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Biopotential of silver nanopeptides synthesized from protein extracts of *Padina*

Abstract

The present study was conducted to observe the bio-reduction of silver ions through the peptide solution of selected *Padina* species viz., *P. pavonica*, *P. tetrastomatica* and *P. gymnospora*. The aqueous silver ions exposed to the protein extracts and the silver nanopeptides synthesis was confirmed by the color change of protein extracts. These environmentally benign silver nanopeptides were further confirmed by using UV-Vis spectroscopy and powder X-ray diffraction analysis. The antibacterial activity of the synthesized nanopeptides against four selected pathogens viz., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus pyogenes* and *Morganella morganii* was carried out. The UV-Vis spectroscopic analysis confirmed the silver nanopeptides formation with the peak at 429 and 435nm. The protein extracts of *P. pavonica*, *P. tetrastomatica* and *P. gymnospora* mediated silver particles showed eight peaks with corresponding lattice planes which confirms the AgNP's and they are crystalline in nature. The results also show that silver nanopeptides have good antibacterial activity against different bacterial species in which the protein extract of *P. pavonica* and *P.* gymnospora mediated silver particles showed the maximum zone of inhibition against *M. morgonii* (17 mm). The protein extract of *P. tetrastomatica* mediated AgNP's showed the bactericidal activity against *M. morganii* (17 mm) and *S. pyogenes* (17 mm). The present study confirmed that the capping of proteins with silver nanoparticles capping is capable of rendering antibacterial efficacy and hence has a great potential in the preparation of therapeutic drugs used against bacterial diseases.

Keywords: *Padina*; Nanopeptides; Powder X-ray diffraction; AgNP's; Antibacterial efficacy

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Abbreviations: XPD, X-ray powder diffraction; PMF, proton motive force; PI, *P. pavonica*; PII, *P. tetrastomatica*; PIII, *P. gymnospora*

Introduction

Nanotechnology is used to design, depict, synthesize and to check the relevance of structures and devices in the nanometer range. In addition, nanotechnology helps to understand the molecular interactions of living cells. The medicinal and technological feature of nanomaterials was analyzed by Mondal et al.[1] Various metallic elements viz., silver, gold, zinc, copper, alginate, titanium and magnesium are extensively used to produce nanoparticles due to their unique physical properties, chemical reactivity and potential applications as catalysts, biosensors, biological markers, antibacterial and antiviral agents and as drug delivery agents.[2] Among the explored metallic nanoparticles, silver nanoparticles has proved to be most effective as it has good antimicrobial efficacy against bacteria, virus and eukaryotic microorganisms due to their large surface area.[3]

Marine algae act as a resource for the segregation of active principles with improved bio-potentials.[4] Indian seaweeds are of great food value and contain 16 to 30% protein on dry weight and have all essential amino acids which are not available in vegetable food materials. Peptides are found in all organisms such as vertebrates, invertebrates and plants. These peptides were isolated for molecular, biochemical as well as structural studies which includes nanosized materials.[5] Sahayaraj et al.,[6] and Jegadeeswaran et al.,[7] reported the extracellular production of bio-silver nanoparticles from *Padina pavonica* (Linn.) and *Padina tetrastomatica*. With this knowledge, the present investigation was conducted to study the bioefficacy of nanopeptides extracted from *Padina* species viz., *P. pavonica*, *P. tetrastomatica* and *P. gymnospora.*

Materials and methods

Collection of plant materials

The selected species of *Padina* viz., *Padina pavonica* (L.), *Padina tetrastomatica* Hauck and *Padina gymnospora* (Kutzing) Sonder were harvested from the natural habitat (Rasthacaud, Kanyakumari district, Tamil Nadu). The collected algae were washed carefully with tap water to wash away the surface contaminants and rinsed with sterile distilled water to eradicate dirt material.

Protein extraction

For the extraction of protein 500 mg of fresh young thallus of *P. pavonica*, *P. tetrastomatica*, *P. gymnospora* were washed in de-ionized water and ground using pre-chilled mortar and pestle with 0.1M Tris buffer (pH 7.0). The resultant slurry was centrifuged at 10,000 rpm for 10 min at 4°C in cooling centrifuge and the supernatant was stored at -70ºC before use.

Synthesis and characterization of silver nanopeptides

The $AgNO_3$ and protein extracts of selected seaweeds *P. pavonica*, *P. tetrastomatica* and *P. gymnospora* were selected for the production of silver nanoparticles. Precisely $AgNO_3$ (17 mg) was dissolved in distilled water (100 ml). Protein extract of Padina species was added to $AgNO_3$ solution in 1:10 ratio. After reduction the incubated solution was centrifuged at 10,000 rpm for 15 min.

