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Green synthesis of silver nanoparticles using Leptadenia pyrotechnica root extract and evaluation of their antimicrobial activities

Munesh Kumar, Minakshi Lalit, Rajesh Thakur*

Abstract— Aqueous root extract of Leptadenia pyrotechnica was used for the synthesis of silver (Ag) nanoparticles. These biosynthesized nanoparticles were characterized with the help of UV–vis spectrophotometer, Particle size analyser (PSA), Fourier transform infrared spectroscopy (FTIR) and Transmission electron microscopy (TEM). UV-Vis spectra of silver nanoparticles has absorbance peak at 440 nm. The nanoparticles formed were relatively spherical in shape with varying sizes. The synthesized silver nanoparticles were found to be highly toxic against some human pathogenic gram-positive as well as gram-negative bacteria..

Index Terms— Antibacterial assays, Biosynthesis, Leptadenia pyrotechnica, Root extract, Silver nitrate.

1 Introduction

Nanocrystalline silver particles have wide applicability in various areas such as electronics [1], diagnostics [2], catalysis [3], antimicrobials and therapeutics [4, 5]. Synthesis of silver nanoparticles can be achieved through different methods. For example, silver ions are reduced by chemical [6], radiation [7], photochemical [8] and biological methods [9]. The chemical methods used for the synthesis of silver nanoparticles make use of toxic chemicals like hydrazine and high boiling solvents [10] and also associated with production of hazardous by-products which may pose potential environmental risks. Recently, the biological methods for nanoparticle synthesis are gaining more importance because they are simple, low cost and eco-friendly. Biosynthesis of nanoparticles uses enzymes [11], microorganisms [12-14] and plant extracts [15-20]. However, the biosynthesis of nanoparticles using plants is more popular than other biological methods because it does not require the elaborate cell culture processes [21]. Silver nanoparticles have been synthesized using extracts of various plants such as *Coriandrum sativum* [22], *Sorbus aucuparia* [23], *Gliricidia sepium* [24], *Acalypha indica* [25], *Camellia sinensis* [26], *Azadirachta indica* [21], *Aloe vera* [27], *Capsicum annuum* [28] and *Argemone maxicana* [29].

Leptadenia pyrotechnica (Forssk.) Decne. (Commonly known as Khimp or Khip) is an erect, branched and leafless shrub belonging to Asclepiadaceae family. It is a perennial plant with green stem and grows up to 0.5 meter to 2.6 meter high [30-32]. It is distributed in desert areas of Africa, Asia, and the Mediterranean region [31]. This plant is traditionally used for the treatment of wounds and some other diseases in India and Pakistan. The fresh juice of this plant is used for inducing abortion [33] while its sap is applied for treating eczema and other skin diseases [34]. Its antimicrobial activities have been reported against some bacteria [35-37] and fungi [37, 38]. However nanoparticle synthesis using this plant is not reported so far.

Here in, we report for the first time synthesis and characterization of silver nanoparticles using *Leptadenia pyrotechnica* roots and also the antimicrobial activities of these nanoparticles against some pathogenic bacteria.

2 Material and Methods

2.1 Collection and identification of plant samples

L. pyrotechnica was collected from outskirts of Mahendergarh district, Haryana, India. It was identified and authenticated by Raw Materials Herbarium & Museum, National Institute of Science Communication And Information Resources (NISCAIR), New Delhi.

2.2 Preparation of extracts

Roots were separated, cut into small pieces and washed in running tap water for some time. 6g of roots were boiled with 100ml distilled water and then centrifuged at 5000 RPM for 10 mins. The supernatant was taken for further use.

2.3 Synthesis of silver nanoparticles

1mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of L.

