GREEN SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLESUSING OF *ECLIPTA ALBA* L. LEAF EXTRACT AGAINST VETERINARY PATHOGENS.

Latha Raju*[1]and Manoharan Manosathiyadevan[2]
[1]Ph.D.,Research Scholar, [2] Assistant Professor,
Department of Zoology,
Chikkaiah Naicker College, Erode -638 004, Tamil Nadu, India

Abstract: The activity of nanoparticles is said to be more effective when compared to that of complex plant materials. Recent advancements in nanotechnology- based compounds have opened a new horizon for combating multi drug resistance in microorganisms. In particular, the use of silver nanoparticles as a potent for antibacterial agent has focused much attention. The most crucial physico-chemical parameters that affect the antimicrobial potential of silver nanoparticles are its size, shape, surface charge, concentration and colloidal state. Silver nanoparticles exhibit their antimicrobial abilities through multifaceted mechanisms. Silver nanoparticles adhesive to microbial cells, penetration inside the cells, and modulation of the microbial signal transduction pathways have been recognized as the most empirical mode of antimicrobial action. Therefore, an attempt was made to produce silver nanoparticles having physicochemical properties. Thus, eco-friendly, non-toxic silver nanoparticles were green synthesized using the leaf extract of *Eclipta alba* L. and their antibacterial activity against veterinary pathogenic bacteria. The synthesis of silver nanoparticles was confirmed by a change in extract color from pale yellow to dark brown and surface plasmon resonance spectra obtained at the range of approximately 357 nm. SEM analyses shows the spherical and cluster shaped structures of 2µm in size. Instrumental techniques were used to characterize the synthesized silver nanoparticles, like SEM, XRD and FTIR analysis. Also the antibacterial activity of the green synthesized silver nanoparticles against bacterial strains of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typii* and *Klebsiella pneumonia* was studied using Well diffusion method, and found to be highly effective.

Index Terms - Silver nanoparticles, green synthesis, *Eclipta alba*, antibacterial activity, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typii* and *Klebsiella pneumonia*

I. INTRODUCTION

Nanoparticles have been expressed significant advances owing to its wide range of applications in the field of bio-medicine, sensors, antimicrobial, catalysts, and optical fibers, agricultural, bio-labeling and in the other areas. The synthesis of metal nanoparticles an important research branch in nano technology. Chemical methods are the most widely used methods for synthesis of metallic nanoparticles Baker and Satish (2012). With the development of new chemical or physical methods, the concern for environmental contaminations have heightened as the chemical procedures involved in the synthesis of nano materials generate a large amount of hazardous by products. Thus, there is a need for 'green chemistry' that includes a clean, nontoxic and environment-friendly method of nanoparticles synthesis Kuzma (2010).

The biological approaches to synthesis of nanoparticles are better than chemical and physical procedures because of low energy and time expenditure. This method requires no toxic solvents and no dangerous material for environment. Green synthesis of nanoparticles is an eco-friendly method and uses natural solvent Karimi and Mohsenzadeh (2013). Nanoparticles produced by plants are more stable, and the rate of synthesis is faster than that in the case of other organisms. Moreover, the nanoparticles are more various in shape and size in comparison with those produced by other organisms Ramesh et al, (2014).The aim of this study was to investigate antibacterial activity of silver nanoparticles synthesized by using *Eclipta alba* leaf extract against bacterial strains of gram positive bacteria :*Staphylococcus aureus*, *Bacillus subtilis*, gram negative bacteria: *Escherichia coli*, *Salmonella typii*, *Klebsiella pneumonia*.The synthesized silver nanoparticles were confirmed by UV-visible spectroscopy, SEM. The XRD and FTIR analysis prove the presence of various biomolecules present in the sample. The peculiar properties of silver nanoparticle are small in size, with high surface area, easy to suspend in liquid medium and has a deep access to cells and organelles that can easily strike on the bacterial cell.

II. MATERIALS AND METHODS

2.1 Collection of plant material

The medicinal plant *Eclipta alba* widely grown in Kolli hill, was collected from Solakkadu region.

2.2 Preparation of leaf extract

The leaves of *Eclipta alba* were washed with running tap water and distilled water. The leaves were shade dried for 20days, the dried leaves was powdered mechanically using electrical stainless steel blender Fig.1. The leaf powder of 10 g were weighed and boiled for 10-15 minutes with 100 mL double distilled water at 60°C and the extracts were filtered through Whatman filter paper No.1. The filtered extract was stored in refrigerator at 4°C for further use in the synthesis of silver nanoparticles.