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UV-Visible spectral analysis

The supernatant containing silver nanopeptides were spectroscopically observed for further confirmation. The synthesized silver nanoparticles were quantified by UV-Vis spectrophotometer at 300-600 nm and the characteristic peaks were detected.

Powder X-ray diffraction analysis

To know the structural characteristics of silver nanoparticles the powder method of diffraction was employed. The peaks of the X-ray diffraction pattern can be compared with the standard available data using Willars Hand book for the confirmation of the structure.

Antibacterial activity

Escherichia coli, Pseudomonas aeruginosa, Staphylococcus pyogenes and *Morganella morganii* were selected for the current investigation. Agar well diffusion method was carried out to observe the antibacterial activity.[8] Nanopeptides of selected seaweeds were poured into the wells at different concentrations viz. 0, 20µl, 40µl, 60µl, 80µl and 100µl. For the positive control of bacteria, silver nitrate solution was applied and for negative control, the distilled water was used. The zone of inhibition was measured (in mm) using a measuring scale.

Results

Synthesis of silver nanoparticles

When the protein extracts of *P. pavonica, P. tetrastomatica* and *P. gymnospora* are mixed with 1 mM $AgNO_3$ solution, the color of the solution changes from pale green to yellowish brown color indicating the synthesis of silver nanopeptides.

UV-Vis analysis

Silver nanoparticles synthesis was confirmed by recording the UV-Vis spectrum of the reaction media. The nanopeptides of *P. pavonica* showed the optical peak at 429nm with the absorption maxima of 2.219, *P. tetrastomatica* showed the optical peak at 429nm with the absorption maxima of 0.915 and *P. gymnospora* showed the optical peak at 435nm with the absorption maxima of 0.696 and the development of peaks specifies that the particles are polydispersed.

Bioefficacy studies on AgNP's extract of Padina species

The silver nanopeptides of *Padina* species showed the antibacterial activities against studied pathogens *E. coli, S. pyogenes, M. margonii* and *P. aeruginosa* with varied degree of zone inhibition (Table 1-3). The AgNP's was used as a negative control and the results were tabulated Table 1-3. The 100 µl silver nanopeptides of *P. pavonica* expressed the highest zone of inhibition against *M. morganii* (17 mm) and *E. coli* (15 mm). Next to that, 80 µl and 100 µl silver nanopeptides of *P. pavonica* demonstrated the inhibition against *S. pyogenes* with 16 mm. The silver nanopeptides of *P. pavonica* failed to show inhibitory activity against *P. aeruginosa* (Table 1).

The silver nanopeptides of *P. tetrastomatica* showed the bactericidal activity against *M. morgonii* (17 mm), *S. pyogenes* (17 mm) at the concentration of 80 µl and 100 µl and *E. coli* (15 mm) at the concentration of 100 µl of *P. tetrastomatica* nanopeptides. The nanopeptides of *P. tetrastomatica* failed to show inhibitory activity against *P. aeruginosa* (Table 2).

Table 1 Bioefficacy of AgNP's synthesized by protein extract of *P. pavonica*

Pathogens	Concentration of AgNP's synthesized by *P. pavonica* in µl (Zones of inhibition in mm)					AgNP's
	20	40	60	80	100	50
E. coli	-	-	14±0.3	14±0.3	15±0.3	4±0.25
S. pyogenes	-	14±0.3	15±0.3	16±0.2	16±0.2	5±0.3
M. morganii	-	14±0.3	15±0.3	16±0.34	17±0.3	5±0.2
P. aeruginosa	-	-	-	-	-	4±0.3

Note: The zone of inhibition excludes the well (6 mm)

Table 2 Bioefficacy of AgNP's synthesized by protein extract of *P. tetrastomatica*

Pathogens	Concentration of AgNP's synthesized by *P. tetrastomatica* in µl (Zones of inhibition in mm)					AgNP's
	20	40	60	80	100	50
E. coli	-	-	14±0.3	14±0.3	15±0.3	4±0.25
S. pyogenes	-	14±0.3	15±0.3	15±0.3	17±0.2	4±0.3
M. morganii	-	14±0.3	15±0.3	17±0.36	17±0.2	5±0.34
P. aeruginosa	-	-	-	-	-	4±0.3

Note: The zone of inhibition excludes the well (6 mm)

Table 3 Bioefficacy of AgNP's synthesized by protein extract of *P. gymnospora*

Pathogens	Concentration of AgNP's synthesized by *P. gymnospora* in µl (Zones of inhibition in mm)					AgNP's
	20	40	60	80	100	50
E. coli	-	-	-	-	-	4±0.25
S. pyogenes	-	-	14±0.3	15±0.3	16±0.2	3±0.3
M. morganii	-	14±0.3	15±0.3	17±0.36	17±0.3	4±0.2
P. aeruginosa	-	12±0.4	14±0.3	16±0.26	-	3±0.3