- *Mr. Munesh Kumar is currently pursuing PhD degree program in Biotechnology at Guru Jambheshwar University of Science & Technology, Hisar, Haryana, India, E-mail: kaliya.manu@gmail.com*
- *Ms. Minakshi Lalit is currently pursuing PhD degree program in Microbiology at Guru Jambheshwar University of Science & Technology, Hisar, Haryana, India, E-mail: pr.minal@gmail.com*
- *Dr. Rajesh Thakur is Assistant Professor in Department of Bio & Nano Technology, Guru Jambheshwar University of Science & Technology, Hisar, Haryana, India, PH- 01662-263514. E-mail: [illegible]*

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pyrotechnica root extract was added into 90 ml of aqueous solution of 1mM silver nitrate in a conical flask wrapped by paper and stirred (750 RPM for 8 hours) at 45°C. The synthesized nanoparticles were dried by lyophilisation.

2.4 UV-Vis Spectra analysis

Silver nanoparticles synthesis was confirmed by sampling the aliquots of the suspension and the absorption maxima was scanned by UV-Vis spectrophotometer (Shimadzu, Model: UV-2450) at the wavelength of 300 – 800 nm.

2.5 PSA Measurements

The particle size distribution of silver nanoparticles was analysed using Zetasizer nano ZS (Malvern instruments, Malvern, UK).

2.6 FTIR Spectral analysis

The dried samples were grinded with KBr to form pellets for FTIR measurements. The spectrum was recorded in the range of 4000 - 400 cm^{-1} using FTIR spectrophotometer (Spectrum BX11, Perkin–Elmer) in the diffuse reflectance mode operating at resolution of 4 cm^{-1}.

2.7 TEM analysis of silver nanoparticles

Shape and size of the silver nanoparticles were investigated by TEM images using Morgagni 268D instrument. TEM samples were prepared by placing a drop of the suspension of nanoparticles solution on carbon-coated copper grids and drying under lamp.

2.8 Antibacterial assays

The antibacterial assays were done on human pathogenic gram-positive (*Staphylococcus aureus*) and gram-negative (*Klebsiella pneumoniae, Enterobacter aerogenes, Escherichia coli* and *Pseudomonas aeruginosa*) bacteria by using agar well diffusion method. Nutrient agar media was prepared, autoclaved at 121°C for 15 minutes and solidified in petri plates. An inoculum (turbidity adjusted to approximately 10^8 CFU/ml of bacterium, compared with 0.5 Mc Farland standards) of each bacterial strain was spread on this media and wells were bored into the solidified media using a sterile 7 mm diameter cork borer. Silver nanoparticles sample and standard antibiotic were added in respective wells. Amikacin was used as standard antibiotic. The plates were incubated at 37°C for 24 hours. Three replicate trials were conducted against each bacteria and the mean values are presented.

3 Results and Discussion

It is well known that in aqueous solution, silver nanoparticles exhibit yellowish brown color due to excitation of surface plasmon vibrations in silver nanoparticles [15]. As the L. pyrotechnica root extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from watery to yellowish brown due to reduction of silver ions (Fig.1); which indicated silver nanoparticles formation.

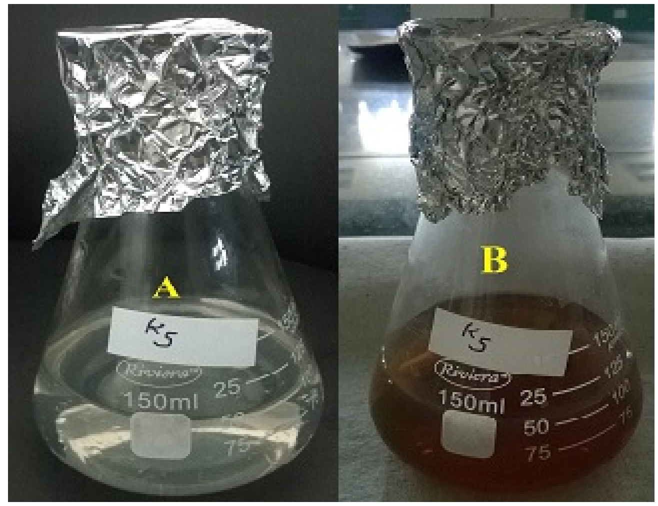

Fig.1. *L. pyrotechnica* root extract solution before (A) and after (B) addition of 1mM $AgNO_3$ solution.

