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## Synthesis and Evaluation of Anti-Inflammatory Activity of Silver Nanoparticles from *Cissus vitiginea* Leaf Extract

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### ARTICLE DETAILS

#### Article history:

Received 20 October 2017

Accepted 28 October 2017

Available online 21 November 2017

#### Keywords:

Nanochemistry

*Cissus vitiginea*

Silver Nanoparticles

SEM

Anti-Inflammatory Activity

### ABSTRACT

To meet the increasing demands for commercial nanoparticles new eco-friendly “green” methods of synthesis are being discovered. Plant mediated synthesis of nanoparticles offers single step, easy extracellular synthesis of nanoparticles. Nanotechnology refers broadly to a field of applied science and technology whose unifying theme is the control of matter on the atomic and molecular scale. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. The silver nanoparticles have various and important applications. In this work, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1 mM AgNO<sub>3</sub> solution through the extract of *Cissus vitiginea* leaf as it acts as a reducing as well as capping agent. Nanoparticles were characterized using UV-Vis absorption spectroscopy and SEM analysis showed the average particle size range 10-40 nm with higher density polydispersed spherical in shape. The synthesized silver nanoparticles exhibited potential anti-inflammatory activity.

### 1. Introduction

Nanotechnology is becoming an innovative area of increasing research and industrial interest since the 1980's. Nanotechnology can be defined as the manipulation of atom by atom from the material world by the combination of engineering, chemical and biological approaches. In the past decade, considerable attention has been paid for the development of novel strategies for the synthesis of different kind of nano-objects. Most of the current strategies are usually working by the use physical or chemical principles to develop a myriad of nano-objects with multiple applications. Main fields of nanotechnology applications range from catalysis, micro- and nano-electronics (semiconductors, single electrons transistors), non-linear optic devices, photo-electrochemistry to biomedicine, diagnostics, foods and environment, chemical analysis and others [1]. Nanochemistry is relatively new areas of science arisen in last decade of past century after discovery of fullerenes and nanotubes. It is introduced into more extent interdisciplinary integrated modern science known now as rapidly developing nanotechnology [2].

Metal nanoparticles can be prepared by physical, chemical and biological routes; the first one is a physical approach that utilizes several methods such as evaporation/condensation and laser ablation. The second one is a chemical approach in which the metal ions in solution is reduced in conditions favoring the subsequent formation of small metal clusters or aggregates [3]. Various metals like copper, titanium, gold, silver and iron were used for the synthesis of nanoparticle. Among the noble metals, silver nanoparticles have become the focus of intensive research due to its wide ranges of application for many sectors of life and industry [4]. Recently, biosynthetic methods employing naturally occurring reducing agents such as polysaccharides, biological microorganism such as bacteria and fungus or plants extract, i.e. green chemistry, have emerged as a simple and viable alternative to more complex physical and chemical synthetic procedures to obtain AgNPs [5].

In the recent decades, increased development of green synthesis of nanoparticles is inevitable because of its incredible applications in all fields of science. There were numerous work have been produced based on the plant and its extract mediated synthesis of nanoparticles. AgNP has been synthesized by using the plant broth from a wide variety of plants such as *Bacopa monnieri* [6], and *Catharanthus roseus* [7]. Keeping in view,

in the present study to explore the novel approaches for the biosynthesis of silver nanoparticles using *Cissus vitiginea* leaf and evaluate the anti-inflammatory activity.

### 2. Experimental Methods

#### 2.1 Chemicals

All the experiments were conducted at room temperature. Materials used for the synthesis of silver nanoparticles are AR grade silver nitrate (AgNO<sub>3</sub>) purchased from Merck, India.

#### 2.2 Collection of Plant Materials

The *Cissus vitiginea* leaves were collected in March 2016 from Thanjavur, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Dr. S. John Britto, the director, Rapinat Herbarium and Center for Molecular Systematics, St. Joseph's college Trichy-Tamil Nadu, India. A voucher specimen has been deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil Nadu, India.

#### 2.3 Preparation of Leaf Extract

The dried leaves were pulverized well with mortar and pestle to make a powder. Twenty grams of powder sample was mixed into 100 mL of deionized water and the mixture was boiled for 10 min. After cooling the leaf extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4 °C for further use.

#### 2.4 Synthesis of Ag Nanoparticles using Leaf Extracts

For the Ag nanoparticles synthesis, 5 mL of *Cissus vitiginea* leaf extract was added to 45 mL of 1 mM aqueous AgNO<sub>3</sub> solution in a 250 mL Erlenmeyer flask. The flask was then incubated in the dark at 5 hrs (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without leaf extract. The Ag nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. Then the Ag nanoparticles were freeze and dried for using SEM analysis [8].

