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Exploitation of Parthenium Hysterophorus L. for the Rapid Biosynthesis of Silver Nanoparticles and Evaluation of their Anti-Microbial Activity

KEYWORDS: Rapid biosynthesis, silver nanoparticles Parthenium hysterophorus L, antibacterial activity

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ABSTRACT *The improvement of rapid and reliable processes for the synthesis of nanosized materials is of great importance in the field of nanotechnology. In this paper, we describe a novel synthesis approach which is rapid, simple and "green" for the synthesis of metallic nanostructures of noble metals such as silver, by using plant extract of Parthenium hysterophorus L treated with 1mM AgNO3 solution and microwave irradiation. It was found that exposure of plant extract of Parthenium hysterophorus L and microwave irradiation to silver ion lead to the formation of silver nanoparticles. The nanoparticles were examined using UV-Visible Spectroscopy, X Ray diffraction, Scanning electron microscopy, and Transmission Electron Microscopy analyses. The formation of nanoparticles by this method is extremely rapid, requires no toxic chemicals and the nanoparticles are stable for few months. Further these biologically synthesized nanoparticles were found to be toxic against different human pathogens namely Escherichia coli L, Pseudomonas aeruginosa L, Klebsiella pneumoniae L. The antimicrobial activity of the silver nanoparticles was determined. Scanning Electron Microscopy micrograph analysis and Transmission Electron Microscopy of the silver nanoparticles indicated that they were well-dispersed and the size was found to be around 10nm.*

INTRODUCTION

The systems being designed and produced at incredibly small scale of atoms and molecules, i.e, nanoscale refers to nanotechnology. This field of science has made its place in production of nanomaterials which are regarded as 'first generation' products that includes nanoparticles, nanocrystals, nanobiomotors, nanowires, quantum dots etc. The worldwide emergence of nanoscale sciences and engineering was marked by the announcement of the National Nanotechnology Initiative in Jan 2000 [1]. Nanomaterials are the leading edge because of their unique properties which has enabled the technology to acquire the superiority in the applied fields and made it indispensible in areas of human activity [2]. A decade ago, nanoparticles were studies because of their size-dependent physical and chemical properties [3], now they have entered a commercial exploration period [4, 5], with the development, nanomaterial level is now the most advanced in both scientific knowledge and commercial applications. Nanoparticles are engineered structures with at least one dimension of 100 nanometers or less. These novel materials are increasingly used for commercial products, including developing new designs for medicinal applications [5].

Metal nanoparticles such as gold and silver have been recognized to be important in the fields of chemistry, physics and biology. These particles are being processed for various purposes because of their remarkable properties such as conductivity, biocompatibility, optical, photothermal, magnetic, catalytic properties and also antimicrobial activity. The size and size distribution of the particles is extremely a critical condition to be considered. Other physiochemical factors which are also important are shape, morphology, charge, area, reactivity and chemical surroundings [7-12].

Silver nanoparticles have received considerable attention due to their attractive physical and chemical properties [13]. Silver is currently used to control bacterial growth in variety of applications. Silver nanoparticles have a potential ability to kill over 650 different bacteria [14,15]. Antimicrobial activity of silver is recently being used in medicine to reduce infections as well as to prevent bacterial colonization on prostheses [16], cathetars [17, 18], vascular grafts [19], dental materials [20], stainless steel materials [21] and human skin [22, 23]. The use of silver nanoparticles as antibacterial agent is relatively new. It is because of their high reactivity due to large surface to volume ratio. Nanoparticles of silver are now used in tooth pastes, soaps, fabrics, disinfectants, house hold appliances, also used as intercalating material, optical receptors, display devices, for biolabelling, for diagnosing biological problems, as antimicrobials, as anti-inflammatory agents [24, 25, 26].