2.3 Synthesis of silver nanoparticles

100 mL (10^{-3} M) aqueous solution of silver nitrate was prepared in Erlenmeyer flask. Then 10 ml of leaf extract and 90ml of double distilled water were added to 60 µl of aqueous silver nitrate solution kept in a separate conical flask of 250ml at room temperature. The conical flask was covered and kept in dark chamber until the solution colour changes from pale yellow to dark yellow. After 30 minutes, the solution turns from pale yellow to dark brown indicating the formation of silver nanoparticles Fig.2. The bio reduction of silver ions was monitored by sampling using UV spectrophotometer.Fig.3.

2.4 Separation of silver nanoparticles

The synthesized silver nanoparticles were separated by centrifugation using a REMI centrifuge at 10,000rpm for 15min. The supernatant liquid was re-suspended in the sterile double distilled water. The process was carried out thrice to get rid of any uncoordinated bio molecules. After, the desired reaction period, the supernatant liquid was discarded and the pellets were collected and stored at 4˚C for further use.

2.5 Lyophilization

The pellet obtained was then lyophilized by using freeze dryer to enhance the stability of silver nanoparticles.

2.6 Characterization of silver nanoparticles

A colour change from pale yellow to dark brown upon incubation due to surface plasma resonance (SPR) vibration was observed indicating the formation of nanoparticles. The optical absorbance between 300 nm and 800 nm with a Shimadzu UV-Visible spectrophotometer (UV-1800, Japan) were performed to investigate the reduction of silver ions by leaf extract. For Scanning electron microscopic studies, the particle size and surface morphology was confirmed using the images of nanoparticles were studied using scanning electron microscopy (SEM; JEOL, Model JFC-1600) and measurements were operated at an accelerating voltage of 120 kV. FTIR spectra of the samples were measured using Perkin-Elmer Spectrum instrument in KBr pellets is used to obtain the infrared spectra of absorption and emission of the formed silver nanoparticles. FTIR spectra were recorded from wave number 600-4000 cm-1. For XRD studies, the spectra were recorded by using Phillips PW 1830 instrument operating at a voltage of 40 kV with CuKα1 radiation.

III. TEST MICROORGANISMS

The bacterial specimens were collected from Acme ProGen Biotech (India) Pvt. Ltd., Balaji Nagar, Salem,Tamil Nadu, INDIA and then pure culture was maintained in nutrient broth and used to check antimicrobial activity.

IV. ANTIMICROBIAL ACTIVITY

Antimicrobial activity of Eclipta alba leaf extract against bacterial strains of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typii* and *Klebsiella pneumonia* was studied using well diffusion method.

V. WELL DIFFUSION METHOD

In this agar well diffusion method, a suitable agar medium was prepared, once the agar is solidified the medium was inoculated and swabbed with bacterial suspension of approximately 1-2 X 108 CFU/mL using cotton swab. The wells were prepared by punching with a six millimeters diameter standard sterile cork borer made up of stainless steel. These wells were filled up with 25 – 100µL of the antimicrobial solutions to be tested Cleidson et al, 2007and Magaldi et al, (2004). The plates were incubated at 35 ± 2°C for 18 – 24 h. The antimicrobial activity is calculated in millimeter by using the expression: ZOI = Total Diameter of growth inhibited zone minus diameter of the well, where, ZOI is Zone of inhibition.

