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Original Research Article

Synthesis of Silver Nanoparticles using *Costusspeciosus* and study of its anti-microbial properties against urinary tract pathogens

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A B S T R A C T

The integration of nanomaterials with biology is finding wide applicability in various areas of medical sciences. Recently, silver nanoparticles have gained attention because of their antimicrobial activity which offers the possibility of their use for medical purposes. Metallic nanoparticles can be obtained by physical, chemical or biological methods. But silver nanoparticles synthesized by chemical reduction method are found to be toxic, flammable and not at all ecofriendly. Therefore, in the present study, an attempt was made to formulate a cost effective and environment friendly technique for green synthesis of silver nanoparticles. The silver nanoparticles were synthesized using extract of *Costus speciosus* with $AgNO_3$ solution. Their antibacterial activity was studied using agar well diffusion method on isolates viz., *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeroginosa, Proteus mirabilis, Enterobacter* obtained from patients suffering from urinary tract infection. The antibacterial efficacy of silver nanoparticles of *Costus speciosus was* found to be superior to that of hot or cold extracts of *Costus speciosus*. Silver nanoparticles were characterized by UV-vis spectroscopy and Dynamic Light Scattering (for particle size analysis). This work demonstrates the possible use of biologically synthesized silver nanoparticles to treat urinary tract infections.

Introduction

Infectious disease is the main cause of mortality in the world and the rapid increase of antibiotic resistance amongst pathogenic bacteria is becoming a serious public health problem. Antimicrobial resistance is becoming a factor in virtually all hospital acquired infections and it is expected that some bacterial infections may soon become untreatable in the near future. These concerns have led to major research efforts to discover alternative strategies that could be used to combat bacterial infections in patients- one of which is the use of nanotechnology.

Urinary tract is the most common site of nosocomial infections. Contrary to the synthetic drugs, antimicrobials of plant

origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Iwu*et al*., 1999). Plant extracts and silver nanoparticles synthesized from plant extracts have been found to be efficient in the treatment of UTIs. Their large surface-to-volume ratio of nanoparticles is important for their antimicrobial efficiency.Nanocrystalline silver particles have found tremendous applications inthe field of high sensitivity biomolecular detection and diagnostics, antimicrobials andtherapeutics (Rai andYadav, 2009; Elechiguerra*et al*.,2005 as cited in Geethalakshmi and Sarada, 2010). The use of environmentally benign materials like plant leaf extracts (Parashar*et al*., 2009), bacteria (Saifuddin*et al*., 2009), fungi (Bhainsa and D'Souza, 2006) and enzymes (Willner*et al*.,2007) for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and otherbiomedical applications as they do not use toxic chemicals for the synthesis protocol (Parashar*et al*. ,2009 as cited in Geethalakshmi and Sarada, 2010). Chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medicalapplications. Green synthesis provides advancement over chemical and physical method as it is cost effective,environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use highpressure, energy, temperature and toxic chemicals.

Furthermore, plants like *Costusspeciosus* or spiral ginger have been used for the treatment of urinary tract infections since time immemorial (Wong, 2007). But, as there are hardly any reports on the use of *Costusspeciosus* silver nanoparticles for prevention of urinary infections.

Therefore in the present study, an attempt was made to study the antimicrobial efficacy of silver nanoparticles of *Costus speciosus* against urinary tract pathogens isolated from samples collected from patients suffering from urinary tract infections.

Materials and Methods

Costus speciosus leaves were taken from the local fields of Keshav Shrishti, Maharashtra, India. All of the reagents and solvents were procured from Himedia laboratories, Mumbai, India. Silver nitrate (AgNO3) was procured from Sigma Aldrich USA. UV-Vis spectrophotometer (Shimadzu 1800), Dynamic Light Scattering (DLS), Scanning Electron Microscope (Hitachi S-4500)were used to investigate morphology and size of the particles.

Preparation of the extract

Cold extract

100g air dried powder of *Costus speciosus* was soaked in 200ml distilled water for 12 h.The extract was then filtered using Whatman filter paper No.1 and stored at 4° C for further analysis.

