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Research Article

BIOSYNTHESIS OF SILVER NANO PARTICLES AND ITS ANTIBACTERIAL ACTIVITY AGAINST HUMAN PATHOGENS

***JOHN DE BRITTO A AND STEENA ROSHAN SEBASTIAN**

Plant Molecular Biology Research Unit, St.Xavier's College, (Autonomous), Palayamkottai, Tamilnadu, India.
Email: bjohndesxc@gmail.com

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ABSTRACT

Research on nanoparticles is currently an area of intense scientific research, due to a wide variety of potential applications in biomedical, optical, and electronic fields. Synthesis of nanoparticles can be carried out by using various chemical and physical methods. But use of such methods is harmful in one or the other way as the chemicals often used are toxic, flammable, not easily disposable due to environmental issues, having low production rate, etc. As a result, a great deal of effort has been put into the search for methods utilizing biological systems, such as the micro-organisms and plants, for the synthesis of metal nanoparticles. Such biologically synthesized silver nanoparticles from Apocynaceae members were evaluated against the human pathogens. The plant based silver nano particles synthesized from *Rauvolfia tetraphylla* was found to be most active against *Shigella dysenteriae* with the highest zone of inhibition.

Keywords: Apocynaceae, Silver Nanoparticles, Antibacterial activity, Human Pathogens.

INTRODUCTION

Nanotechnology can be defined as a research for the design, synthesis, and manipulation of structure of particles with dimension smaller than 100 nm. Nanobiotechnology combines biological principles with physical and chemical procedures to generate nano-sized particles with specific functions. Nanobiotechnology is emerging as the cutting-edge technology, interdisciplinary with physics, chemistry, biology, material science and medicine [1].

Chemical synthesis methods are available for the synthesis of metal nanoparticles, many of the reactants and starting materials used in these methods are toxic and potentially hazardous in concern with biological applications [2]. But soon, an array of biological synthesis protocols leading to the formation of nanostructures has been reported using bacteria [3,4], fungi [5,6] and plants [7,8]. In this context it is noteworthy to mention that synthesis of inorganic nanoparticles by biological systems makes nanoparticles more biocompatible and environmentally benign. Since prehistoric times, among all inorganic antimicrobial agents, silver has been extensively used to resist infections. As silver salts, having an antimicrobial effect [9], are used in a variety of applications including dental work, catheters and burn wounds [10]. Hence synthesis of silver nanoparticles has gained great desire nowadays.

The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources. Until natural products have been approved as new antibacterial drugs, there is an urgent need to identify novel substances active towards highly resistant pathogens [11,12]. The practice of herbal medicine dates back to the very earliest period of known human history. There is evidence of herbs having been used in the treatment of diseases and for revitalizing body system in almost all ancient civilizations, the Egyptian, the Chinese and even Greek and Roman civilizations [13]. Majority of herbal plants are safe and economical. Generally plant extracts have no problem of drug resistance. The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobials [14].

The members of Apocynaceae are well known for their medicinal properties and hence five members of this family were selected for synthesizing plant based silver nanoparticles and their antibacterial activity were evaluated against the human pathogens.

MATERIALS AND METHODS

Collection of plant materials

Fresh leaves of *Cathranthus roseus* L., *Rauvolfia tetraphylla* L., *Ervatamia divaricata* L., *Nerium indicum* Miller and *Thevetia peruviana* (Pers.) K. Schum. were collected randomly from the region of Tirunelveli, Tamilnadu.

Synthesis of Silver nano particles

a) Boiling of the plant materials

The collected plant leaves were thoroughly washed and dried with water absorbent paper and cut into small pieces. The pieces of leaves were dispensed in 100ml of sterile distilled water and boiled for an hour at 80ºC. This extracts were collected in separate conical flasks by standard filtration method.

b) Preparation of Silver nanoparticles

100 ml of 10^{-3} M Silver nitrate solution was added to 5ml of each leaf extract taken in BOD bottle separately. The color change of the leaf extracts was checked periodically. Then the BOD bottles were incubated at room temperature for 28 hrs in dark and it was centrifuged at 10000 rpm for 25 minutes. The obtained pellets were used for antibacterial activity [15].

