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A Facile and Eco-friendly Approach to Synthesize Silver Nanoparticles from *Eclipta prostrata and* Their Anti-bacterial Studies on Isolated Human Pathogens

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Abstract-

Nowadays, nanotechnology has grown to be an important research field in all areas including medicinal chemistry. The size, orientation and physical properties of nanoparticles have reportedly shown to change the performance of any material. For several years, scientists have constantly explored different synthetic methods to synthesize nanoparticles. On the contrary, the green method of synthesis of nanoparticles is easy, efficient, and eco-friendly in comparison to chemical synthesis. The chemical synthesis involves toxic solvents, high pressure, energy and high temperature conversion. Since, green synthesis is the best option to opt for the synthesis of nanoparticles, therefore the nanoparticles were synthesized by using aqueous leaf extract of *Eclipta Prostrata* and metal ions (such as silver). Silver was of particular interest due to its distinctive physical and chemical properties. *Eclipta Prostrata leaf* extract was selected as it is of high medicinal value and it does not require any sample preparation and hence is cost-effective. The fixed ratio of plant extract and silver ions were mixed and kept at room temperature for reduction. The color change from yellow to reddish brown confirmed the formation of nanoparticles. Further, the synthesized nanoparticles were characterized by UV, SEM, TEM and XRD data. The antimicrobial activity of synthesized nanoparticle has also been examined.

Keywords **–** Silver Nanoparticles, Green synthesis, *Eclipta Prostrata, Antibacterial activity*

I.INTRODUCTION

The prospect of exploiting natural resources for metal nanoparticle synthesis has become to be a competent and environmentally benign approach [1]. Green synthesis of nanoparticles is an eco-friendly approach which might pave the way for researchers across the globe to explore the potential of different herbs in order to synthesize nanoparticles [2]. Silver nanoparticles have been reported to be synthesized from various parts of herbal plants viz. bark of Cinnamom, [3] Neem leaves, [4-5] Tannic acid[6] and various plant leaves [7]. Metal nanoparticles have received significant attention in recent years owing to their unique properties and practical applications [8, 9]. In recent times, several groups have been reported to achieve success in the synthesis of Au, Ag and Pd nanoparticles obtained from extracts of plant parts, e.g., leaves [10], lemongrass [11], neem leaves [12-13] and others [14]. These researchers have not only been able to synthesize nanoparticles but also obtained particles of exotic shapes and morphologies [12]. The impressive success in this field has opened up avenues to develop “greener” methods of synthesizing metal nanoparticles with perfect structural properties using mild starting materials. Traditionally, the chemical and physical methods used to synthesize silver nanoparticles are expensive and often raise questions of environmental risk because of involving the use of toxic, hazardous chemicals [15]. Also, majority of the currently prevailing synthetic methods are usually dependent on the use of organic solvents because of hydrophobicity of the capping agents used [16]. Recently, the search for cleaner methods of synthesis has ushered in developing bio-inspired approaches. Bio-inspired methods are advantageous compared to other synthetic methods as, they are economical and restrict the use of toxic chemicals as well as high pressure, energy and temperatures [17]. Nanoparticles may be synthesized either intracellularly or extracellularly employing yeast, fungi bacteria or plant materials which have been found to have diverse applications.

Silver nanoparticles (AgNPs) have been proven to possess immense importance and thus, have been extensively studied [18-20]. AgNPs find use in several applications such as electrical conducting, catalytic, sensing, optical and antimicrobial properties [21]. In the last some years, there has been an upsurge in studying AgNPs on account of their inherent antimicrobial efficacy [22]. They are also being seen as future generation therapeutic agents against several drug-resistant microbes [23]. Physicochemical methods for synthesizing AgNPs thus, pose problems due to use of toxic solvents, high energy consumption and generation of by-products. Accordingly, there is an urgent need to develop environment-friendly procedures for synthesizing AgNPs [24].

Eclipta prostrata (syn. Eclipta alba) commonly known as false daisy, yerba de tago, and bhringraj, is a species of plant in the family Asteraceae. Other common names include kehraj in Assamese and karisalaankanni in Tamil.

This plant has cylindrical, grayish roots. The solitary flower heads are 6–8 mm in diameter, with white florets. The achenes are compressed and narrowly winged.This species grows commonly in moist places as a weed in warm temperate to tropical areas worldwide. It is widely distributedthrough out India, China, Thailand, and Brazil.

