WORLD JOURNAL OF PHARMACEUTICAL RESEARCH

Volume 3, Issue 1, 1339-1351. Research Article ISSN 2277 – 7105

SYNTHESIS OF SILVER NANOPARTICLES OF AQUEOUS EXTRACT OF PLANT *LANTANA CAMARA* AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITY

Nilesh Meghani*, Heena Mankani and Saraswathy Nagendran

SPPSPTM, SVKM's NMIMS, Vile Parle (W), Mumbai, India.

Article Received on
21 October2013

Revised on 23 November 2013,
Accepted on 26 December 2013

DOI: 10.20959/wjpr20141-798

***Correspondence for Author:**

Nilesh Meghani,

SPPSPTM, SVKM's NMIMS, Vile Parle (W), Mumbai, India.

meghnaninilesh@yahoo.com

ABSTRACT

It has been confirmed that aqueous silver ions can be reduced by aqueous extract of plant parts to produce stable silver nanoparticles. Antibacterial effect of nanoparticles of *Lantana Camara* can add on to the antibacterial effect of plant extract apart from being environmental friendly process. In this work, silver nanoparticles of aqueous extract of plant *Lantana Camara* was developed and its antibacterial effect by disk diffusion method was evaluated with *B. Subtilis*, *S. Aureous*, *S. Typhi* and *E.coli*. Silver nanoparticles were synthesized using $AgNO_3$ solution and aqueous extract of *Lantana Camara* with different ratios i.e. 1:4, 3:2, 4:1, 2:3, 2:2 and for different time intervals i.e. 30 minutes, 1, 2, 3, 4, 5, 6, and 24 hours. Characterization of synthesized particles was done using UV visible spectroscopy, Fourier transform infrared spectroscopy, X-ray diffraction, transmission electron microscopy and antibacterial studies. The effect of washing of the diffusion disk with distilled water on antibacterial property was also evaluated. This study shows the possible use of biologically synthesized nanoparticles of aqueous extract of *Lantana Camara* as an antibacterial.

Keywords: UV visible spectroscopy, X-ray diffraction, nanoparticles.

INTRODUCTION

Nanotechnology has demonstrated tremendous potential by producing major advances in energy, including economic solar cells and high-performance batteries; electronics, with ultrahigh density data storage and single-atom transistors; and food and agriculture, offering smart delivery of nutrients and increased screening for contaminants, and health and medicine [1]. There has been no universally accepted classification of the definitions of

"nanotechnology" and "nanomedicine" and continue to be an area of controversy. Nanomedicine is the use of nanotechnology in medicine which utilizes the advanced and novel physical, chemical and biological properties of materials at the nanometric scale [2]. Nanomedicine takes advantage of two general phenomena that occur at the nanoscale: transitions in physiochemical properties and transitions in physiological interactions. Many of the early definitions of nanotechnology employ a cut-off around 100 nm (including that of the National Nanotechnology Initiative (NNI) [3], focusing on the former, where quantum effects are often restricted to structures on the order of ones to tens of nanometers [4,5]. However, unique physiochemical behavior sometimes emerges for nanomaterials with defining features greater than 100 nm (e.g., the plasmon-resonance in 150 nm diameter gold nanoshells that are currently under clinical investigation for cancer thermal therapy [6]. In addition, many of the benefits (and risks) of nanomedicine are related to the unique physiological interactions that appear in the transition between the molecular and microscopic scales. At the systemic level, drug bioavailability is increased due to the high relative surface area of Nanoparticles [7] and greater accumulation in the various pathological conditions like cancerous tissue due to its enhanced permeability and retention effect [8]. The nanoparticles produced by using the nanotechnology is known to produce completely new or improved particles based on specific characteristics such as size, distribution and morphology. Synthesis of silver nanoparticles has drawn particular attention due to their distinct properties like catalysis, magnetic and optical polarizability, electrical conductivity and antimicrobial activity (9). The antibacterial effects of silver salts is well known since ancient times [10], and its use as bactericidal agent in a variety of applications including dental work [11], catheters [12] and burn wounds [13] has been implicated. Silver confers oligodynamic effect on the microbes by reducing Ag ions which incorporates into cell membrane and cause the cell death via leakage of intracellular substances [14,15].