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### 2.5 UV-Vis and FTIR Spectra Analysis

The reduction of pure  $\text{Ag}^+$  ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu). An aliquot of this filtrate containing silver nanoparticles are used for Fourier transmission infrared spectroscopy (FTIR).

### 2.6 SEM Analysis of Silver Nanoparticles

Scanning electron microscopic (SEM) analysis was done using ZEISS machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

### 2.7 In Vitro Anti-Inflammatory Activity

*In vitro* anti-inflammatory activity was carried out by the method of Sangita Chandra *et al.*, [9].

## 3. Results and Discussion

### 3.1 Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles through leaf extracts were carried out. Leaf extract is used as reducing agent as distinctive properties catalytic and chemical stability. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The time duration of change in colour varies from plant to plant. The phytochemicals present in the leaf extract were considered responsible for the reduction of silver ions. It is well known that silver nanoparticles exhibit yellowish - brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. The appearances of yellowish-brown colour (Fig. 1) in the reaction vessels suggest the formation of silver nanoparticles (SNPs) [10].

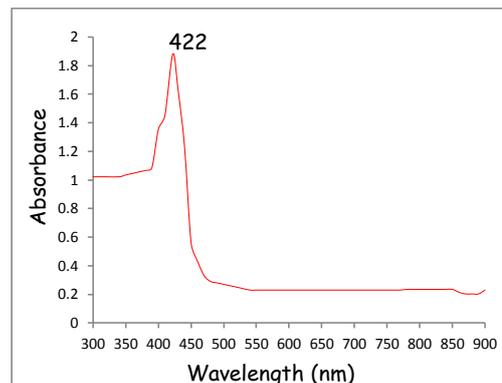


$\text{AgNO}_3$  = 1 mM  $\text{AgNO}_3$  without *Cissus vitiginea* extract.  
 $\text{AgNPs}$  = 1 mM  $\text{AgNO}_3$  with *Cissus vitiginea* leaf extract after 5 hrs of incubation (Brown colour)

**Fig. 1** Formation of brown colour after addition of  $\text{AgNO}_3$  indicate synthesis of AgNPs in the process of reduction of  $\text{Ag}^+$  to Ag nanoparticles and control ( $\text{AgNO}_3$ )

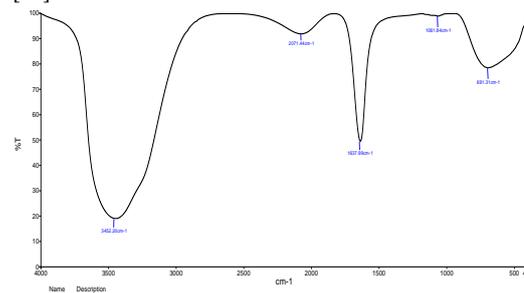
### 3.2 UV-Vis and FTIR Spectra Analysis

It is generally recognized that UV-Vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions. Fig. 2 shows the UV-Vis spectra recorded from the reaction medium after 5 hours. The UV-Vis spectra of the reaction mixture of silver nitrate solution with *Cissus vitiginea* leaf extract at the peaks observed at 422 nm indicate the presence of silver nanoparticles which is synthesized by *Cissus vitiginea* extract, the peak was raised due to the effect of surface plasmon resonance of electrons in the reaction mixture and the broadening of peak indicated that the particles are polydispersed. Appearance of this peak assigned to a surface plasmon, is well-documented for various metal nanoparticles with size ranging from 2 nm to 100 nm [11].



**Fig. 2** UV-Vis absorption spectrum of silver nanoparticles synthesized by treating 1 mM aqueous  $\text{AgNO}_3$  solution with *Cissus vitiginea* leaf extract after 5 hrs

