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ORIGINAL ARTICLE

Antibacterial Activity of Silver Nanoparticles Synthesized from a Few Medicinal Ferns

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ABSTRACT
Biologically synthesized silver nanoparticles are being widely using in the field of medicine. In disease management practices, usage of metal nanoparticles which are synthesized from plants is a global emerging trend. Hence in the present study the silver nanoparticles were synthesized from three medicinal ferns Pteris argyreae, Pteris confusa and Pteris biaurita and their antibacterial activity was tested against five human pathogenic bacteria such as Shigella boydii, Staphylococcus aureus, Klebsiella vulgaris, Shigella dysentriae and Salmonella typhi. The silver nanoparticles exhibited potential bactericidal effects against human pathogenic bacteria. Among the five bacteria K. vulgaris was highly susceptible to the silver nanoparticles. The highest inhibition zone (20 mm) was observed in silver nanoparticles from P. biaurita extract against K. vulgaris. To the best of our knowledge this is the first report on the antibacterial activity of silver nanoparticles synthesized from the ferns.
Key wards: *silver nanoparticles, Pteris argyreae, Pteris confusa and Pteris biaurita*

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INTRODUCTION

Infectious disease still remains an important cause of morbidity and mortality in man, especially in developing countries. The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains. The development of multiple antibiotic resistance organisms has constituted a global problem as far as treatment of some infectious diseases is concerned [1]. Pteridophytes are not infected by microbial pathogens, which may be one of the important factors for the evolutionary success of pteridophytes and the fact that they survived for more than 350 million years. Considering the rich diversity of Indian medicinal plants including Pteridophytes, it is expected that, the screening of plant extract for antibacterial activity may be beneficial for humans and plants diseases. Traditionally people used pteridophytes as medicine and anti bacterial agents [2].

Nanotechnology is expected to be the basis of many main technological innovations in the 21st century. Research and development in this field is growing rapidly throughout the world. The use of nanoparticles is gaining impetus in the present century as they posses defined chemical, optical and mechanical properties. Among them, the metallic nanoparticles are most promising as they contain remarkable antibacterial properties due to their large surface area to volume ratio, which is of interest to researchers due to the growing microbial resistance against metal ions, antibiotics, and the development of resistant strains [3,4]. Traditionally most of the metal and metal oxide nanoparticles were routinely synthesized by various physical and chemical methods. Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. Among the various biosynthetic approaches, the use of plant extracts has advantages such availability, safe to handle and broad viability of metabolites [5]. Hence the present was carried out to synthesis silver nanoparticles from three medicinal ferns *Pteris argyreae*, *Pteris confusa* and *Pteris biaurita* and to explore their bactericidal activities against human pathogenic bacteria *Shigella boydii, Staphylococcus aureus, Klebsiella vulgaris, Shigella dysentriae* and *Salmonella typhi.*

MATERIALS AND METHODS

Collection of plant materials

Healthy, disease free fronds (leaves) of the medicinal ferns *P. argyreae*, *P. confusa* and *P. biaurita* were randomly collected from southern Western Ghats and their identification was confirmed with the help of herbarium specimens in XCH (Xavier's College Herbarium), St. Xavier's college, Palayamkottai.

Boiling of the plant materials

The collected fern leaves were thoroughly washed under running tap water. The cleaned leaves were dried with water absorbent paper. Then they were cut into small pieces. The pieces of leaves were dispensed in 100ml of sterile distilled water and boiled for one hour at 80ºC. After that the leaf extracts were collected in separate conical flasks by standard filtration method.

Synthesis of Silver nanoparticles

10^{-3} M Silver nitrate solution was prepared and stored in brown bottles. 5ml of leaf extract was taken in BOD bottle separately and to this 100ml of $AgNo_3$ solution was added. The colour change of the leaf extracts from pale green to dark brown was checked periodically. Then the BOD bottles were incubated at room temperature for further incubation till 28 hours. The colour change to brown indicated that the silver nanoparticles were synthesized from the leaves and centrifuged at 10000 rpm for 25 minutes where pellets used for antibacterial activity [6].

Bacterial strains

The microorganisms *S. boydii, S. aureus, K. vulgaris, S. dysentriae* and *S. typhi* were procured from Tirunelveli Medical College, Palayamkottai and were maintained at 4 ˚C on nutrient agar slants.

Antibacterial activity

The antibacterial activity of the isolated silver fern nanoparticles pellets was tested by disc diffusion method [7]. Muller Hinton agar medium was seeded with 100µl of each inoculum (1×10^8 CFU/ml). The impregnated discs containing the pellets (100µg/disc) were placed on the agar medium seeded with tested microorganisms. Blank discs (impregnated with $AgNo_3$) were used as negative control. The plates were then incubated at 37 ˚C for 24 h to allow maximum growth of the microorganisms. The antibacterial activity of the test samples was determined by measuring the diameter of zone of inhibition expressed in millimeter. Experiments were done in triplicate and the results were recorded.