VI. RESULT AND DISCUSSION

Fig:1*Eclipta alba* leaf powder

Fig:2 Synthesis of AgNPs

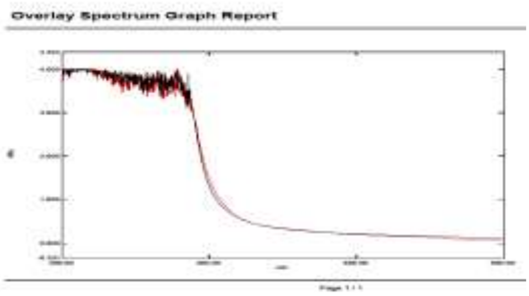


Fig:3 UV-VISIBLE SPECTRSCOPY

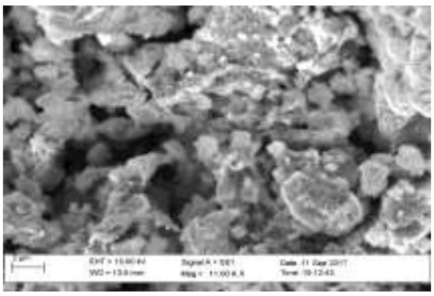


Fig:4 SEM IMAGE

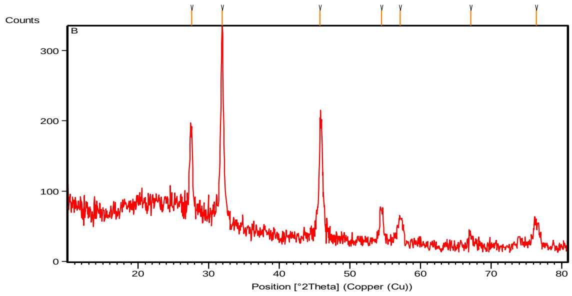


Fig:5 XRD READING

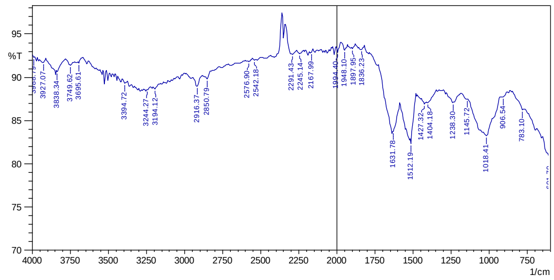


Fig:6 FTIR ANALYSIS

TABLE:I

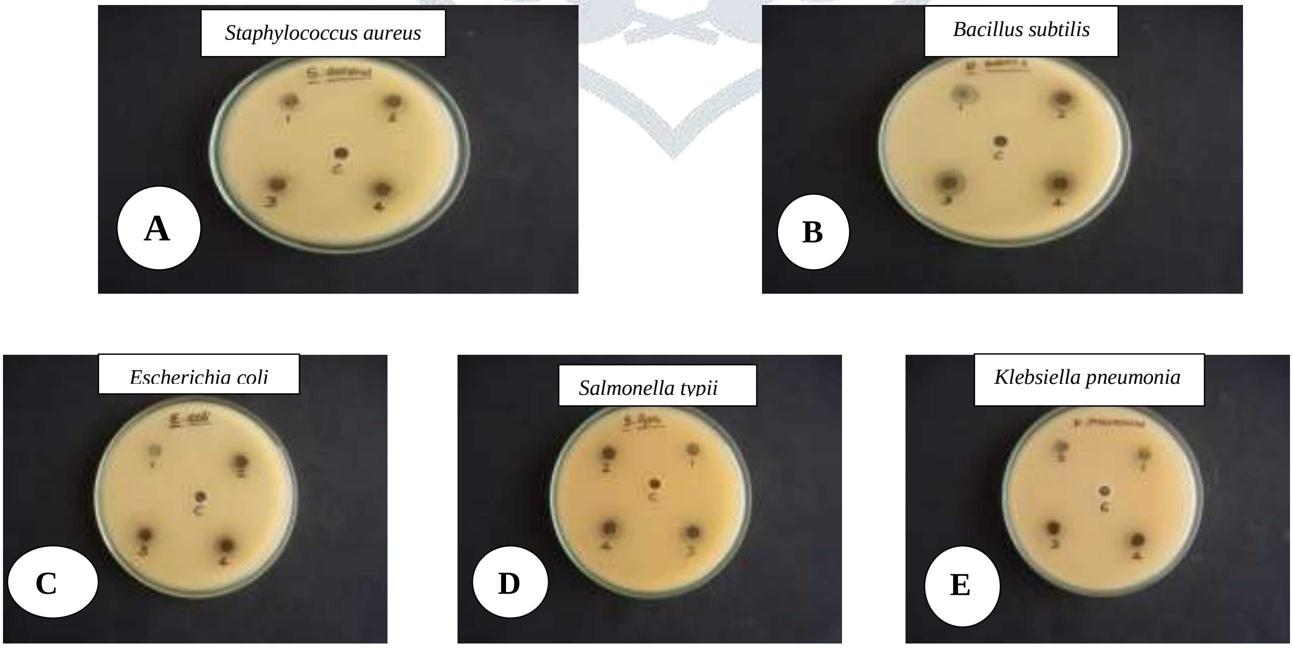
Antibacterial activity of synthesized silver nanoparticles from leaves of *Eclipta alba* against bacterial strains of gram positive bacteria :(*Staphylococcus aureus*, *Bacillus subtilis*), gram negative bacteria: (*Escherichia coli*, *Salmonella typii*, *Klebsiella pneumonia*).

