



## Original article

Biogenic synthesis of silver nanoparticle using *Cissus quadrangularis* extract and its invitro studyS. Kanimozhi<sup>a,\*</sup>, R. Durga<sup>a</sup>, M. Sabithasree<sup>a</sup>, A. Vimal Kumar<sup>a</sup>, A. Sofiavizhimalar<sup>b</sup>, Avinash Ashok Kadam<sup>c</sup>, Rajakrishnan Rajagopal<sup>d</sup>, Rengasamy Sathya<sup>e</sup>, Nur Izyan Wan Azelee<sup>f,g,\*</sup><sup>a</sup> Department of Biotechnology, Bharath Institute of Higher Education and Research, Selaiyur, Chennai, India<sup>b</sup> Department of Biotechnology, Mother Teresa Women's University, Kodaikanal 624101, India<sup>c</sup> Research Institute of Biotechnology and Medical Converged Science, Dongguk University-Seoul, 32 Dongguk-ro, Ilsandong-gu, Goyang-si 10326, Gyeonggi-do, Republic of Korea<sup>d</sup> Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia<sup>e</sup> Department of Microbiology, Centre for Research and Development, PRIST University, Tamilnadu, India<sup>f</sup> School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Skudai 81310, Johor, Malaysia<sup>g</sup> Institute of Bioproduct Development, Universiti Teknologi Malaysia UTM, 81310 Skudai, Johor, Malaysia

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## ABSTRACT

**Objective:** Green synthesis of metallic and metal oxide nanomaterial has received significant importance in various fields. Green synthesis of silver nanoparticles was performed using aqueous extracts of *Cissus quadrangularis*. Aqueous extracts of plants plays a major role to reduce the silver nitrate to silver nanoparticles and also aid as surface stabilizing material for silver nanoparticles production.**Methods:** The synthesized nanoparticles was monitored by physicochemical techniques such as XRD, FT-IR, Uv-Vis, and SEM. Anti-arthritis activity of silver nanoparticles was examined by Egg albumin denaturation and albumin (BSA) denaturation method.**Results:** XRD, FT-IR and Uv-Vis spectrum confirms crystalline nature of silver nanoparticles. SEM images show the spherical shape of the nanoparticles. Egg albumin denaturation method results revealed 81% inhibition and bovine serum albumin method shows 85% inhibition than standard drug.**Conclusion:** It confirms that, the green synthesized silver nanoparticles would be useful in promoting research aiming at the development of new agent for arthritis applications. Hence, the utilization of natural harmless materials like plant extracts for nanoparticles synthesis offers various advantages like eco-friendliness and compatibility for drug and biomedical applications.© 2022 The Authors. Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Nanotechnology has emerged worldwide of modern potentialities by fabricating nanomaterial with suitable size and shapes for various applications including medicine and agriculture field (Hasan, 2015; Khalil et al., 2013; Aref and Salem 2020; Badineni

et al., 2021). Synthesis of nanoparticle has been extended to global attention due to their physicochemical properties. Various methods are used for nanoparticles synthesis. Among these physical and chemical mediated methods are toxic, expensive and dangerous to environment. This problem can be overcome by using non-toxic, economic and eco-friendly greener method (Dhuper et al., 2012; Anand et al., 2017). Therefore green synthesis method was adopted for synthesis of nanomaterial by plants and microorganism. Synthesis of nanoparticle from plants gives a special advantage compared to microorganism due to presence of various biomolecules in the plants which are responsible for stabilization and act as capping agents for nanoparticle synthesis (Iravani, 2011; Anand et al., 2019; Anand et al., 2020). Synthesis of silver nanoparticles using different plant extracts of *Ficus carica*, *Coccoloba nucifera*, *Moringa oleifera*, *Carica papaya*, *Eucalyptus hybrid*, *Allium cepa*, *Aloe vera* and *Basella alba* have been successful and also exhibited various significant biological activities (Geetha

\* Corresponding authors at: School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Skudai 81310, Johor, Malaysia (Nur Izyan Wan Azelee).

