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PHYTOSYNTHESIS OF SILVER NANOPARTICLES USING LEAF EXTRACT OF *WATTAKAKA VOLUBLIS* (L.F.) STAPF. AND THEIR ANTIBACTERIAL ACTIVITY

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Abstract: The synthesis of silver nanoparticles using the leaf extract of *Wattakaka volublis* (L.f.) Stapf is reported in the present investigation. The Silver nanoparticles exhibited characteristics Plasmon resonance at 440 nm in UV-Vis spectrophotometer and High Resolution Scanning electron Microscope. The FTIR data reveals the possible functional groups of biomolecules involved in bioreduction and capping for efficient stabilization of silver nanoparticles. HRSEM studies revealed the nanoparticles are spherical in shape and ranging between 30 and 40 nm in size. The silver nanoparticles synthesized were evaluated for their antibacterial efficacy against the bacteria *Pseudomonas aeruginosa, staphylococcus aureus* and *Escherichia coli*.

Keywords: Antibacterial, *Wattakaka volubilis*., Silver Nanoparticles, XRD & HRSEM.

Introduction

The field of nanotechnology is one of the most active areas of research in modern material science. Nanotechnology mainly deals with the fabrication of nanoparticles having various shapes, sizes and managing their physical and chemical parameters for further use in human benefits [1]. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, morphology and distribution. Synthesis of nanomaterials using living organisms is an emerging area in nanoscience and technology [2] [3] [4]. Biosynthesis of nanoparticles is advantageous over physical and chemical methods as it is a cost-effective and environment friendly method, where it is not necessary to use high pressure, energy, temperature and toxic chemicals [5] [6]. Silver nanoparticles have unique catalytic, optical, electrical and antimicrobial properties [7]. Silver is a nontoxic inorganic antimicrobial agent, which inhibits 650 types of microbe's growth [8]. Many of the reports published involve the biological synthesis of nanomaterials using bacteria, fungi and plant extracts. Silver nanoparticles have been synthesized using various plant extracts such as *Cinnamon camphora* [9], *Cinnamon zeylanicum* [10], Geranium [11], Neem leaf broth [12], *Aloe vera* plant extracts [13], Tamarind leaf extract [14], Phyllostachys sp leaves extract [15] and

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Acalypha indica [16]. The latex of *Jatropha curcas*, a plant whose seeds are used to extract biodiesel has also been used for the synthesis of silver nanoparticles [17]. Li *et al* 2007 [18] synthesized silver nanoparticles using the *Capsicum annum* L. extract. The synthesis of gold and silver nanoparticles has also been reported using black tea leaf extracts. Black tea leaf extracts are known to contain more amounts of flavonones and polyphenols. It was found that the reduction of metal ions was accompanied by oxidation of polyols [19]. In the present study, we are reporting a simple, effective, low cost and environmental safe method for synthesis of silver nanoparticles using leaf extract of *Wattakaka volublis (L.f.) Stapf.*

Material and Methods

Preparation of leaf extract

Leaves of *Wattakaka volubilis*. a medicinal plant were collected from the Jogimatti State Reserve Forest Chitraduga, Karnataka, India. The leaves were washed 2-3 times with tap water followed by Double distilled water to remove dust and impurities. Leaves were shade dried for 5 days and blended using kitchen blender to obtain the powder. The leaf powder was sterilized at 121 ◦C for 15 min. 10g of powder was taken and mixed with 100 ml of double distilled water and kept in shaker for 24h. The extracts were filtered through Wattman No1 filter paper and stored in refrigerator at 4 ◦C for further studies.

Synthesis of Silver nanoparticles

Five millilitre of the filtrate was added to 250ml Erlenmeyer flask containing 100 ml of 3mM aqueous silver nitrate solution. The mixture was subjected for shaking at rotation speed of 200 rpm for 48 hrs at 30°C and the pH was maintained between 6-7. Synthesis of the silver nanoparticles was confirmed by the colour change of mixture from Colorless to dark brown.

Characterization

The bioreduction of silver ions in the solution at different time intervals was monitored by using Uv-Visible spectrophotometer (U-3010). The solution containing bioreduced silver ions was centrifuged at 6000 rpm for 20 min to remove the unwanted biomass residue, the resulting suspension was then dispersed in 10 ml of double distilled water and centrifuged again at the same conditions this redispersion and centrifugation process was repeated for 2-3 times to obtain the pellet of silver nanoparticles free from any biomass residue. The pellet thus obtained was dispersed in double distilled water and oven dried to obtain the powder. The powder thus obtained was used for FTIR, XRD and HRSEM studies. Scanning electron

microscopic studies were carried out using HRSEM to know shape and size of silver nanoparticles.