From various bioreductant, *Lantana camara* leaves extract was selected for this study because (I) it is easily available in the nature (ii) anti-microbial properties of the synthesized silver nanoparticles might be enhanced due to synergistic effects of *Lantana camara* leaves [16].

The principal objective of this work was to study and establish the anti-microbial property of the aqueous extract of leaves of silver nanoparticles of *Lantana camara*.

MATERIALS AND METHODS

SYNTHESIS OF SILVER NANOPARTICLES

Silver nitrate (AgNo3) solution of concentration of 1 mM was freshly prepared .Fresh leaves of *Lantana camara* was washed 3 times washed with distilled water upon collection and was finely cut. Moisture was removed from the leaves with the use of blotting paper. Different concentrations from 5% to 80% of leaves with double distilled water was prepared and boiled for 10 minutes in water bath and mixture was than filtered with 0.45 micron filter paper to obtain aqueous extract.

The extract was immediately mixed with 1mM AgNo3 solution at different ratios i.e. 1:4, 3:2, 4:1, 2:3 and 2:2 make up 100 ml volume in 250 ml volumetric flask and were incubated for different time intervals i.e. 30 minutes, 1, 2, 3, 4, 5, 6, and 24 hours.

PURIFICATION OF SILVER NANOPARTICLES

To obtain he dry powders of the silver nano particles, the broth containing the nanoparticles were centrifuged at 10 000 rpm for 10 minutes, following that the pellets were redispersed in sterile distilled water to get rid of any uncoordinated biological molecules. This process of centrifugation and re-dispersion in sterile deionized distilled water was repeated three times to obtain the better separation of free entities from the metal nanoparticles. The purified pellets than freeze dried using a lyophilizer.

CHARACTERIZATION OF THE SILVER NANOPARTICLES

UV-VISIBLE SPECTROSCOPY

Preliminary identification of the silver nanoparticles was carried out using UV-Visible spectroscopy as noble metal, especially gold (Au) and Silver (Ag) nanoparticles exhibit the unique optical properties because of their surface plasmon resonance (SPR) dependent on size, shape and size distribution of silver nanoparticles (17). The formation of silver nanoparticles was confirmed by measuring the UV-Visible spectra recorded of the solutions after dilution a small aliquot (0.2 ml) of the sample 20 times. UV visible spectra were recorded on double beam spectrophotometer from 300nm to 700nm.

FTIR SPECTROSCOPY

The purified powders of silver nanoparticles of Lantana Camara mixed with completely dried Kbr pellets were subjected to FTIR spectroscopy measurement on Perkin-Elmer spectrum. For comparison plain dried KBr pellets were used.

XRD ANALYSIS

X-ray diffraction (XRD) analysis of drop coated films of silver nanoparticles in sample was prepared for the determination of the formation of silver nanoparticle by JDX 8030 diffractometer operated at a voltage of 40 kV and with 30 mA current. Scanning range was selected from 20° to 80°.

TEM ANALYSIS

The transmission electron micrographs of the purified dried silver nanoparticles were taken using the Tecnai G-20, a 200 kV TEM with a W-source and an ultra high resolution pole piece with a point-point resolution of 1.9 Å.

ANTI-MICROBIAL ANALYSIS

The anti-microbial property of silver nanoparticles coated with paper discs was tested on *B.subtilis, S. Aurious, S. Typhi* and *E.Coli* obtained from National Cell Culture Laboratory, Pune, India by disc diffusion method. The microorganisms were cultured on Mueller Hinton agar at 37 ºC during 24 hours. A sterile cotton swab was used to inoculate the surface of another Mueller Hinton agar plate rotating the plate every 60º for homogenous growth. The nanoparticles coated paper discs were placed on the agar plates. The width of zone of inhibition was measured after 24 h incubation at 37 ºC.

RESULTS AND DISCUSSION

On adding the aqueous extract of the *Lantana camara* leaves to AgNo3 solution, the color of the reaction medium was changed to brown. The appearance of brown color was because of the excitation of surface plasmon vibrations, characteristic of silver nanoparticles [18]. The localized surface Plasmon resonances arise due to collective oscillations of the conduction electrons confined to the metallic nanoparticles. Excitation of localized surface Plasmon results in strong light scattering by an electric field at a wavelength where resonance occurs resulting appearance of a strong surface Plasmon absorbance bands [19].

