

SYNTHESIS, CHARACTERIZATION, AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES USING AQUEOUS EXTRACT OF *ASPARAGUS RACEMOSUS* RHIZOME – AN *IN VITRO* APPROACH

HEMADEVI N¹, VENKATALAKSHMI P²

¹Department of Biochemistry, Sengamala Thayaar Educational Trust Women's College, Mannargudi, Tamil Nadu, India. ²Department of Biochemistry, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India. Email: venkatalakshmisathish@gmail.com

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ABSTRACT

Objective: The proposed study is carried out to synthesize and characterize silver nanoparticles (AgNPs) using rhizomes of *Asparagus racemosus* and to evaluate its antimicrobial activity *in vitro*.

Methods: Aqueous extract of *A. racemosus* rhizomes was prepared and subjected to preliminary phytochemical screening. AgNPs of *A. racemosus* rhizomes were prepared using standard procedure. The synthesized particles were characterized using ultraviolet-visible spectrophotometry, Fourier transform infrared (FTIR) spectroscopy, and scanning electron microscopic (SEM) analysis. Further, these nanoparticles were subjected to *in vitro* antimicrobial studies.

Results: Preliminary phytochemical analysis showed the presence of tannins, phlobatannins, saponins, flavonoids, terpenoids, triterpenoids, alkaloids, carbohydrates, proteins, anthraquinones, polyphenols, and glycosides. The study of synthesized AgNPs showed a sharp absorbance at 410 nm. The results of FTIR analysis confirmed the presence of phenol, alkanes, aliphatic amine, secondary alcohol, alkenes, and aromatic amines. SEM analysis of the AgNPs revealed higher density polydispersed AgNPs of various sizes. The AgNPs synthesized from rhizome extract showed higher toxicity toward tested microorganisms when compared to extract alone.

Conclusion: It has been concluded that silver nanoparticles of *A. racemosus* could be a good source of plant-derived antimicrobials.

Keywords: Antimicrobial, *Asparagus racemosus*, Nanoparticles, Fourier transform infrared, Scanning electron microscopy, Ultraviolet-visible spectrophotometry.

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INTRODUCTION

Traditional medicine has become an indispensable part of human society since ancient times. "There has been a resurging interest in the field of phytotherapeutics in the last few decades [1]." Among the technologies of the new millennium, nanotechnology and nanomaterials have been providing solutions to challenges faced in the areas of environmental and life sciences [2]. Colloidal nanosilver has a broader utility in many consumer products [3]. Synthesis of silver nanoparticles (AgNPs) with the aid of plant extracts has emerged as a thriving branch of nanotechnology [4]. Hence, researchers are interested in synthesizing nanosilver by employing different plant extracts as reducing agents. *Asparagus racemosus* is widely distributed crossways the globe, and it is mainly cultivated in India [5]. The use of *A. racemosus* has been reported in the ancient literature of Ayurveda (*Charaka Samhita*) [6]. It has been regarded as a "Rasayana" in Ayurveda and thereby considered to be useful in promoting general well-being by increasing cellular energy and resistance [7]. Conventionally, *A. racemosus* is designated in epilepsy, Vata disorders [8], and brain tonic, which helps in regulating cardiac diseases and hypertension [9].

It finds applications in treating disorders associated with the male gonads [10]. It has been used in Ayurvedic formulations for digestive discomfort, indigestion, amoebiasis, piles, and debility [11]. Traditional Health practitioners usually prescribe *A. racemosus* to treat uterine disorders [12]. Recent researches disclosed *Shatavari* as antidiarrheic [13], antispasmodic, and aphrodisiac [11], antidiysenteric, demulcent, diuretic [14], galactagogue, nutritive, mucilaginous, refrigerant, stomachic, and works as a tonic for human beings. It acts as an immunomodulator in reinforcing the immune system and also protects vital organs [15,16].

In this perspective, an aqueous extract of *A. racemosus* rhizome is used in the present work to synthesize AgNPs. The synthesized nanoparticles have been subjected to spectrophotometric and electron microscopic characterization. The antimicrobial activity of the synthesized nanoparticles has also been evaluated *in vitro*.