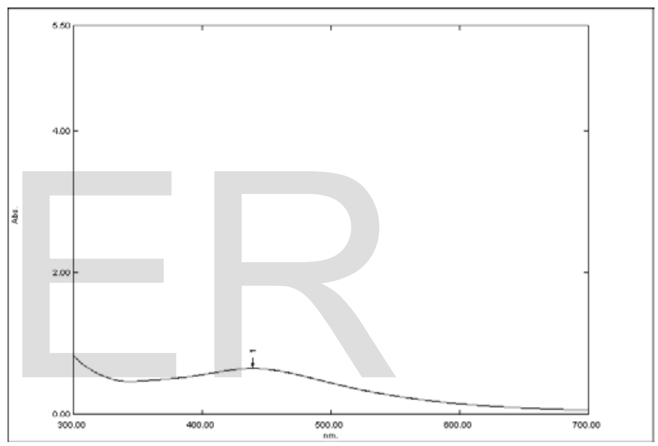

Fig. 2. UV-Vis absorption spectrum of silver nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with *L. pyrotechnica* root extract.

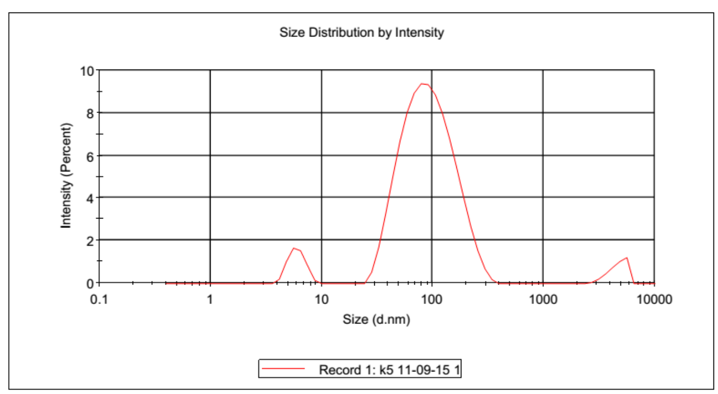

Fig. 3. Particle size analyzer results of aqueous solution of silver nanoparticles synthesized using *L. pyrotechnica* root extract.

UV-Vis spectra of aqueous component of silver nanoparticles is shown in Fig. 2. Absorption spectra of silver nanoparticles synthesized in the reaction media has absorbance peak at 440 nm, broadening of peak indicates that the particles are polydispersed. Fig.3. shows the particle size of the silver nanoparticles as determined by PSA in aqueous solution. The PSA observation shows that the average diameter of silver nanoparticles formed is 68.84nm.

FTIR analysis was used to identify the biomolecules for capping and efficient stabilization of the silver nanoparticles synthesized by L. pyrotechnica root extract (Fig. 4). The band at 3406.93 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The peak at 2926.90 cm^{-1} and 1629.15 cm^{-1} corresponds to O-H stretch carboxylic acids and N-H bend primary amines, respectively. The peak at 1384.74 cm^{-1} corresponds to C-N stretching of aromatic amine group. The 1053.43 cm^{-1} peak indicates C-O stretching. Therefore the synthesized nanoparticles are surrounded by plant metabolites having functional groups of alcohols and carboxylic acids. It is suggested that these biomolecules could possibly perform dual functions of silver nanoparticles formations and their stabilization in the aqueous medium [22]. TEM images of silver nanoparticles derived from L. pyrotechnica root extract are shown in Fig. 5. It is observed that relatively spherical nanoparticles are formed with varying sizes (10-70 nm). Particle size distribution histogram determined from TEM is shown in Fig. 6. The nanoparticles formed are polydisperse and some of them are agglomerated. It is also observed that silver nanoparticles are surrounded by a faint thin layer of other materials, which we suppose are capping organic material from *L. pyrotechnica* root broth.