The plant has traditional uses in Ayurveda. It is bitter, hot, sharp, dry in taste. In India it is known as bhangra, bhringaraj and bhringraja. Wideliacalendulacea is known by the same names, so the white-flowered E. alba is called white bhangra and the yellow-flowered W. calendulacea is called yellow bhangra. It is reported to improve hair growth and color [25-26].

Ecliptaprostrata contains coumestans suchas wedelolactone and demethyl wedelolactone, polypeptides, polyacetylenes, thiophene derivatives, steroids, triterpenes and flavonoids.

II. EXPERIMENTAL

Materials

Chemicals used in the present study were of highest purity and purchased from Sigma-Aldrich (New Delhi, India); Merck and Himedia (Mumbai, India). *Eclipta prostrata* leaves were collected locally from SCET, Narsapur.

Preparation of plant extract

Plant leaf extract *of Eclipta prostrata* was prepared by taking 5 g of the leaves and properly washed in distilled water. They were then cut into fine pieces and taken in a 250 mL Erlenmeyer flask with 100 mL of sterile distilled water. The mixture was boiled for 5 min before finally filtering it. The extract thus obtained was stored at 4 °C and used within a week [7].

Synthesis of silver nanoparticles

The aqueous solution of 1 mM silver nitrate (AgNO3) was prepared to synthesize AgNPs. 190 mL of aqueous solution of 1 mM AgNO3 was slowly added to10 mL of *Eclipta prostrata* aqueous leaf extract while stirring, for reduction into Ag ions and kept at room temperature for 20 h [7, 27].

UV-Vis spectra analysis

UV-Vis spectrum of the reaction medium recorded the reduction of pure Ag+ ions at different hours after diluting the sample with distilled water. UV-Vis spectral analysis was performed by using UV-Vis double beam spectrophotometer.

XRD (X-ray diffraction) measurement

The AgNP solution was repeatedly centrifuged at 5000 rpm for 20 min, re-dispersed with distilled water and lyophilized to obtain pure AgNPs pellets. The dried mixture of AgNPs was collected to determine the formation of AgNPs by X'Pert Pro x-ray diffractometer (PANalytical BV, The Netherlands) operated at a voltage of 30 kV and a current of 30 mA with CuKα radiation in a θ-2θ configuration.

SEM

SEM is a powerful instrument which permits the observation characterization of heterogeneous organic and inorganic materials. In this studies SEM is used to analyze micro structural characteristics of AgNPs. SEM images were obtained from JEOL

JSM – 6610LV.

TEM

TEM technique wasemployed to visualize the size and shape of Ag nanoparticles.The200kV Ultra High Resolution Trans-mission ElectronMicroscope (JEOL-JEM2100F) has been used. TEM grids were prepared by placing a drop of the particle solution on a carbon-coated copper grid and drying under lamp. Fig. 4. shows the typical bright-field TEM micrograph of the synthesized Ag nanoparticles. It is observed that most of the Ag nanoparticles were spherical in shape.

Antimicrobial Activities

Luria Bertani broth (Himedia), Luria Bertani agar (Himedia) standard antibiotic and ciprofloxacin (Himedia) were used in antimicrobial sensitivity testing. Briefly, Luria Bertani (LB) broth/agar medium was used to cultivate bacteria. Fresh overnight cultures of inoculum (50 µl) of each culture were spread on to LB agar plates. Sterile paper discs of 5 mm diameter (containing 30 µl of AgNPs) along with the standard antibiotic, containing discs were placed in each plate. Antimicrobial activities of the synthesized AgNPs were determined, using the agar disc diffusion assay method [27].

III. RESULTS & DISCUSSIONS

UV-Visible studies

UV–Vis spectroscopy is an important technique to establish the formation and stability of metal nanoparticles in aqueous solution [28].The relationship between UV-visible radiation absorbance characteristics and the absorbate's size and shape is well-known. Consequently, shape and size of nanoparticles in aqueous suspension can be assessed by UV-visible absorbance studies.