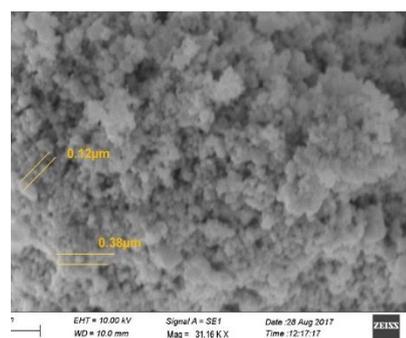
FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules. In the present work, FTIR spectra are used in the identification of biomolecules responsible for capping and stabilizing the silver nanoparticles. The FTIR spectra of the *Cissus vitiginea* is given in the Fig. 3. FTIR spectrum of *Cissus vitiginea* extract shows peak at 891, 1061, 1637, 2071 and 3452  $\text{cm}^{-1}$ . The band appeared at about 1637  $\text{cm}^{-1}$  can be assigned for aromatic rings. The strong broad band appearing at 3452  $\text{cm}^{-1}$  can be associated to the stretching vibrations of alcoholic and phenolic O-H. At 1061  $\text{cm}^{-1}$  a peak is observed that could be for plant ascribed to multiplet C-O group. Therefore, from the results of FTIR analyses of extract mediated synthesized silver nanoparticles it can be concluded that some of the biological molecules of leaf extract such as alkaloids, phenols, flavonoids, amino acids, glycosides, and tannins are responsible for biotransformation of silver ions to silver nanoparticles and its stabilization in aqueous medium. This results agreement with earlier reports [12].



**Fig. 3** FTIR analysis of silver nanoparticles synthesized by treating 1 mM aqueous  $\text{AgNO}_3$  solution with *Cissus vitiginea* extract

### 3.3 Scanning Electron Microscope (SEM)

The surface morphology, size and shape of the silver nanoparticles were analyzed by scanning electron microscope. Fig. 4 shows the SEM image of silver nanoparticles synthesized from leaf extract. The SEM images show individual silver nanoparticles which are higher density polydispersed spherical in shape as well as number of aggregates with no defined morphology. The presences of biomolecules in the leaf extract has resulted in the synthesis of spherical silver nanoparticles and the aggregation may be due to the presence of secondary metabolites in the leaf extract. The SEM image shows the size of the silver nanoparticles ranging from 10 to 40 nm. Similar result of the silver nanoparticles size was reported by using *Coccinia grandis* leaf extract [8], and by using *Allophylus serratus* Leaf [13].



**Fig. 4** High resolution scanning electron microscopic (SEM) image of silver nanoparticles (AgNPs). Polydispersed (Cluster) AgNPs ranged between 10–40 nm

### 3.4 Anti-Inflammatory Activity

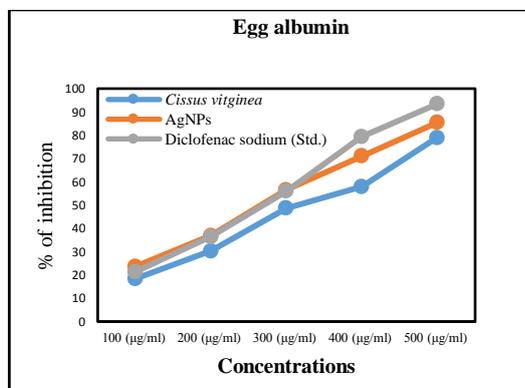
The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair, which are aimed at host, defense and usually activated in most disease condition [14]. Chronic inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population. At present, although synthetic drugs are dominating the market, element of toxicity that these drugs entail, cannot be ruled out. Their prolonged use may cause severe adverse effects on chronic administration [15]. Currently much interest have been paid in the search of nanoparticle with anti-inflammatory activity which may lead to the discovery of new therapeutic agent that is not only used to suppress the inflammation but also used in diverse disease conditions where the inflammation response amplifies the disease process. There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property of *Cissus vitiginea* leaf extract and AgNPs. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins (egg albumin and bovine serum albumin) *in vivo* [16, 17]. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development.

The increments in absorbance of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein denaturation by *Cissus vitiginea* leaf extract, AgNPs and reference drug diclofenac sodium. The present findings exhibited a concentration dependent inhibition of protein denaturation by the *Cissus vitiginea* leaf extract and AgNPs (Table 1 and Fig. 5). The lowest activity of *Cissus vitiginea* leaf extract, AgNPs and diclofenac sodium were 18.42%, 23.68% and 21.37% in the concentration of 100 µg/mL respectively while the highest activity of *Cissus vitiginea* leaf extract, AgNPs and diclofenac sodium were 78.94%, 85.52% and 93.45% in the concentration of 500 µg/mL respectively. The greatest effect of AgNPs (500 µg/mL) was found to be near to standard diclofenac sodium. The half inhibition concentration (IC<sub>50</sub>) of *Cissus vitiginea* leaf extract, AgNPs and diclofenac sodium were 321, 270 µg/mL and 257 µg/mL respectively. From the present study it can be concluded that AgNPs showed marked *in vitro* anti-inflammatory effect against the denaturation of protein (Table 1 and Fig. 1). Our result agrees with the earlier reports [18, 19].

