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GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM ETHANOLIC SEED EXTRACT OF *ACRANYTHES ASPERA* (LINN.) AND ITS ANTI-INFLAMMATORY ACTIVITIES

R.Vijayaraj[1], K.Naresh Kumar[1], P.Mani[2], J.Senthil[3], G.Dinesh Kumar[4] and T.Jayaseelan[4]

[1]Department of Biotechnology, Karunya University, Coimbatore -641114, Tamilnadu, India.
[2]Department of Biotechnology, Annai College of Arts and Science, Kumbakonam, Tamilnadu, India.
[3]Department of Medicinal Plant Biotechnology, Sharmila Institute of Medicinal Products Research Academy, Thanjavur, Tamilnadu, India.
[4]P.G.and Research Department of Zoology and Biotechnology, A.V.V.M. Sri Pushpam College, Poondi-613 503, Thanjavur District, Tamil Nadu, India.

ABSTRACT

Nanoparticles have a promising action in a variety of areas and fields. Plant based nanoparticle synthesis become advantageous than chemical synthesis. The present study involves green approach for the synthesis of silver nanoparticles (AgNPs) using *Acranythes aspera* as the source of phytochemicals for reducing and capping agent for the reduction of silver ions to silver atoms and stabilization of the particles. The nanoparticles were synthesized and characterized by using UV-Vis spectroscopy, Scanning Electron Microscopy (SEM) and FTIR analysis. Based on the findings, silver nanoparticle synthesizing *A.aspera* seed has the ability and capacity to act as an anti-inflammatory activity against carrageenan induced albino rats.

Key Words:- *Acranythes aspera*, Silver Nano, UV, FTIR, SEM, Anti-inflammatory activity.

INTRODUCTION

Plants are a big source of various therapeutic compounds which have been exploited for so many years in the field of medicine. In the past few decades there has been tremendous growth in the field of herbal medicine because of their natural origin with no side effect. The use of nanotechnology in field of medicine results in targeted and specific drug delivery with no toxicity and still maintaining good therapeutic effects (Kumaradevan *et al.*, 2015). The World Health Organization (WHO) has listed 21000 plants that have been used for medicinal purpose.

Corresponding Author

P.Mani
Email:- master.maniji@gmail.com

Extracts from plants usually contain various polyphenols such as flavonoids which may prove to be excellent reducing agents useful for the synthesis of silver nanoparticles (Sharma *et al.*, 2007).

The field of nanotechnology is one of the most active areas of research in modern medicinal science. Nanotechnology applications are highly suitable for biological molecules because of their exclusive properties. The biological molecules undergo highly controlled assembly for making them suitable for the metal nanoparticle synthesis which is found to be reliable and eco-friendly. Silver nanoparticles have ample application including skin ointments and creams containing silver to prevent infection of burns and open wounds (Ip M *et al.*, 2006). Plants provide a better platform for nanoparticle synthesis as they are free from toxic chemicals as well as provide natural capping agents. Synthesis of nanoparticles using plant extract is the most adopted method of eco-friendly production and also has a special advantage that the plants are widely distributed, easily available, much safer to handle and act as a source of several metabolites (Vaidyanathan *et al.*, 2006). The plant-mediated synthesis is a rapid, flexible and suitable process for large-scale production of nanoparticles. Nowadays, plant parts like seed, leaf, bark, stem and fruit (Ankamwar *et al.*, 2006, Sathishkumar *et al.*, 2009) extracts have been effectively used for synthesis of nanoparticles. Among nanoparticles, silver nanoparticles have been used enormously due to their potent anti-inflammatory activity. Inflammatory diseases are major worldwide problem. Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain (Bar H *et al.,* 2009)

Inflammation is either acute or chronic. Acute inflammation may be an initial response of the body to harmful stimuli. In chronic inflammation, the inflammatory response is out of proportion resulting in damage to the body. Cyclooxygenase (COX) is the key enzymes in the synthesis of prostaglandins, Prostacyclins and Thromboxanes which are involved in inflammation, pain and platelet aggregation. Plant based Silver Nanoparticles have effective application to treat inflammation and open wounds (Pilotto *et.al.*,2010)

Achyranthes aspera(L.)belongs to the family Amaranthaceae. It is an annual, stiff erect herb and commonly found as a weed throughout India. It is an important medicinal plants having many therapeutic uses against inflammation, microbes, odontalgic, Rheumatism, Bronchitis, skin disease and rabies (Girach *et al.,*1992). The main objectives of the present study focuses on the synthesis, characterization of silver nanoparticles and also to prove that the silver nanaoparticles having potent anti-inflammatory activity.