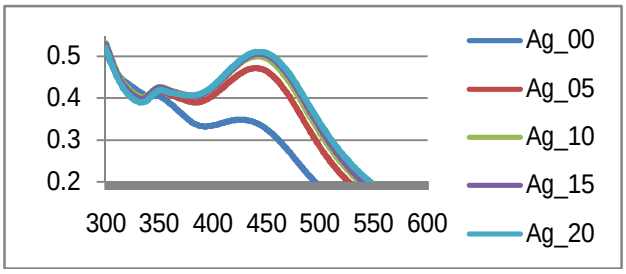

Fig. 1. UV-Vis absorption spectrum of AgNPs synthesized by treating 1mM $AgNO_3$ solution with *Eclipta prostrata* leaf extract at different time periods.

Fig. 1 depicts the absorbance spectra of reaction mixture containing aqueous silver nitrate solution (1 mM) and *Eclipta prostrata* (prepared from 5 g leaf material). The absorption spectra obtained reveal the production of AgNPs within 5 h. On adding the afore-mentioned plant broth to AgNO3 solution, the solution changed from yellowish orange to brown. The final color turns deep and finally, brownish with passage of

time. The intensity of the absorbance was found to increase as the reaction proceeded further.

AgNPs displaying intense yellowish brown colour in water arises from the surface plasmons. This is due to the dipole oscillation arising when an electromagnetic field in the visible range is coupled to the collective oscillations of conduction electrons. It is an established fact that metal nanoparticles ranging from 2 to 100 nm in size demonstrate strong and broad surface plasmon peak [29]. The optical absorption spectra of metal nanoparticles that are governed by surface plasmon resonances (SPR), move towards elongated wavelengths, with the increase in particle size. The absorption band position is also strongly dependent on dielectric constant of the medium and surface-adsorbed species [30-31].

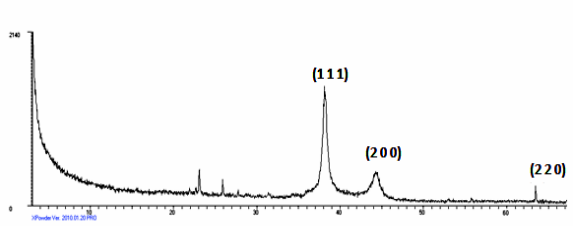

Fig. 2. shows the XRD-spectrum of purified sample of AgNPs.

XRD studies

The peaks observed in the spectrum at 2θ values of 38.07°, 44.15° and 64.49° corresponds to (111), (200) and (220) planes for silver, respectively (Fig. 2.)[15]. A peak at 46º is possibly due to crystalline nature of the capping agent [32, 10]. This clearly shows that the AgNPs are crystalline in nature due to reduction of Ag+ ions by *Eclipta prostrata* leaf extract. The AgNPs were centrifuged and redispersed in distilled water several times before XRD analysis. This excludes the possibility of any free compound/protein present that might lead to independent crystallization and thus, resulting in Bragg's reflections. Usually, the particle size is responsible for the broadening of peaks in the XRD pattern of solids [33]. The noise due to the protein shell surrounding the nanoparticles is visible from the spectrum [34].

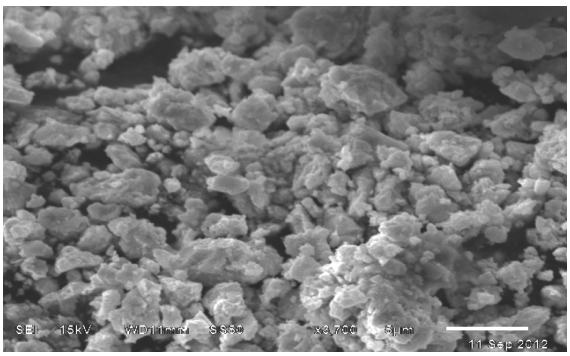

Fig. 3. shows the *SEM image* of purified sample of AgNPs.

SEM Studies

The SEM (Scanning Electron Microscopy) image for silver nanoparticles was presented in Fig. 3. Form this figure it was observed that the formed nanoparticles are crystalline in nature with certain degree of porocity. The schererr rings, which are characteristics of FCC for nanoparticles, were clearly observed. From this the structures seen in SEM image reveals that synthesized nanoparticles are nanocrystalline in nature.

It was observed that silver nanoparticles were scattered over the surface and no aggregation was noticed.

