ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Green Synthesis of plant-mediated silver nanoparticles using *Withania somnifera* leaf extract and evaluation of their antimicrobial activity

Veera babu Nagati[1], Rama Koyyati[1], Rajkiran Banala[2,] Jahnavi Alwala[1], Manisha R Donda[1], Pratap Rudra Manthur Padigya[1]*

1. Department of Biochemistry, Osmania University, Hyderabad- 500 007, India.
2. Department of Zoology, Osmania University, Hyderabad- 500 007, India.

Manuscript Info

Manuscript History:

Received: 05 October 2013
Final Accepted: 22 October 2013
Published Online: November 2013

Key words:
Withania somnifera, Fourier Transform Infrared (FTIR), Transmission electron microscope (TEM), Scanning Electron Microscopy (SEM), Ashwagandha silver nanoparticles (AsAgNPs)

Corresponding Author

Pratap Rudra Manthur Padigya

Abstract

To develop a reliable, eco-friendly and easy process for the synthesis of silver nanoparticles using leaf extract of medicinal plant '*Withania Somnifera*' and evaluate its anti-microbial properties. The synthesis and characterization of silver nanoparticles was confirmed by UV-Visible spectrophotometer, Fourier Transform Infrared (FTIR), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Disc diffusion assay method was used to confirm the antibacterial activity of silver nanoparticles. UV-Visible absorption spectra of the reaction medium containing silver nanoparticles showed maximum absorbance at 455 nm. FTIR analysis confirmed reduction of Ag^+ ions to Ag^0 ions in synthesized silver nanoparticles. The SEM and TEM analysis showed the particle size between 5-40nm, and spherical in structure. The silver nanoparticles have shown bactericidal effects against *Escherichia coli and Staphylococcus aureus*. The leaf extract of *W. somnifera* quickly reduces Ag^+ to Ag^0 and enhances synthesis of silver nanoparticles with highly anti-microbial activity.

1. Introduction

Nanoparticles are being considered as fundamental building blocks in nanotechnology. The most important and distinct property of nanoparticles is that they exhibit larger surface area to volume ratio. Metal nanoparticles have tremendous applications in science and technology. The most effectively studied nanoparticles today are those made from noble metals (Sintubin L et al., 2009) in particular Ag, Pt, Au and Pd. Among the above four, silver nanoparticles play a significant role in the field of biology and medicine. A variety of techniques including physical and chemical methods have been developed to synthesize silver nanoparticles. The physical methods are highly expensive and chemical methods are harmful to the environment (Yang W.T.et al., 2011). Therefore, there is a growing need to develop ecofriendly nanoparticles that do not use toxic chemicals in the synthesis (Elumalai E.K. et al., 2010; Mohsen Zargar et al., 2010 and Rajesh W. Raut et al., 2010). Silver nanoparticles are having high toxicity to various micro-organisms (Kim et al., 2011; Saad S et al., 2011 and Swarna Jaiswala et al., 2012). The recent reports include the synthesis of nanoparticles using medicinal plants (Mukunthan KS et al., 2011; Prasad TNVKV et al., 2011). This stands as a great application in the field of nano-medicine. Medicinal property of the extract and nano-silver could play vital role in treatment of many diseases (Akhil Gupta et al., 2011; Shreesh Kumar Ojha and Dharamvir Singh Arya 2011). Most of the reported green synthesis methods using plants (Begum N. A et al. 2009) took more than 1 hour for the formation of colloidal silver. In the present study, the silver nanoparticles were synthesized in less time using the leaf extracts of the plant *Withania somnifera*.

