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Research Article

ANTIMICROBIAL POTENTIAL OF BIOSYNTHESIZED SILVER NANOPARTICLE AGAINST HUMAN PATHOGEN *PSEUDOMONAS AERUGINOSA* AND *KLEBSIELLA PNEUMONIAE*

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ABSTRACT

Objectives: The main objective of this investigation was to evaluate the potential of water extract from pteridophytes (*Adiantum raddianum*, *Acrostichum aureum,* and *Christella dentata*) in synthesizing silver nanoparticles (AgNPs) through eco-friendly routes and accessing their antibacterial activity.

Methods: Water extract from pteridophytes (*A. raddianum*, *A. aureum,* and *C. dentata*) was used for synthesizing AgNPs. AgNPs formation was confirmed from the plasmonic absorbance spectra. Antimicrobial potential of synthesized particles was evaluated against human bacterial pathogens *Staphylococcus aureus*, *Proteus* species, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Escherichia coli*. The results were statistically validated using two-way ANOVA. Most active AgNPs were characterized using high-resolution transmission electron microscopy to access the morphology and size.

Results: Water extract from the pteridophytes is capable of synthesizing AgNPs through green synthetic routes. AgNPs synthesized using water extract from *A. raddianum* showed a significant activity against *P. aeruginosa* followed by *K. pneumoniae*. These AgNPs possessed spherical morphology with size ranging between 10 and 20 nm.

Conclusion: Novel bioactive formulations, particularly effective against *P. aeruginosa* and *K. pneumoniae,* can be developed through eco-friendly methods using water extract of *A. raddianum*.

Keywords: Biosynthesis, Silver nanoparticle, Antimicrobial, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Adiantum raddianum.*

INTRODUCTION

Potential of nanotechnology is evolving day-by-day, with the aid of novel synthetic methods [1]. However, toxic chemicals involved in these process, for generating unique molecular units, are causing a serious threat to the environment and workforce involved [2]. Hence, academic and industrial researchers in this field are actively involved in the standardization of novel eco-friendly synthetic methods. The potential of polar groups in humic acid isolated from mangrove ecosystems had been used in the green synthesis of silver nanoplates as described by Prashob *et al*. [3]. These humic acid synthesized silver nanoplates were very effective in suppressing the proliferation of Dalton lymphoma ascites. Even though green synthetic methods for producing nanocolloids with unique shapes and properties for clinical and industrial applications are progressing. [4,5]. Much work needs to be done to identify novel sources and methods for generating nanoparticles with distinctive properties. In this regard, biogenic sources, used for synthesizing nanoparticles, are eco-friendly prospects for functionalizing nanoparticles with novel properties [6].

Among biogenic sources, leaf extracts and microbes isolated from marine and mangrove sediments, nodules, etc., are extensively used for the synthesis and functionalization of particles [7-14]. These biosynthetic eco-friendly methods have opened new avenues for applications in non-linear optics, suppressing pathogens, and therapeutic treatments [4,8,15]. These methods were successful in generating particles with uniform shape and size distribution. However, rate of biosynthesizing nanoparticles using leaf extracts are comparatively faster than fungi and other microbes [4,16]. So far, extracts of pteridophytes *Adiantum raddianum*, *Acrostichum aureum,* and *Christella dentata* were not investigated for developing nanoparticles useful in therapeutic applications. Considering these facts, herein, the prospective of aqueous extract to synthesize silver nanoparticles (AgNPs) with potential to suppress pathogens was studied. The results will be useful for developing novel therapeutic combinations to contain microbial infections.

To biosynthesis AgNPs, aqueous extracts collected from the fronds of pteridophytes *A. raddianum*, *A. aureum,* and *C. dentata* were used. *A. raddianum* is a common fern, in which frond cell walls are enriched with Type III mannan [17]. *A. aureum*, a commonly observed abundant mangrove fern in the wetlands of Kerala, India, is known for its cytotoxic and other biological activity [18]. *C. dentata* is another fern common in the tropical environment, with unique biological activity [19]. Synthesized particles were characterized using ultraviolet (UV)-visible spectrometer and high-resolution transmission electron microscopy (HRTEM). Antimicrobial studies were performed against Gram-positive and Gram-negative human pathogens using disc diffusion assay.