Antibacterial activity

The antimicrobial method employed in the present study is based on the disc diffusion method [20]. This method depends on the radial diffusion of an antibiotic or extract, from the disc through semisolid agar layer in Petri plate, which prevents the growth of micro organisms in a circular area or the zone around the disc.

The hot sterile medium was poured into the sterile Petri plates to form 2-3 mm thick uniform layer and allowed to solidify. These plates were later lawn cultured with bacterial broth suspension. The sterile discs impregnated with 5µl, 10µl and 15µl of the extract were placed on the media with flamed forceps and gently pressed down to ensure contact with the suspension. To increase the efficiency (sensitivity) of the test system the plates were kept for 30 minutes at room temperature. The plates were incubated at 37^0 C for 24hrs and zone of inhibition (including the diameter of sterile disc) was measured using Hi-Antibiotic Zone scale.

Results and Discussion

Addition of leaf extract of *Wattakaka volublis* (L.f.) Stapf to 3mM aqueous $AgNo_3$ changed the color of extract from colorless to dark brown after 30minutes of shaking on the shaker. The color change was due to excitation of surface plasmon vibrations [21]. The intensity of color increased with the increase in time duration indicating the continuous reduction of silver ions. The formation of silver nanoparticles in the reaction mixture was confirmed by the Plasmon resonance of silver nanoparticles at 440 nm by Uv-Vis spectrophotometer (Fig.1).

FTIR spectrum of the silver nanoparticles (Fig.2) showed peaks at 3391cm^{-1}, 2134cm^{-1}, 1644cm^{-1}, 1049cm^{-1}, 742cm^{-1} and 475cm^{-1} which correspond to Stretching frequency of O-H band, Stretching vibration in S-H, tertiary amides, C-N stretching vibration of amide, Si-CH and alkyl halides respectively. These above functional groups might have been involved in reduction of silver ions and their capping in order to achieve stability.

The HRSEM micrograph of (Fig.3) revealed the spherical shape of silver nanoparticles ranging between 30 and 40 nm in size. The elemental nature and purity of the sample was confirmed by EDAX (Fig.4).

The antibacterial activity of silver nanoparticles tested against bacteria *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* showed inhibition zones of 7 mm, 6 mm and 7 mm for concentrations of 5µl, 10µl and 15 µl respectively against *Pseudomonas aeruginosa.* The inhibition zones of 7 mm, 7 mm and 8 mm were observed against s*taphylococcus aureus* for concentrations of 5µl, 10µl and 15 µl respectively. No inhibition zone is reported against the bacterium *E.coli* (Fig.5 & 6).

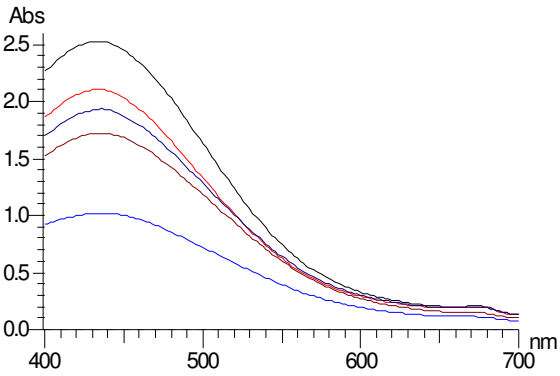

Fig.1. UV-Vis Absorption spectrum of silver nanoparticles synthesized using *Wattakaka volublis* (L.f.) Stapf. leaf extract

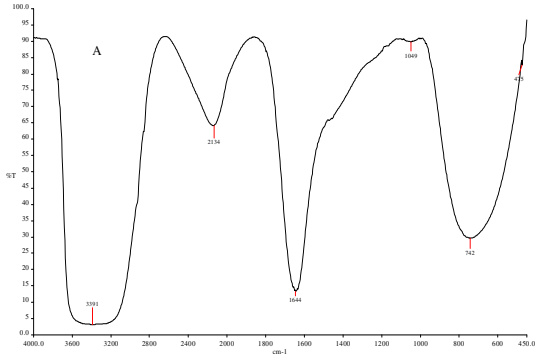

Fig.2. FTIR spectra of silver nanoparticles synthesized using *Wattakaka volublis* (L.f.) Stapf. leaf extract