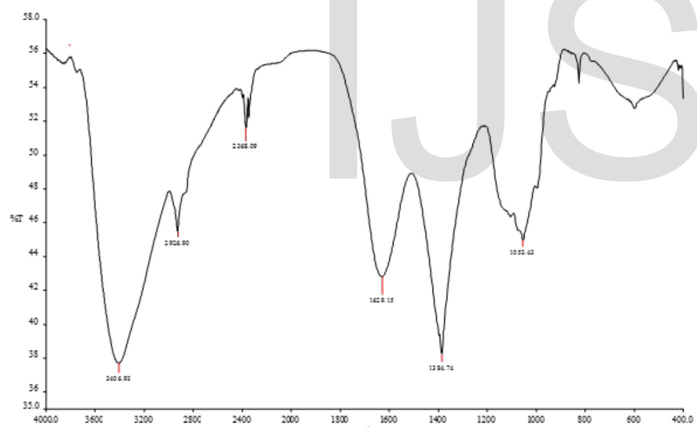

Fig. 4. FTIR spectra of freeze dried silver nanoparticles synthesized using *L. pyrotechnica* root extract.

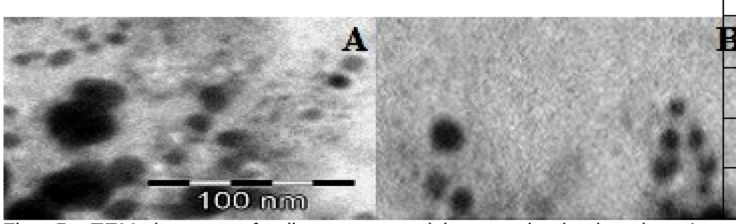

Fig. 5. TEM images of silver nanoparticles synthesized using *L. pyrotechnica* root extract

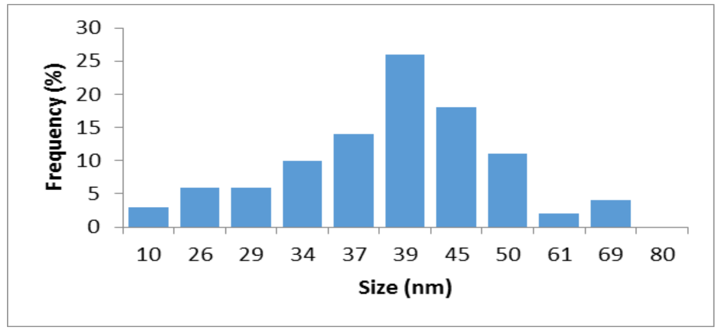

Fig. 6. Particle size histogram of biosynthesized silver nanoparticles.

The antibacterial assays results (Fig. 7) show that silver nanoparticles synthesized using L. pyrotechnica root extract have potential antibacterial properties against human pathogenic bacteria S. aureus, E. coli, E. aerogenes, P. aeruginosa and K. pneumoniae. Zone of inhibition of silver nanoparticles and standard antibiotic drug are shown in table 1. The activity index (A.I.) and percent inhibition (P.I.) were calculated at a concentration of 50mg/ml of aqueous silver nanoparticles solution using the following formula:

$$A.I. = \frac{\text{Mean zone of inhibition of silver nanoparticles solution}}{\text{Zone of inhibition obtained for standard antibiotic}}$$

P.I. = Activity index x 100

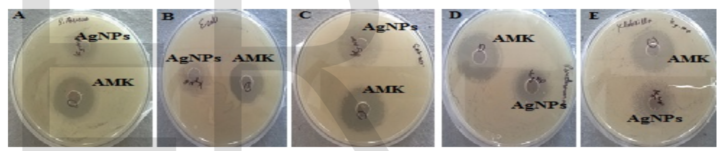

Fig. 7. Antibacterial activity of silver nanoparticles (AgNPs) against (A) *Staphylococcus aureus*, (B) *Escherichia coli*, (C) *Enterobacter aerogenes*, (D) *Pseudomonas aeruginosa* and (E) *Klebsiella pneumoniae*. Standard antibiotic Amikacin (AMK) (85 µg/ml) was used as positive control.

