

ABSTRACT

Nano-biotechnology gives emphasis for the synthesis of nanoparticles using living organisms such as microorganisms, plant extracts or plant biomass in an eco-friendly way. Among the various agents used for nanoparticle synthesis, plants and the extracts from various parts of the plant have found to possess important applications. The biomolecules found in plants induce the reduction of Ag^+ *ions from silver nitrate to silver nanoparticles (AgNPs). The Caesalpinia bonduc stem bark extract was used as reducing and stabilizing agent for the synthesis of silver nanoparticles. Synthesized nanoparticle is confirmed by the change of color from transparent yellow to dark brown indicates the formation of silver nanoparticles. UV-Vis absorption spectroscopy was used to monitor the quantitative formation of silver nanoparticles. The maximum Peak was observed at 490 nm and also characterized by SEM, TEM and the average particle size was found to be in between 5-50nm respectively. The antibacterial assay was done on Gram positive bacteria like Staphylococcus aureus, Basillus subtilis and Gram negative bacteria like Escherichia coli. The plant based route could be considered to be an environmental friendly, safe and economic biological method for the silver nanoparticles production.*

Keywords: Silver nanoparticles, *Caesalpinia bonduc*, Green synthesis, Eco-friendly, Antibacterial activity.

INTRODUCTION

Nanotechnology is a rapidly growing science of producing and utilizing nano-sized particles. It can be defined as the science and engineering involved in the design, synthesis, characterization and application of materials and devices whose smallest functional organization in at least one dimension is on the nanometer scale [1]. Over the past few years, synthesis of metal nanoparticles has become an important research topic in modern material science due to their distinctive potential applications in the field of electronics, magnetics, optoelectronics, information storage and drug delivery [2–6]. The synthesis of metallic nanoparticles is an active area of academic research in the field of nanotechnology. A variety of chemical and physical procedures could be used for synthesis of metallic nanoparticles.

However, these methods are fraught with many problems including use of toxic solvents, generation of hazardous byproducts, and high energy consumption. Accordingly, there is an essential need to develop environmentally benign methods for synthesis of metallic nanoparticles. A promising approach to achieve this objective is to exploit the array of biological resources in nature. Indeed, over the past several years, plants, algae, fungi, bacteria, and viruses have been used for the production of low cost, energy efficient, and nontoxic metallic nanoparticles [7].

Among the different living organisms used for nanoparticles synthesis, plants are of particular interest in metal nanoparticles synthesis because of its advantage over other environmentally benign biological process as it eliminates the elaborate process of maintaining cell cultures [8 – 10]. Plant mediated synthesis of nanoparticles is gaining importance due to its simplicity and ecofriendliness Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process [11, 12]. The most important application of silver nanoparticles in medical industry is topical ointments to prevent infection against burn and open wounds [13]. The reduction of Ag+ ions by combinations of bio molecules found in the extracts such as vitamins, enzymes/proteins, organic acids such as citrates, amino acids, and polysaccharides [13]. Currently, the investigation of this fact has regained importance due to the increased bacterial resistance to antibiotics, caused by their over usage.

Caesalpinia bonduc (L.) Roxb. is found throughout the hotter parts of India, Common in West Bengal, South India and Maharashtra. It is often grown as hedge plant. An armed liana, up to 15 m in height, found wild throughout the plains of India and up to an altitude of 1,000 m in the Himalayas; it is also found in deltaic regions of western, eastern and southern India. Branchlets glossy, black armed with recurved prickles at the base of pinnae and elsewhere [14].Seeds contain bonducin, saponin, a bitter substance phytosterinin, a thick yellow fatty oil (20-24%) having a disagreeable odour of the following fatty acid composition: palmitic,[14,15]; stearic,[7.5]; oleic,29.0; linoleic, 59.0%, and lignoceric in traces. The defatted kernels contain α-,β-,γ-,δ- and ε-caesalpins[15], furanoditerpenoidcaesalpinF[16], homoisoflavonebonducillin[17], Cassanefurano diterpenoid (Bonducellpin E, F and G) [18] and an amorphous glycoside, Bonducin.

