Int. J. Nano Dimens., 13 (3): 329-334, Summer 2022

ORIGINAL ARTICLE

Microwave-assisted greener synthesis of Silver nanoparticles using *Entada rheedii* leaf extract and investigation of its anticancer and antimicrobial properties

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Received 11 March 2022; revised 28 April 2022; accepted 14 May 2022; available online 19 May 2022

Abstract

In the current investigation phytochemically mediated, easy, efficient, and eco-friendly green synthesis of silver nanoparticles (AgNPs) was carried out using Entada rheedii leaf extract as a reducing and capping agent in a microwave-assisted synthetic pathway. UV-Visible spectroscopy, IR spectroscopy, scanning electron microscope (SEM), and transmission electron microscopic (TEM) techniques were used to confirm the formation of silver nanoparticles. The functional groups present in the capping agent were identified by FTIR analysis. SEM and TEM analysis studied the surface morphology of the biosynthesized AgNPs. AgNPs also showed significant antibacterial effects against four different bacterial pathogens *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Vibrio cholera*. Additionally, the prepared AgNPs exhibited solid anticancer activity against Dalton's lymphoma ascites (DLA) cells.

Keywords: *Anticancer Activity; Anti-Microbial Activity; Entada Rheedii; IR Spectroscopy; SEM; TEM.*

How to cite this article

Mathew A., Analiparambil Ravindran R., Vazhanthodi A.R., Valiyaparambil Sivadasan B., Ovungal S., Madathilpadi Subrahmanian S., Chittadimangalath P., Anthyalam Parambil A. Microwave-assisted greener synthesis of Silver nanoparticles using Entada rheedii leaf extract and investigation of its anticancer and antimicrobial properties. Int. J. Nano Dimens., 2022; 13(3): 329-334.

INTRODUCTION

Nowadays metal nanoparticles have been a field of extreme research interest due to their potential applications in areas such as catalysis, cancer therapy, sensing, biomedical, drug delivery, electronics, etc. [1–6] Among the several metal nanoparticles such as platinum (Pt), palladium (Pd), gold (Au) and silver (Ag), the studies on silver nanoparticles (AgNPs) have been carried out most extensively because of its easy method of synthesis and lower cost. Even though several methodologies like chemical, electrochemical, photochemical and micro emulsion, etc. methods have been used for the production of silver nanoparticles [7, 8], many of them suffer from harsh reaction conditions and use of harmful chemicals. So, scientists are in quest of new techniques for the synthesis of these nanoparticles.

Recently, bio-inspired synthesis of metal nanoparticles is developed as a substitute to chemical method and different research groups have demonstrated the biosynthesis of metal nanoparticles by means of bacteria, plant extracts,

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fungi and yeast [9–17]. Among these the plant-based synthesis is very simple, economic, fast, and greener as compared to other methods [13, 14]. However, the phytochemical mediated synthesis of silver nanoparticles is time consuming as in comparison to the conventional chemical reduction method. This disadvantage can be minimized by performing the plant-mediated synthesis under microwave (MW) condition as MW heating is uniform and faster. Several papers are available in the literature describing the plant mediated synthesis of nanoparticles under MW condition [18–20].

In this report, we introduce a new plant *Entada rheedii* for the synthesis of silver nanoparticles by MW assistance. *Entada rheedii*, commonly known as African dream herb is a large woody climber belonging to Mimosaceae family and traditionally it has high medicinal value. The phytochemical screening studies of *E. rheedii* leaf extract revealed the presence of numerous poly-functional molecules like terpenoids, flavonoids and tannins [21]. We have demonstrated the ability of these phytochemicals both for the reduction of silver ions to metallic silver and for stabilizing the biosynthesized silver nanoparticles. These silver nanoparticles were characterized by UV-Visible spectroscopy, scanning electron microscopic (SEM) and transmission electron microscopic (TEM) techniques. The cytotoxicity of silver nanoparticles was investigated using Dalton's lymphoma ascites cells. By using agar well diffusion method, the antimicrobial studies were performed against both gram positive and gram-negative bacterial strains.

MATERIALS AND METHODS

Silver nitrate ($AgNO_3$) was purchased from Merck Chemicals (99.8%) and used without further purification. The absorption spectra of aqueous leaf extract and synthesized silver nanoparticles were recorded in the range 300-700 nm with UV-Vis spectrometer (PG Instruments Ltd T90+). The FTIR spectra of leaf extracts and synthesized silver nanoparticles were recorded on a Shimadzu IR Prestige-21 FTIR spectrometer in the range 4000–500 cm^{-1}. The surface morphology was analysed by Scanning Electron Microscope (SEM) using VEGA3 TESCAN. Transmission electron microscopic images were obtained using a JEOLJEM-2100 microscope.