The plant also known as Ashwagandha or Indian ginseng belongs to Solanaceae (nightshade) family (Sushma Jain et al., 2001; Elsakka M et al., 1990). It is a well known plant for promoting vigor and vitality (Akhil Gupta et al., 2011). An antioxidant (Vidhi Mehrotra et al., 2011) adaptogenic (Bhattacharya SK; Muruganandam AV 2003 and Singh B et al., 2001) (having capability to increase body's resistance to stress), aphrodisiac, anti-

inflammatory, sedative, mood elevator [Bhattacharya SK et al., 2000] etc. are some of the properties of this plant. The biochemical constituents of Ashwagandha plant are called withanolides (Elsakka M et al., 1990) until today, 12 alkaloids, 35 withanolides, and several sitoindosides from this plant have been isolated and studied. Ashwagandha is believed as general energy-promoting and has disease prevention property and improves the immunity (Satish K. Verma et al., 2012).

2. Materials and Methods:

Withania somnifera leaves were taken from the Local fields of Khammam, Andhra Pradesh, India. All of the reagents and solvents were procured from Himedia laboratories, Mumbai, India. Silver nitrate ($AgNO_3$) was procured from Sigma Aldrich USA.

2.1 Plant material and preparation of the Extract:

Fresh leaves of Ashwagandha (*Withania somnifera*) were used to make the aqueous extract. 25 gms of fresh green leaves were thoroughly washed thrice with distilled water followed by double distilled water to remove the dust particles and other contaminants. This plant material was chopped into fine pieces and was taken in a clean 250 ml Erlenmeyer conical flask and 100 ml of sterile double distilled water that was added and incubated on a sand bath for 30 mins to facilitate the formation of aqueous leaf extract. The extract was then filtered using Whatman No. 1 filter paper. The plant extract is used for the synthesis of silver nanoparticles and the extract can be stored at 4^0C for further use.

2.2 Preparation of 1mM Silver nitrate solution:

For the preparation of 1mM Silver nitrate ($AgNO_3$) 0.0421gms of $AgNO_3$ was added to 100 ml of double distilled water. The solution was mixed thoroughly and stored in an amber colored bottle in order to prevent auto oxidation of silver.

2.3 Synthesis of Silver nanoparticles:

For the synthesis of plant mediated silver nanoparticles, the leaf extract and 1mM silver nitrate solution were taken in 1:4 ratio respectively and continuously stirring on the hot plate at 60^0C for 30 minutes until the color change was observed. This indicates the preliminary confirmation for the formation of Ashwagandha silver nanoparticles (As-Ag Nanoparticles).

2.4 UV-Vis Spectra analysis:

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after 30 min. UV-Vis spectrophotometer is procured from ELICO SL-159. A small aliquot of the sample was taken for UV-Vis spectrum analysis (350-750 nm). The maximum absorbance spectrum of As-Ag nanoparticles was observed at 455 nm.

2.5 FTIR analysis of As-Ag nanoparticles:

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 15000 rpm for 10 mins and the resulting suspension was washed with sterile distilled water. Thereafter, the purified suspension was dried to obtain stable powder. Finally, the dried nanoparticles were analyzed by FTIR Nicolet Avatar 660 (Nicolet, USA).

2.6 SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of synthesized and stabilized silver nanoparticles were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid and sample was analyzed for morphology and size of the silver nanoparticles.

2.7 TEM analysis of silver nanoparticles:

The distribution of Ag nanoparticles throughout the Ashwagandha was evident from the transmission electron microscope (TEM) image. The synthesized silver nanoparticles were used for analysis of particle size. TEM specimens were prepared by drop casting one or two drops of aqueous solution onto carbon coated copper grids, which were allowed to dry at room temperature overnight. Transmission electron microscope (TEM) (Philips Tacna G2 FEI F12, operating at 80–100 kV) was used to investigate morphology and size of the particles.

2.8 Antibacterial assays:

The antibacterial assays were done on human pathogens such as *Staphylococcus aureus* and *Escherichia coli* by standard disc diffusion method. Luria Bertani (LB) broth/agar medium was used to grow bacteria. Fresh inoculums (100 µl) of overnight cultures were spread on to LB agar plates. Sterile Whatman No.1 paper discs of 5mm diameter (containing 10µg of As-Ag nanoparticles) along with Ampicillin standard antibiotic containing discs were placed on each plate. After incubation overnight at 37^0C, zone of inhibition was measured (mm in diameter).

