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Research Article

GREEN SYNTHESIS OF SILVER NANO PARTICLES AND THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

Biocontrol of pathogens is an important and useful way for avoiding the side effects caused by synthetic antibiotics and chemicals and controlling the emergence of antibiotic resistant pathogens. Hence the present study was focused to control the five harmful human pathogens *Shigella boydii, Staphylococcus aureus, Klebsiella vulgaris, Shigella dysentriae Salmonella typhi* in bio control methods using five common medicinal plants *Cassia auriculata, Cassia alata, Cassia occidentalis, Cassia roxburghii* and *Caesalpinia pulcherrima* by screening their antibacterial activity, synthesizing the silver nano particles and its antimicrobial activity. The synthesis of plant based silver nano particles were confirmed by the colour change from pale green and dark orange. Among the five plants *C. auriculata* extract nanoparticles showed maximum inhibitory effect compared with other four plants based nano particles. The antibacterial activity exhibited by the silver nano particles of all the selected medicinal plants was very high compared to the inhibition of positive control ciprofloxacin. In plant silver nano particles all the inhibition zones were observed in between 14 mm – 22 mm.

Keywords: Green synthesis, Silver Nano Particles, human pathogens and antibacterial activity

INTRODUCTION

Diseases that remain most challenging in today's healthcare system tend to be complex for involving multiple mechanisms, targets and drugs for effective disease management. In contrast to current combination therapies, however, plant based drugs contain a mixture of multiple components thereby saving considerable time and expense[1]. Infectious diseases are leading cause of death world-wide. Bacterial diseases occur when pathogenic bacteria get into the body and begin to reproduce and crowd out healthy bacteria, or to grow in tissues that are normally sterile. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases[2]. Although synthetic and semi synthetic antimicrobial drugs abound in various markets today, there is need for continuous search for new ones to cope with the increased evolution of multiple antimicrobial resistant strains of organisms[3]. Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value[4].

Nanotechnology is the application of science and technology to control matter at the molecular level. At the nanoscale level, the properties of matter are significantly different from their macroscopic bulk properties. Nanotechnology is also referred to the ability for designing, characterization, production and application of structures, devices and systems by controlling shape and size at the nanometer scale[5]. Nanoparticles are viewed as the fundamental building blocks of nanotechnology[6]. They are the starting points for preparing many nano structured materials and devices. Their synthesis is an important component of the rapidly growing research efforts in nanoscience and nanoengineering. Due to the emergence of antibiotic resistant pathogens, there is a pressing need to search for new antimicrobial agents from natural and inorganic substances[7]. Among inorganic antimicrobial agents, silver has been employed most widely since ancient times to fight infections. Silver nano particles act as an antimicrobial, anti-bacterial, anti-viral, anti-biotic and anti-fungal agent when incorporated in coatings, nano fiber, first aid bandages, plastics soap and textiles, in self cleaning fabrics and as conductive filler. Hence the present study was focused to

synthesize the Silver nano particles from five medicinal plants like *Cassia auriculata* L., *Cassia alata* L., *Cassia occidentalis* L., *Cassia roxburghii* DC., *Caesalpinia pulcherrima* (L.) Sw and screen their antibacterial activity against five human pathogens *Shigella boydii, Staphylococcus aureus, Klebsiella vulgaris, Shigella dysentriae Salmonella typhi.*

MATERIALS AND METHODS

Collection of plant materials

Fresh plant/ plant parts were collected randomly from the region of Tirunelveli, India and their identification was confirmed with the help of herbarium specimens in XCH (Xavier's College Herbarium), St. Xavier's college, Palayamkottai.

SYNTHESIS OF SILVER NANO PARTICLES

Boiling of the plant materials

The collected plant 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 each leaf extract was taken in BOD bottle separately and to this 100ml of $AgNo_3$ solution was added. The same protocol was followed for other four leaf extracts. The color 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 color change to brown indicated that the silver nano particles were synthesized from the leaves and centrifuged at 10000rpm for 25 minutes where pellets used for antibacterial activity[8,9].

Bacterial strain for antibacterial activity of silver nano particles

The microorganisms used to examine the antibacterial activity were, *Shigella boydii Staphylococcus aureus Klebsiella vulgaris Shigella dysentriae, Salmonella typhi.* The microorganisms were procured from Tirunelveli Medical College, Palayamkottai and were maintained at 4 ^{0}C on nutrient agar slants.

Antibacterial activity

The antibacterial activity of the isolated plant-silver based nanoparticles pellets was tested by disc diffusion method [10]. 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 ^{0}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 repeated twice and mean of the three experiments was recorded.

Relative Percentage Inhibition (RPI)

The relative percentage inhibition of the test extracts with respects to positive control (Ciprofloxacin) was calculated by using the following formula [11, 12].

$$\text{Relative percentage inhibition of the test extract} = \frac{100\ X\ (x-\ y)}{(z-y)}$$

Where,

x: total area of inhibition of the test extract
y: total area of inhibition of the solvent
z: total area of inhibition of the standard drug

The total area of inhibition was calculated by using area = $\pi r2$; where, r = radius of zone of inhibition.