Antibacterial Activity

S. No	Sample	Concen-trations (µL)	Zone of inhibition (mm) in different concentration				
			B.subtilis	*S.aureus*	*K.pneu-moniae*	*S. typhi*	*E.coli*
1	Control	DMSO					
2	*Eclipta alba*	25	6mm	5mm	4mm	4mm	NA
3		50	7mm	6mm	5mm	6mm	5mm
4		75	8mm	7mm	6mm	6mm	6mm
5		100	9mm	8mm	7mm	7mm	7mm

*DMSO-Dimethyl sulfoxide, NA - NO ACTIVITY

Fig-7 Antibacterial activity of gram positive bacterial strains :a) *Staphylococcus aureus*, b) *Bacillus subtilis* and gram negative bacterial strains: c) *Escherichia coli*, d) *Salmonella typii*, e) *Klebsiella pneumonia*



Note: C- Control,1-- 25µL , 2--50 µL, 3--75 µL, 4--100 µL

Synthesis and characterization of silver nanoparticles, it is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. The UV-visible spectra was observed at the maximum absorbance occurs at 357 nm Fig:3. The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles Sun, et al, (2001), indicating the presence of spherical Ag nanoparticles. The excitation of Surface Plasmon Resonance (SPR) band at 410 to 430 nm confirmed the synthesis of Ag NPs at plant products Sathishkumar, (2009). The SEM image of silver nanoparticles was due to interactions of hydrogen bond and electrostatic interactions between the organic capping molecules bound to the AgNPs. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent Priya et al, (2011). SEM analysis shows high-density AgNPs synthesized shown that relatively spherical and uniform AgNPs were formed with diameter of 2µm Fig:4. The larger silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements. Kumar and Yadav, (2009) reported the unidentified crystalline peaks (27.89°, 32.30°, 46.26°, 54.79°) are also apparent in many works in which the XRD pattern includes the relevant 2θ range. These peaks are due to the organic compounds which are present in the extract. Fig:5, shows the XRD pattern of AgNPs and peak values at 2θ degrees of 27.6°, 31.9°, 45.7°, 54.4°, 57.1°67.0°, 76.4° corresponding to (311), (100), (111), (022), (020), (222), (131) Whereas XRD examination produces a diffraction pattern that is subsequently compared with data contained in a standard crystallographic database to determine structural information. Analysis of the XRD data identifies crystallite size, structure, preferred crystal orientation, and phases present in samples Klug and Alexander (1974), Barrett et al, (1986). FT-IR spectroscopy can be used to investigate surface chemistry and identify surface residues such as functional groups like carbonyls and hydroxyls moieties that attach to the surface during nanoparticle synthesis Poinern, (2014). FTIR spectrum indicated the clear peaks with (1018.41, 1512.19, 1631.78, 2916.37, 3838.34 cm^-1) different values in the above peak values they corresponded to functional groups like, (C-N stretching amine group1018.41 cm^-1), (N=O nitroso group 1512.19 cm^-1), (C=N amine group 1631.78 cm^-1), (C-H alkane group 2916.37 cm^-1), (O-H alcohol group 3838.34 cm^-1) Fig.6. FTIR spectrum the most intense band at 1620cm^{-1} 1636 cm^{-1} represent carbonyl groups from polyphenols ; the results suggest that molecules attached with Ag NPs have free and bound amide groups. These amide groups may also be in the aromatic rings. This concludes that the compounds attached with the Ag NPs could be polyphenols with an aromatic ring and bound amide region Rai et al, (2009). The functional groups such as alcohol, amines, amides, alkenes, methyl, aliphatic and halides confirmed their presence in AgNPs and these are the main classes in most of the functional groups. They were denoted as possible biomolecules responsible for stabilizing, capping and reducing agents of the AgNPs Cho et al, (2005), Srinivasan et al, (2014) and Vijayaraghavan, (2012).