Hot extract

2 g of air dried powder of *Costus speciosus* was boiled in 20ml of distilled water in a water bath for 1 h. This extract was then filtered using Whatman filter paper No.1 and stored at 4° C for further analysis.

Synthesis of Silver nano particles

25 gm. fresh green leaves of *Costus speciosus* were thoroughly washed thrice

with distilled water followed by double distilled water to remove the dust particles and other contaminants. The plant material was chopped into fine pieces and taken in a clean 250 ml Erlenmeyer flask and 100 ml of sterile double distilled water was added and boiled for10 min. to facilitate the formation of aqueous leaf extract. The extract was then filtered using Whatman No. 1 filter paper. This aqueous leaf extract was mixed with 1mM silver nitrate solution in a ratio 1:10and incubated on a magnetic stirrer at 60^{o}-80^{o}Cfor 5 hours until a color change was observed.

Visual inspection of colour

Change in colour of the nanoparticle solution after reduction gives a preliminary confirmation for the formation of *Costus speciosus* silver nanoparticles.

UV-Vis Spectra analysis

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after 30 min.

SEM analysis of silver nanoparticles

Thin films of synthesized and stabilized silver nanoparticles were prepared on a carbon coated copper grid by analyzing a very small amount of the sample placed on the grid. Scanning electron microscopic analysis assisted in the study of the morphology and size of the silver nanoparticles of *Costus speciosus*.

Dynamic light scattering of Silver nanoparticles

Costus speciosus silver nanoparticle solution was diluted with double distilled water and placed in cuvette of particle size analyzer.

Antibacterial assays

Urinary tract pathogens (*viz. Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Enterobacter* spp.)were isolated from samples collected from patients suffering from urinary tract infection and the antibacterial activity of hot and cold extracts of *Costus speciosus* along with their silver nanoparticles was studied against these pathogenic isolates using Agar Well Diffusion Method.18 h cultures (0.1ml each)of the pathogenic isolates were spread on Sterile NutrientAgar plates. Four wells were punched in each plate using sterile cork borer (6mm diameter).The wells were then filled up to the brim with the hot and cold extract of crude *Costus speciosus* as well as its silver nanoparticles under aseptic conditions. The plates were then incubated at 36±1ºC for 24h in an upright position. The results were recorded and analyzed in terms of the zones of inhibition formed around each well.

Results and Discussion

It is well known that silver nanoparticles exhibit reddish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Shankar *et al*.2004 as cited in Geethalakshmi *et al*., 2010). The *Costus speciosus* leaf extract on mixing with aqueous solution of the silver ion complexalso showed a change in color from yellowish green to reddish brown indicating the reduction of silver ions and formation of silver nanoparticles and which was further confirmed by UV-Vis spectroscopy. Absorption spectra of silver nanoparticles formed in the reaction media showed maximum absorbance at 406 nm. Broadening of peak indicated that the

particles are poly-dispersed. The high density nanoparticles observed under the scanning electron microscopeas well as the peak at 50% using dynamic light scattering technique confirmed the development of *Costus speciosus* silver nanostructures.

Normally antimicrobial sensitivity testing using Kirby-Bauer method relies on the near relationship that exists for most antimicrobials between diameters of zone of inhibition and minimum inhibitory concentration (MIC) as per the international collaborative study method. According to Kirby-Bauer method the organisms are generally reported as 'Sensitive', 'Intermediate' or 'Resistant' on comparison with the standard chart. But these standard values are available only for commonly available antimicrobial agents. To date there is no such report on such standard values for medicinal plants for a comparative study.

A study using crude extract of the medicinal plant *Costusspeciosusas* well as its silver nanoparticles was carried out against urinary tract pathogens using Agar well diffusion method. The results obtained were tabulated and analyzed (Table.1).

The data obtained indicated that hot and cold extract of *Costus specious* as well as its silver nanoparticles were inhibitory towards all the pathogenic isolates used for the study viz. *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Enterobacter* sp., *Pseudomonas aeruginosa*. A comparative study between the various extracts of *Costus speciosus* and the silver nanoparticles of *Costus speciosus* with respect to their antimicrobial efficacy against the urinary pathogens used in the study indicated that silver nanoparticles of *Costus speciosus* were the most bactericidal of all the extracts used in the study. Smaller particles with larger surface to volume ratios are found to possess greater antibacterial activity (Duran *et al*., 2010). This is justified by the results obtained in the present study.