3. Results:

It is well known that silver nanoparticles exhibit reddish brown color in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles. The Ashwagandha leaf extract was mixed to the aqueous solution of the silver ion complex; the change in color is observed from yellowish green to reddish brown indicating the reduction of silver ion (Fig.1B-C); and formation of silver nanoparticles and which is confirmed by UV–Vis spectroscopy. The UV-Vis spectra were recorded for the reaction medium after 30 mins by continuously stirring on the hot plate at 60^0C. Absorption spectra of silver nanoparticles formed in the reaction media has shown maximum absorbance at 455 nm (Fig.2A) broadening of peak indicated that the particles are poly-dispersed. The green synthesized silver nanoparticles by employing Ashwagandha leaf extract was further demonstrated using Fourier Transform Infra red spectroscopy (FTIR) (Fig.3) showed a strong peak at *1612*cm^{-1} silver nanoparticles is due to the reduction by capping material of plant extract corresponding to Methyl 7-oxooctadecanoate involved in reduction of $AgNO_3$ (Ag^+ to Ag^0). Scanning Electron Microscopy (SEM) image showing the high density silver nanoparticles synthesized in the presence of Ashwagandha leaf extract was further confirmed by the development of silver nanostructures (Fig.2B). The TEM analysis (Fig.4A-C) showed the particle size between 5-40 nm as well the spherical structure of the nanoparticles. In the figures (Fig.5A &B), the antibacterial activity of silver nano particles (5 µg and 10 µg), leaf extract and Ampicillin (10 µg) on *Staphylococcus aureus* and *Escherichia coli*, shows clear increase in inhibition zones with increase dose and its evident in comparison to leaf extract alone and 10µg concentration of silver nanoparticles shows similar effects as 10 µg Ampicillin (Table 1).

Fig.1 *(*A) *Withania somnifera* (Ashwagandha) Leaves and (B) Digital photographs of Ashwagandha Leaf extract and Ashwagandha silver nanoparticles.

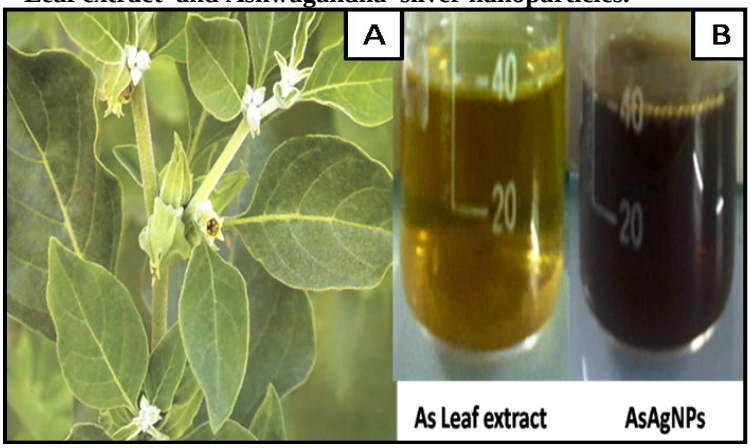

Fig. 2 (A) UV-Visible absorption spectrum and (B) SEM analysis of silver nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with *Withania somnifera* leaf extract.