METHODS

Sampling

The frond samples of pteridophytes *A. raddianum*, *A. aureum,* and *C.dentata* were collected from the wetlands of Kochi, Kerala, along the fringes of Cochin estuary. The samples were identified by refereeing to pteridophyte flora of India. These zones which harbor a variety of pteridophytes are threatened by a lot of developmental pressure and need immediate measure to conserve these biodiversity. Frond samples were packed in self-sealing covers and preserved in the deep freezer till further analysis.

Preparation of aqueous extract

Leaf samples (10 g) from *A. raddianum*, *A. aureum,* and *C. dentata* were grinded to fine paste at room temperature. These fine frond paste was then mixed with 100 ml Milli-Q water at room temperature. Supernatant solution was recovered through centrifugation and passed through nylon membrane filter paper to remove fine particles. Aqueous extract thus obtained were dialyzed to remove the inorganic ions. Ion free aqueous extracts were then dried in the freeze drier, and approximately 115 mg of residue was obtained. An appropriate quantity of crude was redissolved in Milli-Q water to prepare 1000 ppm stock solution of aqueous extract.

Synthesis of AgNPs

Final working solution for the biosynthesis of AgNPs consists of 10 ppm aqueous extract and silver nitrate (AR grade) ranging between 0.001 and 0.01 mol/L. In the reaction media aqueous extract function both as reducing and capping agent for the syntheses of AgNPs. A uniform temperature of 75°C was maintained throughout the experiment, using a hot air oven. Reduction of Ag ions to Ag nanoparticles was inferred from the color change, colorless to pale yellow [5,6].

Characterization of AgNPs

Biosynthesized AgNPs were characterized using UV-visible spectrometer (Analytik Jena Specord 200 Plus). Plasmonic absorbance maxima of the particles were measured in the absorption band, ranging between 200 and 700 nm. Further, HRTEM (Jeol/JEM 2100) was used to take the high-resolution images of AgNPs. AgNPs with potential antibacterial activity were characterized using HRTEM. Before HRTEM imaging, AgNPs were drop casted on copper grids and allowed to dry overnight under vacuum. Representative images of AgNPs were collected by scanning various zones [16].

Antibacterial study

Five human pathogens such as *Staphylococcus aureus*, *Proteus* species, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Escherichia coli* were selected for the screening. Antibacterial potential of AgNPs was investigated using disc diffusion assay. Each disc was uniformly dispersed with AgNPs to obtain a particle concentration of 1 nmol/mL. Silver-free aqueous extracts of *A. raddianum*, *A. aureum,* and *C. dentata* were used as control. Average of triplicate analyses was used to evaluate the potential of AgNPs to suppress the viability of pathogens [6].

Statistical validation

Two-way ANOVA was performed using SPSS Version 11. Mean values were compared using Tukey's test ($p<0.05$).

RESULTS

Results of the surface plasmon absorbance of biosynthesized AgNPs are shown in Fig. 1. These plasmonic absorbance data reveal the potential of aqueous extract solution to function as both reducing and capping agent to synthesize AgNPs. The rate of reduction of silver ions to Ag metal and consequent stabilization of nanosize was comparatively fast. These biosynthesized particles were stable for months.

Results of antimicrobial potential of AgNPs against *S. aureus*, *Proteus* sp., *K. pneumoniae*, *P. aeruginosa,* and *E. coli* using aqueous extracts of *A. raddianum*, *A. aureum,* and *C. dentata* are shown in Table 1. All AgNPs in nmol/L concentrations were comparatively active against Gram-negative bacteria, whereas moderate activity was shown against Gram-positive bacterium. Among the biosynthesized AgNPs, nanoparticles synthesized using the extract of *A. raddianum* was comparatively the most active followed by *C. dentate* and *A. aureum*. AgNPs synthesized using *A. raddianum* showed a significant inhibition of both *P. aeruginosa* and *K. pneumoniae*. Whereas, AgNPs synthesized using the extracts of *C. dentate* and *A. aureum* manifested comparable potential to suppress *K. pneumoniae*. However, their activities were only moderate. Likewise, against Gram-positive *S. aureus,* all the biosynthesized particles showed only moderate activity. Finally, against *Proteus* sp., even though

Table 1: Antibacterial activity of biosynthesized AgNPs against Gram-negative and Gram-positive bacteria