Preparation of Entada rheedi leaf extract

Fresh *E. rheedii* leaves were collected from Kerala (India) and used to prepare aqueous extracts. The leaves were first thoroughly rinsed with tap water, followed by distilled water to remove the dust particles. Then the leaves were air-dried at room temperature to remove water from the surface and further cut into small pieces. 5 g leaves were weighed and transferred into 500 mL round bottom flask containing 100 mL distilled water and boiled for 2 hours. The raw extract obtained was filtered in hot condition using Whatman No. 1 filter paper to remove particulate matter and to get a clear solution. The clear extracts were then refrigerated and further used for the synthesis of silver nanoparticles.

Synthesis of silver nanoparticles using E. rheedi leaf extracts

Silver nanoparticles were synthesized using microwave heating using a domestic microwave oven (LG-MS-2029 UW) operating at a power of 1200 W and frequency 2450 MHz). 2 mL of aqueous extract solution was added to 1 mL of $1x10^{-3}$ M aqueous silver nitrate solution and diluted to 30 mL with distilled water. This solution exposed to microwave heating for 4 minutes. After microwave heating, solutions were allowed to natural cooling to room temperature. The change in color of mixture of $AgNO_3$ and leaf extract from yellow to brown indicates the formation of silver nanoparticles. Same procedure followed for the synthesis of silver nanoparticles using *E. rheedii* leaf extract.

Cytotoxicity of AgNp in cancer cell lines

Dalton's lymphoma ascites cells (DLA cells) were used to evaluate the cytotoxicity of silver nanoparticles. DLA cells were aspirated from the peritoneal cavity of tumor-induced mice, washed several times with normal saline, and a viable cell suspension of 1106 cells in 0.1 ml was transferred to tubes containing various concentrations of AgNPs, viz 10µg, 20µg, 50µg, 100µg, and 200µg. Phosphate buffered saline (PBS) was used to get the volume up to 1 ml. In the control group, the same concentrations of leaf extracts were being used. The assay mixtures were incubated for 3 hours at 37 °C before becoming mixed with 0.1 ml of 1% trypan blue and counting the number of dead and living cells on a haemocytometer after

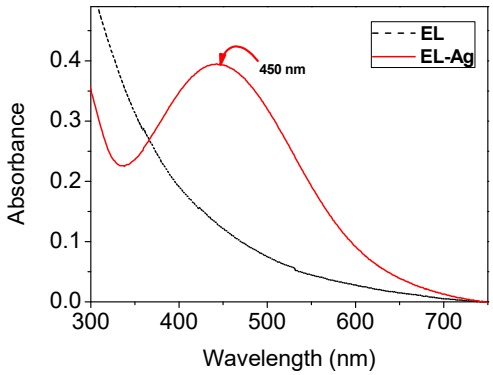

Fig. 1. Absorption spectra of *E. rheedii* leaf extract (EL) and silver nanoparticles synthesized using *E. rheedii* leaf extract (EL-Ag).

three minutes. The percentage of cytotoxicity was calculated by using the formula

$$\text{Percentage of toxicity} = \frac{\text{Number of dead cells}}{\text{Number of live cells} + \text{number of dead cells}} \text{X100}$$

Antimicrobial effects of AgNps on bacterial pathogens

The antimicrobial properties of green synthesized AgNPs were tested against four different bacterial pathogens, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Vibrio cholerae* by Agar well diffusion method [14, 22-23]. The four bacteria strains were applied on Mueller-Hinton agar using a sterilized cotton swab (MHA). A sterile blank antimicrobial susceptibility disc was used in the test. The discs were loaded with 10 µl of AgNPs as an experiment (represented as S); *Entada* leaf extracts were utilized as a control (represented as C), and the antibiotic ampicillin was employed as a positive control (represented as AB). The discs were then placed on the agar plate and incubated at 37 °C for 24 hours. The inhibitory zone was examined after 24 hours of incubation.

RESULTS AND DISCUSSION

UV–Vis spectral analysis

The brownish color of silver nanoparticles in aqueous solution is due to the surface plasmon resonance (SPR) [24]. The change in colour of mixture of aqueous leaf extract and silver nitrate ($AgNO_3$) from light yellowish to brown upon microwave heating clearly indicate the formation of silver nanoparticles. The formation of silver nanoparticles was further confirmed by using UV–Vis spectroscopic technique, a frequently used technique to characterize the metallic nanoparticles. The strong surface plasmon resonance in UV–Vis spectra (band at 450 nm), confirm the formation of silver nanoparticles in the synthesis using *E. rheedi* leaf extract (Fig. 1). However, no such characteristic absorption bands were present for the pure aqueous extracts of *E. rheedi* leaf extracts (Fig. 1).