E-mail addresses: [kanimozhi.vs@gmail.com](mailto:kanimozhi.vs@gmail.com) (S. Kanimozhi), [nur.izyan@utm.my](mailto:nur.izyan@utm.my) (N.I.W. Azelee).

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et al., 2014; Mariselvam et al., 2014; Prasad and Elumalai, 2011; Jain et al., 2009; Mani et al., 2021a; Dubey et al., 2009; Chandran et al., 2006; Ahmed et al., 2016; Mani et al., 2021b). *Cissus quadrangularis* is a vining plant which is found in India and also Africa. *Cissus quadrangularis* comes under the family of Vitaceae and also called as *Vitis quadrangularis* has been used medicinal purposes for centuries (Patil et al., 2017). It has been used for treating various diseases including helminthic, dyspeptic, ulcer, tonic seizure, irregular menses and respiratory diseases (Patil et al., 2017; Oben et al., 2007). *Cissus quadrangularis* was used as a reducing material for synthesizing zinc oxide nanoparticles that demonstrated potential inhibitory activity against various microbes like *Pseudomonas aeruginosa*, *Escherichia coli*, *Acetobacter baumannii*, *Bacillus cereus*, *Mycobacterium smegmatis* (Sathappan et al., 2021). Synthesis of silver nanoparticles from *Cissus quadrangularis* stem extract showed antibacterial activity (Kalpana et al., 2017a; 2017b). Valli and vaseeharan (2012) synthesized silver nanoparticles from *Cissus quadrangularis* that significantly inhibited the development gram positive and negative bacteria at low inhibitory concentrations of 10 µg/ml. *Cissus quadrangularis* mediated CaO nanoparticles has been examined for antibacterial activity. Highest zone of inhibition was obtained for *Escherichia coli* than other bacterial strains of *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Shigella dysenteriae* and *Salmonella* sp. (Marquis et al., 2016). Among the other metal nanoparticles, silver nanoparticles plays a major role in various activities such as antibacterial, antioxidant, antifungal and cytotoxic activities. The silver nanoparticles were examined for different applications especially in medical fields such as photosensitizers, radio sensitizers, antiviral agents, and used as therapeutic agents to treat the skin, oral, breast, lung, and leukemia cancer due to their intrinsic therapeutic properties (Kumar et al., 2014; Wei et al., 2015; Nakkala et al., 2014). Rheumatoid Arthritis is an immune system illness characterized by chronic joint inflammation, infiltration of immune cells, destruction in bones and cartilage. There are several studies which proposed that, macrophages, B cells and T cells contribute to severe destruction in joints and damages occur in tissues by releasing various prostanooids and cytokines (Al-Zifzaf et al., 2015). A previous study reported that 82% of inhibition was obtained on BSA for anti-arthritis activity of zinc oxide nanoparticles prepared using *Lagenaria siceraria* extracts (Kalpana et al., 2017a). *Luffa acutangula* peel extract mediated ZnO nanoparticles were tested for their anti-arthritis property. The studies revealed that ZnO nanoparticles showed anti-arthritis activity by efficiently inhibiting the denaturation of both albumin and bovine serum albumin (Ananthalakshmi et al., 2021). Studies of Senthilkumar et al., 2017 suggested that, maximum inhibition of 90% was achieved for anti-arthritis activity of ZnO nanoparticles from leaf extracts of *Tectona grandis*. Hence, in the present study, aqueous extracts of *Cissus quadrangularis* was used as a reducing material for silver nanoparticles synthesis. The synthesized silver nanoparticles were monitored by XRD, FT-IR, UV-Vis, and SEM techniques. The activity of silver nanoparticles was evaluated for anti-arthritis studies. This invitro anti-arthritis activity was determined by egg albumin and bovine serum protein (BSA) denaturation method.