Synthesis of Ag nanoparticles can be achieved by chemical routes[27], or by means like sol-process, sol-gel process, pyrolysis[28], chemical vapor deposition, gas condensation, co-condensation[29],thermal decomposition, radiation assisted, microwave radiation assisted process or by bio-based protocols using either microbial or plant extract. In the process of synthesis, aqueous solution of silver nitrate is reduced to silver nanoparticles by the reducing agent used in the corresponding method adopted. Some of the chemicals used by researches are citric acid, trisodium citrate [30], borohyride [6, 31], ethanol for the purpose of reduction. Although chemical method is the simple one [21] the use of environmentally benign materials like certain plant, bacteria or fungi extracts for the synthesis offers numerous benefits of eco-friendliness, cost-effectiveness, elimination of high pressure, high energy as well.

We have used *Parthenium hysterophorus L.* is an obnoxious weed which is popularly called as congress weed in India. This was introduced in India in 1956 and spread over most part of the country and it is known for causing skin itching just by touch for which its considered regardless. We have worked to get the best out of this undesirable weed, for synthesis of silver nanoparticles on reduction of silver ions present in aqueous solution of silver complex in *Parthenium hysterophorus L* extract and which can be demonstrated with the change in color due to formation of nanoparticles.

MATERIALS AND METHODS

Preparation of leaf broth

In the present investigation the broth was prepared using 25g of fresh *from Parthenium hysterophorus L* shown in Fig1, washed thoroughly, dried, cut into fine pieces and boiled in 100ml Millipore water for 10 min. The broth was filtered using Whatman's filter paper and filtrate was used as reducing agent to reduce silver.

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Figure 1. *Parthenium hysterophorus* plant.

Biosynthesis and characterization of silver nanoparticle
Conventional method of biosynthesis of silver nanoparticles using extract of *Parthenium hysterophorus L*

In a typical reaction procedure, 1mM silver nitrate solution was prepared by dissolving 0.16g of AgNO3 in 1000ml Millipore water. The Leaf broth was added to silver nitrate in the ratio of 1:5 and the mixture is incubated under dark conditions at room temperature for 5 days to facilitating the formation of silver nanoparticles. Control experiments were conducted with uninoculated broth, to check for the role of broth in the synthesis of nanoparticles.

Rapid method of biosynthesis of silver nanoparticles using extract of *Parthenium hysterophorus*
Leaf broth treated with 1mM AgNO3 in the ratio of 1:5, the reaction mixture was subjected to several short burst of microwave irradiation in a cyclic mode. A cycle constituting 15 seconds exposure to microwave radiation and 20seconds of non-exposure to prevent over heating as well as aggregation of metals. The reaction mixture was monitored by sampling of aliquot (1ml) of solution after 5, 7,9,12 and 15 cycles, control experiments were conducted with uninoculated broth. Suspension is centrifuged at a speed of 1000rpm for 30 minutes, pellets were collected, and collected pellets were washed and dried in a hot air oven.

UV-Vis Spectrophotometric analysis
UV–vis spectroscopic studies were carried out using Shimadzu UV Vis 1601 Shimadzu Spectrophotometer. The reduction of Ag+ ions was monitored by sampling an aliquot (1 mL) of the solution at intervals of each cycle and measured the UV-Vis spectra of the solution. The spectra were recorded at room temperature using a one-centimetre quartz cuvette1.

XRD analysis of silver naoparticles
XRD analysis is one of the preferred analytical tool for estimating the size of crystalline particle. The purified silver nanoparticles were characterized by X-ray diffraction method and SEM. The nanoparticle powder which is obtained is subjected to screening by X ray diffraction studies using XRD JOEL-JDX8030 and diffffractometer with CuK tange.t The crystalline domain size was calculated from XRD peaks using Debye-Scherrer formula

SEM and TEM analysis of silver nanoparticles
Powder extracted was subjected to electron microscopy studies to determine the morphology and size of the synthesized silver nanoparticles. SEM data helped us to figure out the morphology wherein TEM gives the size.