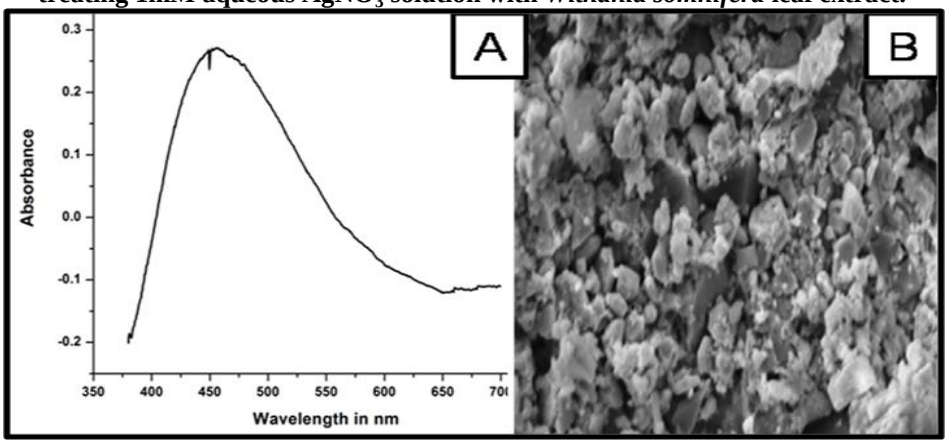

Fig.3: FTIR spectra of vacuum dried powder of synthesized *As-Ag nanoparticles*

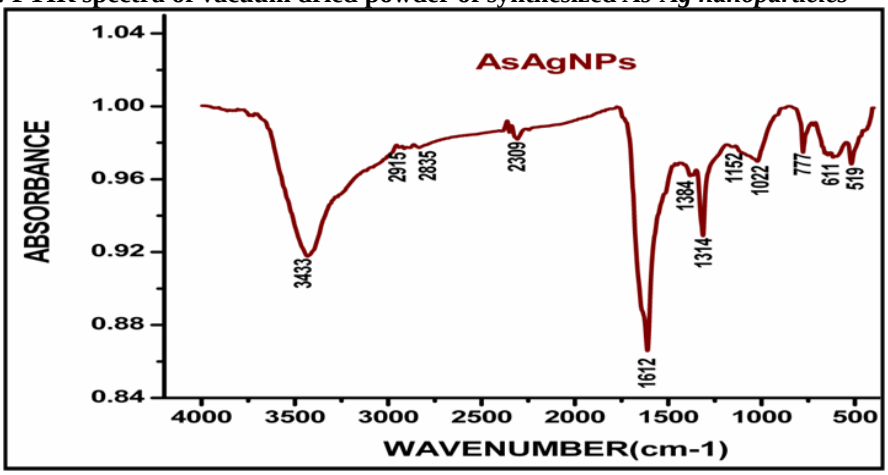

Fig.4: (A) TEM image of Ag nanoparticles dispersed in Ashwagandha matrix; (B) Selected area of electron diffraction pattern of silver Nanoparticles; (C) Corresponding to the histogram showing particle size distribution.