Note: The zone of inhibition excludes the well (6 mm)

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The nanopeptides of *P. gymnospora* showed the maximum bactericidal activity against *M. morgonii* (17 mm), *S. pyogenes* (16 mm) and *P. aeruginosa* at the concentration of 80 µl and 100 µl of the nanopeptides sample. The nanopeptides of *P. gymnospora* failed to show inhibitory activity against *E. coli* (Table 4). The negative control of AgNP's expressed the maximum zone of inhibition (7 mm) against *M. morganii.*

Table 4 XRD analysis of selected *Padina* species

2- Theta°	Intensity count	Intensity (%)	PI	PII	PIII
27.82	768	45.7	+	+	+
32.24	1681	100	+	+	+
40.55	257	15.3	-	+	-
46.22	833	49.6	+	-	+
54.78	275	16.4	-	+	+
57.44	272	16.2	+	+	+
66.46	94.7	5.6	-	-	+
67.41	187	11.1	+	+	-
74.39	114	6.8	+	+	+
76.67	256	15.2	+	+	+

Note: PI, *P. pavonica*; PII, *P. tetrastomatica*; PIII, *P. gymnospora*

XRD analysis

The XRD pattern of *P. pavonica, P. tetrastomatica* and *P. gymnospora* nanopeptides are illustrated in Figure 1-3. The synthesized silver nanopeptides of *P. pavonica* showed eight peaks at 27.82º, 32.24º, 46.22º, 54.78º, 57.44º, 67.41º, 74.39º and 76.67º due to reflections from 768, 1681, 833, 275, 272, 187, 114 and 256 planes of silver respectively (Table 4, Figure 1). The silver nanopeptides of *P. tetrastomatica* displayed eight peaks at 27.82º, 32.24º, 40.55º, 54.78º, 57.44º, 67.41º, 74.39º and 76.67º due to reflections from 768, 1681, 257, 275, 272, 187, 114 and 256 planes of silver respectively (Table 4, Figure 2). The silver nanopeptides of *P. gymnospora* demonstrated eight peaks at 27.82º, 32.24º, 46.22º, 54.78º, 57.44º, 66.46º, 74.39º and 76.67º due to reflections from 768, 1681, 833, 275, 272, 94.7, 114 and 256 planes of silver respectively (Table 4, Figure 3). A comparison of the XRD pattern with standard confirmed that silver particles are formed in the XRD analysis.

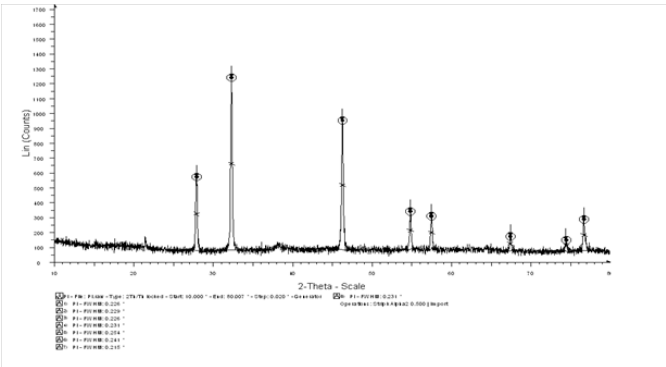

Figure 1 XRD analysis of AgNP's synthesized by protein extract of *P. pavonica.*

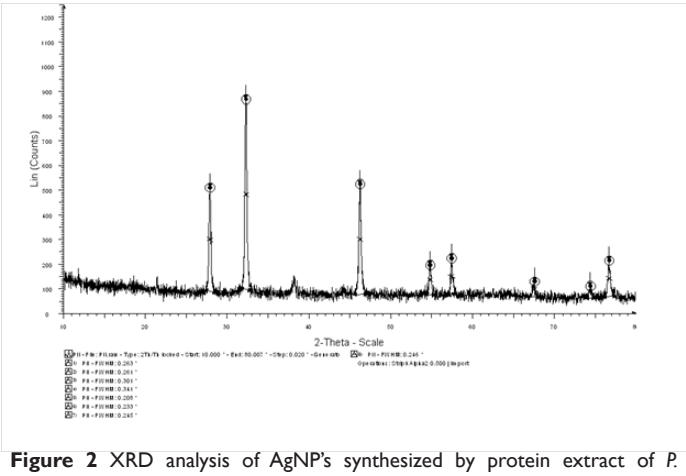

Figure 2 XRD analysis of AgNP's synthesized by protein extract of *P. tetrastomatica.*