Antibacterial assay
Silver nanoparticles synthesized from *Parthenium hysterophorus L broth* were tested against against *E. coli L, Psuedomonas aueroginosa L and Klebsiella pnuemoniae L* by well diffusion method . Pure cultures were subcultued in Muller Hinton broth for 24 h at 37°C. Wells of 5 mm diameter were made on Muller Hinton agar plates using gel puncture. Each strain was swabbed uniformly into the individual plates using sterile cotton swabs. Using sterile micropipette 20 µl (0.002mg) of the sample of nanoparticle solution was poured onto each of the wells at the centre in all the plates. After incubation at 35 °C for 24 h the different levels of zone of inhibition were measured using the Hi antibiotic zone scale.

RESULT AND DISCUSSION
Parthenium hysterophorus L leaf extract appears green in color. This extract treated with 1mM $AgNO_3$ and incubated at room temperature for 7 days, the color change in the suspension to reddish brown is the primary indication of the formation of silver nanoparticles. Change in color which is due to the excitation of Suface Plasmon Resonance (SPR) (29). In metal nanoparticles such as silver, the conduction band and the valence band lie very close to each other in which electrons move freely (29). These free electrons give rise to SPR absorption band occurring due to the collective oscillation of electrons of silver nanoparticles in resonance with light wave. Classically, the electric field of an incoming wave induces a polarization of the electrons with respect to much heavier ionic core of silver nanoparticles. As a result a net charge difference occurs which in turn acts as a restoring force. This creates a dipolar oscillation of all the electrons with the same phase. When the frequency of the electromagnetic field becomes resonant with the coherent electron motion, a strong absorption takes place, which is the origin of the observed color Fig.2

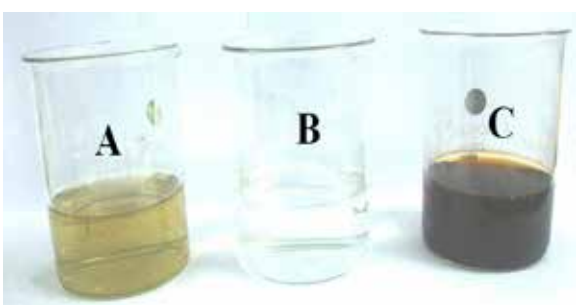

Figure 2.A. Picture showing the leaf extract of *Parthenium hysterophorus*, .B. Picture showing 1mM $AgNO_3$ solution without the leaf extract .2.C. Picture showing the resulting mixture of plant extract and silver nitrate (1:5) after 5 days of incubation.

Rapid biosynthesis method, where in the mixture is irradiated with 5,7,9,12 and 15 cycles, Fig.3 shows the gradient in the color formation from yellow to reddish brown respectively. The reduction of silver to nanoparticles increasingly proceed with the increase number of cycles in other words increase in time of exposure to radiation.

Figure3. Picture showing the samples after rapid biosynthesis of silver nanoparticles using *Parthenium hystero-*

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***phorus* leaf extract at the end of 5th cycle, 7th cycle, 9th cycle, 12th cycle and 15th cycle respectively.**

UV-Vis absorbance spectra of silver nanoparticles biosynthesized conventionally by treating 1ml aqueous AgNO3 solution with leaf broth of *Parthenium hysterophorus L* is shown in Fig.4. The wavelength, λ_{max} is obtained at 426 nm with absorbance value 2.731.The sharp peak and the Surface Plasmon Resonance band in the silver nanoparticles for 5th,7th,9th,12 and 15th cycles also remain close to 420nm suggests that particles are monodispersed and distributed with no evidence of aggregation Fig.5. The absorption intensities were ranged from 1.38 and increased upto 2.49 which is higher than 1.5 reported with plant leaf extract earlier [11], suggesting that the conversion of silver is higher. The bands obtained are nearly symmetrical reflecting more on uniform distribution.