All parts of *Caesalpinia bonduc* (bark, leaves, roots, seed) are used in traditional system of medicines. The roasted seed powder is used as an anti leprotic. The seeds are useful as antidiabetic, antiperiodic, antipyretic [19] . Fixed oil expressed from the seeds is a remedy in discharges from the ear; is used as an embrocation in rheumatism, and to remove freckles from the face as a cosmetic [20]. The objective of the present study was the synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the aqueous extract of medicinal plants and evaluation of antibacterial activity of synthesized silver nanoparticles.

MATERIAL AND METHODS

***2.1 Medicinal Plant Extract Preparation*:** The fresh and healthy stem bark (20g) of *Caesalpinia bonduc* sample was collected from Tank of Rampet Village, Warangal District, Telangana state, India, Collected material was washed, cleaned, finely chopped and soaked in 100ml milli Q water. Further the material was boiled in conical flask for 15-20 min. Obtained stem bark extract was filtered through Whatmann's filter paper no.1 and stored in refrigerator at 4ºC for further experiment.

***2.2 Synthesis of silver nanoparticles*:** 1 mM $AgNO_3$ solution was prepared and stored in amber colour bottle and used in future experiment work and Silver nitrate ($AgNO_3$) was obtained from Sigma Aldrich. 10 ml plant material broth was added to 90ml 1 mM aqueous silver nitrate with constant stirring and allowed to react at ambient conditions for reduction into Ag^+ ions. The observed color change of reaction mixture from transparent yellow to dark brown indicates the formation of silver nanoparticles from the stem bark extract of *Caesalpinia bonduc*. The content was centrifuged at 20,000 rpm for 20 minutes. The supernatant obtained was used for the analysis like antibacterial activity.

2.3 Characterization of *Caesalpinia bonduc* silver nanoparticles (CbAgNPs):
An ELICO SL-159 UV-Vis spectrophotometer was used for the spectrometric analysis to confirm silver nanoparticles formation. The stem bark extract was used as reference blank. The purified suspension was oven dried and the powder was subjected to FTIR spectroscopy analysis (Bruker α- spectrophotometer) in the diffuse reflectance mode at a resolution of $4cm^{-1}$ in KBr pellets. Further the morphology of synthesized AgNPs was determined by using SEM in Zeiss 700 Scanning electron microscope. In addition to SEM, Transmission electron microscope (TEM) was used for characterizing size and shape of green synthesized silver nanoparticles in Philips model CM 200 instrument operated at an accelerating voltage at 200 kV.

2.4 Antibacterial activity using Disc Diffusion method: The antibacterial activity of synthesized silver nanoparticles was determined using disc diffusion assay method. Luria Bertani media was prepared and poured into sterilized Petri plates and then plates were spreaded with *Staphylococcus aureus*, *Basillus subtilis* and *Escherichia coli*, separately. Then sterile discs were kept and the samples were added in different concentrations to the disc and plates were incubated at 37^0C for 24 hours. Then zone of inhibition was measured.

RESULTS AND DISCUSSION

The synthesis of silver nanoparticles is an advanced technique in modern nanotechnology and is evolving as an important branch of nanotechnology. This study deals with the synthesis and characterization of silver nanoparticles using stem bark extract of *Caesalpinia bonduc*. Synthesized silver nanoparticles were reddish brown in color. The color of the extract was changed from light yellowish to reddish brown after addition of $AgNO_3$ and on incubation for 15min at 65^0C. The coloration was due to the excitation of the surface Plasmon vibration in the silver nanoparticles [21]. The reduction rate and formation of nanoparticles can be increased further by increase in temperature [22].The bactericidal effect [23-25] of CbAgNPs was measured by disc diffusion method.

3.1 UV-Vis spectrophotometer: The UV-Vis spectroscopy was the preliminary technique for the characterization of the silver nanoparticles. The UV-Vis absorption was analyzed after centrifuging and redispensing the particles in deionized water, the maximum broad absorption peak was observed at 470nm was confirmed that polydispersed nanoparticles were formed.