Determination of antibacterial activity

a) Microorganisms

The microorganisms used to examine the antibacterial activity were, *Shigella boydii, Shigella dysenteriae, Klebsiella vulgaris, Staphylococcus aureus* and *Salmonella typhi* obtained from Microbial Type Culture Collection (MTCC), Chandigarh. The bacterial strain was cultured in nutrient broth at 37°C and maintained on nutrient agar (HiMedia) slant at 4°C.

b) Inoculum

The microorganism was inoculated into nutrient broth and incubated at 35 ± 2 °C for 4 h. The turbidity of the resulting suspensions was diluted with nutrient broth to obtain a transmittance of 25.0 at 580 nm. That percentage was found spectrophotometrically comparable to 0.5 McFarland turbidity standards. This level of turbidity is equivalent to approximately 3.0 × 10^8 cfu/ml. The UV1100 spectrophotometer was used to adjust the transmittance of the working suspensions.

c) Antibacterial activity

The antibacterial activity of the isolated plant based silver nanoparticles pellets were tested by disc diffusion method [16]. Mueller 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 and Ciprofloxacin (5 mcg / disc) was used as positive control. The plates were then incubated at 37ºC 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. The entire test was performed in triplicate.

d) Determination of % of Relative Inhibition Zone Diameter

The antimicrobial activity was calculated by applying the expression: % RIZD = (IZD sample-IZD negative control)/IZD antibiotic standard×100, where RIZD is the relative inhibition zone diameter and IZD is the inhibition zone diameter (mm) [17].

RESULTS AND DISCUSSION

Confirmation of metal-plant extracts interaction

It was found that aqueous silver ions when exposed to herbal extracts were reduced in solution, thereby leading to the formation of silver hydrosol. The plant extracts were pale green in color before addition of Ag^+ ions and this changed to brownish color suggested the formation of silver nanoparticles. The bottles were observed periodically for change in color from green to different shades of brown (Table 1). The time duration of change in colour varies from plant to plant. The green coloured solution changed into yellow colour within 1 hour. Yellow coloured solution changed into orange colour within 8 hours. Finally orange coloured solution changed into brown colour within 28 hours. The brown coloured solution indicated the formation of silver nanoparticles.

Table 1: Periodical colour change of plant extracts with Silver nitrate

Time	*Catharanthus roseus*	*Rauvolfia tetraphylla*	*Ervatamia divaricata*	*Nerium indicum*	*Thevetia peruviana*
0 min	Green	Green	Green	Green	Green
10 min	Light yellow	Light green	Light green	Light green	Light green
30 min	Yellow	Light yellow	Light yellow	Light yellow	Light yellow
1 hr	Dark yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
2 hr	Orange	Pale orange	Light orange	Pale orange	Light orange
3 hr	Dark orange	Orange	Orange	Orange	Orange
4 hr	Reddish orange	Dark orange	Dark orange	Dark orange	Dark orange
8 hr	Reddish orange	Dark orange	Dark orange	Dark orange	Dark orange
16 hr	Brown	Pale brown	Pale brown	Pale brown	Pale brown
24 hr	Dark brown	Light brown	Light brown	Light brown	Light brown
28 hr	Reddish brown	Dark brown	Dark brown	Dark brown	Dark brown

It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [18]. As the extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from watery to yellowish brown due to reduction of silver ion which indicated formation of silver nanoparticles. It is generally recognized that UV-Vis spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspensions [19]. In the process of dissociation of silver nitrate, it appears that a reductase enzyme (nitrate reductase) is responsible for the reduction of Ag+ ions and the subsequent formation of metallic silver nanoparticles.

Antibacterial activity of plant based Silver Nanoparticles (SNPs)

The plant based silver nanoparticles showed efficient antibacterial activity towards the selected human pathogens. The silver nanoparticles of all the plants had good antibacterial activity towards all the selected human pathogens. The silver nanoparticles of *Rauvolfia tetraphylla* showed highest activity towards *Shigella dysenteriae* with an inhibition zone of 36.6 mm. It also showed activity against *Shigella boydii* with an inhibition zone of 32.2 mm. The highest zone of inhibition showed by silver nanoparticles of *Nerium indicum* was 30.1 mm against *Shigella boydii.*

The silver nanoparticles of *Ervatamia divaricata* showed activity towards all the pathogens, of that 28.0 mm was found to be efficient zone of inhibition against *Shigella dysenteriae*. The silver nanoparticles of all the plants were active against *Klebsiella vulgaris*, in which the highest zone of inhibition (23.7 mm) was found by the silver nanoparticles of *Nerium indicum*. The least zone of inhibition was 14.3 mm by the silver nanoparticles of *Thevetia peruviana* against *Staphylococcus aureus* (Table 2).