FTIR studies

Fig. 2 shows the FTIR spectra of pure *E. rheedii* leaf extract and silver nanoparticles synthesized using *Entada rheedii* leaf extract. FTIR spectra of pure *E. rheedii* leaf extract showed peaks at 3315, 2120 and 1640 cm^{-1}. The silver nanoparticles synthesized using *Entada rheedii* leaf extract also showed similar peaks, represent the presence of same functional groups in nanoparticle system [22].

SEM analysis

Scanning microscopic studies were used to investigate the surface morphology and appearance of the nanoparticle. Figs. 3a, and 3b depicts the SEM images of *Entada rheedii* leaf extract stabilized AgNPs under different magnifications. The small AgNPs are covered by a thin network of biomolecules that derived from the mucilage or gum of the fruit, this further prevent the particle from aggregation. Such observations were reported during plant extract mediated synthesis of metal nanoparticle by some other research groups [2, 5].

TEM analysis

The size, morphology and crystal structure of synthesized silver nano particles were further studied in detail by transmission electron

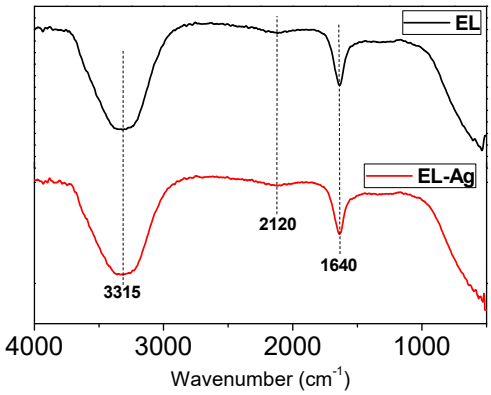

Fig. 2. FTIR spectra of *E. rheedii* leaf extract (EL) and silver nanoparticles synthesized using *E. rheedii* leaf extract (EL-Ag).

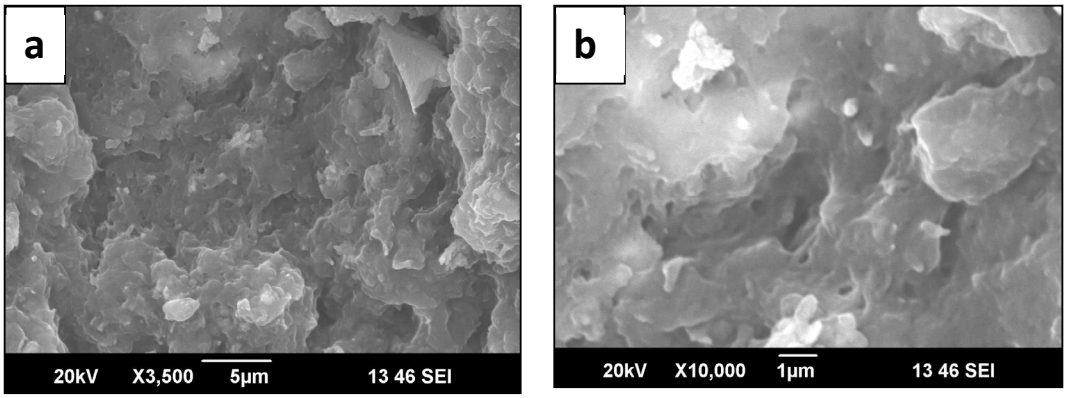

Fig. 3. SEM image of AgNPs prepared using Entada leaf extract under different magnification.

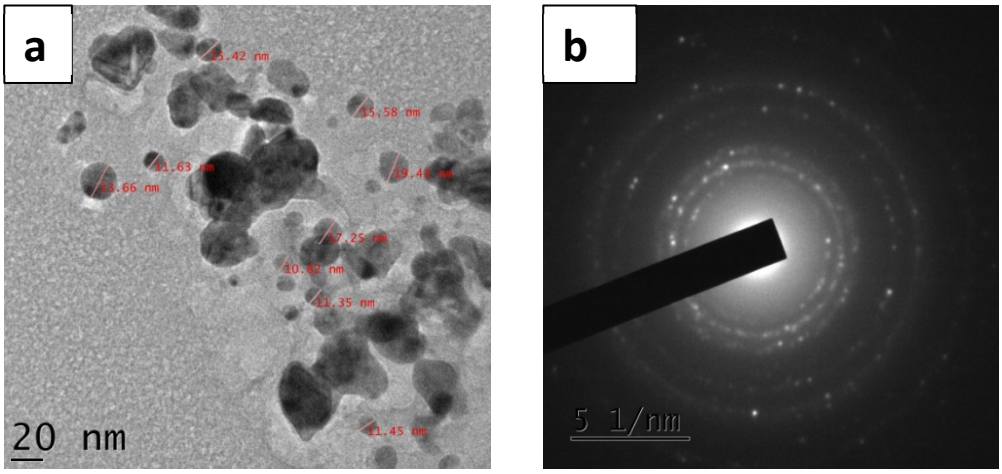

Fig. 4. (a) Transmission Electron Microscopic (TEM) images and (b) SAED pattern of AgNPs prepared using Entada leaf extract.