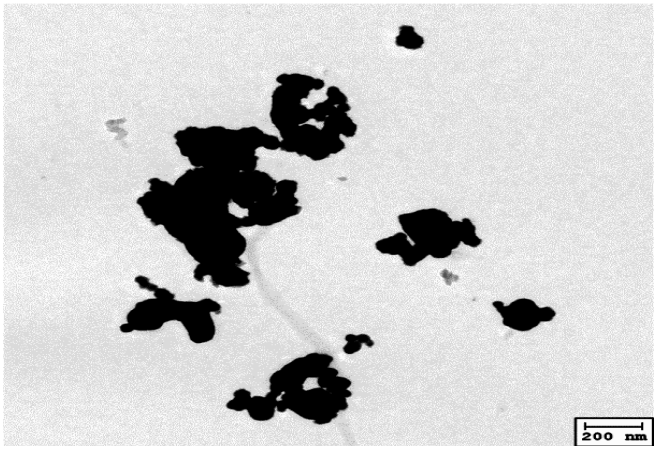

Fig. 4. shows the *TEM image* of purified sample of AgNPs

TEM studies

Silver nanoparticles are further examined using transmission electron microscopy (TEM). The TEM images of synthesized silver nanoparticles was recorded and presented in Fig. 4. The average size of silver nanoparticles was observed as 15±2 nm which was in agreement with Scherers equation.

Antimicrobial Activities

Table. I. Zone of Inhibition of nano metals by agar well diffusion method

S.No	Human Pathogenic Bacteria	Disease	Zone of Inhibition (mm)*		
			Ag	extract	Antibiotic+
1	Salmonella typhi	Typhoid	12	7	17
2	Vibrio cholerae	Cholera	11	7	18
3	Shigelladysenteria	Shigellosis	9	8	18
4	Enterococcus faecalis	Gastrointestinal infection	10	7	19

Well size 6mm, *zones at 30µg/ml concentration, +Ciprofloxacin.

The antibacterial activity of AgNPs was determined against hugman gastrointestinal pathogens viz., *S. typhi, V. cholerae, S. dysenteriae, E. faecalis* (Table

I). The nanoparticles showed consistently moderate antibacterial activity when compared to ciprofloxacin. The zone of inhibitions of nano particles found to be in the range between 9-12mm. 10-100 mg/ml was determined minimum inhibitory concentration (MIC) of nano, which required to inhibit the bacterial growth. 12 mm was highest zone of inhibition of Ag particles on *S. typhi* with lowest MIC (1mg/ml).

IV. CONCLUSIONS

The present study represents a clean, non-toxic as well as eco-friendly procedure for synthesizing AgNPs. The capping around each particle provides regular chemical environment formed by the bio-organic compound present in the *Eclipta prostrata* leaf extract, which may be chiefly responsible for the particles to become stabilized. This technique gives us a simple and efficient way for the synthesis of nanoparticles with tunable optical properties governed by particle size. From the of nanotechnology point of view, this is a noteworthy development for synthesizing AgNPs economically. In conclusion, this green chemistry approach toward the synthesis of AgNPs possesses several advantages viz, easy process by which this may be scaled up, economic viability, etc. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially stimulating for the large-scale synthesis of other inorganic materials, like nanomaterials.

REFERENCES

[1]. Bhattacharya, D.; Gupta, R. K.; Crit. Rev. Biotechnol. 2005, 25, 199.
[2]. Savithramma, N.; Rao, M. L.; Devi, P. S. J. Biol. Sci. 2011, 11, 39.
[3]. Sathishkumar, M.; Sneha, K.; Won, S. W.; Cho, C. W.; Kim, S.; Yun Y. S. Colloids Surf. B 2009, 73, 332.
[4]. Tripathi, A.; Chandrasekaran, N.; Raichur, A. M.; Mukherjee, A. J. Biomed. Nanotechnol.2009, 5, 93.
[5]. Prathna, T. C.; Chandrasekaran, N.; Raichur A. M.; Mukherjee, A. Colloids Surf. B 2011, 82, 152.
[6]. Sivaraman, S. K.; Elango, I.; Kumar, S.; Santhanam, V. Curr.Sci. 2009, 97, 1055.
[7]. Song, J. Y.; Kim B. S. Bioproc. Biosyst.Eng. 2009, 32, 79.
[8]. Ahmad, A.; Mukherjee, P.; Senapati, S.; Mandal, D.; Khan, M. I.; Kumar, R.; Sastry, M. Colloids Surf. B 2003, 28, 313.
[9]. Shahverdi, A.R.; Minaeian, S.; Shahverdi, H.R.; Jamalifar, H.; Nohi, A.A. Proc. Biochem. 2007, 2, 919.
[10]. Shankar, S. S.; Ahmad, A.; Sastry, M. Biotechnol. Prog.2003, 19, 1627.
[11]. Shankar, S. S.; Rai, A.; Ahmad, A.; Sastry, M. J. Colloid Interf. Sci. 2004, 75, 496.
[12]. Shankar, S. S.; Rai, A.; Ahmad, A.; Sastry, M. Chem. Mater. 2005, 17, 566.
[13]. Chandran, S.P.; Chaudhary, M.; Pasricha, R.; Ahmad, A.; Sastry, M.