## 2. Materials and methods

### 2.1. Synthesis of silver nanoparticles

Green synthesis of silver nanoparticles was performed using stem extracts of *Cissus quadrangularis*. Young stems of *Cissus quadrangularis* were washed and crushed with 100 ml of deionised water to prepare the extract. 5 mM silver nitrate solution was

added to extract of *Cissus quadrangularis*. The solution was stirred in a magnetic stirrer for 10 h at room temperature. It was centrifuged to collect the silver nanoparticles.

### 2.2. Characterization

XRD analysis was performed using X-ray 7000, Shimadzu to confirm the amorphous/crystalline character of the materials. Diffraction patterns were measured using Cu, K-alpha radiation at 30 kV and 30 mA in, with 2θ range of 0° to 80°. The synthesized nanoparticle was characterized using Shimadzu FTIR spectrometer. Diffuse reflectance FTIR spectrum for silver nanoparticles was measured from 4000 to 400 cm<sup>-1</sup>. Surface morphology of nanoparticles was obtained from scanning electron microscopy (SEM). SEM analysis was performed using JEOL JM 5600 equipped with 20 kV voltage and wavelength range of 5–6 nm. UV-Vis spectrum was obtained from Jasco V650 Vis spectrophotometer with the wavelength ranging from 200 to 600 nm.

### 2.3. Anti-arthritis activity

The in-vitro study of the synthesized silver nanoparticles was tested by anti-arthritis activity. It was determined by two methods such as BSA denaturation and albumin denaturation method.

#### 2.3.1. Albumin denaturation

Eggs albumin, phosphate buffered saline (PBS, pH6.4) were added to 1 ml of different concentrations of samples (100–500 µg) except blank. It was incubated and heated at 70 °C. Then the absorbance of the solution was measured at 660 nm. The protein denaturation (% inhibition) of the sample was calculated.

#### 2.3.2. BSA denaturation

Different concentrations of samples (100–500 µg), phosphate buffer and 0.5% BSA were added in the test tubes and incubated in the incubator for 20 min at 37 °C. Absorbance was measured at 255 nm.

## 3. Results and discussion

### 3.1. XRD

Fig. 1 specifies the XRD patterns for silver nanoparticles from *Cissus quadrangularis* extract. XRD pattern revealed crystalline nature of silver nanoparticles with Bragg reflection 2θ values of 38.18°,

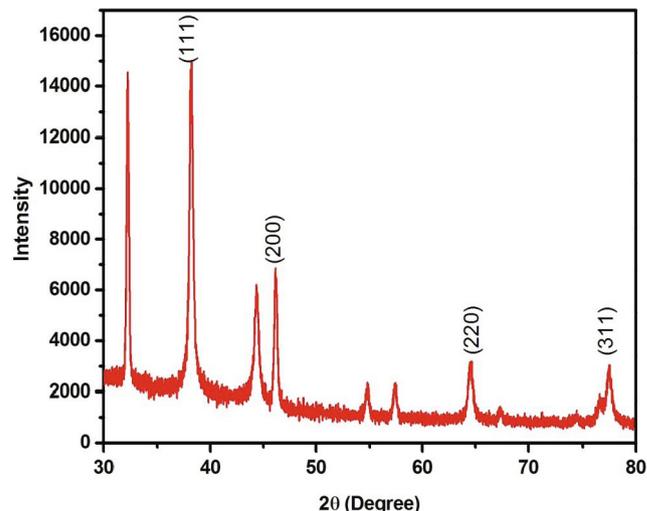


Fig. 1. XRD pattern of Silver Nanoparticle from *Cissus Quadrangularis* Extract.