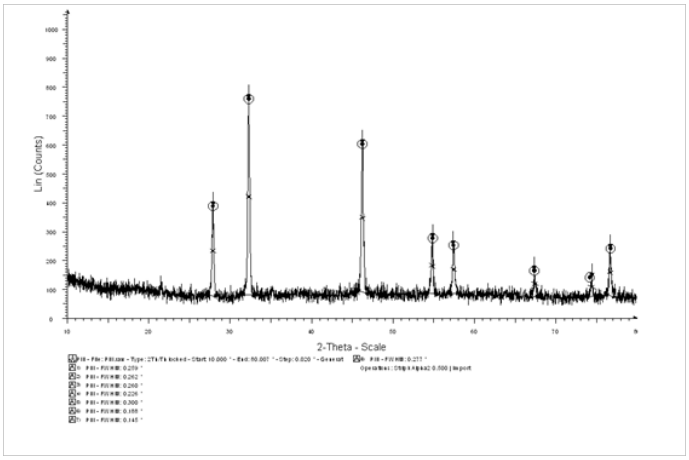

Figure 3 XRD analysis of AgNP's synthesized by protein extract of *P. gymnospora.*

Discussion

The application of nanotechnology has increased in the twenty-first century. Nanotechnology has the ability to manipulate the hardness properties of assemblies that are at the nanosize scale of various biomolecules. Synthesis of nanoparticles can be attained by physical, chemical and biological methods.[9] The study of nanoparticles biosynthesis offers an important to nanobiotechnology. *Padina* species proved to be vital for the extra-cellular biosynthesis of AgNP's. Biosynthesis of silver nanoparticles exhibits a brown color in protein solution which indicates the acceleration of surface plasmon vibrations in metal nanopeptides.[10] The synthesis of silver nanopeptides of *Padina* species were displayed the UV-Vis spectroscopic peak at 429 nm which is a distinct peak of silver nanoparticles.[11] The spectrum of nanopeptides develop into bell shaped curve indicates the presence of polydispersed materials.[12] In the present study, the protein extracts of *P. pavonica* and *P. tetrastomatica* mediated silver particles showed the optical peaks at 429 nm, *P. gymnospora* showed the optical peak at 435 nm and thus the extension of peaks showed that the particles are polydispersed. The XRD analysis explained the crystalline nature of silver.[7] The protein extracts of *P. pavonica, P. tetrastomatica* and *P. gymnospora* mediated silver particles showed eight peaks with corresponding lattice planes confirms the crystalline nature of AgNP's.

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Nanoparticles exhibit various physiochemical properties with same composition results in different toxicity mechanisms to the natural systems. The main goal of silver nanoparticles is to combat the microbial action on external and internal cell surface of the cell. Earlier studies denoted the attachment of AgNP's on cell surface, dissipate the ATP pool and Proton Motive Force (PMF) and finally results in cell death.[13] Silver ions reacts with thiol (-SH) groups and generate Reactive Oxygen Species and hence neutralizes cellular enzymes and DNA.[14] Because of such advantages, silver nanoparticles can be employed as a antibacterial agent against *E. coli, S. pyogenes*, *M. morganii* and *P. aeruginosa* and there is an elevated scope to generate a new anti-bacterial agent. Silver reduces the respiration process of bacteria by binding with bacterial cell wall and cell membrane.[9] Sahayaraj et al.,[6] synthesized the AgNP's from the aqueous extracts of *P. pavonica*. Both the algal extract and silver-based nanoparticles of *P. pavonica* were tested against three important pathogens. Bio-silver nanoparticles show the highest zone of inhibition against *F. oxysporum* (12.33±0.33). In the present study, the nanopeptides of *P. pavonica* and *P. tetrastomatica* explored an effective antibacterial activity (17 mm) against *M. morganii* and *S. pyogenes*. The nanopeptides of *P. pavonica* and *P. tetrastomatica* failed to show activity against *P. aeruginosa.* The AgNP's developed by *P. gymnospora* possessed antibacterial activity against *S. pyogenes*, *M. morganii* and *P. aeruginosa* but failed to show activity against *E. coli*.

Conclusion

In this study, bioreduction of silver ions using protein extracts of *P. pavonica, P. tetrastomatica* and *P. gymnospora* have been successfully carried out. The study showed that the selected three seaweeds are good resources to develop silver nanoparticles at faster rate. Analytical techniques, such as UV-Vis spectroscopy and X-ray powder diffraction (XPD) were applied to characterize the nanoparticles morphology. In addition, the results presented good antibacterial activity besides different bacterial species and thus concluded that capping of silver nanoparticles with proteins shows antibacterial efficacy. The green synthesis of silver nanopeptides improves the economic viability. Exploitation of synthesized capped nanopeptides would enable us to know the nature of capping agent and utilize them for medicinal and biomedical applications.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

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