Statistical analysis

All data were expressed as mean ± SD. Statistical analyses were evaluated by one-way ANOVA followed by Tukey HSD test. Values with $P< 0.05$ were considered statistically significant. Mean and standard deviation were also calculated using the Microsoft Excel sheet, Office Edition 2007.

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles from plants

In this test the metal – plant extracts interaction was confirmed. It was found that silver ions when exposed to herbal extracts were reduced in solution, thereby leading to the formation of silver hydrosol. The ferns extracts were pale green in colour before addition of Ag+ ions and this changed to reddish brown colour suggested the formation of silver nanoparticles. The bottles were observed periodically for change in colour from pale green to different shades of brown (Table 1).

Antibacterial activity

The antibacterial activities of synthesized fern based silver nanoparticles were tabulated (Table 2). The inhibition rate of silver nanoparticles of *P. argyreae*, *P. confusa* and *P. biaurita* extracts against *S. boydii* was 10 mm, 9 mm and 12 mm respectively. The inhibition rate against *S. aureus* showed was 13 mm in all the three ferns silver nanoparticles. The inhibition rate of silver nanoparticles of *P. argyreae*, *P. confusa* and *P. biaurita* extracts was 15 mm, 17 mm and 20 mm against *K. vulgaris* respectively. The inhibition rate against *S. dysentriae* and *S. typhi* showed was 11 mm and 14 mm respectively in all the three ferns silver nanoparticles.

The ANOVA analysis of the data revealed that *P. biaurita* ($p<0.05$) showed highly significant activity against the tested pathogens (Table 2). Tukey HSD analysis of the data revealed that *K. vulgaris* was highly susceptible.

The search for antimicrobial agent has continued to be concentrated on lower plants, fungi and bacteria. In this study ferns are used as the source of silver nanoparticles synthesis. The ferns and their medicinal properties were already discussed in varieties of Ayurvedic studies. Different solvent extracts of ferns exhibited efficient antibacterial activity [8]. But there is no record for bioactivity of fern nanoparticles. Recently nanoparticles synthesis were achieved with bacteria, fungi, actinomycetes [9,10] and use of plant extract such as neem, *Camellia sinensis*, coriandrum, nelumbo lucifera, ocimum sanctum and several others which is compatible with the green chemistry principles [11,12,13]. The main phytochemicals responsible for the synthesis of nanoparticles are terpenoids, flavones, ketones, aldehydes amides etc. In the present study fern silver nanoparticles showed high antibacterial activity against the selected human

pathogenic bacteria. It may due to the presence of various phytochemicals (secondary metabolites) in the tested ferns.

Table 1: Confirmation tests for silver nanoparticles in ferns extracts

Time	*P. argyreae*	*P. confusa*	*P. biaurita*
0 min	Green	Pale green	Pale green
10 min	Light yellow	Yellow	Yellow
30 min	Yellow	Yellow	Yellow
1 hr	Yellow	Dark yellow	Dark yellow
2 hrs	Orange	Orange	Orange
3 hrs	Dark orange	Dark orange	Dark orange
4 hrs	Dark orange	Reddish orange	Reddish orange
8 hrs	Reddish orange	Reddish orange	Reddish orange
16 hrs	Brown	Brown	Brown
24 hrs	Dark brown	Dark brown	Dark brown
28 hrs	Reddish brown	Reddish brown	Reddish brown

Table 2: Antimicrobial activity of three fern silver nanoparticles

Ferns	Inhibition Zone in mm				
	S. boydii	***S. aureus***	***K. vulgaris***	***S. dysentriae***	***S. typhi***
P. argyreae	10.25±0.51	13.35±0.70	15.30±0.25	11.15±0.90	14.60±0.35
P. confusa	09.33±0.15	13.45±0.65	17.10±0.55	11.85±0.80	14.90±0.20
P. biaurita	12.05±0.09	13.20±0.10	20.75±0.20	11.25±0.40	14.75±0.85
Blank disc ($AgNo_3$)	-	-	-	-	-

CONCLUSIONS

Hence the present study clearly indicates that the silver nanoparticles which derived from medicinal fern *P. biaurita* are successfully controlled the selected human pathogens compared with other two ferns silver nanoparticles. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antibacterial agents of natural origin for the treatment of bacterial infections in human beings after proper *in vivo* studies and clinical trials.

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