METHODS

Collection of plant materials

The rhizomes of *A. racemosus* were being purchased from an Ayurvedic medical shop, Thanjavur, Tamil Nadu, India. The powdered material has been used for further studies.

Preparation of aqueous extract and preliminary phytochemical screening

Two grams of the test powder and 100 ml of water were taken in a conical flask and shaken well for 30 min. After 24 h, the extract has been filtered using Whatman filter paper No.1. The filtrate has been transferred into a china dish and dried over a water bath at 45°C. The obtained extract has been used for phytochemical analysis [17-19].

Synthesis of AgNPs from *A. racemosus* rhizomes extract

About 5 ml of *A. racemosus* rhizomes extract was taken in a 250 ml Erlenmeyer flask and added with 45 ml of 1 mM aqueous silver nitrate (AgNO₃) solution. It was then incubated in the dark for 5 h (to minimize the photoactivation of AgNO₃), at room temperature. A control setup has also been maintained without rhizome extract. Purification of the obtained AgNP solution was carried out by successive centrifugation for 15 min, at 10,000 rpm followed by re-dispersion of the pellet in de-ionized water. Then, the AgNPs were freeze-dried [20].

Ultraviolet (UV) and Fourier transform infrared (FTIR) spectroscopic analysis

The synthesized AgNPs were scanned in the wavelength ranging from 300 to 800 nm using the PerkinElmer spectrophotometer, and the characteristic peaks were detected. FTIR analysis has been performed using a PerkinElmer spectrophotometer system to identify the unique peaks ranging from 400 to 4000 cm^{-1} and their functional groups.

Scanning electron microscopy (SEM)

In this research work, the Jeol JSM-6480 LV SEM machine was being used to characterize the mean particle size and morphology of nanoparticles. The freeze-dried sample of AgNPs solution has been sonicated with distilled water. A drop was kept on a glass slide and allowed to dry. A thin platinum coating was applied to make the samples conductive. JEOL JSM-6480 LV SEM machine has been operated at a vacuum of the order of 10–15 torr with an accelerating voltage of 10–20 kV.

Antimicrobial assay

Six-millimeter (diameter) discs of Whatman No. 1 filter paper were impregnated with different concentrations of the test substances after sterilization by autoclaving at 121°C and drying in a hot air oven at 50°C. Two Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*; one Gram-negative bacteria *Escherichia coli*; and two fungi *Aspergillus flavus* and *Candida albicans* were being selected for evaluating antimicrobial efficacy of the synthesized AgNPs. The bacterial isolates were first subcultured in a nutrient broth and incubated at 37°C for 18 h, while the fungal isolates were subcultured in Potato dextrose agar (PDA) for 72 h at 25°C.

Antibacterial activity

Antibacterial activity was carried out using the modified method initially described by Bauer *et al.* [21]. Mueller–Hinton agar was prepared then autoclaved at 15 lbs pressure aimed at 20 min and cooling to 45°C. The air-conditioned media were poured on to germ-free Petri plates and allowed for solidification. The plates with media were sowed with the respective bacterial suspensions using a sterile swab. The test drug-coated discs were placed suitably on Petri plates along with control and standard (gentamicin A [10 μg] for bacteria) discs. The plates had been incubated at 37°C for 24 h. After the incubation period, the diameter of the zone formed around the paper disc was measured and expressed in mm [22].

Antifungal activity

PDA was ready and autoclaved at 15 lbs weight for 20 min and cooling to 45°C. The cooling media have been added with 10 ml/L tartaric acid (10%), which acted as a sterile agent and poured, on to sterile Petri plates and allowed for solidification. The dishes with media were seeded with the respective fungal suspensions using a sterile swab. The test drug-coated discs were placed suitably on Petri plates along with control and standard (amphotericin B [20 μg]) discs. The plates were being incubated at 28°C for 72 h. After the incubation period, the diameter of the zone formed around the discs was measured and expressed in mm [21,22].