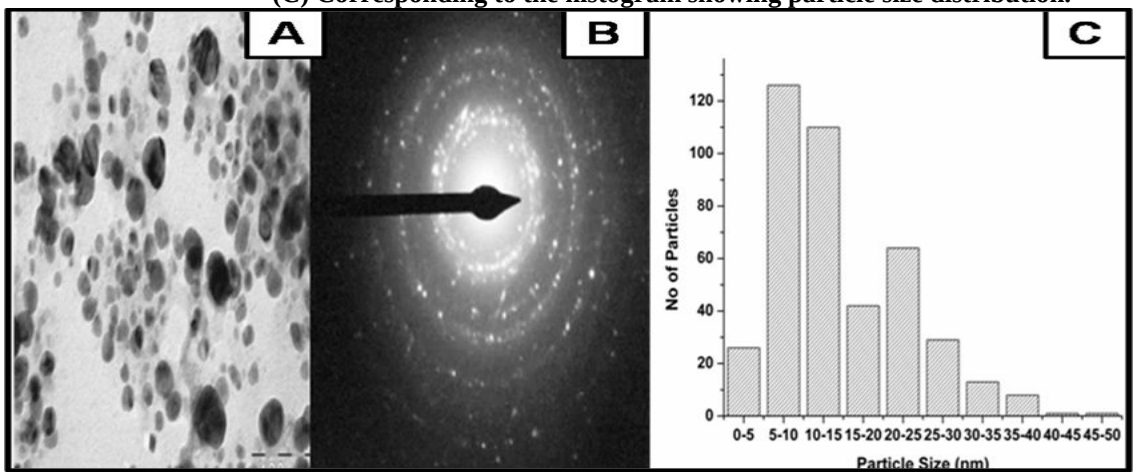

Fig.5: Antibacterial activity of silver nanoparticles on (A) *Escherichia coli* and (B) *Staphylococcus aureus.* *(1mg/ml solution: discs containing 1= 5µgr of AsAgNPs, 2=10µl of AsAgNPs, 3=10µl of leaf extract, A=5µl of Ampicillin)*

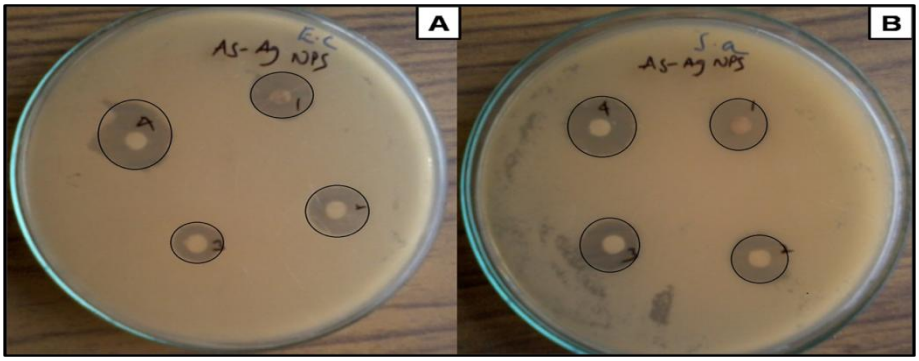

Table 1 Antimicrobial activity of the *AsAgNps* (Ashwagandha silver nanoparticles) synthesized using *Withania somnifera* leaf extract by Disc diffusion method.

Concentration added to each disc (1mg/ml)	Zone of inhibition of AsAgNps in mm	
	E. coli	*Staphylococcus aureus*
(1) 5µl AsAgNps (5µg)	14	8
(2) 10µl AsAgNps (10µg)	15	13
(A) Ampicillin (10µg)	16	14
(3) Leaf Extract (10µl)	5	6

Discussion and Conclusion:

In the present we have demonstrated the potential of leaf extract of the Ashwagandha plant in reducing aqueous Ag^{+} to Ag^{0} ions and the formation of eco-friendly silver nanoparticles with fairly well-defined dimensions . The present study provides evidence that the leaves are good source for synthesizing stable silver nanoparticles in lesser time. This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic, shelf life and viability, etc. These eco-friendly nanoparticles could be used as an excellent source against multi drug resistant bacteria, enhancing wound healing process, and act as anticancer, anti-stress agent. The green synthesis of nanoparticles can also be used in large-scale for synthesizing nanoparticles from other inorganic materials. Though there is a report describing synthesis of gold and silver nanoparticles using Ashwagandha root and dried leaf extracts, but the present study used fresh leaves as a source which is economic and easily available for synthesis. The Silver nanoparticles synthesized via green route are highly toxic to multidrug resistant bacteria (Fig.5A-B, table-1) and due to its great potential it can be considered as one of biomedical application in near future. The present study showed a simple, rapid and economical route to synthesize silver nanoparticles.

Acknowledgements:

The authors acknowledge to the UCG New Delhi for the funding of the Major research project UGC F. No. 34-289/2008 (SR) dated 02-01-2009 and CFRD Osmania University, Hyderabad for the FTIR analysis.

References:

Ai Lan Chew, Jeyanthi James Antony Jessica, Sreenivasan Sasidharan. (2012): Antioxidant and antibacterial activity of different parts of Leucas aspera. Asian Pacific Journal of Tropical Biomedicine; 2(3): 176-180.