TABLE 1
ANTIBACTERIAL ACTIVITY OF AQUEOUS SILVER NANOPARTICLES SYNTHESIZED USING *L. PYROTECHNICA* ROOT EXTRACT AT CONCENTRATION OF 50 MG/L

Bacterial Strain	Zone of inhibition (mm) of AgNPs	Zone of inhibition of standard drug (mm)	Activity Index	% Inhibition
S. aureus	20	25	0.80	80
E.coli	21	25	0.84	84
E. aerogenes	25	26	0.96	96
P. aeruginosa	23	27	0.85	85
K. pneumoniae	22	25	0.88	88

Well diameter – 7 mm, Standard drug amikacin concentration – 85 µg/ml

4 Conclusion

Silver nanoparticles with spherical shapes and varying sizes (10-70 nm) were synthesized using aqueous root extract of *Leptadenia pyrotechnica*. Silver nanoparticles were characterized by UV-Visible, PSA, FTIR and TEM measurements. Biosynthesis of silver nanoparticles using resources like *Leptadenia pyrotechnica* is a better alternative to chemical synthesis, since this method of synthesis is pollutant free and eco-friendly. The results suggested that *Leptadenia pyrotechnica* plays an important role in the reduction of silver and stabilization of synthesized silver nanoparticles. Study also found that these nanoparticles show potent antibacterial activity on both gram-positive and gram-negative bacteria and should be explored further for antimicrobial applications.

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References

[1] Y. Cui, C.M. Lieber, "Functional nanoscale electronic devices assembled using silicon nanowire building blocks," *Science*, Vol. 291, No. 5505, pp. 851-853. (2001)

[2] S. Schultz, D.R. Smith, J.J. Mock, D.A. Schultz, "Single-target molecule detection with nonbleaching multicolor optical immunolabels," *Proc Natl Acad Sci*, Vol. 97, No. 3, pp. 996-1001. (2000)

[3] R.M. Crooks, M. Zhao, L. Sun, V. Chechik, L.K. Yeung, "Dendrimer-encapsulated metal nanoparticles: synthesis, characterization, and applications to catalysis," *Acc Chem Res*, Vol. 34, No. 3, pp. 181-190. (2001)

[4] I. Sondi, B. Salopek-Sondi, "Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria," *J Colloid Interf Sci*, Vol. 275, No. 1, pp. 177-182. (2004)

[5] J.L. Elechiguerra, J.L. Burt, J.R. Morones, A. Camacho-Bragado, X. Gao, H.H. Lara, M.J. Yacaman, "Interaction of silver nanoparticles with HIV-1," *J Nanobiotechnol*, Vol. 3, No. 6, pp. 1-10. (2005)

[6] Y. Sun, Y. Yin, B.T. Mayers, T. Herricks, Y. Xia, "Uniform silver nanowires synthesis by reducing $AgNO_3$ with ethylene glycol in the presence of seeds and poly (vinyl pyrrolidone)," *Chem Mater*, Vol. 14, No. 11, pp. 4736-4745. (2002)

[7] N.M. Dimitrijevic, D.M. Bartels, C.D. Jonah, K. Takahashi, T. Rajh, "Radiolytically induced formation and optical absorption spectra of colloidal silver nanoparticles in supercritical ethane," *J Phys Chem*, Vol. 105, No. 5, pp. 954-959. (2001)