S. No	Bacteria	Zone of inhibition		
		A. raddianum	***A. aureum***	***C. dentata***
1	*E. coli*	3±1 mm (−)	2±1 mm (−)	2±1 mm (−)
2	*K. pneumoniae*	8±2 mm (++)	6±1 mm (+)	6±2 mm (+)
3	*Proteus* sp.	3±1 mm (−)	2±1 mm (−)	2±1 mm (−)
4	*P. aeruginosa*	10±1 mm (++)	4±1 mm (+)	4±2 mm (+)
5	*S. aureus*	4±1 mm (+)	5±1 mm (+)	4±2 mm (+)

n=3, ++significant, +moderate, −insignificant. *E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae, P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus, A. raddianum: Adiantum raddianum, A. aureum: Acrostichum aureum, C. dentate: Christella dentata*

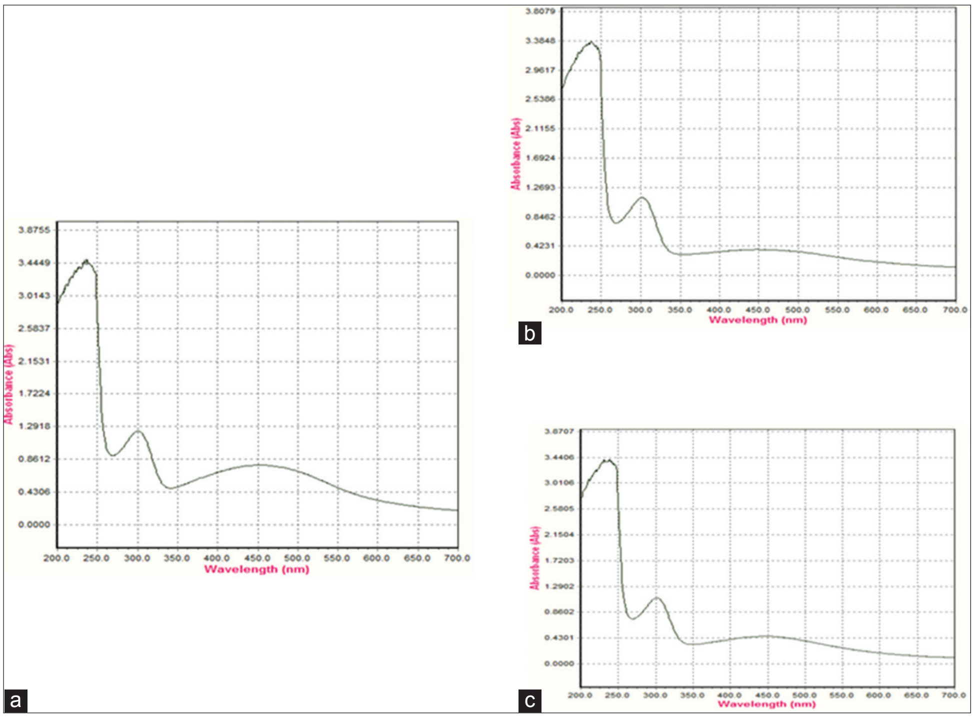

Fig. 1: Surface plasmon absorbance of biosynthesized silver nanoparticles (AgNPs). (a) Corresponds to AgNPs synthesized by *Adiantum raddianum*, (b) corresponds to AgNPs synthesized by *Acrostichum aureum* and (c) corresponds to AgNPs synthesized by *Christella dentata*

biosynthesized AgNPs suppressed the pathogen activity, the extent of inhibition was insignificant.

Two-way ANOVA was performed to statistically validate the effect of AgNPs synthesized using water extract of different pteridophytes on Gram-positive and Gram-negative bacteria and also to evaluate how AgNP activity varied within the pathogens. As per the results, statistically, the mechanism of pathogen inhibition shown by AgNPs synthesized using *A. raddianum* is significantly different from those synthesized by *A. aureum* ($p=0.004$) and *C. dentata* ($p=0.002$) at $p<0.0005$. Statistically, antibacterial activity shown by AgNPs on *K. pneumoniae* is significantly different from *E. coli* and *Proteus* sp. at $p<0.0005$. While comparing the activity of AgNPs between *K. pneumoniae and S. aureus,* the effect was statistically significant at $p=0.012$. Whereas, no statistically significant difference was observed in the antibacterial activity of AgNPs between *K. pneumoniae* and *P. aeruginosa* ($p=0.864$).