MATERIALS AND METHODS

Collection and Authentication of Experimental Plant:

Fresh healthy and young seeds (3 to 6 month old) of *A. aspera* L. were collected from their natural habitat of Perugavazhndan in Thiruvarur district, Tamilnadu, India, and authenticated by professionals in Department of Botany, St. Joseph's College, Tiruchirappalli. The herbarium number of the plant (RVR001).

Preparation of Extraction:

The dried and powdered seed of *A.aspera* (500 g) was extracted using soxhlet extractor by evaporating with 75% ethanol. The soxhlet extraction was carried out for 3 days and the extract was collected. The excess ethanol was evaporated by using vacuum evaporator. The sample is evaporated to dryness under boiling water bath at 55^0C.

Animals:

Albino rats 4 week old weighing 150-200 g were used for the present study. To maintain the animal house under the standard condition of temperature (24±2°C) and relative humidity (30% to 70%) with a 12:12 light: dark cycle. The animals were fed with standard pellet diet and water. The animal handling was performed according to Good Laboratory Practice (GLP). Ethical clearance was obtained from institutional animal ethical committee and conducted according to the Indian National Science academy guidelines for the use and care of experimental animal (CPCSEA/265).

Biosynthesis of silver nanoparticles:

Aqueous solution (1mM) of silver nitrate ($AgNO_3$) was prepared and used for biosynthesis of silver nano particles. 10 ml of *A.aspera* ethanolic seeds extract was added into 95 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag+ ions. It is then boiled for 15 minutes at 80°C. Reduction of silver nitrate to silver ions was confirmed by the color change from light to dark brown. The formation of silver nanoparticles was also confirmed by UV-visible spectrophotometric analysis. The fully reduced solution was centrifuged at 5000rpm for 20 minutes. The particles settled down are thoroughly washed with distilled water for 2 or3 times to remove the extract from it and dried in hot air oven. The prepared silver nanoparticles are then stored for further purposes (Rajasekar *et.al.*,2013)

Characterization of Silver Nanoparticles

UV-VIS Analysis

The optical property of silver nanoparticles was determined by UV-VIS spectrophotometer (perkin Elmer Lamda 35, Germany). The UV-VIS spectra of samples were measured at 2600nm.The bio reduction of silver ions in aqueous solution was monitored by UV-VIS spectra between 300-700nm.

SEM Analysis

The morphological features of synthesized silver nanoparticles from plant extract were studied by Scanning Electron Microscope. SEM analysis is done after drying the extract and dried powder is used for SEM Images.

FTIR Analysis

The chemical composition of the synthesized silver nanoparticles was studied using FTIR spectrometer

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(Perkin – Elmer L5 55 – Luminescence spectrometer). The solution for FTIR analysis was prepared by mixing plant extract with 1mM silver nitrate. The samples were scanned using infrared in range of 1950-600 cm-1 using FTIR. The spectrum obtained was compared with reference chart.

Carrageenan induced inflammation in Albino rats:

Paw edema was induced in male albino rats (150–200g,4weeks old) by the injection of 100 μl carrageenan 1% (λ-carrageenan, type IV) in the right hind foot pad (4). The left hind foot pad was injected with the same volume of saline solution (Patil *et al.,* 2009).

Evaluation of Anti-Inflammatory Activity:

Ethanolic extract of *A.aspera* and AgNPs of *A.aspera* was tested for Anti-Inflammatory activity against carrageenan induced paw edema in rats. The reductions of paw edema of rats are compared with the standard drug i.e. indomethacin.