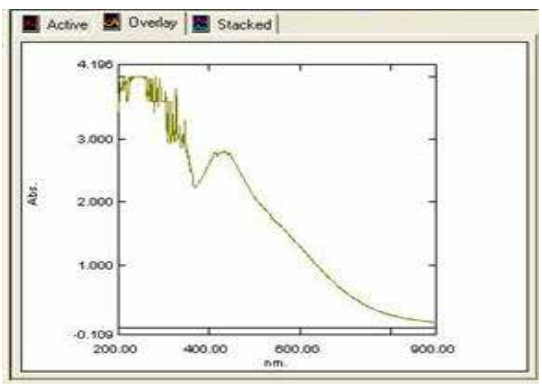


Figure 4. UV-Vis absorbance spectra of silver nanoparticles biosynthesized conventionally.

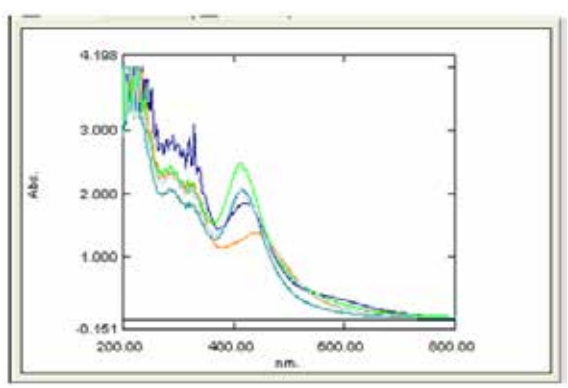


Figure 5. UV-Vis absorption spectra of silver naoparticles derived using rapid biosynthesis method.

One major advantage of this rapid biosynthesis method is time required for the formation of nanoparticles. Conventional incubation method takes 7days where in with the help of irradiation, silver nanoparticles can be synthesized within few sec. The other advantages of using microwave radiation is that it provides uniform heating around the nanoparticles and can assist the digestive ripening of such particles without aggregation. The microwave radiation heats up a material through its dielectric loss, which converts the radiation energy into thermal energy. Rapid microwave heating also provides uniform nucleation and growth conditions, leading to homogeneous nanomaterials with smaller sizes. Power dissipation is fairly uniform throughout with "deep" inside-out heating of the polar solvents, which leads to a better crystallinity.

XRD patterns obtained for silver nanoparticles synthesized by rapid method using extract of *Parthenium hysterophorus L* at 12th cycle marked (111) indexed based had the cubic structure. The XRD pattern of Ag ions is known to display peak at 2λ= 7.9λ,11.9λ,17.8λ,30λ,38λ,44λFig.6.

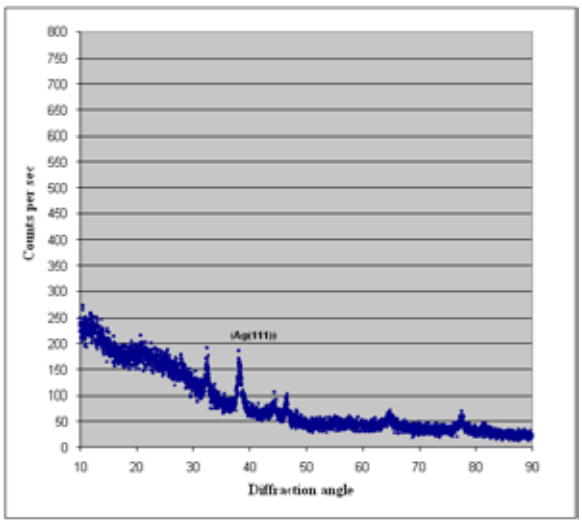


Fig.6. XRD pattern of silver nanoparticles obtained at 12th cycle of rapidly biosynthesis.