44.37°, 66.46° and 77.44° which could be indexed in (111), (200), (220), and (311) planes respectively. This confirmed the crystalline nature of silver nanoparticles. The same experimental results were reported by Nalvothu et al. (2014). The data was compared with JCPDS file no. 87-0720 which was good agreement with standard values. A high intensity diffraction peak at 38° which was indexed as (111) plane indicated the crystalline silver nanoparticles. Non indexed peak at 32° and 46° were related to bio organic molecules crystallization on the surface of nanoparticle (Gomathi et al., 2017; Mallikarjuna et al., 2014). The crystallite sizes of the nanoparticle are calculated by Debye-Scherrer formula.

$$D = \frac{0.9\lambda}{\beta \cos \theta}$$

$\lambda$  represents X-ray wavelength,  $\beta$  represents diffraction peak of full width at half maximum (FWHM) and  $\theta$  represents the Bragg's reflection peak. Particle size of the silver nanoparticles was calculated and tabulated (Table 1). The average crystallite size of silver nanoparticles was 24 nm.

### 3.2. FT-IR spectroscopy

FTIR spectrum of silver nanoparticles synthesized from *Cissus quadrangularis* is depicted in Fig. 2. FTIR spectrum was used to find the functional groups present in the nanoparticles. Broad band at 3378  $\text{cm}^{-1}$  was related to water molecules of O-H stretching vibration which might be due to presence of phenols and alcohols; stretching vibration of C-H was found in the range of 2923  $\text{cm}^{-1}$  arisen from aromatic compounds. The band at 1735  $\text{cm}^{-1}$  attributed to C=O stretching vibration of carboxylic acid or ester, strong band at 1622  $\text{cm}^{-1}$  was assigned to bending vibration of amide group in proteins. Lower bands occur at 1365  $\text{cm}^{-1}$  and 1054  $\text{cm}^{-1}$  denoted  $\text{CH}_2$  alkanes and stretching of C-O ester or ether in the silver nanoparticles (Raja et al., 2017).

### 3.3. UV-Vis spectroscopy

Fig. 3 shows the UV-Vis spectrum of *Cissus quadrangularis* mediated silver nanoparticles. The metal nanoparticle contains free electron that provides surface plasmon resonance (SPR) absorption band which is due to the combination of electrons with light wave. A yellowish-brown colour can be evidenced for the reduced nature of silver ions from silver nitrate. The silver SPR band appeared in the range of 410 nm which confirmed the successful synthesis of silver nanoparticles.

### 3.4. Scanning electron microscopy

Morphological characteristics of synthesised silver nanoparticles were monitored using SEM (Fig. 4). SEM images of nanoparticles showed the spherical shape of poly dispersed nanoparticles with agglomeration. Agglomeration of nanoparticles might be due to presence of *Cissus quadrangularis* extracts.

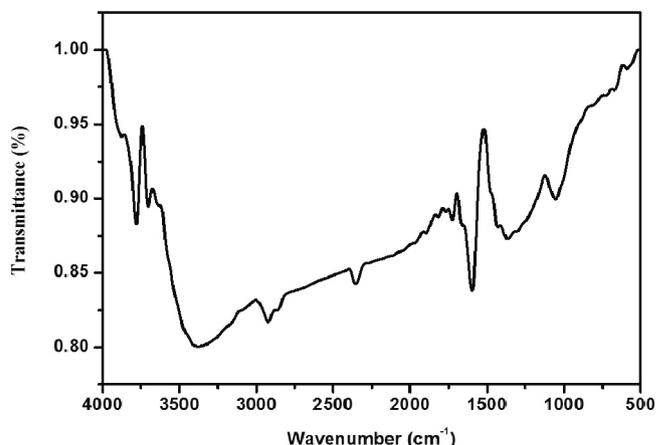
### 3.5. Evaluation of anti-arthritis activity

Synovial disease of arthritis is characterized by chronic inflammation in the joints and it could lead to destruction in the joints