The UV visible spectrum was obtained on varying the concentration of the *Lantana Camara* leaves extract. The particles synthesized with 5% of broth concentration gave a very weak plasmon resonance band (absorbance value 0.11) at 446.85 nm. On increasing the concentration to 20%, λmax increased to 453.75 nm (sharp peak, figure 1) with an absorbance of 0.46. For the 50 % and 80% concentration, there was no change in the value of λmax i.e. 463.05 nm (flat peak).

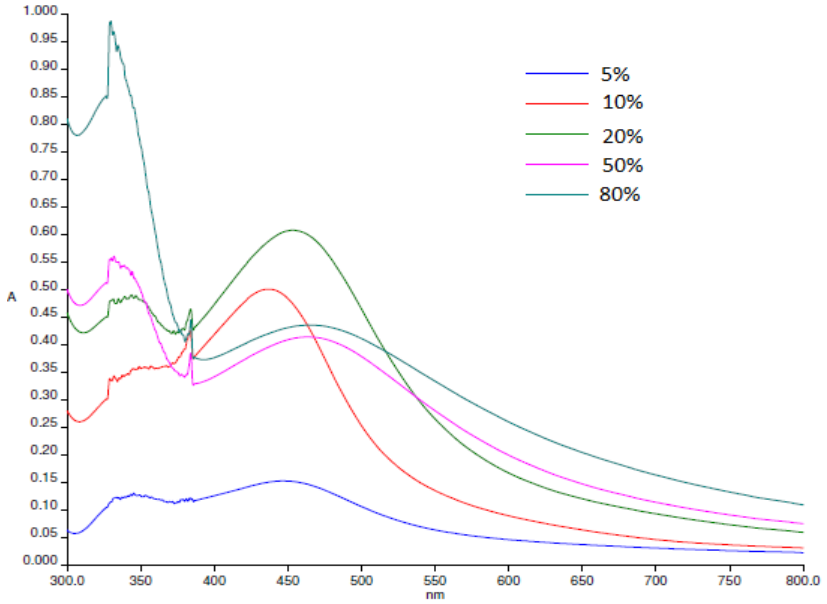

Figure 1: UV analysis of the sample solution after 4 hours of incubation at different concentrations

Table 1: UV analysis of sample solution after 4 hours of incubation at different concentration

Concentration (%)	λmax (nm)	Absorbance
5	446.85	0.11
10	437.50	0.28
20	453.75	0.46
50	463.05	0.49
80	463.05	0.51

The results of UV analysis of sample solution after 4 hours of incubation at different concentration are given in below table 1 and figure 1.

Since, from the above experiments, sharp peak was achieved at 20% concentrations, trial was preceded with the concentration range between 20% and 50%, where sharp peak was founded at 40% concentration. Further experiments were carried out with 20% and 40% of the *Lantana camara* extract as the absorbance was highest with sharp peak as compared to other concentration. The different ratios of the mixture of AgNo3 solution and extract of *Lantana Camara* was chosen, i.e. 1:4, 3:2, 4:1, 2:3 and 2:2. At 20% concentration, the maximum absorbance was obtained at 462 nm (λmax) for 1:4 ratio. For, ratios 2:3 and 2:2, no peaks

were found. Similarly, at 40% concentration, the maximum absorbance was obtained at 468 nm (λmax) with 1:4 ratio. For, ratios 2:3 and 2:2, no peaks were found.

As from the above data, the 40% concentration yielded maximum absorbance and so further analysis was carried out at different time points of incubation, i.e., 30 min, 1 , 2 3, 6, 24, and 48 hours. On increasing interaction time, from 30 min to 3 hours, a shift in λmax from 450 nm to 464 nm was noted. On increasing interaction time further upto 24 hours, the maximum absorbance was observed with sharp peak at 471.07 nm. For 48 hours, absorbance was than decreased.

Further, we evaluated the different ratio of Plant extract and Silver nitrate solution for the 20% and 40% after 4 hours of incubation, as the absorbance were highest at these concentrations compared to other concentration.

For 20% concentration, results of UV analysis for the different ratios are given in table 2 and figure 2.