[8] A. Callegari, D. Tonti, M. Chergui, "Photochemically grown silver nanoparticles with wavelength-controlled size and shape," *Nano Lett*, Vol. 3, No. 11, pp. 1565-1568. (2003)

[9] R.R. Naik, S.J. Stringer, G. Agarwal, S.E. Jones, M.O. Stone, "Biomimetic synthesis and patterning of silver nanoparticles," *Nat Mater*, Vol. 1, No. 3, pp. 169-172. (2002)

[10] K.J. Lee, B.H. Jun, J. Choi, Y.I. Lee, J. Joung, Y.S. Oh, "Environmentally friendly synthesis of organic-soluble silver nanoparticles for printed electronics," *Nanotechnology*, Vol. 18, No. 33, pp. 335601. (2007)

[11] I. Willner, R. Baron, B. Willner, "Growing metal nanoparticles by enzymes," *Adv Mater*, Vol. 18, No. 9, pp. 1109-1120. (2006)

[12] T. Klaus, R. Joerger, E. Olsson, C.G. Granqvist, "Silver-based crystalline nanoparticles, microbially fabricated," *Proc Natl Acad Sci*, Vol. 96, No. 24, pp. 13611-13614. (1999)

[13] Y. Konishi, K. Ohno, N. Saitoh, T. Nomura, S. Nagamine, H. Hishida, T. Uruga, "Bioreductive deposition of platinum nanoparticles on the bacterium *Shewanella algae*," *J Biotechnol*, Vol. 128, No. 3, pp. 648-653. (2007)

[14] B. Nair, T. Pradeep, "Coalescence of nanoclusters and formation of submicron crystallites assisted by *Lactobacillus* strains," *Cryst Growth Des*, Vol. 2, No. 4, pp. 293-298. (2002)

[15] S.S. Shankar, A/ Rai, B. Ankamwar, A. Singh, A. Ahmad, M. Sastry, "Biological synthesis of triangular gold nanoprisms," *Nat Mater*, Vol. 3, No. 7, pp. 482-488. (2004)

[16] D. Philip, C. Unni, S.A. Aromal, V.K. Vidhu, "*Murraya koenigii* leaf-assisted rapid green synthesis of silver and gold nanoparticles," *Spectrochim Acta A Mol Biomol Spectrosc*, Vol. 78, No. 2, pp. 899-904. (2011)

[17] R. Veerasamy R, T.Z. Xin, S. Gunasagaran, T.F.W. Xiang, E.F.C. Yang, N. Jeyakumar, S.A. Dhanaraj, "Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities," *J Saudi Chem Soc*, Vol. 15, No. 2, pp. 113-120. (2011)

[18] D. Philip, "*Mangifera Indica* leaf-assisted biosynthesis of well-dispersed silver nanoparticles," *Spectrochim Acta A Mol Biomol Spectrosc*, Vol. 78, No. 1, pp. 327-331. (2011)

[19] S.P. Dubey, M. Lahtinen, M. Sillanpaa, "Tansy fruit mediated greener synthesis of silver and gold nanoparticles," *Process Biochem*, Vol. 45, No. 7, pp. 1065-1071. (2010)

[20] S.L. Smitha, D. Philip, K.G. Gopchandran, "Green synthesis of gold nanoparticles using *Cinnamomum zeylanicum* leaf broth," *Spectrochim Acta A Mol Biomol Spectrosc*, Vol. 74, No. 3, pp. 735-739. (2009)

[21] S.S. Shankar, A. Rai, A. Ahmad, M. Sastry, "Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth," *J Colloid Interf Sci*, Vol. 275, No. 2, pp. 496-502. (2004)

[22] R. Sathyavathi, M.B. Krishna, S.V. Rao, R. Saritha, D.N. Rao, "Biosynthesis of silver nanoparticles using *Coriandrum sativum* leaf extract and their application in nonlinear optics," *Adv Sci Lett*, Vol. 3, No. 2, pp. 138-143.