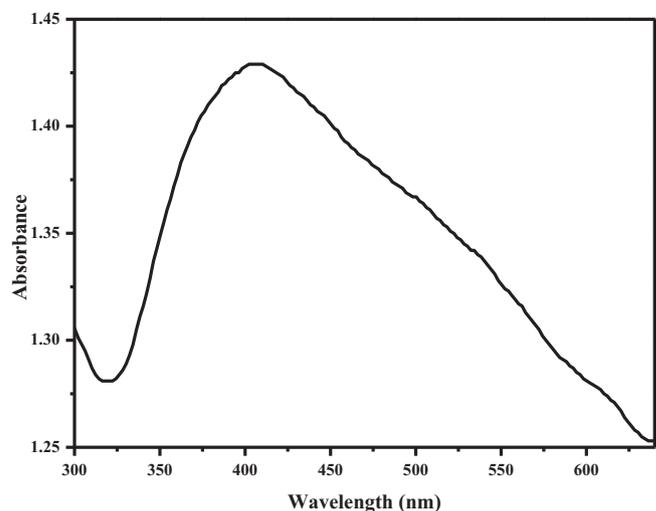
**Table 1**

Calculated particle size of Silver nanoparticle.

Miller indices (hkl) Plane	Peak position 2 $\theta$ value	Height	width	Crystallite size
(111)	38.18	11564.25	0.37067	24.97
(200)	44.37	254.89	0.00339	28.68
(220)	66.46	1944.53	0.44756	22.23
(311)	77.48	1744.65	0.51389	20.15



**Fig. 2.** FTIR spectrum of Silver nanoparticle from *Cissus Quadrangularis* plant extract.



**Fig. 3.** Uv-Vis spectrum of Silver nanoparticle from *Cissus Quadrangularis* aqueous extract.

(Jayaprakasam and Ravi, 2012). Arthritic patients have the common problem of inflammation that occurs in the living tissues. Inflammation is caused by denaturation of protein. Steroids can decrease the inflammation in the tissues and also reduce immune activity which is responsible for inflammation. The anti-arthritis activities of silver nanoparticles of various concentrations 25, 50, 100, 150 and 200  $\mu\text{g/ml}$ , were determined by albumin denaturation and BSA denaturation method. The anti-arthritis activity of synthesized samples of silver nanoparticles by albumin denaturation and BSA denaturation method are shown in Figs. 5 and 6 respectively.

The silver nanoparticles displayed 25, 39, 56, 77, and 81% inhibition of denaturation of albumin and the standard powerful non-steroidal Diclofenac sodium drug (anti-inflammatory drug) exhibited 21, 31, 47, 57, and 73% inhibition of denaturation of albumin.

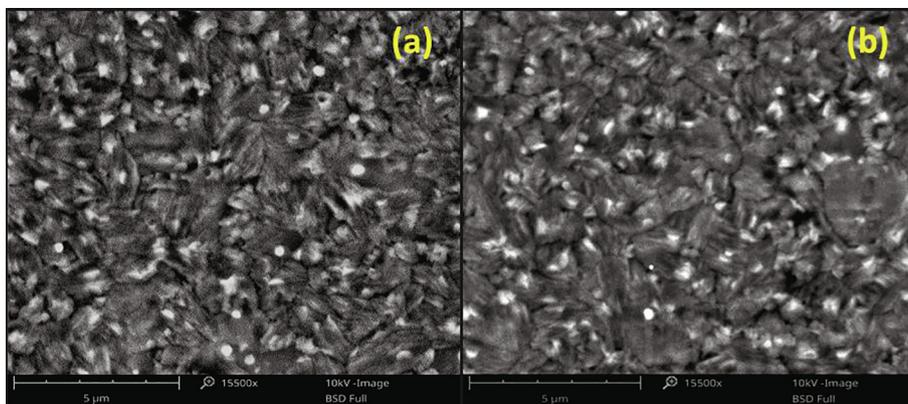


Fig. 4. SEM images of Silver nanoparticle from *Cissus Quadrangularis* plant extract.