3.2 FTIR analysis of silver nanoparticles: The FTIR analysis is the technique used for the identification of change in functional groups. The reduced sample was centrifuged and powdered to stable form. FTIR study showed peaks at $3415cm^{-1}$,$3018cm^{-1}$, $2880cm^{-1}$, aliphatic amine (C-H), $2372cm^{-1}$, $1616cm^{-1}$ carboxylic acid, 1436 cm^{-1}, aromatic amine (C-N), $1373cm^{-1}$, $1218cm^{-1}$, $1069cm^{-1}$, $603cm^{-1}$. FTIR spectrum showed the aliphatic amine, and aliphatic alkenes of alkaloids and terpenoids bound on the surface of CbAgNPs.

3.3 TEM analysis: Transmission Electron Microscopy gave a detailed descriptive image of the silver nanoparticles synthesized with their structural details and their size. The synthesized silver nanoparticles were scanned using TEM from which we conclude that the average mean size of silver nanoparticles was in between 5-60 nm (Fig. 1, 2). The silver particles are crystalline, as can be seen from the selected area diffraction pattern recorded from one of the nanoparticles in the aggregates as shown in Fig. 3.

3.4 SEM analysis: The silver nanoparticles synthesized by using Cajanus Cajan leaf extract were scanned using Scanning Electron Microscope. The images showed relatively spherical shaped nanoparticles were observed at a magnification of x4.06 K (Fig. 3). The high density crystalline powdered silver nanoparticles synthesized by the stem bark extract was found to be 5-60 nm (Fig .4). This confirms the silver nanoparticles were formed by *Caesalpinia bonduc* stem bark extract.

3.5 Antibacterial activity by disc diffusion technique: Antibacterial activity of green synthesized silver nanoparticles against Gram positive *Staphylococcus aureu, Basillus subtilis and* Gram negative *Escherichia coli* [26] bacteria at different concentrations showed that they revealed a strong dose dependent antibacterial activity against the test microorganisms. It was seen that, as the concentration of green synthesized nanoparticles were increased, bacterial growth decreases in both the cases. The zone of inhibition of silver nanoparticles against Gram positive bacteria *Staphylococcus aureus, Bacillus subtilis* and Gram negative bacteria *Escherichia coli* shown in and (Table 1). The results indicated that silver nanoparticles synthesized from *Caesalpinia bonduc* stem bark extract showed effective antibacterial activity in Gram positive than in Gram negative bacteria respectively.