Table 2: UV analysis for the 20% concentration with different ratios

Ratio	λmax (nm)	Absorbance
1:4	462.02	0.80
3:2	454.90	0.68
4:1	453.20	0.48
2:3	No peak	-
2:2	No peak	-

As per the data shown in the above table, the maximum absorbance (λmax) was observed at 1:4. For, ratios 2:3 and 2:2, no peaks were found.

Table 3: UV analysis for the 40% concentration with different ratios

Ratio	λmax (nm)	Absorbance
1:4	468.14	0.90
3:2	454.90	0.80
4:1	459.16	0.68
2:3	No peak	-
2:2	No peak	-

As per the data shown in the above table, the maximum absorbance (λmax) was observed at 1:4 ratio. For, ratios 2:3 and 2:2, no peaks were found.

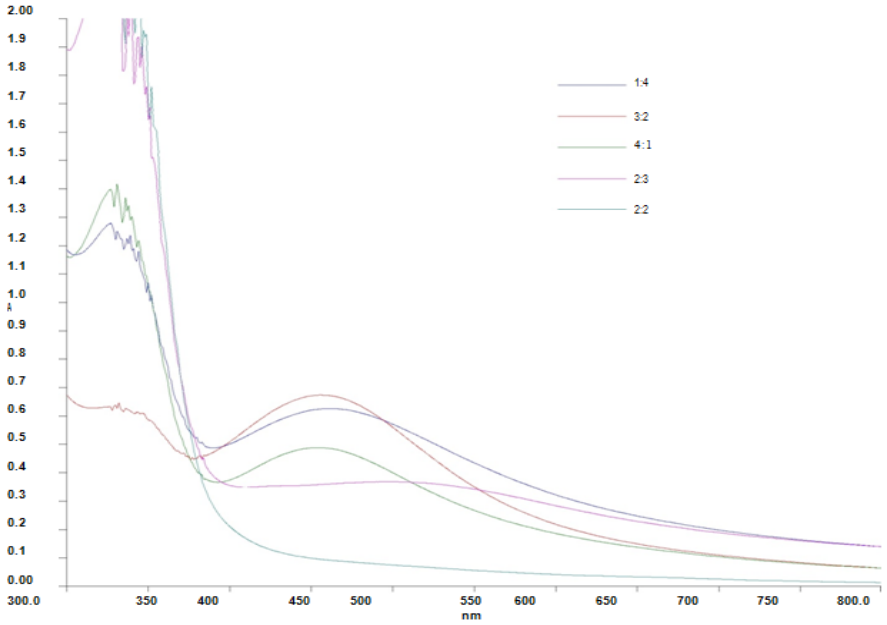

Figure 2: UV analysis for the 20% concentration with different ratios

For 40% concentration, results of UV analysis for the different ratios are given in table 3 and figure 3.

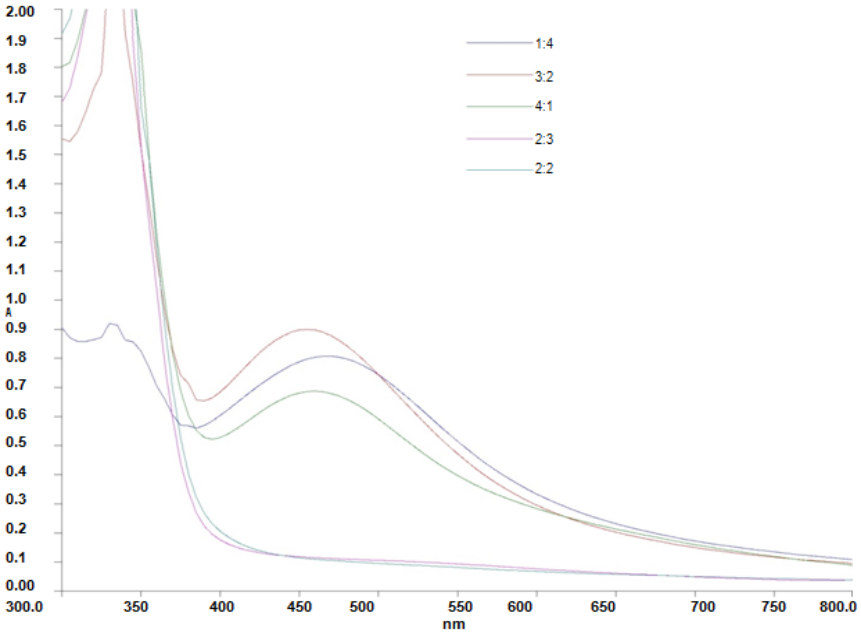

Figure 3: UV analysis for the 40% concentration with different ratios

As from the above data, the 40% concentration yielded maximum absorbance and so further analysis was carried out at different time points of incubation, i.e., 30 min, 1 , 2 3, 6, 24, and 48 hours. Results are given in the below table 4 and figure 4.