**Table 1** Effect of *Cissus vitiginea*, AgNPs and diclofenac sodium on protein denaturation (Fresh egg albumin)

Concentrations	% of inhibition		
	<i>Cissus vitiginea</i>	AgNPs	Diclofenac sodium (Std.)
100 µg/mL	18.42±1.28	23.68±1.65	21.37±1.98
200 µg/mL	30.26±2.15	36.84±2.57	36.45±2.37
300 µg/mL	48.68±3.40	56.57±3.95	55.94±3.47
400 µg/mL	57.89±4.05	71.05±4.97	79.45±4.65
500 µg/mL	78.94±5.52	85.52±5.98	93.45±6.84
IC <sub>50</sub> (µg/mL)	321.20	270.01	257.26

Values are expressed as Mean ± SD for triplicates



**Fig. 5** Effect of *Cissus vitiginea*, AgNPs and diclofenac sodium on protein denaturation (Fresh egg albumin)

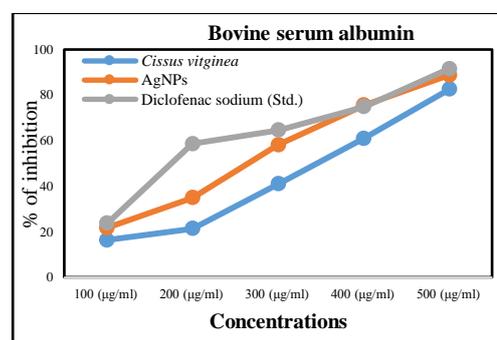
The present findings exhibited a concentration dependent inhibition of protein (bovine serum albumin) denaturation by the *Cissus vitiginea* leaf extract and AgNPs (Table 2 and Fig. 6). The lowest activity of *Cissus vitiginea* leaf extract, AgNPs and diclofenac sodium were 16.31%, 21.78%

and 23.75% in the concentration of 100 µg/mL respectively while the highest activity of *Cissus vitiginea* leaf extract, AgNPs and diclofenac sodium were 82.52%, 88.78% and 91.52% in the concentration of 500 µg/mL respectively. The half inhibition concentration (IC<sub>50</sub>) of *Cissus vitiginea* leaf extract, AgNPs and diclofenac were 332, 266 µg/mL and 216 µg/mL respectively. The greatest effect of AgNPs (500 µg/mL) was found to be near to standard diclofenac sodium. From the present study it can be concluded that AgNPs showed marked *in vitro* anti-inflammatory effect against the denaturation of protein (Table 2 and Fig. 2). Our result agrees with the earlier report [18, 19].

**Table 2** Effect of *Cissus vitiginea*, AgNPs and Diclofenac sodium on protein denaturation (Bovine serum albumin)

Concentrations	% of inhibition		
	<i>Cissus vitiginea</i>	AgNPs	Diclofenac sodium (Std.)
100 µg/mL	16.31±1.14	21.78±1.52	23.75±1.66
200 µg/mL	21.36±1.49	34.94±2.44	58.65±4.10
300 µg/mL	41.05±2.87	58.21±4.07	64.54±4.51
400 µg/mL	60.94±4.26	75.57±5.28	74.83±5.23
500 µg/mL	82.52±5.77	88.78±6.42	91.52±6.40
IC <sub>50</sub> (µg/mL)	332.34	266.51	216.61

Values are expressed as Mean ± SD for triplicates



**Fig. 6** Effect of *Cissus vitiginea*, AgNPs and diclofenac sodium on protein denaturation (Bovine serum albumin)

### 4. Conclusion

Medicinal plants have medicinally important compounds in their different parts. The synthesis of nanoparticles using plants depends on the nature of plant such as its phytochemical content, special adaptation, and medicinal importance. In this study, we investigated eco-friendly and cost-effective green synthesis of silver nanoparticles using leaf extract of medicinal plant *Cissus vitiginea*. Water soluble organic compounds present in the leaf extract was mainly responsible for synthesis of silver nanoparticles by reducing silver ions to nanosized silver particles. The UV-visible spectroscopy, FTIR and SEM studies of the synthesized silver nanoparticles elucidated that the silver nanoparticles were crystalline in nature, spherical in shape with size ranging between 10 and 40 nm and stable. The synthesized silver nanoparticles exhibited anti-inflammatory activity. This finding suggests that the synthesis of AgNPs using *Cissus vitiginea* leaf extract could be a good source for developing green nanomedicine for the management of inflammation.

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