RESULTS AND DISCUSSION

Natural products such as plant extracts provide a wide range of opportunities for new drug discoveries because of chemical diversity, either as pure compounds or as standardized extracts [23]. Recent evidences suggest that herbal-based products are a precious source for the production of chemical entities that could be utilized for the treatment of some complex diseases [24]. The phytochemicals present in herbal products help in building immunity against microbial infections [25]. Besides, phytochemicals in the plant extracts can act as reducing and capping agents in reducing silver in AgNO_3 to AgNPs [26] and thereby extends the application of plant extracts in the biosynthesis of metal nanoparticles. In the present study, qualitative phytochemical analysis of an aqueous extract of *A. racemosus* rhizomes revealed the presence of flavonoids, tannin, saponins, glycosides, carbohydrate, terpenoids, alkaloids, polyphenol triterpenoids, phlobatannins, amino acid, and anthraquinone while steroid was absent (Table 1).

In the present study, change in coloration of AgNO_3 incubated with the selected plant extract from yellow to brown, which was noted after 5 h, whereas no color change has been observed in AgNO_3 without plant extract. The appearance of brown color in plant extract treated flask indicated the formation of AgNPs. The present finding was in agreement with Satyavani *et al.* [27], who observed the brown color in the reaction mixture during the synthesis of AgNPs from *Citrullus colocynthis* stem-derived callus extract with one mM AgNO_3 solution (Fig. 1).

The nanosilver particles of Shatavari rhizome extract synthesized by this method had been further subjected to UV-visible spectrophotometric characterization in a suitable wavelength range of 400–450 nm [28,29]. Sharp absorbance peak at 410 nm represented the AgNPs (Fig. 2). The presence of surface plasmon resonance of nanosilver particles of Shatavari rhizome extract has become evident from the UV-visible absorption band in the current visible light region (380–460 nm), suggesting the presence of nanosilver particles with size ranging from 2 nm to 100 nm [30,31]. The reduction has been attributed to the presence of phenolics, terpenoids, polysaccharides, and flavones in the extract [32] (Table 2).

FTIR measurements signify the role of phytochemicals in the stabilization of AgNPs through reduction and capping [33]. In the present study, the FTIR spectrum showed peaks at 891, 1061, 1637, 2071, and 3452 cm^{-1} . The band appeared at about 1637 cm^{-1} can be assigned for aromatic rings. The broader band appearing at 3452 cm^{-1} can be associated with the stretching vibrations of alcoholic

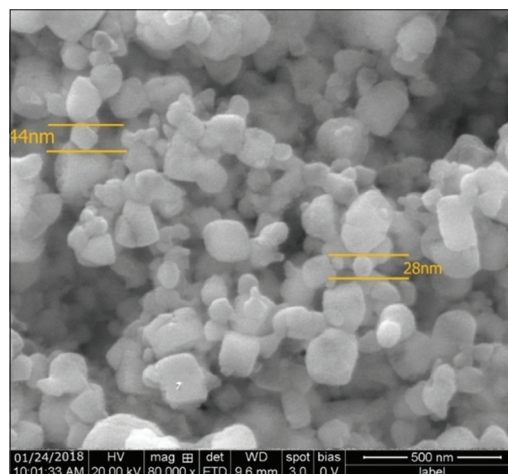


Fig. 1: High-resolution scanning electron microscopic image of silver nanoparticles. Polydispersed (Cluster) AgNPs ranged between 28 and 44 nm

Table 1: Preliminary phytochemical analysis of *A. racemosus* rhizomes

S. No.	Secondary metabolites	Aqueous extract
1.	Tannin	+
2.	Phlobatannins	+
3.	Saponin	+
4.	Flavonoids	+
5.	Steroids	-
6.	Terpenoids	+
7.	Triterpenoids	+
8.	Alkaloids	+
9.	Carbohydrate	+
10.	Amino acid	+
11.	Anthraquinone	+
12.	Polyphenol	+
13.	Glycoside	+

“+” indicates the presence of the compounds, “-” indicates the absence of the compounds. *A. racemosus*: *Asparagus racemosus*

and phenolic O-H. At 1061 cm^{-1} , a peak is observed that could be for multiple C-O groups. The results of FTIR analysis confirmed the presence of phenol, alkanes, aliphatic amine, secondary alcohol, alkenes, and aromatic amines compounds (Table 3 and Fig. 3). This has suggested the attachment of some polyphenolic components to AgNPs. The peaks at $1000\text{--}1200\text{ cm}^{-1}$ indicate C-O single bond, and peaks at $1620\text{--}1646\text{ cm}^{-1}$ represent carbonyl groups (C = O) from polyphenols such as catechin gallate, epicatechin gallate, and theaflavin [34].