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 nano particles from plants

In this test the metal – plant extracts interaction was confirmed. 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 duration of change in colour varies from plant to plant where *Cassia auriculata* obtained colour change within 10 minutes where as the other four plants obtained colour within 30 minutes to change. The green coloured solution was changed into yellow colour within 1 hour. Yellow coloured solution was changed into orange colour within 8 hours. Finally orange coloured solution was changed into brown colour within 28 hours. The brown coloured solution indicated the presence of silver nano particles.

Antibacterial activity of plant silver nano particles

To control the harmful pathogens *S. boydii, S. aureus, K. vulgaris, S. dysentriae* and *S. typhi* in biocontrol method plant silver nano particles derived from five different medicinal plants *C. auriculata, C. alata, C. occidentalis, C. roxburghii* and *C. pulcherrima* were evaluated for their antibacterial activity in disc diffusion method. Plant silver nano particles showed marked inhibitory effects against the isolated bacteria. Among these *C. auriculata* extract nanoparticles showed significant ($p<0.05$) inhibitory effect compared to the other four extracts nano particles. Tukey HSD analysis of the data revealed that *S. dysenteriae* was highly susceptible.

The silver nano particles derived from *C. auriculata* extract exhibited maximum inhibitory effects against the pathogen *S. boydii* (22 mm) and *S. dysentriae* (21mm). It also controlled the pathogens *K. vulgaris, S. aureus* and *S. typhi* effectively (18 mm) (Table 2). But the standard antibiotic Ciprofloxacin (positive control) exhibited the inhibition zone 11-15 mm against all the tested bacteria. Hence the selected medicinal

plant *C. auriculata* showed highest inhibitory effects compared to the standard antibiotics (Table 4).

C. occidentalis nano particles showed the highest inhibition zone (20mm) against *S. dysenteriae*. It also showed moderate inhibition (18mm and 16mm) against *S. boydii* and *K. vulgaris*. The lowest inhibition was obtained against *S. aureus* and *S. typhi* (14mm). Hence the inhibition was considered as high compared to positive control (Table 2 & 4).

Silver nano particles extracted from *C. pulcherrima* exhibited marked inhibition against all the pathogens. The highest inhibition zone was 20mm against *S. boydii*. 18 mm inhibition zone was observed against *S. dysenteriae* and *K. vulgaris* (Table 2). The lowest inhibition zones were observed against *S. aureus* and *S. typhi* (16 mm and 17mm).

C. roxburghii based silver nano particles inhibited the selected human pathogens moderately. Among these, the maximum inhibition zone was observed against *S. dysenteriae* (19 mm). These silver nano particles controlled the pathogens *S. boydii K. vulgaris* and *S. typhi* moderately (17mm). The minimum inhibition (16 mm) was observed against *S. aureus* (Table 2).

C. alata extract nano particles showed the highest inhibition (18 mm) against *S. dysenteriae and K. vulgaris*. 17 mm inhibition zone was observed against *S. boydii* and *S. typhi* (Table 2). The lowest inhibition was observed against *S. aureus* (16 mm).

Relative Percentage Inhibition (RPI)

The results of antimicrobial activity of methanol extracts and plant silver nano particles were compared with the positive control (Ciprofloxacin) for evaluating their relative percentage inhibition (Table 3). The silver nano particles of *C. auriculata* exhibited maximum relative percentage inhibition (175%) against the test inoculum *S. dysenteriae* followed by *S. typhi* (165.4%), *S. boydii* (146.6%), *S. aureus* (124%) and *K. vulgaris* (112.5%) respectively. *C. occidentalis* exhibited maximum relative percentage inhibition against the test inoculum *S. dysenteriae* (170.0%) followed by *S. typhi* (127.2%), *S. boydii* (120.0%), *K. vulgaris* (100.0%) and *S. aureus* (93.3%) respectively. *C. pulcherrima* exhibited maximum relative percentage inhibition against the test inoculum *S. typhi* (154.5%) followed by *S. dysenteriae* (152.5%), *S. boydii* (134.0%), *S. aureus* (106.6%) and *K. vulgaris* (100.0%). *C. roxburghii* exhibited maximum relative percentage inhibition against the test inoculum *S. dysenteriae* (158.3%) followed by *S. typhi* (158.1%), *S. boydii* (113.0%), *S. aureus* (106.6%) and *K. vulgaris* (100.0%). *C. alata* exhibited maximum relative percentage inhibition against the test inoculum *S. typhi* (154.5%) followed by *S. dysenteriae* (150.0%), *S. boydii* (116.0%), *S. aureus* (108.6%) and *K. vulgaris* (101.8%) respectively (Table 3).