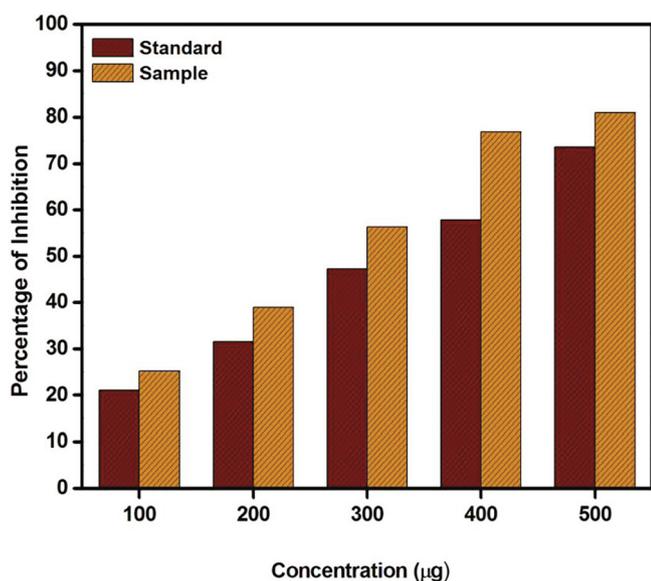


Fig. 5. Anti-arthritis activity of Silver nanoparticle by albumin denaturation method (Standard- Diclofenac sodium drug and sample- Silver nanoparticle).

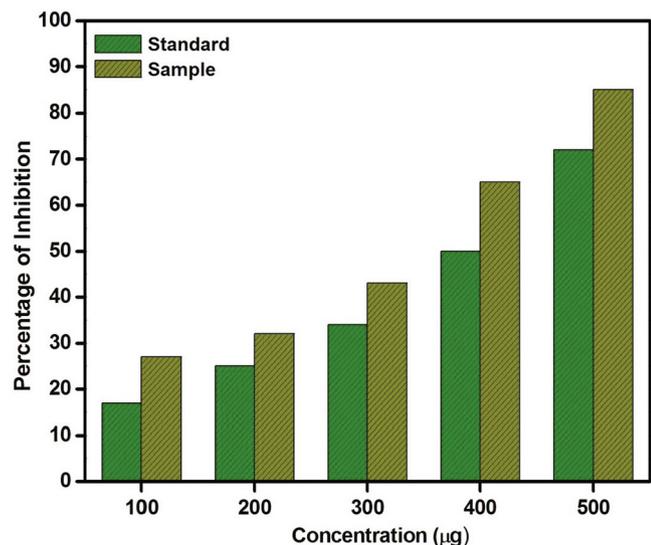


Fig. 6. Anti-arthritis activity of Silver nanoparticle by BSA denaturation method (Standard- Diclofenac sodium drug and sample- Silver nanoparticle).

Inhibition of protein denaturation by silver nanoparticles showed better result compared to reference standard drug. The present investigation demonstrated 81% inhibition of egg albumin denaturation and 85% inhibition of bovine serum albumin. Protein denaturation was decreased by increasing concentrations of silver nanoparticles. Therefore, the result showed that silver nanoparticles can inhibit the denaturation of BSA and albumin (Senthilkumar et al., 2017). Hence, the present study revealed that, silver nanoparticles are capable of inhibiting the denaturation of albumin and BSA in Rheumatic arthritic diseases.

#### 4. Conclusion

A simple, eco-friendly, economic, non-toxic greener method was used for this study. Aqueous extracts of *Cissus quadrangularis* acted as stabilizing and reducing agents for synthesis of silver nanoparticles. The average size of the Silver nanoparticles was 24 nm and the assistance of plant extract in the reduction of silver ions to silver nanoparticles was evident from the functional groups detected in the FTIR spectrum. Uv-Vis spectrum displayed strong absorption band at 410 nm which confirmed the reduction of silver nanoparticles. SEM images displayed the spherical shape of the nanoparticles. The silver nanoparticles exhibited a remarkable efficacy for anti-arthritis activity. Hence, the result obtained from this study will be assisting the pathway to extend the performance of green synthesized silver nanoparticles from *Cissus quadrangularis* extracts in arthritis investigations and develop appropriate drugs.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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