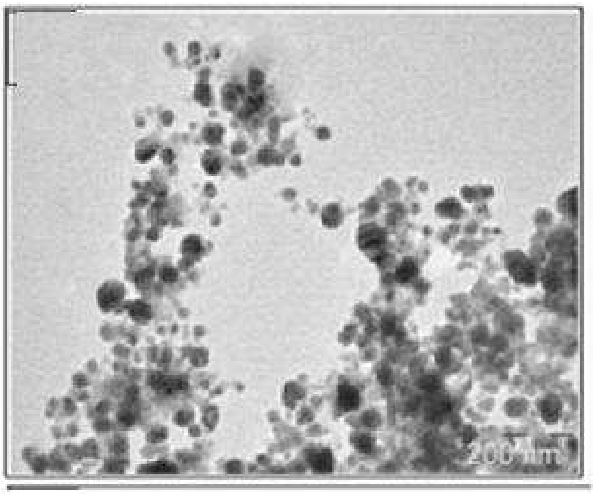

Fig . 1

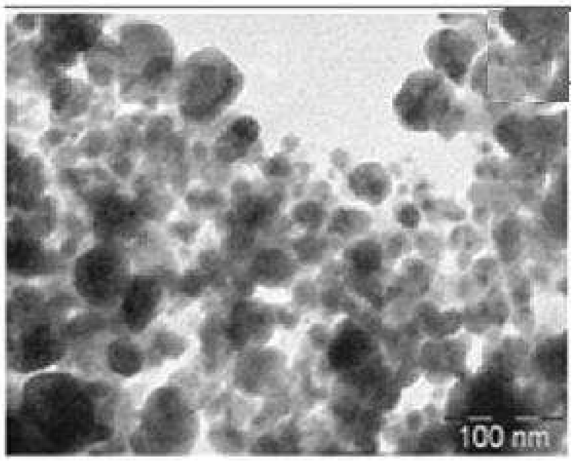

Fig . 2

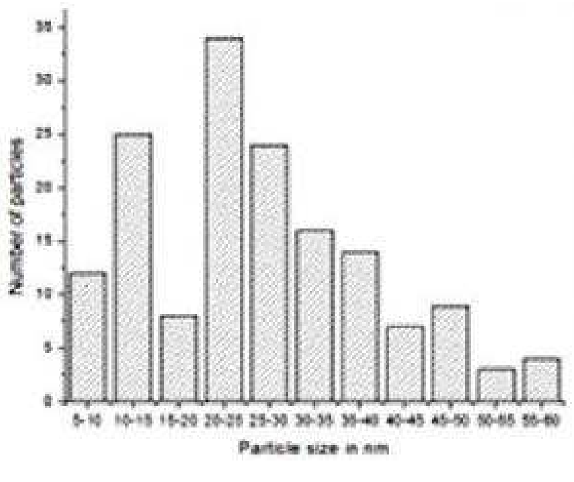

Fig. 3

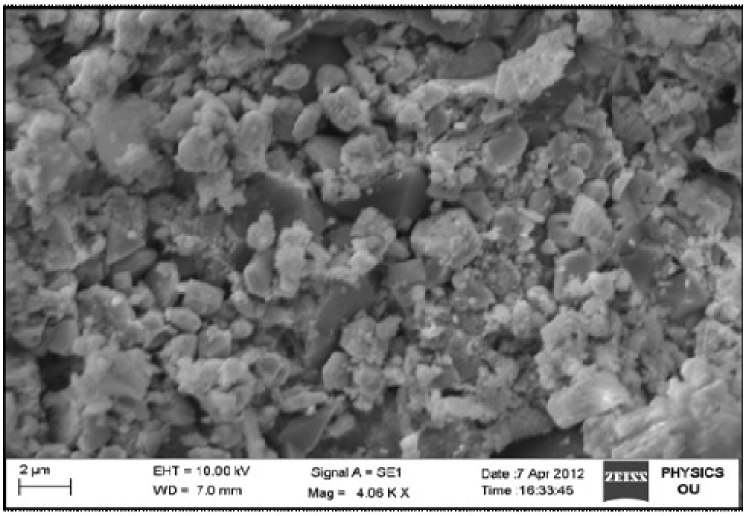

Fig 4: SEM image of silver nanoparticles synthesized from *Caesalpinia bonduc*stem bark extract at magnification of x4.06 K

Table-1: Antimicrobial activity of stem bark extract of *Caesalpinia bonduc*

S. No.	Bacterial species	Inhibition zone (mm)			
		Control (Plant extracts)	CbAgNPs	silver nitrate	Ampicilline
1.	*Escherichia coli*(Gram negative)	7	15	9	20
2.	*Staphylococcus aureus*(Gram positive)	9	16	11	28
3.	*Bacillus subtilis*(Gram positive)	10	18	10	28

CONCLUSION

In this study, silver nanoparticles which were synthesized from *Caesalpinia bonduc* stem bark extract showed antibacterial activity against Gram positive and Gram negative bacterial strains. Further, Zone of inhibition was performed against Ampicillin and leaf extract which was used as a control respectively. Thus it is proven from this study that the silver nanoparticles synthesized from *Caesalpinia bonduc* stem bark extract seems to be promising and effective antibacterial agent against bacterial strains. This green chemistry approach towards the synthesis of silver nanoparticles is highly essential effort being addressed in nanomedicine because of its varied advantages. Plant extract being very ecofriendly and cost effective can be used for the large scale synthesis of silver nanoparticles in nanotechnology processing industries.