Table 4: UV analysis for the 40% concentration at different time interval

Time points	λmax (nm)
30 minutes	450.16
1 hour	456.75
2 hour	461.33
3 hour	464.39
6 hour	465.22
24 hour	471.07
48 hour	446.00

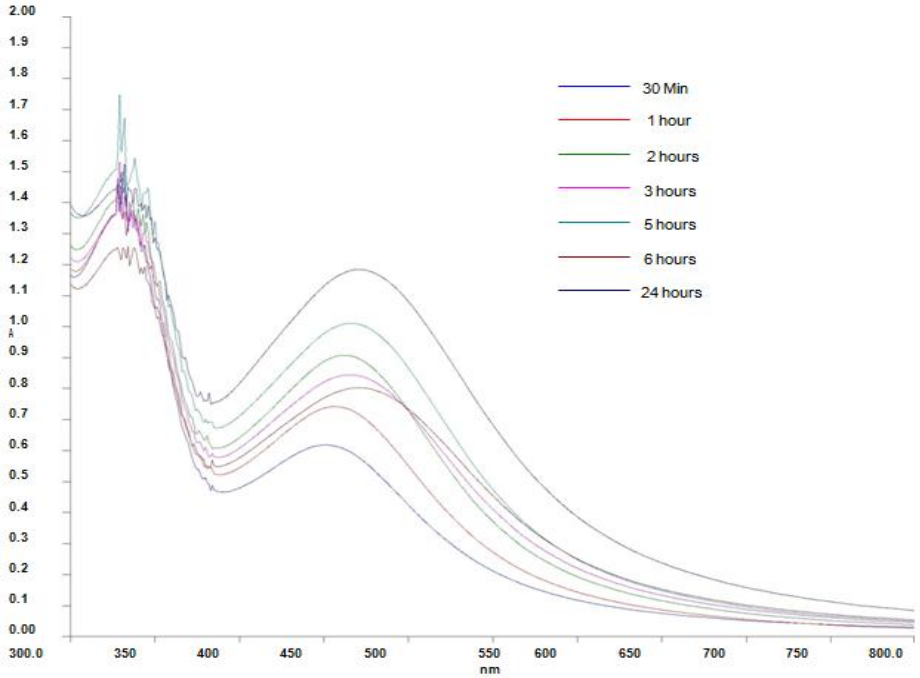

Figure 4: UV analysis for 40% concentration at different time interval

From the above table 4, it is clear that maximum absorbance (λmax) was observed at 24 hour incubation time i.e, 471.07 nm.

FTIR spectra (figure 5) of the *Lantana camara* extract with the silver nanoparticles showed the presence of bonds due to the O–H stretching (around 1417cm^{-1}), secondary amine (around 3427 cm^{-1}), C=O group (around 1632 cm^{-1}), alkyene group (around 2107 cm^{-1}) and S=O group (around 1030 cm^{-1}). These bonds are indicative of lantadenes, alkaloids, terpenoids, and phenolics of compound present in the aqueous *Lantana Camara* extract [20] and

formation of sulfoxide bond also indicated the interaction between silver nanoparticles and extract of *Lantana Camara*.

In the solution, when metal nanoparticles are dispersed, they must be stabilized against the Van der Waals force that may cause coagulation or agglomeration. The stabilizing agent in this case might be phytochemical components from the *Lantana Camara* extract. The nanoparticles were not in direct contacts even within the aggregates which indicate the stability of the nanoparticles as supported by the previous study on *F. oxysporum* [21].