Table 2: Antibacterial activity of plant based silver nanoparticles

S. No.	Plants	Inhibition Zone Diameter (mm)				
		B_1	B_2	B_3	B_4	B_5
1.	*Catharanthus roseus*	20.3±0.1	16.5±0.3	18.1±0.1	16.0±0.1	15.2±0.3
2.	*Rauvolfia tetraphylla*	32.2±0.4	36.6±0.2	22.3±0.3	22.7±0.2	18.0±0.3
3.	*Ervatamia divaricata*	26.0±0.1	28.0±0.1	14.0±0.1	18.4±0.2	16.2±0.1
4.	*Nerium indicum*	30.1±0.2	16.1±0.5	23.7±0.2	27.0±0.1	18.4±0.1
5.	*Thevetia peruviana*	16.3±0.5	20.0±0.1	18.5±0.3	14.3±0.2	25.2±0.1

B_1 - *Shigella boydii*; B_2 - *Shigella dysenteriae*; B_3 - *Klebsiella vulgaris*; B_4 - *Staphylococcus aureus*; B_5 - *Salmonella typhii*

Table 3: Relative Inhibition Zone Diameter (%) of plant based silver nanoparticles

S. No.	Plants	Relative Inhibition Zone Diameter (%)				
		B_1	B_2	B_3	B_4	B_5
1.	*Catharanthus roseus*	120	133.3	112.5	133.3	163.6
2.	*Rauvolfia tetraphylla*	133.3	283.6	137.5	146.6	400.0
3.	*Ervatamia divaricata*	93.3	116.6	175.0	106.6	163.6
4.	*Nerium indicum*	118.1	133.3	120.8	300.0	218.1
5.	*Thevetia peruviana*	106.6	166.6	112.5	93.3	243.1

B_1 - *Shigella boydii*; B_2 - *Shigella dysenteriae*; B_3 - *Klebsiella vulgaris*; B_4 - *Staphylococcus aureus*; B_5 - *Salmonella typhii*

Relative Inhibition Zone Diameter (%)

The results of antibacterial activity by the plant based silver nanoparticles of the selected plants against the human pathogens were compared with the positive and negative controls and given in the form of Relative Inhibition Zone of Diameter (%) in the table 3.

Silver has been known to exhibit strong toxicity to wide range of microorganisms (antibacterial applications). It was shown that the antibacterial activity of silver nanoparticles was size dependent. The bactericidal effect of silver and silver nanoparticles can be attributed to the attachment of silver nanoparticles to the surface of the cell membrane disturbing permeability and respiration functions of the cell [20]. It is also stated that silver nanoparticles not only interact with the surface of membrane, but can also penetrate inside the bacteria [21]. Smaller silver nanoparticles having the large surface area available for interaction would give more bactericidal effect than the larger silver nanoparticles [20]. Additionally reports suggest that ionic silver strongly interacts with thiol group of vital enzymes and inactivates them [22,23,24]. Experimental evidence also proposes that DNA may lose its replication ability once the bacteria have been treated with silver ions [25].

The antibacterial activity of plant based silver nanoparticles of *Ocimum sanctum* and *Vitex negundo* were tested against *Staphylococcus aureus, Vibrio cholerae, Proteus vulgaris* and *Pseudomonas aeruginosa*, for which significant results were observed [26]. Antibacterial activity of silver nanoparticles against *Staphyloccocus aureus, Pseudomonas aeruginosa* and *Escherichia coli* has been investigated [27]. The antibacterial properties of the biosynthesized silver nanoparticles when incorporated on textile fabric were investigated [28]. Silver impregnated medical devices like surgical masks and implantable devices showed significant antimicrobial efficiency [29]. The current investigation suggests that, use of silver ion or metallic silver as well as silver nanoparticles can be exploited in medicine for burn treatment, dental materials, coating stainless steel materials, textile fabrics, water treatment, sunscreen lotions, etc. [30]. Antibacterial activity of cotton fabric coated silver nanoparticles showed distinct bactericidal effect against *Staphylococcus aureus* and *E.coli* with all the tested concentration [31].

Seven Apocynaceae members were studied for their antibacterial activity against ten pathogens, of which *Plumeria alba* showed efficient antibacterial activity and *Rauvolfia tetraphylla* showed moderate activity against most of the pathogens [32]. But in this study the plant based Silver nanoparticles synthesized from *Rauvolfia tetraphylla* was active against the pathogens studied. Hence the plant based Silver nanoparticles are found to be more efficient than the plant extracts that have been used since time immortal.

CONCLUSION

The silver nanoparticles synthesized and investigated in this study establish a stronger antibacterial potency which was efficient against most of the human pathogens studied. The green chemistry approach addressed in the present work on the synthesis of silver nanoparticles is simple, cost effective and the resultant nanoparticles are highly stable and reproducible. This approach can be further capitalized to rapidly screen plants used in traditional medicines for ailments resulting from microorganism as well as in the extraction of potential molecules that could be used in future therapeutics.

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