Biotechnol. Prog.2006, 22, 577.
[14]. Huang, J.; Li, Q.; Sun, D.; Lu, Y.; Su, Y.; Yang, X.; Wang, H.; Wang, Y.; Shao, W.; He, N.; Hong, J.; Chen, C. Nanotechnology 2007, 18, 105104.
[15]. Tripathy, A.; Raichur, A. M.; Chandrasekaran, N.; Prathna, T.C.; Mukherjee, A.; J. Nanopart. Res. 2010, 12, 237.
[16]. Raveendran, P.; Fu, J.; Wallen, S.L. J. Am. Chem. Soc. 2003, 125, 13940.
[17]. Parashar, U. K.; Saxena, P. S.; Srivastava, A. Dig. J. Nanomater. Bios.2009, 4, 159.
[18]. Nair, L. S.; Laurencin, C. T. J. Biomed. Nanotechnol.2007, 3, 301.
[19]. Zhang, Y. W.; Peng, H. S.; Huang, W.; Zhou, Y. F.; Yan, D. Y. J. Colloid Interf. Sci. 2008, 325, 371.
[20]. Sharma, V.K.; Yngard, R.A.; Lin, Y. Adv. Colloid Interf. Sci. 2009, 145, 83.
[21]. Abou El-Nour MM, Eftaiha A, Al-Warthan A, Ammar RAA. Arab J. Chem. 2010, 3: 182, 135.
[22]. Choi, O.; Deng, K.K.; Kim, N.J.; Ross Jr., L.; Surampalli, R.Y.; Hu, Z. Water Res. 2008, 42, 3066.
[23]. Rai, M.; Yadav, A.; Gade, A. Biotechnol. Adv. 2009, 27, 76.
[24]. Thakkar, K. N.; Mhatre, S. S.; Parikh, R.Y. Nanomedicine NBM 2010, 6, 257.
[25]. Kritikar, KR., Basu, BD. 1975.Chronica Botanica Indian Medicinal plants. New Delhi.
[26]. Chopra, RN., Nayar, SL., Chopra, IC., 1955. Glossary of Indian Medicinal plants. C.S.I.R., New Delh.
[27]. Jain, D.; Daima, H. K.; Kachhwaha, S.; Kothari S. L. Dig. J. Nanomater. Bios.2009, 4, 557.
[28]. Philip, D.; Unni, C.; Aromal, S. A.; Vidhu V. K. Spectrochim. Acta A 2011, 78, 899.
[29]. Prathap, S. C.; Chaudhary, M.; Pasricha, R.; Ahmad A.; Sastry, M. Biotechnol. Prog.2006, 22, 577.
[30]. Xia, Y.; Halas, N. J. Mrs. Bull. 2005, 30, 338.
[31]. Mie, G.; Ann. D. Physik. 1908, 25, 377.
[32]. Mukherjee, P.; Roy, M.; Mandal, B. P.; Dey, G. K.; Mukherjee, P. K.; Ghatak, J.; Tyagi, A. K.; Kale, S. P. Nanotechnology 2008, 19, 075103.
[33]. Jenkins, R.; Snyder, R. L. Introduction to X-ray powder diffractometry; Winefordner, J. D. (Ed.); Wiley: USA, 1996, pp. 403+xxiii.
[34]. Vigneshwaran, N.; Ashtaputre, N. M.; Varadarajan, P. V.; Nachane, R. P.; Paralikar, K. M.; Balasubramanya, R. H. Mater. Lett.2007, 61, 1413.