Furthermore, out of the five pathogenic isolates, *Pseudomonas aeruginosa* was found to be the most susceptible organism. These results were also confirmed by statistical analysis where it was observed that null hypothesis was rejected as there was significant difference among the various extract for the zone of inhibition($P<0.05$) and there was difference in effectiveness of various extract on comparison with each other. The mean average zone of inhibition of one extract may not be directly compared with that of another extract because different extracts may diffuse at different rate altogether. After applying ANOVA or one way analysis of variance it was observed that since the 'f' value was more than the 'critical f' value, the null hypothesis was rejected.

There is a growing concern about the emergence and re-emergence of drug-resistant pathogens. Therefore, the development of new antimicrobial compounds or the modification of those available to improve antimicrobial activity for therapy, antisepsis or disinfection is a high priority area of research. In this endeavor, nanotechnology provides a means to modify key features of different materials, including metal nanoparticles. The inhibitory and bactericidal activities of silver ions have long been known. Some forms of silver have been demonstrated to be effective against

Table.1 Antimicrobial sensitivity of hot, cold extracts as well as silver nanoparticles of *Costus speciosus by* Agar well diffusion method

Isolates	Zone of inhibition (mm)		
	Costus speciosus		
	Hot Extract	**Cold Extract**	**Silver nanoparticles**
Escherichia coli	11	10	12
Klebsiella pneumonia	9	8	18
Proteus mirabilis	10	8	15
Enterobacter sp.	11	9	15
Pseudomonas aeruginosa	12	10	20

urinary tract infections and central venous catheter infections. There has been resurgence in the use of silver-based antiseptics that may be linked to broad-spectrum activity and far lower propensityto induce microbial resistance than antibiotics.

Silver nanoparticles are already known to be very efficient antimicrobials and are being now used in diagnostics and therapeutics. Furthermore, the use of medicinal herbs, especially *Costus speciosus* in the treatment of urinary infections is well known since ages. But there are hardly any reports stating the use of silver nanoparticles of *Costus speciosus* for treatment of urinary tract infections.

The present study is therefore a step towards demonstrating the potential of green synthesis of eco-friendly, stable silver nanoparticles nanoparticles from *Costus speciosus* plant with fairly well-defined dimensions and with an antibacterial efficacy towards urinary tract pathogens. These nanoparticles exhibit a great potential in treatment of patients suffering from urinary tract infections.

References

Bhainsa K. C., and D' souza S. F. 2006. Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigates*. Coll Surf B Bioint., 47 :160-164.

Duran N., Priscyla D. M., Conti R. D., Alves O.L., Costab F. T. M. and Brocchib, M. 2010. Potential use of silver nanoparticle on pathogenic bacteria,their toxicity and possible mechanism of action. J.Braz.Chem.Soc., (21)6: 949-959.

Geethalakshmi R.L. and Sarada D. V. L. 2010. Synthesis of plant mediated silver nanoparticles using *Trianthema decandra* extract and evaluation of their anti microbial activities. Inter. J. Engineer. Sci. Technol. (2) 5: 970-975.

IwuM.W., Duncan A.R. and Okunji C.O. 1999. New Antimicrobials of Plant Origin. In: Janick J. (ed.): Perspectives on New Crops and New Uses. ASHS Press, Alexandria, VA : 457–462.

Parashar V., Parashar R., Sharma B. and Pandey A. C. 2009. Parthenium leaf Extract mediated synthesis of silver nanoparticles: a novel approach towards Weed utilization. Digest J. Nanomat. Biostruct. 4(1): 45 – 50.

Saifuddin, N., Wong C.W. and Yasumira A. A. 2009 . Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. E- J. Chem. (6) 1: 61-70.

Willner B., Basnar B. and Willner B . 2007. Nanoparticle–enzyme hybrid systems for nanobiotechnology. FEBS J. (274): 302–309.