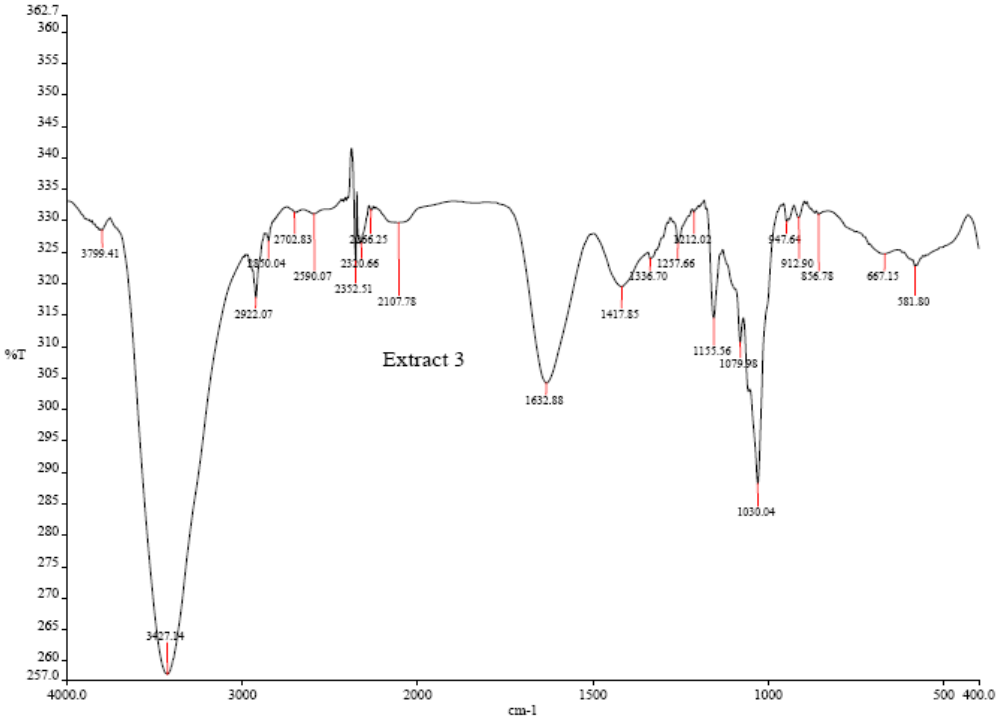

Figure 5: FT-IR spectra of lantana camara extract with silver nanoparticle

X-ray diffraction studies demonstrated the peak point at 38 (figure 6) that indicated the crystalline nature of the silver nanoparticles. The TEM analysis showed multi shaped nanoparticles (spherical and crystalline shaped) for the incubation period of 24 hour in the range of 0.5 µm to 50 nm for the resolution of ×30,000 to ×500 respectively (figure 7). The beginning of transverse Plasmon vibration can be clarified in terms of departure from spherical shape for a small number of particles (22).