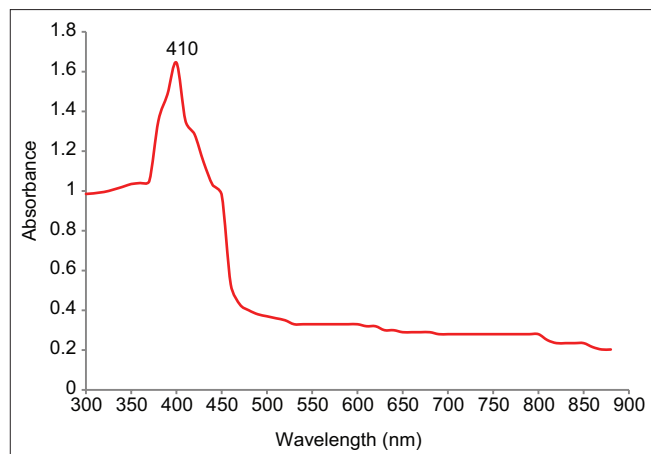


Fig. 2: Ultraviolet-visible spectral analysis of silver nanoparticles

SEM is a valuable tool to evaluate the surface morphology, size, and form of the green AgNPs synthesized. The SEM images have shown individual AgNPs, which are predominantly spherical in shape and a number of aggregates with no defined morphology. The presence of phytochemicals in the rhizome extract of *A. racemosus* might have contributed to the synthesis of spherical AgNPs. The SEM image shows the size of the AgNPs, ranging from 28 to 44 nm. The present findings on the size of the obtained nanoparticles are in par with previous studies reported by authors on *Aloe vera* extracts [35] and *Euphorbia hirta* extracts [36].

Most of the nanosilver particles of Shatavari rhizome extract had been found in aggregates and a few scattered, as observed under SEM. In our study, SEM images were verified with multiple trials and evidenced that there was a disparity in particle sizes (28–44 nm), which fall closer to many of the AgNPs produced using other plant materials [37,38].

Tona *et al.* (1998) rightly pointed out that “Plants are an essential source of potentially useful structures that could be effectively utilized to develop new therapeutic entities and assessment of antimicrobial activity of the plant extracts through *in vitro* assay procedures is the preliminary step to achieve this goal [39,40].” AgNPs biosynthesized from *A. racemosus* rhizomes extract were tested against selected microorganisms for antimicrobial activity using the agar disc diffusion method. The inhibitory effect of AgNPs from *A. racemosus* rhizomes extract was compared to *A. racemosus* rhizomes extract alone and standard chloramphenicol through zone of inhibition after 24 h of incubation. AgNPs from *A. racemosus* rhizomes extract showed about 3.28 mm inhibition zone against the test organisms *E. coli*; 2.30 mm and