microscopy. HRTEM images of AgNPs were shown in the Fig. 4 a. The TEM images confirm the formation of spherical silver nanoparticles. The particles are well isolated from each other by the capping phytochemicals; hence no particle aggregation was observed which is shown in the

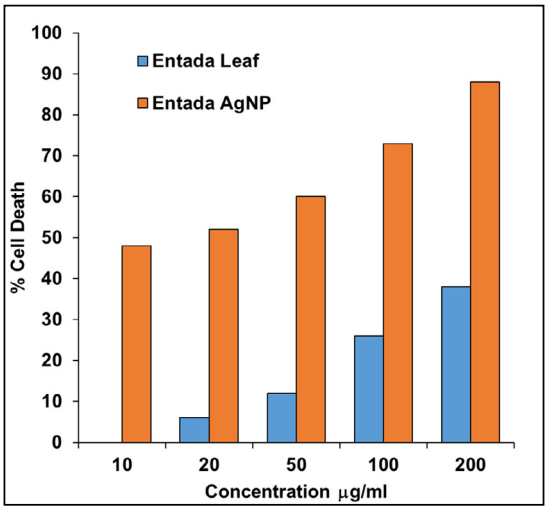

Fig. 5. The anticancer effect of green synthesized AgNPs on DLA cells.

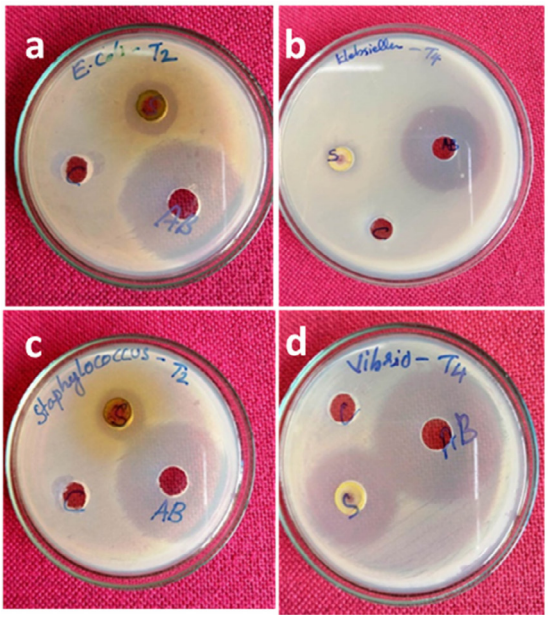

Fig. 6. The antimicrobial properties of AgNPs against four different bacterial pathogen species: (a) *E. Coli,* (b) *K. pneumonia*, (c) *S. aureus*, (d) *V. Cholerae*. Entada leaf extract (control), AgNPs (experimental) and antibiotic (positive control) are represented as C, S and AB respectively.

TEM image suggesting the high capping ability of the *Entada* leaf metabolites. The crystalline nature of the synthesized Ag-NPs was revealed by selected area electron diffraction (SAED) pattern which shows concentric rings Fig. 4b.

Anticancer activity of silver nanoparticles

The anticancer property of green synthesized AgNPs was investigaed with DLA cells, the result of the study is presented in Fig. 5. The result shows that AgNps reduce the viability of DLA cells in a dose dependant manner.

Antibacterial effect of silver nanoparticles

AgNPs were tested for antibacterial activity against four different bacterial pathogens, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Vibrio cholerae*. The

disc diffusion test findings are represented in the Figs. 6a, 6b, 6c, 6d. For the disc diffusion test, the presence of a clean zone surrounding the AgNPs disc indicated that the biogenic AgNPs displayed excellent antibacterial activity, inhibiting the proliferation of bacterial pathogens.

CONCLUSION

The current investigation reveals that *E. rhedii* is an excellent reducing and capping agent for the synthesis of silver nanoparticles. The biosynthesised AgNPs are characterized by using several techniques like Uv-Vis., FTIR, SEM and TEM. The silver nano particle synthesized by the leaf extract of *E. rhedii* has excellent anticancer activity in Dalton's lymphoma ascites cells. Nanoparticles also show remarkable antimicrobial properties. Owing to these properties, synthesized AgNPs have significant applications in biomedical field. Further inversigations are necessary to analyse medicinal value of the synthesized silver nanoparticle.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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