From this study the peak was obtained at 38˚. FWHM for the peak is estimated as 0.0157 rad. Average particle size can be estimated using Debye-Scherrer formula given by

D = 0.9 λ / W Cos λ
where D is the particle size

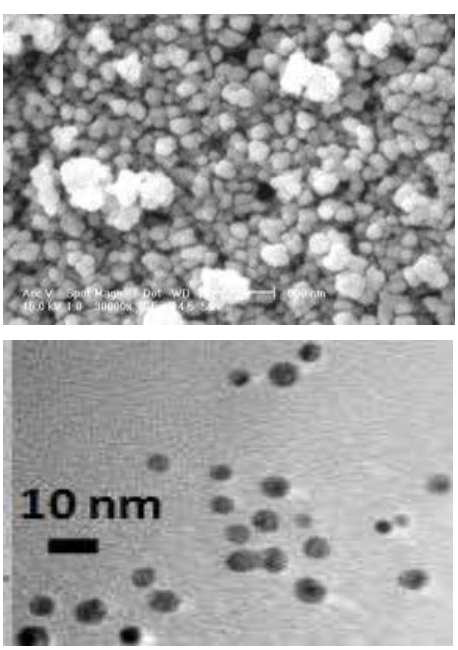
λ is the wavelength of X ray= 0.1541nm

W is Full Width at Half Maximum (FWHM)

Calculation
D = (0.9 * 0.1541)/ 0.0157 *Cos (19)

= **9.3 nm**

Hence theoretical value of the particle size is found to be 9.3nm.



Figur.7. SEM micrograph Figure8. TEM micrograph
A typical TEM and SEM micrographs are shown in the Fig.7&8

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respectively of silver nanoaprticles obtained by the synthesis of *Parthenium hysterophorus L* leaf broth. With the help of these micrographs, the average particlese size of silver nanoparticles is around 10nm and are cubical in shape. TEM analysis helps us to determine the size of the particles this is due to the fact that during transmission electron microscopy the electrons penetrate through the particle and the beam of electron are analyzed.SEM ananlysis helps us to determine the morphology of the particle since the electrons from the surface are reflected and the beam is of these reflected electrons are scanned. The size value is in an accordance with the theoretical value of size of the silver naoparticles as per XRD analysis.

The anti microbial activity was tested against *E. coli, Psuedomonas aueroginosa L and Klebsiella pnuemon L* using disc diffusion method. Control is also maintained in which no zone of inhibition is observed. The diameter of the zone of inhibition around each disc with silver nanoparticles is represented Fig.9.

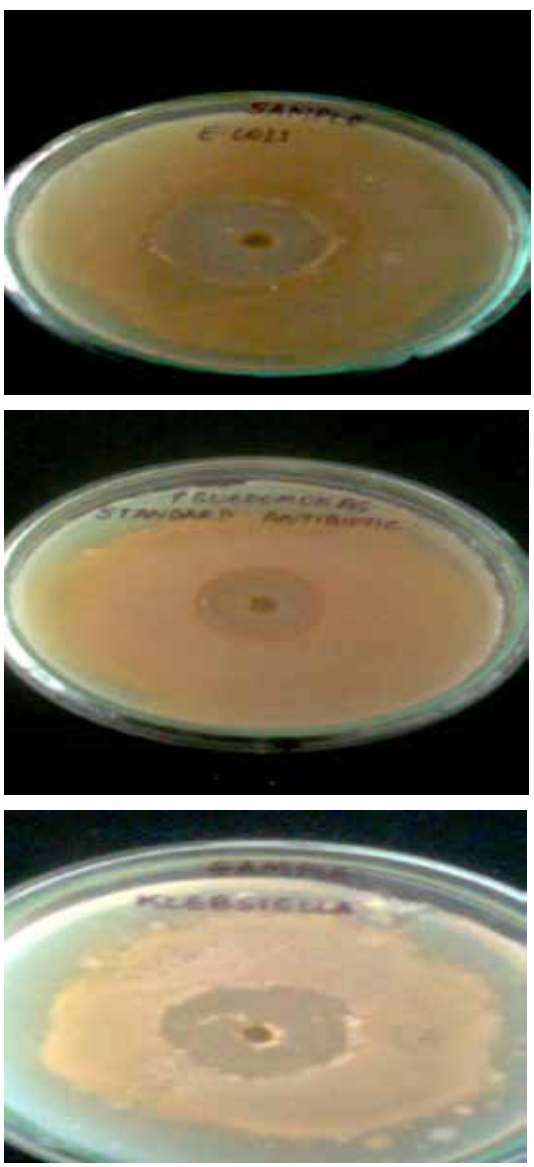
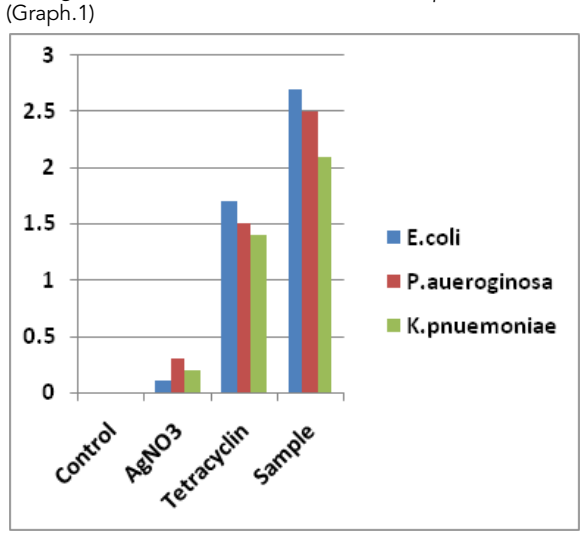


