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Green synthesis of silver nanoparticles using Hygrophila auriculata seed extract with antibacterial activities

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Abstract
In the present study, biosynthesis of silver nanoparticles (AgNPs) using the aqueous extract of Hygrophila Auriculata plant seed act as a reducing and capping agent has been reported in the present work. Biosynthesized AgNPs were primarily confirmed by change in colour from colourless to brown. UV-Vis spectrum of the AgNPs showed surface plasmon resonance (SPR) peak at 443 nm. Fourier transform infrared spectroscopy (FTIR) was used to key out the specific functional groups responsible for the reduction of silver nitrate to form silver nanoparticles and the capping agents present in the seed extract. The field emission scanning electron microscopy (FESEM) analyses revealed that the synthesized AgNPs were shape and the particle size was estimated in the range1-100 nm. The energy dispersive X-ray analysis (EDX) spectrum showed peaks for the presence in the range 5 Kev. The X-ray powder diffraction (XRD). The structural phase of the AgNPs was found in the form of face centred cubic (FCC).The antibacterial effect of nanoparticles produced Hygrophila Auriculata was studied using different pathogenic bacteria such as Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. From the disc diffusion methods, the synthesized silver nanoparticles showed an antibacterial activity.

Keywords: Hygrophila Auriculata, silver nanoparticles, Antibacterial properties

1. Introduction
The silver nanoparticles (AgNPs) has received special interest worldwide from scientific community in the field of nanotechnology due to their unique properties and biological applications. When compared to other physical and chemical methods, Green synthesis of nanomaterials is considered as a clean, non-toxic and environmental-friendly method [1]. Nanotechnology is a combination of two words; nano and technology. The term nano originate from Greek word nanos meaning extremely small or dwarf i.e. one billionth part (10^{-9}). Nanoparticles as particles having various dimensions and size ranging from 1 to 100 nm [2]. Metal nanoparticles are good chemically reactive as compared to bulk solid counterparts. They are widely used for fabrication and preparation of metal nanoparticles have become increasingly popular in recent years [3, 4]. They have numerous superior properties and are widely applied in various fields such as medicine, photo catalysis, lithograpy, electronics, surface enhanced resonance Raman scattering, optical biosensors, etc [5-8]. The silver nanoparticles have got much recognition because nanomaterials have a long list of applicability in improving human life and its environment [9]. The way in which a biological system fabricates and reproduces structural and functional inorganic materials with precise dimensions and controlled morphology has attracted nanotechnologist [10].

Nanoparticles bridges the gap between bulk level and atomic-molecular level. To utilize and optimize chemical and physical properties of nano-sized metal particles, a large spectrum of research has been focused to control the size and shape which crucial in tunning their properties [11]. Among these methods, the chemical reduction of silver ions to metallic silver in the presence of capping agents is the most popular method for the preparation of silver nanoparticles [12]. Surfactants and polymers are the most commonly used capping agents [13, 14]. However, most of the capping agents and reducing agents used in the chemical methods involve in toxicity and other hazards and may inflict continuous risks on the environment [15]. Furthermore, the use of environmental toxicity or biological hazards may limit the application of silver nanoparticles. The researchers are showing an upsurge of interest in exploring new protocols to prepare silver nanoparticles using green technology to eliminate the effects of hazards as well as chemical on the environment and to maximize safety and efficiency, [16]. Besides this, it has been found that 5000 years old Indian system of Medicine Ayurveda had some knowledge of nano-scale fabrication used for medicinal purposes [17].

The medicinal herb Hygrophila Auriculata (HA) belongs to the family of Acanthaceae. The different parts of HA (root, flower, stem, fruit, and leaves) have been traditionally used to treat various health issues, including urinary calculi and cystisis [18]. A survey of the literature shows that the root, seeds, and aerial parts of the plant are widley used in the traditional system of medicine for the treatment of jaundice, hepatic obstruction, rheumatism, inflammation, pain, urinary infection, edema, gout, malaria, impotence and as an aphrodisiac. The seed are used as ingredients in various aphrodisiacs and tonic confections, and in the treatment of blood disorders, biliousness, gonorrhoea, spermatorrhea and fever.

In this study, we conducted a biosynthesis of Ag-NPs extract of H. auriculata. The obtained Ag-NPs were characterized by UV-Vis spectrophotometry (UV-Vis), X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Field emission scanning electron microscopy (FESEM) and (EDX), and antibacterial activity.

2. Experimental Methods

2.1 Chemical and Glassware

Silver Nitrate (Analytical grade) was purchased from sigma Aldrich whereas Hygrophila Auriculata seeds (Fig.1) were purchased from a local market in Trichy, Tamil Nadu, India.