The percentage of inhibition of paw-edema is calculated by:

$$\%\text{ inhibition of Paw edema} = \frac{C\text{-}T}{C\text{-}T \times 100}$$

C=increase in paw edema volume of control groups
T=increase in paw edema volume after administration of extract.

RESULTS

Photochemical analysis:

The preliminary phytochemical screening results of *A.aspera* showed various bioactive secondary metabolites constituents such as alkaloids, flavonoids, steroids, cardiac gylcosides, reducing sugars, anthraquinones, saponins, tannins and terpenoids.

UV-Vis Spectra analysis:

The reduction of silver is confirmed in the samples by visual observation. The sample exhibited dark brown. This colour variation may be attributed to excitation of surface plasmon vibration in silver nanoparticles. After 24 hrs incubation in dark room condition, the light coloured reaction mixtures turned into dark brown for indicating silver nanoparticle formation. (Fig 2). In the present result, the Surface Plasmon Resonance (SPR) of AgNPs produced a peak at 420nm, which suggests the dispersal of silver nanoparticles.

FTIR analysis

In the present work, FTIR spectra are used in the identification of biomolecules responsible for capping and stabilizing the silver nanoparticles. The FTIR spectra of the sample given in the fig. 3 exhibited peaks at 3452cm^{-1}, 2086cm^{1}, 1640cm^{-1} and 638 cm^{-1} which indicates the functional group of the plant component involves in the reduction and stabilization of AgNPs. The transmittance attributes O-H stretch, C=C bond and C-H which reveals that the water soluble heterocyclic components, polyols and certain proteins present in the extract which are involved in the reduction of silver nitrate.

SEM analysis of silver nanoparticles

SEM analysis shows uniformly distributed silver nanoparticles on the surfaces of the cells (Figure 4). SEM analysis reveals individual spherical polydispersed AgNPs as well as number of aggregates, which were irregular in shape. The size of the silver nanoparticles was found to be 5-50 nm, with an average size of 20 nm. The larger silver particles may be due to the aggregation of the smaller ones.

Anti-inflammatory effect of *Achyranthes aspera*

In carrageenan induced paw edema in rats, oral administration of ethanolic extracts of *A.aspera* showed inhibition of paw edema at 3 h after carrageenan injection and was compared with standard Indomethacin (Table1).The orally administered ethanolic seed extracts of *Achyranthes aspera* (100 and 200 mg/kg) (Fig.5) and silver nanoparticles of *Achyranthes aspera* (100 mg/kg) showed significant inhibition.The anti-inflammatory effect induced by Indomethacin progressively increased and reached a maximum of 44.12% after 3hrs ($p<0.001$). Silver nanoparticles of *Achyranthes aspera* (100 mg/kg), showed better inhibition of edema.

Table 1. **Anti-inflammatory effect of *Achyranthes aspera***

S.no	Group/Treatment	Mean Paw edema	%edema
1.	Control	-	-
2.	Carrageenan alone	0.63±0.08	-
3.	Carrageenan+*A.apera* extract 100mg/kg	0.45±0.02	53.3±1.61
4.	Carrageenan+*A.apera* extract 200mg/kg	0.42±0.02	49.05±5.74
5.	Carrageenan+AgNPs extract 100mg/kg	0.39±0.18	47.05±5.74
6.	Indomethacin	0.37±0.05	44.12±2.11

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Figure 1. Biosynthesis of silver nanoparticle from plant extract

AgNO3= 1mM AgNO3without extract.
AgNPs (S1)=1 mM AgNO3 with A.aspera extract after 5 h of incubation (Brown colour).