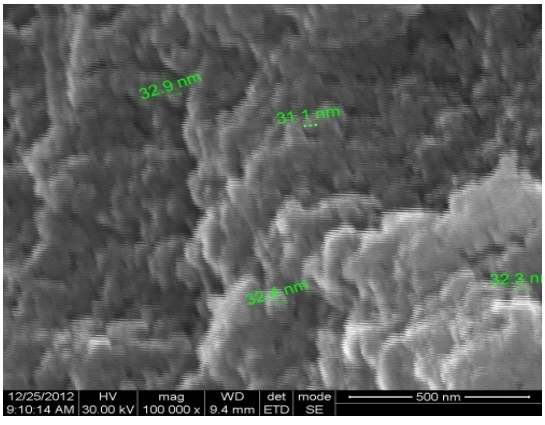

Fig.3. HR-SEM micrograph of silver nanoparticles synthesized using *volublis* (L.f.) Stapf. leaf extract

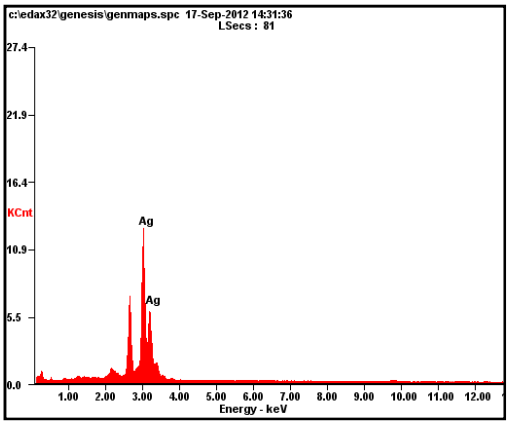

Fig.4. EDAX spectrum of silver *Wattakaka* nanoparticles synthesized using *Wattakaka volublis* (L.f.) Stapf. leaf

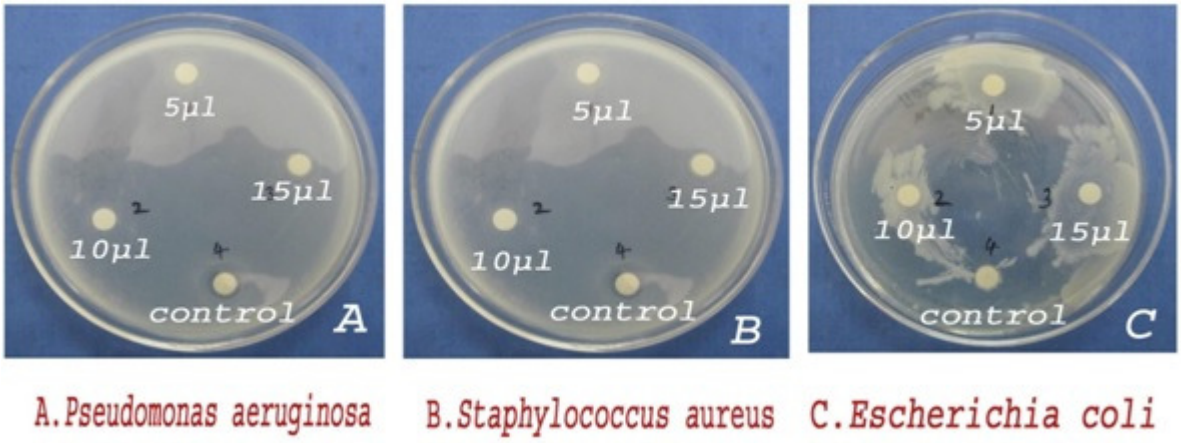

Fig.5. Antibacterial activity of silver nanoparticles synthesized using *Wattakaka volublis* (L.f.) Stapf. leaf extract.

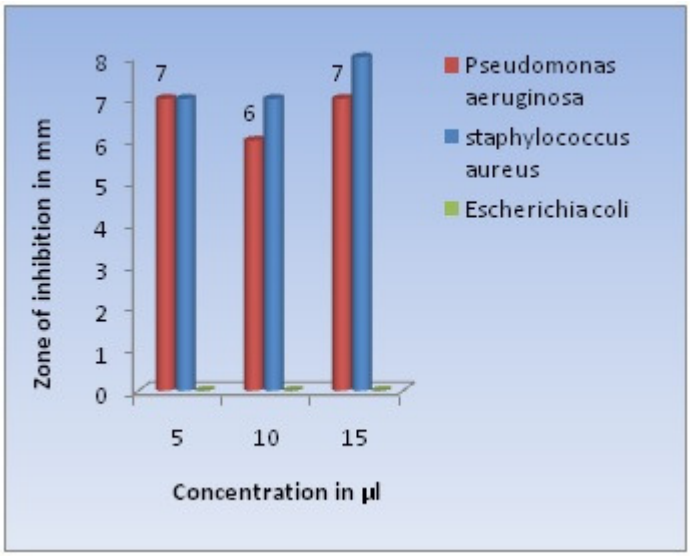

Fig.6. Graph showing the zones of inhibition against the tested bacteria

Conclusions

Synthesis of silver nanoparticles by using leaf extract of *Wattakaka volublis* (L.f.) Stapf. a medicinal plant has been. Demonstrated in present investigation. The reduction of Silver ions and their capping was achieved by organic molecules present in the leaf extract. The HRSEM results revealed that the Silver nanoparticles are spherical in shape and ranging between 30 and 40 nm in size. Silver nanoparticles showed good antibacterial activity against the bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus* but no inhibition was reported against bacterium *Escherichia coli.*