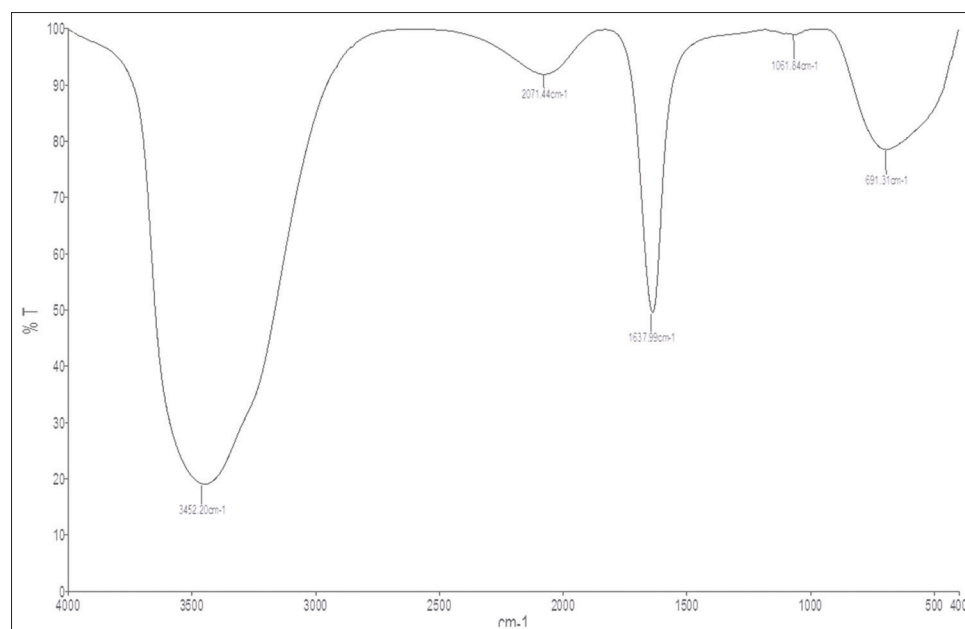


Fig. 3: Fourier transform infrared spectrum of Ag nanoparticles synthesized by reduction of Ag^+ ions by *Asparagus racemosus* rhizomes extract

Table 2: Antimicrobial activity of AgNPs, AgNO_3 , and *A. racemosus* rhizome extract

Samples (30 $\mu\text{g/ml}$)	ZOI (mm)				
	<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>C. albicans</i>	<i>A. flavus</i>
AgNO_3	1.58 \pm 0.11	1.76 \pm 0.12	1.68 \pm 0.11	1.40 \pm 0.12	1.10 \pm 0.10
<i>A. racemosus</i> rhizomes	1.06 \pm 0.07	0.82 \pm 0.05	0.75 \pm 0.05	1.80 \pm 0.08	1.30 \pm 0.13
AgNPs	3.28 \pm 0.22	2.30 \pm 0.16	2.11 \pm 0.14	4.20 \pm 0.25	3.40 \pm 0.16
Standard (chloramphenicol)	6.29 \pm 0.44	5.78 \pm 0.40	5.71 \pm 0.39	6.10 \pm 0.46	4.20 \pm 0.40

Values were expressed as mean \pm SD for triplicate. *A. racemosus*: *Asparagus racemosus*, AgNPs: Silver nanoparticles, AgNO_3 : Silver nitrate, *S. aureus*: *Staphylococcus aureus*, *B. subtilis*: *Bacillus subtilis*, *E. coli*: *Escherichia coli*, *A. flavus*: *Aspergillus flavus*, *C. albicans*: *Candida albicans*, ZOI: Zone of inhibition

Table 3: FTIR profile of AgNPs and their functional groups

Peak values	Functional groups
3452.20	Alcohol, phenol
2071.44	Alkenes
1433.58	Aldehyde
1637.90	Aromatic
691.31	Alkanes

FTIR: Fourier transform infrared, AgNPs: Silver nanoparticles

2.11 mm against test organisms: *S. aureus* and *B. subtilis*, respectively. Considerable antifungal activity of the synthesized nanoparticles has also been reported against *C. albicans* and *A. flavus*, which indicate the microbicidal activities of the silver.

The AgNPs synthesized that from rhizome extract showed higher toxicity toward tested organisms when compared to extract alone. The augmented microbicidal activity of nanosilver particles compared to the AgNO₃ solution is due in part to their larger surface area and enhanced surface contact with microorganisms [41] and also due to the synergistic effect between particles and natural compounds [42]. The biological synthesis of AgNPs using *A. racemosus* rhizome extract provided an eco-friendly, simple, and efficient route for the synthesis of benign nanoparticles that could find potential applications in the biomedical field.

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AUTHORS' CONTRIBUTIONS

Author NH performed experiments and analyzed the data. PV is a research guide who has contributed to the structuring of the work and helped in writing and revising the manuscript.

CONFLICTS OF INTEREST

Authors N Hemadevi and P Venkatalakshmi declare that they have no conflicts of interest.

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