Morphological characters

The plant is a sub shrub, usually growing in marshy places along water courses. The stem is reddish brown and the shoot has 8 leaves and 6 thorns at each node. The leaves occur in whorls, the outer pair of leaves is larger, lanceolate, scalerous, margins, minutely denate, subsessile, thorns strong straight or curved. Flowers occur in axillary whorls, bract and bracteoles leafy. Calyx four lobed, lobes unequal. Corolla,-5 petals gamopetalous, unequally 2-lipped, middle lobe of the lower lip with yellow palate; corolla purple coloured; stamens – four, in two pair, filaments unequal; anthers divergent; ovary two celled; four ovules in each cell; fruit a dehiscent capsule.

2.2 Preparation of Seed extract

20 gram of Hygrophila Auriculata seeds were gathered and washed with double distilled to remove dirty particles. The seeds were dried and grained with mortar. The mashed sample was mingled with 100 ml of double distilled water in a 500 ml beaker and kept at 60 $^{\circ}$C in haeating mantle. Using Whatman No.1 filter paper, the sample was filtered. Then the final extract of seed was stored.

2.3 Synthesis of Silver nanoparticles

A 50 ml of aqueous solution was prepared which consists of 1mM Silver nitrate is (AgNPs) and this solution is used for the synthesis of silver nanoparticles. To achieve efficient synthesis, 5 ml of H. Auriculata seed extract was added into aqueous solution of 1 mM silver nitrate with constant stirring on a magnetic stirrer for 1 hour. The colour change of solution was monitored. At constant 8000 rpm, the fully condensed solution was centrifuged for about 10 minutes. The brown colour of prepared nanoparticles from colourless indicates 100% conversion of silver ions in silver nanoparticles as shown in Fig.2.

3. Charcterization of Silver Nanoparticles

3.1 UV-Vis Spectroscopy

The UV-Visible absorption peaks were observed in the visible range 443 nm by using UV-VISIBLE Spectrophotometer lambda 35 Perkin Elmer which range between 190-1100 nm [19].

3.2 FTIR Spcetrophotometry

FTIR Spectrum 1000 Perkin Elmer Spectrometer has recorded IR absorption spectra on thoroughly dried samples using Kbr pellets which ranges from 4000 cm^{-1} to 400 cm^{-1}.

3.3 Powder X-ray diffraction (XRD)

The structure of prepared silver nanoparticles has investigated and powdered XRD patterns of the silver nanoparticles were recorded by XRDX “X” PERT PRO diffractro meter [20].

3.4 Fesem and Edax

Field emission scanning electron microscopy (FESEM) has used to visualize the as-synthesized nanoparticles. On a glass slide having 10 mm by 10 mm dimensions, one drop of purified nanoparticles solution was dried for 24 hours. The film which forms on the glass surface was made use of to examine the morphology of the nanoparticles using the model were performed on FEI QUANTA-250 FEG equipped with an EDAX instrument [21].

3.5 Antibacterial activity of nanoparticles

Antibacterial activity of synthesized nanoparticles was tested compounds against bacterial pathogens [22]. In current investigation, biologically synthesized silver nanobactericides exposed bactericidal activity in various test pathogens such as E.Coli, pseudomonas aerugonisa, S. aureus. Further disc diffusion method showed significant activity of silver nanoparticles [23].

4. Results and Discussion

4.1 UV-Visible spectroscopy

A significant colour change was visible after the addition of seed extract to silver nitrate, a change of colourless to brown which indicates formation of silver nanoparticles from the prepared mixture. Performing UV-Visible absorption spectroscopy is to view the process of reduction. And it shows the absorption of synthesized AgNPs in experimenting mixture. UV-Visible absorption spectroscopy confirms the AgNPs formation in the silver nitrate reaction medium by producing a peak at 443 nm. As shown in Fig.3. We experimented that the absorbance happened due to possible reaction between seed extract and silver ions. At an absorption band of 398 nm, residues of protein are attributed which involve the mechanisms of bio-reduction process [24].

4.2 FTIR analysis

FTIR analysis were performed to know the responsible biomolecules which causes of the Ag^{+} ions in silver nitrate and capping of the bio reduced silver nanoparticles synthesized by raw plant extract. The result of FTIR displays an intensive peaks at 3408.12 cm^{-1}, 2927.78 cm^{-1}, 2054.78 cm^{-1}, 1654.59 cm^{-1}, 1542.27 cm^{-1}, 1384.78 cm^{-1}, 1238.30 cm^{-1}, 1047.10 cm^{-1}, and 618.30 cm^{-1} as shown in Table.1. Which corresponds to functional groups in the synthesis and stability of silver nanoparticles [25]. As shown in Fig.4.