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References

[1] S. Chandan, S. Vineet, N. Pradeep, K. Vikas Singh, H. *Digest Journal of Nanomaterials and Biostructures* Vol. 6, No 2, (2011) 535-542.

[2] K. Kathiresan Potential of marine bio-nanoparticles for application in nanotechnology Proceedings of 5th National Conference of Indian Association of Applied Microbiologists on emerging trends & evolving technologies in applied microbiology with special reference to microbial nanotechnology, 11-12 January 2007, Kanchipuram. pp.37-40, 2007.

[3] K. Kathiresan, S. Manivannan, M.A. Nabeal, B. Dhivya. *Colloids and Surfaces B: Biointerfaces,* **71** (2009) 133-137.

[4] A. Tripathy, A.M. Raichur, N. Chandrasekaran, T.C. Prathna, A. Mukherjee. *Journal of Nanoparticle Research.* (2009).

[5] Z.P. Xu, Q.H.Zeng, G.Q.Lu, & A.B. Yu. *Chemical Engineering Science.* 61 (2006) p. 1027- 1040.

[6] D.S. Goodsell. *Bionanotechnology: Lessons from Nature*. John Wiley & Sons Inc. Publication (2004).

[7] S. Sinha, P. Chattopadhyay, I. Pan, S. Chatterjee, P. Chanda, D.Bandyopadhyay, K. Das and S.K. Sen. *African Journal of Biotechnology* 8 (2009), p. 6016-6027.

[8] M. Singh, S. Singh, S. Prasad, I.S. Gambhir. *Digest Journal of Nanomaterials and Biostructures* **3 (**2008) p.115.

[9] J. Huang, Q. Li, D. Sun, Y. Lu, Y. Su, X. Yang, H. Wang, Wang, Y.W. Shau, N. He, J. Hong, C. Chen. *Nanotechnology,* **18** (2007) 1-11.

[10] M. Sathishkumar, K. Sneha, S.W. Won, C.W. Cho, S. Kim Y.S. Yun. *Colloids and Surfaces B: Biointerfaces* 73 (2009) p.332.

[11] S. Shankar, A. Ahmad, M. Sastry. *Biotechnol. Prog*., 19 (6), 2003. P.1627- 1631.

[12] S. Shankar, A. Rai, A. Ahmad, M. Sastry. *J Colloid Interface Sci* 275 (2004) p.496–502

[13] S.C. Prathap, M. Chaudhary R. Pasricha, A. Ahmad M. Sastry. *Biotechnol. Prog***.** 22 (2006**) p.**577.

[14] B. Ankamwar, M. Chaudhary, M. Sastry. Synthesis and Reactivity in Inorganic, Metal-organic and Nano-metal Chemistry, 35 (2005) 19-26.

[15] A. Jegan, A. Ramasubbu, S. Vasanth Kumar, M. Balamurugan S. Saravanan. Digest Journal of Nanomaterials and Biostructures Vol. 6, No 1, January-March 2011, p. 325-330.

[16] C. Krishnaraj, E.G. Jagan, S. Rajasekar, P. Selvakumar, P.T. Kalaichelvan, N. Mohan. Colloids and Surfaces B: Biointerfaces 76 (2010) p.50.

[17] H. Bar, D.K. Bhui, G.P. Sahoo, P. Sarkar, S.P. De, A. Misra. Colloids and surfaces A: Physicochemical and engineering aspects 339 (2009) 134- 139.

[18] S. Li, Y. Shen, A. Xie, X. Yu, L. Qui, L. Zhang, Q. Zhang. Green Chemistry. 9 (2007) p. 852-858.

[19] N.A. Begum, S. Mondal, S. Basu, R.A. Laskar, D. Mandal. Colloids and surfaces B: Biointerfaces. 71(2009) 113- 118.

[20] A.W. Bauer, W.M. Kirby, J.C. Sherries, M. Truck. American journal of Clinical Pathology 33(1966) pp.493-96.

[21] A. Rai, A. Singh, A. Ahmad M. Sastry. Langmuir 22 (2006) p.736–741.