Figure 2. UV-Vis absorption spectrum of nanoparticles

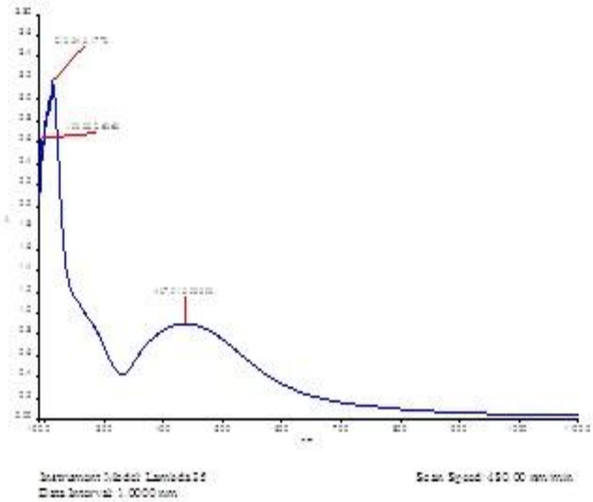

Figure 3. FTIR analysis of silver silvernano particles

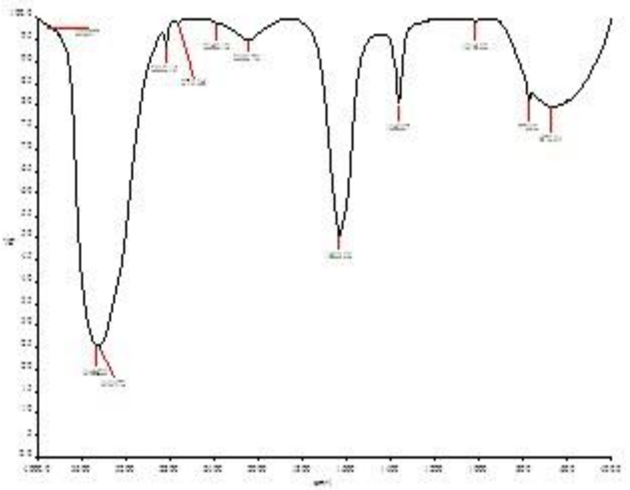

Figure 4. Image of silvernano particles in SEM

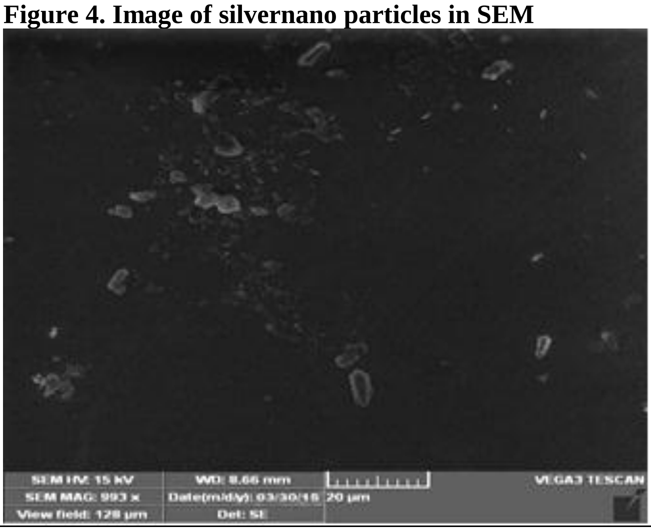

Figure 5(a). Inflammation induced paw edema

Figure 5(b). Treated paw edema

DISCUSSION

The development of green processes for the production of nanoparticles is evolving into a significant branch of nanotechnology (Raveendran *et al.*, 2006). Nanotechnology is expected to be the basis of many technological innovations in the 21st century. The synthesis of nanoparticles is a promising research field due to the possible applications for the extension of novel technologies (Virender *et al.*, 2009).

Biological methods of nanoparticles synthesis using plant or plant extract have been suggested as possible eco-friendly alternatives to chemical and physical methods.Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required. At the same time the biologically synthesized silver nanoparticles has many applications in the field of medicine and agriculture (Ahmad *et al.*, 2004)

The change of colour indicates the formation of silver nanoparticles (SNPs) (Shankar *et al.*, 2004). Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The synthesis of SNPs had been confirmed by measuring the UV-Vis spectrum of the reaction media (Fig.1).