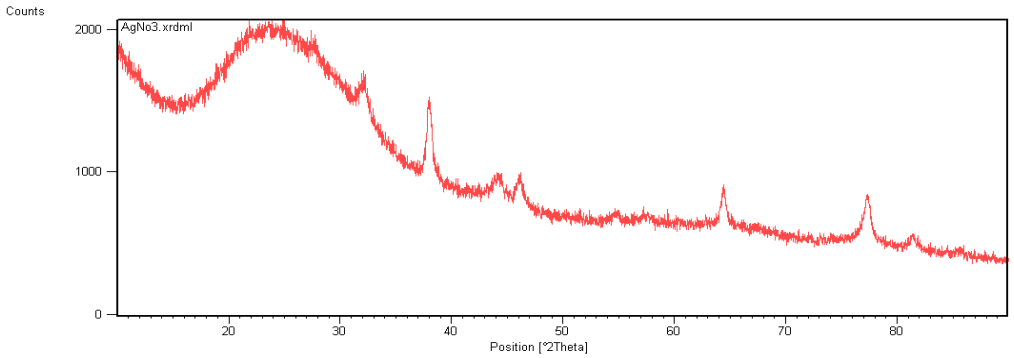

Figure 6: X-ray diffraction analysis

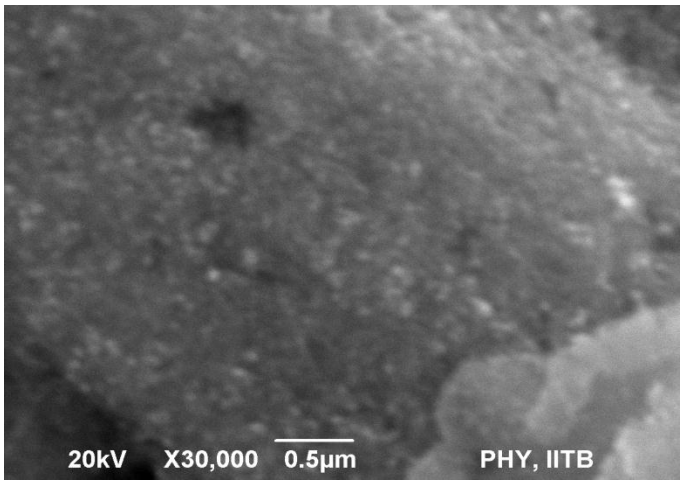

Figure 7: TEM analysis

ANTIMICROBIAL ACTIVITY

The disk diffusion method was used for this study. The inhibition rate of 20 µl of the silver nanoparticles of Lantana Camara extract was highest for concentration of 20 µl i.e. 9 mm for B. Subtilis, 10 mm for S. Aureus, 11 mm for S. Typhi and 12 mm for E.Coli. However, the inhibition rate for the concentration of 5 µl, 10 µl and 15 µl was lower than that of 20 µl in all the group of micro-organisms.

The washing effect on the disk was checked by applying direct wash, single wash and double washes of the aqueous extract of the silver nanoparticles of Lantana Camara. The best result was obtained when aqueous extract of the silver nano particles of Lantana Camara was applied directly. The inhibition rate in this group was 10 mm, 9 mm, 12 mm and 12 mm for B. Subtilis, S. Aureous, S. Typhi and E. coli respectively. On application of single washing with double distilled water to the disc dipped in aqueous extract of the silver nanoparticles of Lantana Camara, the results obtained were 9 mm, 7 mm, 12 mm and 12 mm for B. Subtilis,

S. Aureous, S.Typhi and E.Coli respectively. The results were inferior when double washing was applied compared to direct wash and single wash group.

Furthermore, aqueous extract of the silver nano particles of Lantana Camara was used in milli molar (mM) concentration, i.e, 50 mM, 100 mM, 250 mM and 500 mM to evaluate its anti-microbial activity at different concentration. These concentration ranges was used for all the four groups of micro organisms, i.e B. Subtilis, S. Aureous, S. Typhi and E.coli. There was no zone of inhibition observed in the B.subtilis group. For S. aureus group, the zone of inhibition observed was 8 mm for 50 mM and 100 mM, 9 mm for 250 mM and 11 mm for 500 mM. The zone of inhibition for S. typhi was no zone for 50 mM, 6 mm for 100 mM, 7 mm for 250 mM and 500 mM. For E.coli, the inhibition zone obtained was no zone for 50 mM, 7 mm for 100 mM and 250 mM and 8 mm for 500 mM. The results are shown in the below table 5. Further studies to evaluate the mechanism of bactericidal properties of disk coated with silver nanoparticles are expected in near future.

Table 5: Anti-microbial activity of the mixture of silver Nanoparticle and plant extract

	Zone of Inhibition (mm)			
	B. Subtilis	S. Aurious	S. Typhi	E.coli
5µl/ml	6	8	8	8
10 µl/ml	8	9	9	8
15 µl/ml	8	10	10	10
20 µl/ml	9	10	11	12
Sample with different washing				
Direct	10	9	12	12
Single wash	9	7	12	12
Double wash	9	6	11	10
Different Concentration of Nanoparticle Solution				
50 mM	NS	8	NS	NS
100 mM	NS	8	6	7
250 mM	NS	9	7	7
500 mM	NS	11	7	8

NS: Zone of inhibition not observed

CONCLUSION

It is confirmed that silver nanoparticles of the extract of *Lantana Camara* leaves were synthesized and found stable in the solution. Also, the anti-microbial effects against various types of microorganisms were successfully demonstrated. However, the mechanistic study on bactericidal properties of the extract will be performed in the future.

REFERENCES

(1) Etheridge M.L., Campbell S.A., Erdman A.G., Haynes C.L., Wolf S.M., McCullough J., The big picture of nanomedicine: thestate of investigational and approved nanomedicine products, Nanomedicine: NBM,2013; 9:1-14.