[23] S.P. Dubey, M. Lahtinen, H. Sarkka, M. Sillanpaa, "Bioprospective of *Sorbus aucuparia* leaf extract in development of silver and gold nanocolloids," *Colloid Surf B*, Vol. 80, No. 1, pp. 26-33. (2010)

[24] W. Rout Rajesh, R. Lakkakula Jaya, S. Kolekar Niranjan, D. Mendhulkar Vijay, B.S. Kashid, "Phytosynthesis of silver nanoparticle using *Gliricidia sepium* (Jacq.)," *Curr Nanosci*, Vol. 5, pp. 117-122. (2009)

[25] C. Krishnaraj, E.G. Jagan, S. Rajasekar, P. Selvakumar, P.T. Kalaichelvan, N. Mohan, "Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens," *Colloid Surf B*, Vol. 76, No. 1, pp. 50-56. (2010)

[26] Vilchis-Nestor, R. Alfredo, "Solventless synthesis and optical properties of Au and Ag nanoparticles using *Camellia sinensis* extract.," *Mater Lett*, Vol. 62, No. 17, pp. 3103-3105. (2008)

[27] S.P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad, M. Sastry, “Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract,” *Biotechnol Prog*, Vol. 22, No. 2, pp. 577-583. (2006)

[28] S. Li, Y. Shen, A. Xie, X. Yu, L. Qiu, L. Zhang, Q. Zhang, “Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract,” *Green Chem*, Vol. 9, No. 8, pp. 852-858. (2007)

[29] A. Singh, D. Jain, M.K. Upadhyay, N. Khandelwal, H.N. Verma, “Green synthesis of silver nanoparticles using *Argemone mexicana* leaf extract and evaluation of their antimicrobial activities,” *Dig J Nanomater Bios*, Vol. 5, No. 2, pp. 483-489. (2010)

[30] S.K. Bhaduri, P. Mojumder, “Medium density particle board from Khimp plant,” *Nat Prod Radiance*, Vol. 7, No. 2, pp. 106-110. (2008)

[31] S.P. McLaughlin, “Vascular floras of Sonoita Creek State Natural Area and San Rafael State Park: Arizona's first natural-area parks,” Org/SIDA 22, pp. 661-704. (2006)

[32] B.V. Shetty, V. Singh, “Flora of India Series-2: Flora of Rajasthan,” *Botanical Survey of India, Calcutta, India.* (1991)

[33] R.S. Patel, K.V. Kanjaria, K.C. Patel, “Investigation on climber resources used by tribal inhabitants of Ambaji forest of Banaskantha district (North Gujarat),” *Life Sci Leafl*, Vol. 10, pp. 252-257. (2010)

[34] S.S. Katewa, P.K. Galav, “Additions to the traditional folk herbal medicines from Shekhawati region of Rajasthan,” *Indian J Tradit Know*, Vol. 5 No. 4, pp. 494-500. (2006)

[35] M. Munazir, R. Qureshi, M. Arshad, M. Gulfraz, “Antibacterial activity of root and fruit extracts of *Leptadenia pyrotechnica* (Asclepiadaceae) From Pakistan,” *Pak J Bot*, Vol. 44, No. 4, pp. 1209-1213. (2012)

[36] D. Gehlot, A. Bohra, “Antibacterial effect of some leaf extracts on *Salmonella typhi*,” *Indian J Med Sci*, Vol. 54, No. 3, pp. 102. (2000)

[37] M. Al-Fatimi, M. Wurster, G. Schroder, U. Lindequist, “Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen,” *J Ethnopharmacol*, Vol. 111, No. 3, pp. 657-666. (2007)

[38] M.T. Ghaneian, M.H. Ehrampoush, A. Jebali, S.H. Hekmati Moghaddam, M. Mahmoudi, “Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant,” *Environ Health Eng Manage J*, Vol. 2, No. 1, pp. 13-16. (2015)