In the present findings the UV-Vis spectrum of silver nanoparticles synthesized from seed of *A.aspera* have strong absorbance peaks at 420 nm (Fig-2) and the broadening of peaks indicated that the particles are poly-dispersed. Almost all similar results were observed in *Cleodendrum inerme*, *Euphorbia hirta* and *Argimone maxicana* (Elumalai *et al.*, 2010, Farooqui *et al.*, 2010, Khandelwal *et al.*, 2010). The weak absorption peaks at shorter wavelengths are due to the presence of several organic compounds which interacts with silver ions (Savithramma *et al.*, 2011). Silver nanoparticles have free electrons, which give rise to an SPR absorption band due to the combined vibration of electrons of metal nanoparticles in resonance with the light wave (Nath *et al.*, 2007, Noginov *et al.*, 2006, Dubey *et al.*, 2009).

The silver nanoparticles are cubical, rectangular, triangular and spherical in shape with uniform distribution. However, on most occasions, agglomeration of the particles was observed probably due to the presence of a weak capping agent which moderately stabilize the nanoparticles (Nethradevi *et al.*, 2009).The measured sizes of the agglomerated nanoparticles were in the range 287.5–293.2 nm. However, the average size of an individual particle is estimated to be 70 nm. In the present study SEM analysis provides the morphology and size details of the nanoparticles. The high density AgNPs synthesised by the plant extract of *A.aspera* confirms the presence of AgNPs of size ranging from 20-35nm. Particle size, size distribution and shape of silver nano particles are the important parameters that govern the properties and hence it has wide applications in medicinal fields.

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules (Janakiraman *et al.*, 2009). FTIR gives the information about functional group present in the synthesized silver nanoparticles for understanding their transformation from simple inorganic $AgNo_3$ to elemental silver. Our study suggested that the FTIR analysis confirmed that the bioreduction of silver ions to AgNPs is due to the reduction by capping material of the plant extract. The FTIR spectra of the sample given in the fig. 3 shows the presence of silver nanoparticles, with peaks at 3452cm^{-1}, 2086cm^{1}, 1640cm^{-1} and 638 cm^{-1} indicates the functional group of the plant component involved in the reduction and stabilization of AgNPs. The transmittance attributes O-H stretch, C=C bond and C-H revealed that the water soluble heterocyclic components, polyols and certain proteins present in the extract are involved in the reduction of silver nitrate. These absorbance peaks are related to flavonoids, proteins and carbohydrates.

Biological synthesis of AgNPs is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phytotoxic effects (Gardea-Torresdey *et al.,* 2003). The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogen. Silver sulfadazine depicts better healing of burn wounds due to its slow and steady reaction with serum and other body fluids (Fox *et al.,* 1974). The silver nanoparticles are reported to show better wound healing capacity, better cosmetic appearance and scar less healing when tested using an animal model (Tian *et.al.,*2007). The present finding suggested that significant anti-inflammatory effect was observed in silver nano particles synthesized seed extract of *A.aspera* when compare to normal synthetic drug against inflammatory rats. Our findings not only provided experimental evidence for an anti-inflammatory mechanism but were also beneficial to future research about the effect of mentioned seed extract on other diseases. The present work supports the medicinal values of this plant and revealed that a simple, rapid and economical route to synthesis of silver nanoparticles has the capability of rendering the anti-inflammatory efficacy. Moreover the synthesized SNPs enhance the therapeutic efficacy and strengthen the medicinal values of *A.aspera* .

CONCLUSION

The medicinal plant could be used as an excellent and resourceful green material for the rapid and consistent synthesis of silver nanoparticles. Biological synthesis of nanoparticles has upsurge in the field of nano biotechnology to create novel materials that are eco-friendly, cost effective and stable. These nanoparticles have a great importance in the areas of medicine and agriculture. During the current scenario, nanotechnology motivates progress in all sphere of life, hence biosynthetic route of nanoparticles synthesis will emerge as safer and best alternative to conventional methods. Though various biological entities have been exploited for the production of nanoparticles, the use of plants for the facile robust synthesis of nanoparticles is a tremendous. This study opens up a new opportunity of very conveniently synthesizing Ag nanoparticles using natural products which could be useful in various applications. Our findings could be targeted for the promising potential applications including drug formulation and biomedical applications in future.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

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