(2) Volker Wagner, Barbel Husing, Sibylle Gaisser, Anne-Katrin Bock, Nanomedicine: Drivers for development and possible impacts, 2006.

(3) NNI What is nanotechnology. National Nanotechnology Initiative 2010. Available from: http://www.nano.gov/html/facts/whatIsNano.html, 2011.

(4) Smith AM, Nie S. Semiconductor nanocrystals: structure, properties, and band gap engineering. Acc Chem Res, 2010; 43(2):190-2000.

(5) Daniel M-C, Astruc D. Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. Chem Revs,2004; 104(1): 293-346.

(6) NCT00848042: Pilot study of Aurolaser™ therapy in refractory and/or recurrent tumors of the head and neck. Clinical Trials.gov.

(7) Van Eerdenbrugh B, Van den Mooter G, Augustijns P. Top-down production of drug nanocrystals: nanosuspension stabilization, miniaturization and transformation into solid products. Int J Pharma, 2008; 364 (1):64-75.

(8) V. Torchilin, Tumor delivery of macromolecular drugs based on the EPR effect, Adv. Drug Delivery Rev,2011; 63, 131 – 135.

(9) A. Tripathy, Ashok Raichur, N. Chandrasekaran, T.C. Prathna, Amitava Mukherjee, Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of azadirachta indica (neem) leaves, J nanopart Res, 2010; 237-246.

(10) S. Silver and L.T Phung, Bacterial heavy metal resistance: New surprises, Annu. Rev. Microbiol. 1996;50: 753.

(11) Rosenblatt, A.; Stamford, T. C. M.; Niederman, R., Silver Diamine Fluoride: A Caries "Silver-Fluoride Bullet", Journal of Dental Research,2009; 88 (2): 116–125.

(12) Bouadma, L; Wolff, M; Lucet, JC, Ventilator-associated pneumonia and its prevention, Current opinion in infectious diseases, 2012;**25** (4): 395–404.

(13) D.M. Caruso, K.N. Foster, M.H. Hermans and C.R. Aquacel, Ag in the management of partial thickness burns: Results of a clinical trial, J. Burn Care Rehabil,2004;25, 89..

(14) N. Prabu, Divya T. Raj, Yamuna Gowri K, Ayisha Siddiqua S, Joseph Pushpa D, Synthesis Of Silver Phyto Nanoparticles And Their Antibacterial Efficacy, Digest Journal of Nanomaterials and Biostructures,2010; 5(1), 185-189.

(15) K. Cho, J. Park, T. Osaka and S. Park, The study of antimicrobial activity and preservative effects of nanosilver ingredient, Electrochim. Acta,2005; 51: 956.

(16) Dr. K.V. Sankaran, Fact sheet – Lantana Camara, Asia - Pacific Forest Invasive Species Network

(17) MA Bratescu, N Saito, H Mori, O Takai, Localised surface plasmon resonance of silicon compounds adsorbed on silver nanoparticles, Surf. Sci, 2007;601: 3886.

(18) Ahmed A, Mukhrjee p, Senapati S, Mandal D, Khan MI, Kumar R, Sastry M, Extracellular biosynthesis of silver nanoparticles using the fungus. Fusarium oxysporum. Colloids Surf B Biointerfaces, 2003;28: 313-318.

(19) A. Tripathy, N. Chandrasekaran, A. M. Raichur, A. Mukherjee, Antibacterial applications of Silver Nanoparticles Synthesized by Aqueous Extract of Azadirachta Indica (Neem) Leaves, J. Biomed. Nanotechnol,2009;5(1):93-98.

(20) Ganjewala D, Sam S, Khan KH, Biochemical compositions and antibacterial activities of Lantana camara plants with yellow, lavender, red and white flowers. EurAsia J BioSc.,2009; 3: 69-77 .

(21) A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M. I. Khan, R. Kumar and Murali Sastry., Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Coll. and Surf. B: Biointerfaces 2003; 28: 313, 2003.

(22) J.J Mock, M. Barbic, D.R. Smith, D.A. Shultz and S. Shultz, shape effects in Plasmon resonance of individual colloidal silver nanoparticles, J. Chem. Phy, 2009; 116: 6755.