6.1 Antibacterial Efficacy

The leaf extract of *Eclipta alba* synthesized silver nano particles shows a strong antimicrobial activity against veterinary pathogenic bacterial strains like *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typii* and *Klebsiella pneumonia* . From the present investigation it shows that gram positive bacterial strains exhibit more zone of inhibition maximum at 100µL and minimum at 25 µL the recorded values were 5mm to 9mm.The gram negative bacterial strains showed much lesser effect than the gram positive bacterial strains the observed zone of inhibition was from 4mm to 7mm Fig:7. These findings are in accordance to the previously published reports from Mahesh and Satish, (2008) who investigated their study plant efficiency against Staphylococcus aureus and E. coli having a similar zone of inhibition to the present study. Barbour et al, (2004) determined Screening of selected indigenous plants of Lebanon for antimicrobial activity. Ansari et al. in (2011) Examined antibacterial effect of silver nanoparticles synthesis by chemical methods) against *Staphylococcus aureus* and results showed that AgNPs exhibit bacterio static and bactericidal effect towards all clinical isolates. Also, Sadeghi et al. (2011) investigated antibacterial activity of silver nanoparticles (synthesis by chemical methods) against *S aureus* and *E coli* and results showed that the antibacterial activity against *E coli* is lower than that against *S aureus*, probably because of the difference in cell walls between gram positive and gram negative bacteria. The results of this study are in agreement with our studies. Zarei et al, (2014) tested antibacterial effect of silver nanoparticles (synthesis by chemical methods) against four food borne pathogens (*Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhi murium* and *Vibrio para haemolyticus*) that silver nanoparticles showed great antibacterial effectiveness on four important food borne pathogens. Complete inhibition was reported at a high concentration of 200g/ml when compared to a least concentration of 1.25 g/ml that was tested. At 200 g/ml an inhibition of 88% for E. coli, 86% for *P. aeroginosa* and 94% for *K. pneumonia* was found. The plant *E. hirta* has previously shown to have antibacterial activity both in its aqueous and methanol extracts El-Mahmood Muhammad Abubakar, (2009) and Saravanan, (2012). Hence it can be concluded that the plant material has adhered in some form to the AuNPs and its antibacterial activity is due to the synergistic effect of the two combined . Biradar, et al (2012) and Bhattacharya et al, (2012). Thus the invention of new synthesized nanoparticles from leaf extract of *Eclipta alba* can thus replace synthetic antibacterial products.

VII.CONCLUSION

Green synthesize of silver nano particles of *Eclipta alba* leaf extract resulted in the formation of stable nanoparticles. The spectroscopic observations using various analytical instruments helped in proving the composition of the nanoparticles and also in confirming their size and shape. FTIR evidence show the formation and stability of the biosynthesized silver nano particles to understand the possible chemical and molecular interactions which could be responsible for nanoparticle synthesis. The antimicrobial activity of these nanoparticles was well developed against both gram positive and gram negative bacterial strains. The study also confirms that gram positive bacteria are relatively more resistant to the bactericidal action than gram negative bacteria. The antibacterial activity of the silver nanoparticles synthesized from the leaf extract of *Eclipta alba* had a significant antibacterial effect at very low concentrations of nano powder when compared to the large amount of plant material required to produce the same. Green synthesis of silver nanoparticles can further be applied in various biomedical and biotechnological fields and their properties and application scan further be explored. The microorganisms are unlikely to develop resistance against silver as compared to antibiotics as silver attacks a broad range of targets in the microbes. By using such plant extracts we can develop nano medicine against various human and veterinary pathogens.

ACKNOWLEDGMENT

The authors convey their thanks to the Acme ProGen Biotech, Salem, Tamil nadu, India for providing technical support.

REFERENCES:-

[1] Baker S, Satish S. Endophytes: towards a vision in synthesis of nanoparticle for future therapeutic agents. *Int J Bio-InorgHybdNanomat.*2012;**1**(2):67–77.

[2] Kuzma J. Nanotechnology in animal production—Upstream assessment of applications. *Livestock Sci.* 2010;**130**(1-3):14–24. doi: 10.1016/j.livsci.2010.02.006.

[3] Karimi J, Mohsenzadeh S. Plant synthesis of silver nanoparticles byAchilleawilhelmsii Pharmaceutical plant. *Razi J Med Sci.* 2013;**20**(111):64–9.

[4] Ramesh P, Rajendran A, Meenakshisundaram M. Green Syntheis of Zinc Oxide Nanoparticles Using Flower Extract Cassia Auriculata. *J Nano SciNanoTechnol.* 2014;**2**(1):41–5.

[5] Cleidson V, Simone MDS, Elza FAS, Artur SJ. Braz J Microbiol, 2007; 38: 369-380.