4.3 Powder XRD analysis

The phase of the prepared nanoparticles was determined using X-ray diffraction technique and corresponding XRD patterns are shown in Fig.5. Moreover the resultant particles in the silver nanoparticles having clear peaks of cubic faces (JCPDS File No. 04-0783). 2θ range is used to record the diffracted intensities. The range covers from 30° to 80°. At a sharp peak of 38.5 corresponding to (111), (200), (220), (311) Bragg reflections may be linked with plane of face centered cubic phase of silver. It suggests that the prepared silver nanoparticles having two phases are crystalline and amorphous organic phases. In our experiment, the formation of nano crystals are indicated by the reduction in Ag^+ ions and slight variations in the peak levels.

4.4 Fesem and Edax

The size and shape of the synthesized HA-AgNPs showed a spherical morphology with sizes ranging from 10-80 nm range, and shape of the nanoparticles is strongly produced cloudy form [26]. As shown in Fig.6 (a). EDAX analysis has confirmed zero-valent silver which is present in the sample. The spectrum represents a distinctive peak at 5 keV that corresponds to silver. The glass made samples holder is thought to account for the silicon peak. As shown in Fig.6 (b).

4.5 Antibacterial activity

The silver nanoparticles showed antibacterial activity against E. Coli, P. aeruginosa, S. aureus. As shown in Fig.7. The extracts of these plants were compared with each other and with selected antibiotics for susceptibility of microorganisms. This *in vitro* studies are antibacterial activity of AgNPs against E. Coli, P. aeruginosa, S. aureus as shown in Table.2 [27].