REFERENCES

[1] P S. Gavarkar, R S. Adnaik, S K. Mohite, C S. Magdum; *International Journal of Universal Pharmacy and Bio Sciences* **2014**, 3(4), 392-396.
[2] J Palanivelu, M M Kunjumon, A Suresh, A Nair, C Ramalingam *; J. Pharm. Sci.& Res.*, 2015, 7(9),690-695.
[3] Y Cui, C M Lieber. *Sci* **2001**, 291, 851-853.
[4] T Tuutijarvi, J Lu, M Sillanpaa, G Chen. *J. Haz Mat* **2009**; 166, 1415–1420.
[5] K T Mater. *Mat. Let.*, **2007**, 61, 23-24.
[6] H-W Zhang,Yi Liu, S-H Sun. *Front Phy., in China* **2010**, 5*, 347-356.*
[7] D R Bhumkar, H M Joshi, M Sastry, P V B. Chitosan; *Pharma Res* **2007**, 24, 9257-9.
[8] K Mallikarjuna, G Narasimhan, G R Praveenb, B Sreedhar, C Sreelakshmi, BVS Reddy, D Raju; *Digest J Nano and Biostruct.*, **2010**, 6, 181-186.
[9] N. Kaushik, M. S. Thakkar, S. Snehit, M. S. Mhatre, Y. Rasesh and M. S. Parikh, *J. Nanomedicine*, **2010**, 6, 257.
[10] A. Leela and M. Vivekanandan, *African J. Biotechnol*, **2008**, 7, 3162. & SJ Klaine , PJ Alvarez ,GE Batley, TF Fernandes, RD Handy, DY Lyon et al, *Environ Toxicol Chem*., **2008**, 1825–51.
[11]Government of India Department of AYUSH, Agaru, In: The ayurvedic pharmacopoeia of India, Part 1, Volume II, and First Edition, The Controller of Publications, **2004**, 25–27.

[12]S.S. Shankar, A. Rai, A. Ahmad, M. Sastry, *J. Colloid Interface Sci*., **2004**, 275, 496–502.
[13]JR Soberon , MA Sgariglia , DA Sampietro , EN Quiroga , J Vattuone, *J. Appl. Microbiol*., **2006**, 102, 1450-1461.
[14] C.P Khare, Springer Publication, **2007**, 116.
[15] Anonymous, The Wealth of India, The Dictionary of Indian Raw Materials and Industrial Products, Ca - Ci revised Ed., New Delhi (India), **1999**, 3, 6 – 17.
[16] L. Canonica, G. Jommi, P. Manitto, F. Pelizzoni, *Tetrahedron Letters*, **1963**, 29, 2079 - 2086.
[17] K.O Pascoe, B A Burke, W.A Chan, F Caesalpin, *J. Nat. Prod*., **1986**, 49, 913 – 915.
[18] K K Purushottaman, K. Kalyani,K Subramainan,S.P Shanmuganathan,*Indian J. Chem*.,**1982**,21B, 383.
[19] K Pudhom, D Sommit, N. Suwankitti, A Petsom, *J. Nat. Prod.,* **2007**, 70, 1542-1544.
[20]K M Nadkarni, Indian Materia Medica. IIIrd Ed., Vol - I, India, Popular Book Depot, **1954**, 229. 10. R N Chopra, S L Nayar, I C Chopra, CSIR Publication, **1956**, 43-44.
[21] S. Shankar, A. Rai, B. Ankamwar, A. Singh, A. Ahmad, and M. Sastry, *Nature Materials*.**2014**, 3, 488.
[22] F Martinez-Gutierrez, P L. Olive, A Banuelos, E Orrantia, N Nino, E M Sanchez, F Ruiz, H Bach, Y Av-Gay, *Nanomedicine: Nanotechnology Biology and Medicine*. **2010**, 6, 681–688.
[23]C. Krishnaraj, E.G. Jagan,f, et.al. *Colloids and Surfaces B: Biointerfaces*. **2010**,76 , 50–56.
[24] R Roy, M R Hoover, A S Bhalla, T Slaweekl, S. W Dey, W Cao et al, *Materials Research Innovations*. **2008**, 11, 3–18.
[25] A. Rai, A. Singh, A. Ahmad, and M. Sastry, *Langmuir*. **2006**, 22, 736–741.
[26] M. Singh, S. Singh, S. Prasad, and I. S. Gambhir, *Journal of Nanomaterials and Biostructures*. **2007**, 115–122.