[6] Magaldi S, Mata-Essayag S, Hartung de CC, Perez C, Colella MT, Olaizola C, Ontiveros Y. Int J Infect Dis, 2004; 8(1): 39-45.

[7] Sun, Y.P., Atorngitjawat, P. and Meziani, M.J., "Preparation of Silver Nanoparticles Via Rapid Expansion of Water in Carbon Dioxide Microemulsion into Reductant Solution", Langmuir, 17, 5707-5710, 2001.

[8] Sathishkumar M, Sneha K, Won SW, Cho CWS, Kim Yun YS. *Cinnamon zeylanicum*bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. Colloids Surf B Biointerfaces2009; 73: 332-338.

[9] A. M. Priya, R. K. Selvan, B. Senthilkumar, M. K. Satheeshkumar, and C. Sanjeeviraja, "Synthesis and characterization of CdWO4 nanocrystals," *Ceramics International*, vol. 37, no. 7, pp. 2485–2488, 2011.

[10] Kumar V, Yadav SK (2009) Plant-mediated synthesis of silver and gold nanoparticles and their applications. J of ChemTechnol and Biotechnol 84: 151-157.

[11] Klug, H.P.; Alexander, L.E. X-ray Diffraction Procedures for Poly-crystallite and Amorphous Materials, 2nd ed.; Wiley: New York, NY, USA, 1974.

[12] Barrett, C.S.; Cohen, J.B.; Faber, J.; Jenkins, R.; Leyden, D.E.; Russ, J.C.; Predecki, P.K. Advances in X-ray. Analysis; Plenum Press: New York, NY, USA, 1986; Volume 29.

[13] Poinern, G.E.J. A Laboratory Course in Nanoscience and Nanotechnology, 1st ed.; CRC Press Taylor & Francis: Boca Raton, FL, USA, 2014.

[14] Rai, M., Yadav, A., Gade, A., 2009. Silver nanoparticles as a new generation of antimicrobials.Biotechnol. Adv. 27, 76–83.

[15] Cho, K.H., Park, J.E., Osaka, T., Park, S.G., 2005. The study of antimicrobial activity and preservative effects of nanosilver ingredient.Electrochim.Acta 51, 956–960.

[16] Srinivasan, B., Muthukumaraswamy, S., Mohanraj, J., 2014. Biosynthesis of silver nanoparticles from mangrove plant

(*Avicennia marina*) extract and their potential mosquito larvicidal property.Parasit.Dis. http://dx.doi.org/10.1007/s12639-014- 0621-5.

[17] Vijayaraghavan, K.S.P., Nalini, K., UdayaPrakash, N., Madhankumar, D., 2012. Biomimetic synthesis of silver nanoparticles by aqueous extract of *Syzygium aromaticum*. Mater.Lett. 75, 33–35.

[18] Mahesh B, Satish S (2008) Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens. World Journal of Agricultural Sciences 4(S): 839-843.

[19] Barbour EK, Mohammed AS, Sagherian VK, Habre AN, Talhouk SN (2004) Screening of selected indigenous plants of Lebanon for antimicrobial activity. Journal of EthnopharmacologyPp 1-7.

[20] Ansari MA, Khan HM, Khan AA, Malik A, Sultan A, Shahid M, et al. Evaluation of antibacterial activity of silver nanoparticles against MSSA and MRSA on isolates from skin infections. *Biol Med.* 2011;**3**(2):141–6.

[21] Sadeghi B, Jamali M, Kia S, Ghafari S. Synthesis and characterization of silver nanoparticles for antibacterial activity. *Int J Nano Dimens.* 2011;**1**(2):119–24.

[22] Zarei M, Jamnejad A, Khajehali E. Antibacterial effect of silver nanoparticles against four foodborne pathogens. *Jundishapur J Microbiol.*2014;**7**(1):e8720. doi: 10.5812/jjm.8720. [PubMed: 25147658].

[23] El-Mahmood Muhammad Abubakar, J. Med. Plants Res. 3 (2009) 498.

[24] R. Saravanan, D. Dhachinamoorthi, K. Senthilkumar, M. Srilakshmi, T. Divya Sri, Int. J. Res. Ayurveda Pharmacy 3 (2012) 439.

[25] D. Biradar, K. Lingappa, A. Dayanand, J. Ecobiotechnol. 4 (2012) 43.

[26] D. Bhattacharya, B. Saha, A. Mukherjee, C.R. Santra, P. Karmakar, Nanosci. Nanotechnol.2 (2012) 14.