Fig 1: Hygrophila Auriculata

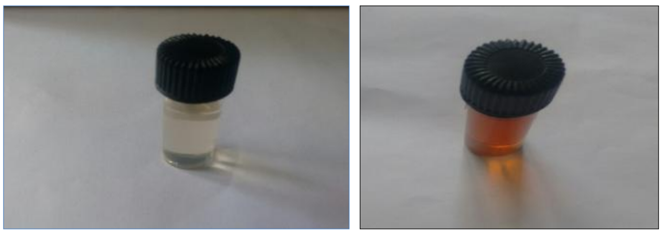

Fig 2: The colour change of seed extract after the addition of $AgNO_3$

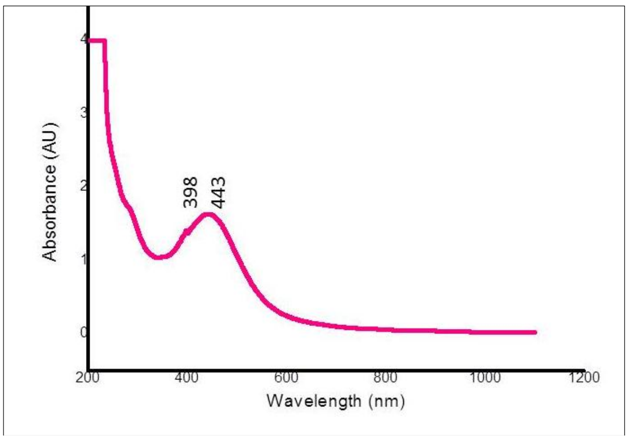

Fig 3: UV-Visible spectroscopy

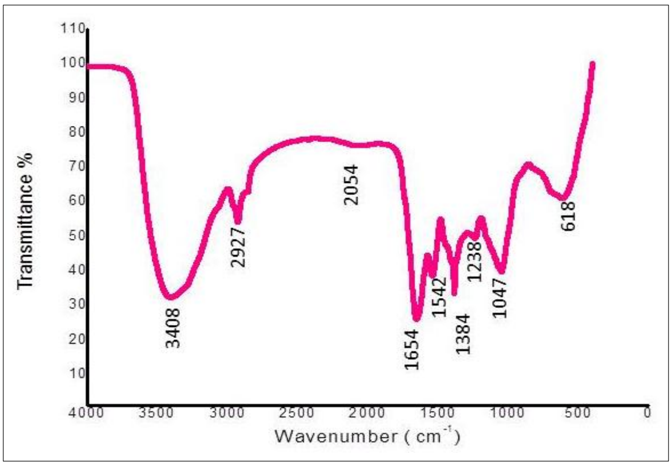

Fig 4: FTIR analysis

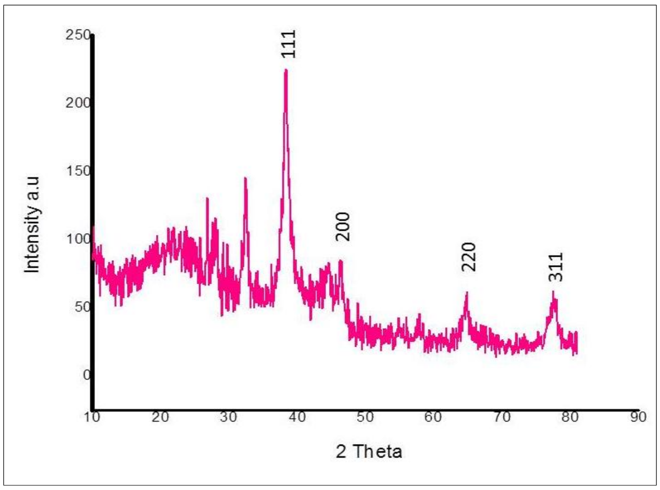

Fig 5: XRD analysis

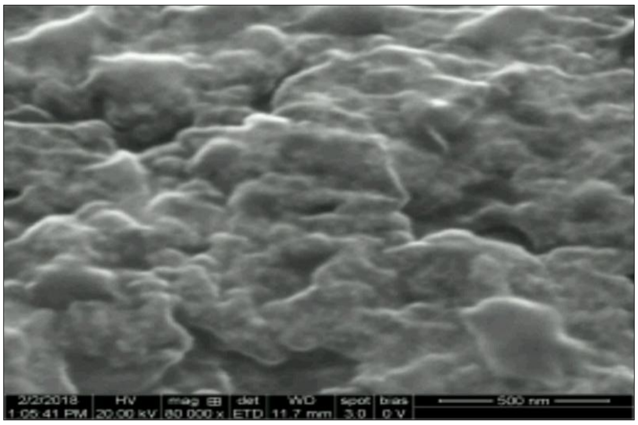

Fig 6(a): FESEM analysis

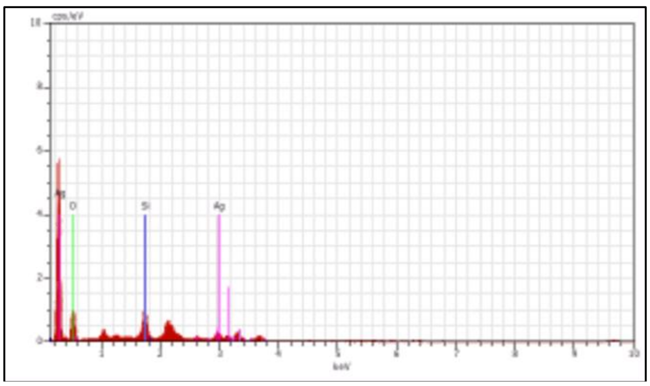

Fig 6(b): EDAX analysis

Fig 7: Antibacterial activity

Table 1: FTIR analysis

Wavenumber peaks (cm^{-1})	Type of vibration	Functional group
3408	Stretching	-OH (H-bonded)
2927	Stretching	C-H
1654	Bending	-OH
1542	Stretching	N-O
1384	Bending	Planar CH
1238	Stretching	Asymmetrical C-O-C
1047	Vibration	C-C, C-OH, C-H
618	Stretching	C-Cl

Table 2: Antibacterial activity

Organism	(Diameter of zone of inhibition in mm)		
	$AgNO_3$	AgNP	HA extract
E.coli	10	12	-
Pseudomonas aerugonisa	12	12	-
S.aureus	8	12	-

5. Conclusion

The field of nanotechnology is new and upcoming research area in the Indian Scenario. Much has already been studied in synthesis of nanomaterials through physical and chemical process. Nanotechnology has dynamically as an important field of modern research with potential effects in electronic and medicine. In recent researches in nanotechnology, the main aspect is the growth of green process for the synthesis of silver nanoparticles. The use of green plants for synthesis of Ag NPs among the various familiar methods are eco-friendly, cost effective, human therapeutic uses etc. A single step methods for bio-synthesis process is also considerable. The silver nanoparticles synthesized by Hygrophila Auriculata seed extract were characterized by UV-Visible spectra has confirmed the reduction of Ag^+ ions 443 nm. The presence of functional groups was confirmed FTIR. The shape of the nanoparticles was strongly produced by cloudy form of the Field emission scanning electron microscopy coupled and Energy-Dispersive X-ray spectroscopy was confirmed the presence of absorption peak at 5 keV. XRD analysis confirms the crystalline face centered cubic structure. In our study, we came to know that the silver nanoparticles has synthesized effectively resistant to the growth and increase of pathogenic bacteria like Staphylococcus aureus, Pseudomonas aeruginosa, E. coli characterization to be done for the synthesized nanoparticles. As a result it is observed that a fine tuning of process variables may give the end product with typical physical characteristics.

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