Figure9. Anti microbial activity against E.coli, *Psuedomonas aueroginosa, Klebsiella pnuemoniae* of silver nanoparticle

The highest antimicrobial activity was observed in *E.coli L,* followed by *Pseudomonas aueroginosa L* which is in turn followed by *Klebsiella pnuemoniae L*. Silver has been known for its well known anti microbial properties since roman time however the advances in generating silver nanoparticles has made possible a revival of the use of silver as a powerful bactericide. Many researchers have used *E.coli* as a model for gram negative bacteria and proved that silver nanoparticles may be used as an anti microbial agent. In the present study 20µL of the nanoparticles was taken as the final product for the antimicrobial assay. The anti bacterial activity of the Silver nanoaprticles for *E.coli L* was maximum (2.7cm) followed by *Pseudomonas aueroginosa L* (2.5 cm) followed by *Klebsiella pnuemoniae L* (2.1cm). However the control showed no zone of inhibition, whereas silver nitrate showed neglible zone of inhibition when compared to the zone of inhibition observed by virtue of silver nanoaprticles. Silver nitrate showed a zone of inhibition of (0.1 cm) in *E.coli*, (0.3 cm) in *Pseudomonas aueroginosa L* and (0.2 cm) in *Klebsiella pnuemoniae L.* (Graph.1)



Graph.1. Anti microbial activity of silver nanoparticles, tetracycline, silver nitrate and control (leaf extract) against E.coli, *Psuedomonas aueroginosa, Klebsiella pnuemoniae* of silver nanoparticle.

CONCLUSION:

In conclusion, the biosynthesis of silver nanoparticles by reducing Ag+ using the leaf extract of the plant *Parthenium hysterophorus L* has been demonstrated. Green synthesis approach for synthesis is advantageous over chemical methods.The formation of silver nanoparticles is faster with rapid biosynthesis method compared to conventional incubation method. The dimension of nanopartilce was in accordance with the microscopy results. Antimicrobial activity of pathogens like *E,coli, Pseudomonas* and *Klebsiella* species is studied. Ag nanoparticles prepared in this process are quiet stable and remain intact for nearly 12 weeks if it protected under light proof conditions. In future, it would be important to understand the biochemical and molecular mechanism of the synthesis of the nanoparticles by the leaf extract in order to achieve better control over size and polydispersity of the nanoparticles.

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