Based on this data, aqueous extract of *A. raddianum* is an effective precursor for the eco-friendly synthesis of AgNPs, for evolving antimicrobial combination to suppress *P. aeruginosa* and *K. pneumoniae*. Based on the antibacterial activity results, AgNPs biosynthesized using aqueous extracts of *A. raddianum* were further characterized using HRTEM to study the morphology, size, and distribution.

HRTEM results of AgNPs biosynthesized using the aqueous extract of *A. raddianum* are shown in Fig. 2. This high-resolution image reveals the formation of spherical AgNPs. A uniform size distribution was observed and size ranged between 10 and 20 nm. HTREM images also reveal a uniform spacing between the particles and surface covered with thin layer of aqueous extract. Recently, highly monodispersed spherical AgNPs were synthesized using aqueous leaf extract of *Erythrina indica*. Spherical nature of these particles was attributed to the unique combination of biomolecules, which function as both reducing and stabilizing agent [20]. Considering this fact, the presence of hydrophilic biomolecules in *A. raddianum*, with polar and reducing functional group, maybe function as both reducing and capping agents to synthesize and stabilize spherical AgNPs.

DISCUSSION

Shape and size are major factors influencing the biostatic potential of AgNPs against pathogens. Depending on the shape and size of AgNPs, respiration rate and permeability of the cells are adversely affected [21]. Significant activity against Gram-negative bacteria has been shown by AgNPs with cationic nature [22]. Even though bacterial remnants and inactive bacteria could inhibit the antibacterial potential of AgNPs through bacteria-adsorbing effect, nature of the polymer used for the functionalizing has shown to prolong the process of inhibition effectively [23]. Recently, fucoidan synthesized AgNPs showcased this mode of persistent activity against Gram-negative bacteria [22]. Chitosan films coated with AgNPs and AgNPs functionalized with gelatin are other examples which inhibit pathogens through this mechanism of action [24,25]. Further, in Gram-negative bacteria, levels of adenosine triphosphate concentration are depleted by the destabilization of cell membrane and plasma membrane [26]. Relatively, moderate activity of AgNPs observed against Gram-positive bacteria, in the present study, may be attributed to the presence of polymer peptidoglycan on the cell membrane. These polymers suppress the antibacterial activity of AgNPs by blocking their entry across the cell membrane [27]. These observations in previously reported works and comparable results observed in the present study emphasize the importance of AgNPs biosynthesized using aqueous extract from *A. raddianum*, *A. aureum,* and *C. dentata* for developing novel treatment methods to contain pathogenic outbreak and infections.

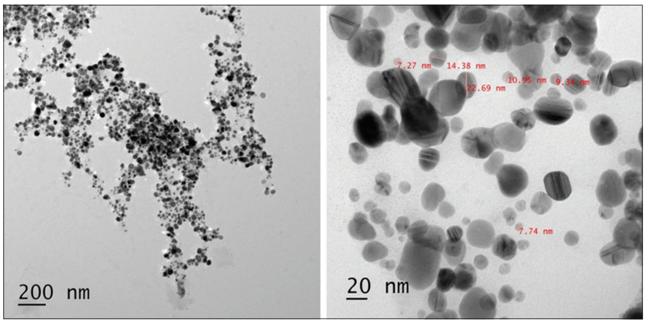

Fig. 2: High-resolution transmission electron microscopy images of silver nanoparticles biosynthesized by aqueous extract of *Adiantum raddianum*

CONCLUSION

Aqueous extract recovered from *A. raddianum*, *A. aureum,* and *C. dentate* is useful for the rapid synthesis of eco-friendly AgNPs. AgNPs biosynthesized using *A. raddianum* was very effective against *P. aeruginosa* and *K. pneumoniae*. Plasmonic absorbance data coupled with HRTEM images confirmed the formation of spherical AgNPs. It can be inferred that AgNPs functionalized with aqueous extract of *A. raddianum* are capable of extended activity, particularly, against Gram-negative bacteria. Results were statistically validated. Summing up, *A. raddianum* is a potential source of biomass and useful for the synthesis of AgNPs for developing novel eco-friendly biocidal formulations. Furthermore, more research is necessary to identify novel, eco-friendly, and cheap sources and synthetic methods to prepare antibacterial formulations to suppress the pathogen strains that are evolving.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest to declare.

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