Akhil Gupta, Anuj Mittal, Ashok Kumar. (2011): Nature's treasurer: plants acting on colon cancer. Journal of Stress Physiology & Biochemistry; 7(4): 217-231.
Anal K. Jha, K. Prasad (2010): Green Synthesis of Silver Nanoparticles Using *Cycas* Leaf *International Journal of Green Nanotechnology: Physics and Chemistry*, 1: 110–117.
Bankura K.P., D. Maity. Synthesis, characterization and antimicrobial activity of dextran stabilized silver nanoparticles in aqueous medium. Carbohydrate Polymers 2012; 89: 1159– 1165.
Bhattacharya A, Ghosal S, Bhattacharya SK. (2001): Antioxidant effect of *Withania somnifera* glycowithanolides in chronic footshock stress induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. *J Ethnopharmacol*; 74: 1-6.
Bhattacharya SK, Bhattacharya A, Sairam K, Ghosal S. (2000): Anxiolytic-antidepressant activity of *Withania somnifera* glycowithanolides: an experimental study. *Phytomedicine*; 7: 463-469.
Bhattarcharya SK, Muruganandam AV. (2003): Adaptogenic activity of *Withania somnifera*: an experimental study using a rat model of chronic stress. *Pharmacol Biochem Behav*; 75: 547-555.
Bharani M., Thirunethiran Karpagam et.al. (2012): Synthesis and Characterization of Silver Nano Particles from *Wrightia Tinctoria.* International Journal of Applied Biology and Pharmaceutical Technology; 3: 58-63
Bogdan C. Nitric oxide and the immune response. *Nature Immunol* 2001; 2: 907-916.
Das R., S. S. Nath, et.al. (2009): Preparation of Silver Nanoparticles and Their Characterization. A.Zojono, Journal of nanotechnology.
Dravyaguna, Bhaisajya vyakhyana, (2006): Ayurvedic herbal pharmacology and pharmacy; Elsevier, pg: 76. Chapter 3.
Elsakka M, Grigorescu E, Stanescu U, et al. (1990): New data referring to chemistry of Withania somnifera species. Rev Med Chir Soc Med Nat Iasi; 94: 385-387.
Elumalai E.K., T.N.V.K.V. Prasad, V. Kambala, et.al. (2010): Green synthesis of silver nanoparticle using *Euphorbia hirta* L. and their antifungal activities, Arch. Appl. Sci. Res; 2(6): 76-81.
Iuvone T, Esposito G, Capasso F, Izzo A. (2003): Induction of nitric oxide synthase expression by *Withania somnifera* in macrophages. *Life Sci*; 72: 1617-1625.
Fidel Martinez-Gutierrez, M.Sc, Peggy L. Olive, Ph.D, et.al. (2010): Synthesis characterization and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. Nanomedicine: Nanotechnology, Biology, and Medicine; 6: 681–688.
Jae Yong Song A, Beom Soo Kim. (2009): Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst Eng; 32: 79–84.
Justin Packia Jacob S., J.S. Finub, Anand Narayanan, (2012): Synthesis of silver nanoparticles using Piper longum leaf extracts and its cytotoxic activity against Hep-2 cell line. Colloids and Surfaces B: Biointerfaces; 91: 212– 214.
Khandelwal N, Singh A, Jain D, Upadhyay MK and Verma HN: (2010): Green synthesis of silver nanoparticles using *Argimone mexicana* leaf extract and Evaluation of their antimicrobial activities, Digest J Nanomater Biostruct; 5: 483-489.
Kim, Soo-Hwan, Hyeong-Seon Lee, (2011): Antibacterial Activity of Silver-nanoparticles Against Staphylococcus aureus and Escherichia coli Korean J. Microbiol. Biotechnol; 39(1): 77–85.
Krishnaraj C., E.G. Jagan,f, et.al. (2010): Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. Colloids and Surfaces B: Biointerfaces; 76: 50–56.
Mohsen Zargar, Azizah Abdul Hamid , Fatima Abu Bakar , Mariana Nor Shamsudin. (2011): Green Synthesis and Antibacterial Effect of Silver Nanoparticles Using *Vitex Negundo* L. *Molecules*; *16*: 6667-6676.
Mukunthan KS, Elumalai EK, et.al, (2011): Catharanthus roseus: A natural source for synthesis of silver nanoparticles. Asian pacific journal of tropical biomedicine; 1(4): 270-74.
Prasad TNVKV, Elumalai EK, et.al, (2011): Evaluation of the antimicrobial efficacy of phytogenic silver nanoparticles. Asian pacific journal of tropical biomedicine; 1: S82-S85.
Rajesh W. Raut, Niranjan S. Kolekar, Jaya R. Lakkakula, Vijay D. Mendhulkar and Sahebrao B. Kashid, (2010): "Extracellular Synthesis of Silver Nanoparticles Using Dried Leaves of Pongamia pinnata (L) Pierre", Nano-Micro Lett.; 2: 106-113.
Saad S, Taher M, Susanti D, Qaralleh H, Rahim NABA. (2011): Antimicrobial activity of mangrove plant (Lumnitzera littorea). Asian Pac J Trop Med; 4(7): 523-52.
Satish K. Verma, Asima Shaban, Reena Purohit, Madhvi Let al. (2012): Immunomodulatory activity of Withania somnifera (L.) Journal of Chemical and Pharmaceutical Research,; 4(1): 559-561.