The bactericidal effect of silver nano particles can be attributed to the attachment of AgNPs to the surface of the cell membrane disturbing permeability and respiration functions of the cell [13]. It is also possible that AgNPs not only interact with the surface of membrane, but can also penetrate inside the bacteria[14]. Smaller AgNPs having the large surface area available for interaction would give more bactericidal effect than the larger AgNPs**.** Additionally reports suggest that ionic silver strongly interacts with thiol group of vital enzymes and inactivates them. Experimental evidence also proposes that DNA may lose its replication ability once the bacteria have been treated with silver ions[15]. The differences between gram-positive and gram-negative bacteria essentially rest in the structure of their respective cell walls. The gram negative bacteria have a layer of lipopolysaccharide at the exterior, followed underneath by a thin (about 7–8 nm) layer of peptidoglycan consisting of linear polysaccharide chains cross-linked by short peptides to form a three dimensional rigid structure [16]. Although the lipopolysaccharides are composed of covalently linked lipids and polysaccharides, they lack strength and rigidity. Negative charges on the lipopolysaccharides are attracted towards weak positive charges available on silver nanoparticle thereby contributing to the sequestration of free Ag+ ions [17]. Thus the relations between nanoparticles and the cell wall of bacteria would be facilitated by the relative wealth of negative charges on the gram-negative bacteria, which was amiable to the fact that growth of gram-negative bacteria was supplementary. Once inside the cell, nanoparticles would impede with the bacterial growth signaling pathway by amending tyrosine phosphorylation of putative peptide substrates decisive for cell viability and division. Thus, gram-positive bacteria may allow less Ag+ to reach the cytoplasmic membrane than the gram-negative bacteria.

Hence the present study clearly indicated that the selected human pathogens were successfully controlled by silver nano particles which derived from five medicinal plants of Caesalpiniaceae family compared with methanol extracts of plants. The maximum inhibition was observed against gram negative pathogens compared with gram positive pathogens.

CONCLUSION

This study has confirmed the antibacterial potentials of plant based silver nano particles, thus supporting their application as a biocontrol herbal remedy for bacterial infection in human beings. With these, there is need for the preparation of different formulations towards ensuring acceptable dosing to *invivo* trials. 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.

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Table 1: Periodical colour change from green to brown of plant extracts with 10^{-3} M $AgNo_3$

Time	Medicinal plant extracts				
	C. auriculata	***C. alata***	***C. occidentalis***	***C. roxburghii***	***C. pulcherrima***
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 hrs	Orange	Pale orange	Light orange	Pale orange	Light orange
3 hrs	Dark orange	Orange	Orange	Orange	Orange
4 hrs	Reddish orange	Dark orange	Dark orange	Dark orange	Dark orange
8 hrs	Reddish orange	Dark orange	Dark orange	Dark orange	Dark orange
16 hrs	Brown	Pale brown	Pale brown	Pale brown	Pale brown
24 hrs	Dark brown	Light brown	Light brown	Light brown	Light brown
28 hrs	Reddish brown	Dark brown	Dark brown	Dark brown	Dark brown

Table 2: Antimicrobial activity of plant silver nanoparticles

Sl. No	Plants	Name of the bacteria				
		S. boydii	***S. dysenteriae***	***K. vulgaris***	***S. aureus***	***S. typhi***
1.	*C. occidentalis*	18.0±0.3	20.4±0.1	16.0±0.2	14.0±0.3	14 .0±0.4
2.	*C. auriculata*	22.0±0.2	21.0±0.3	18.0±0.3	18.6±0.4	18.2±0.2
3.	*C. pulcherrima*	20.1±0.2	18.3±0.4	18.0±0.4	16.0±0.2	17.0±0.1
4.	*C. roxburghii*	17.0±0.5	19.0±0.3	17.0±0.5	16.0±0.1	17.4±0.3
5.	*C. alata*	17.5±0.3	18.0±0.1	18.5±0.2	16.3±0.3	17.0± 0.4

Table 3: Relative Inhibition Zone Diameter of plant silver nano particles

Sl. No	Plants	Name of the bacteria				
		S. boydii	***S. dysenteriae***	***K. vulgaris***	***S. aureus***	***S. typhi***
1.	*C. occidentalis*	120.0	170.0	100.0	93.3	127.2
2.	*C. auriculata*	146.6	175.0	112.5	124.0	165.4
3.	*C. pulcherrima*	134.0	152.5	100.0	106.6	154.5
4.	*C. roxburghii*	113.0	158.3	100.0	106.6	158.1
5.	*C. alata*	116.6	150.0	101.8	108.6	154.5

Table 4: Positive and negative controls

S. No	Bacteria	Positive control (Ciprofloxacin)	Negative control (Silver nitrate)
1.	*S. boydii*	15.0±0.8	0.0±0.0
2.	*S. dysenteriae*	12.0±0.5	0.0±0.0
3.	*K. vulgaris*	16.0±0.3	0.0±0.0
4.	*S. aureus*	15.0±0.1	0.0±0.0
5.	*S. typhi*	11.0±0.2	0.0±0.0

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