Shreesh Kumar Ojha and Dharamvir Singh Arya. (2009): Withania somnifera Dunal (Ashwagandha): A Promising Remedy for Cardiovascular Diseases. World Journal of Medical Sciences; 4 (2): 156-158.

Singh B, Saxena AK, Chandan BK, et al. (2001): Adaptogenic activity of a novel, withanolide-free aqueous fraction from the root of *Withania somnifera*. *Phytother Res*; 15: 311-318.
Sintubin L, De Windt W, Dick J, Mast J, Ha DV, Verstraete W, Boon N. (2009): Lactic acid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles. Appl Microbiol Biotechnol; 84(4):741–749.
Sukumaran S, Kiruba S, Mahesh M, Nisha SR, Miller PZ, Ben CP,et al. (2011): Phytochemical constituents and antibacterial efficacy of the flowers of Peltophorum pterocarpum (DC.) Baker ex Heyne. Asian Pac J Trop Med; 4(9): 735-738.
Sushma Jain, Sunil Dutt Shukla, Kanika Sharma, Maheep Bhatnagar. (2001): Neuroprotective Effects of *Withania somnifera* Dunn. in Hippocampal Sub-regions of Female Albino Rat. . Phytotherapy Research; 15: 544-548.
Swarna Jaiswala,, Patrick McHale, Brendan Duffy, (2012): Preparation and rapid analysis of antibacterial silver, copper and zinc doped sol–gel surfaces. Colloids and Surfaces B: Biointerfaces; 94: 170– 176.
Vaidyanathan R, Gopalram S, Kalishwaralal K, Deepak V, Pandian SRK, Gurunathan S (2010): Enhanced silver nanoparticle synthesis by optimization of nitrate reductase activity. Colloids Surf B; 75(1): 335–341.

Vidhi Mehrotra, Shubhi Mehrotra,Vandna Kirar, Radhey Shyam,et al. (2011): Antioxidant and antimicrobial activities of aqueousextract of Withania somnifera against methicillin-resistant Staphylococcus aureus J. Microbiol. Biotech. Res; 1 (1): 40-45.
Yang W. T., H. Li, Y. Gong, W. Y. Chen, C. Gaidau. (2011): Preparation of silver nanoparticles of enhanced antibacterial effect with benzalkonium bromide. Journal of optoelectronics and advanced materials; 13(6): 661 - 665.
Ziauddin M, Phansalkar N, Patki P, et al. (1996): Studies on the immunomodulatory effects of Ashwagandha. *J Ethnopharmacol*; 50: 69-76.