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Original Article

NANO FABRICATION OF CATHETER WITH BIOCOMPATIBLE BIOGENIC SILVER NANOPARTICLES INCORPORATED MEDICINAL PLANT EXTRACTS FOR THE ANTI BIOFILM ACTIVITY AGAINST PYOGENIC BACTERIA

S.KARTHICK RAJA NAMASIVAYAM*, BENINTON.B.CHRISTO, R.S.ARVIND BHARANI S. M. KARTHIGAI ARASU K. ARUN MUTHU KUMAR & K. DEEPAK

Department of Biotechnology, Sathyabama University, Chennai 119, Tamil Nadu, India. Corresponding author.
Email: biologiask@gmail.com

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ABSTRACT

Objective: Nanotechnology is an important area of various field of science and technology due to their amenability to biological functionalization. The present study is undertaken to evaluate anti biofilm effect of biogenic silver nanoparticles incorporated medicinal plants fabricated catheter against human pathogenic bacteria

Methods: Silver nanoparticles were synthesized by leaf extract broth of *Azadirhacta indica* and the synthesized nanoparticles were incorporated with crude organic solvent extract of *Vitex negundo* and *Allium sativum*. Biofilm inhibition was carried out with the preparation adopting modified method of biofilm spectrophotometric assay. Effect of the nanoparticles incorporated plant extracts on the biochemical composition of biofilm matrix mainly total carbohydrates and total protein was also studied

Results: Synthesized silver nanoparticles, together with plant products showed a maximum inhibitory effect against both the tested clinical isolates and maximum inhibition was recorded in nanoparticles incorporated *Vitex negundo* extract and the biochemical composition of biofilm matrix was also highly reduced in silver nanoparticles incorporated both the plant extracts.

Conclusion: The present study demonstrated that silver nanoparticles incorporated plant extracts was found to exhibit enhanced antibiofilm activity. This study can further be used to prevent or minimize bacterial infections leading to the development of new generation of antimicrobial agents.

Keywords: Catheter, Plant extracts, Biofilm, Silver Nanoparticles

INTRODUCTION

Plant extracts and other biologically active compounds isolated from plants have gained widespread interest in this regard as they have been known to cure diseases and illnesses since ancient times[1].Modern science and technological advances are accelerating the discovery and development of innovative pharmaceuticals with improved therapeutic activity and reduced side-effects from plants. Plant compounds are widely accepted due to the perception that they are safe and they have a long history of use in folk medicine as immune boosters and for the prevention and treatment of several diseases [2] .Over the years, the use of medicinal plants, which forms the backbone of traditional medicine, has grown with an estimated 80% of the populations, mostly in developing countries, relying on traditional medicines for their primary health care [3]. Plant-derived substances under intensive research for possible applications in the pharmaceutical industry include crude extracts of leaves, roots, stems and individual compounds isolated from these, essential oils and essential oil components [4]. Although a lot of research on plants and the active constituents is currently underway, the focus is mainly on the antimicrobial properties against planktonicor biofilm forming bacteria [5].Biofilms are universal, complex, interdependent communities of surface -associated microorganisms. The organisms are enclosed in an exopolysaccharide matrix occurring on any surface, particularly aquatic and industrial water systems as well as medical devices. As such, biofilms are highly relevant for public health [6].Biofilm, likely the predominant mode of device related microbial infection exhibit resistance to antimicrobial agents[7]. A biofilm serves to promote bacteria persistence by resisting antibiotic treatment and host immune responses. Antibiotics are rendered ineffective when biofilms form due to their relative impermeability, the variable physiological status of microorganisms, subpopulations of persistent strains, and variations of phenotypes present they can serve as hides for disease and are often associated with high level antimicrobial resistance of the associated organisms. Nanoscience and Nanotechnology (N&N) are new approaches to research phenomena at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale [8].This new approach can be applied to microbial biofilms [9] .The application of such a technology can be used for the inhibition of biofilm formation which are of higher threat in the process of treatments [10],Combination therapy of nanoparticles with plant extracts is now being extensively used to fight against life threatening biofilm. In the present study, improved anti biofilm effect of silver nanoparticles incorporated extracts of *Vitex negundu* and *Allium sativum* fabricated on catheter against clinical isolate of *E.coli* and *Pseudomonas aeruginosa* biofilm was discussed.

MATERIALS AND METHODS

Synthesis of biogenic silver nanoparticles

Biogenic silver nanoparticle was synthesized from leaf extract broth of *Azadiracta indica* (neem) 100gm of dried leaf material was homogenized finely in domestic mixer and 1gm of homogenized material was dissolved in 100ml deionised water and filtered through crude filter paper 50ml of collected filtrate was transferred to 100 ml of beaker and 100ml of 0.1mM silver nitrate was added and the preparation was kept under magnetic stirrer. Conversion of reaction mixture from pale green to dark brown indicates synthesis of silver nanoparticle and further conformation was carried out by uv- visible spectroscopy, Scanning Electron Microscopy and Energy Dispersive Atomic spectroscopy (EDX) the characterized particle was used for further studies.

Plant materials

Healthy and fresh leaves of *Vitex negundu* and *Allium sativum* were collected home garden and local shop. Collected materials were washed in tap water followed by successive washing in distilled water. Washed materials were shade dried. Dried material was homogenized in domestic mixture into fine powder, stored in plastic container at room temperature used for further studies.

Crude extraction

Crude extraction of the respective plant material was carried out by extraction of the dried materials [11].The dried powder of respective plant material (50 g) was soaked separately with 250 mL of methanol in a 500 mL conical flask for 48 hours at room temperature, without shaking. The solvent was filtered through Whatman filter paper No. 1 and concentrated on a rotary vacuum evaporator. Concentrated crude extract was reconstituted in dimethyl sulfoxide (DMSO) to make a stock solution of 10,25,50,75 and 100 µg/mL and stored at−20 °C until use.

Anti biofilm inhibition study

Bacterial stains

Clinical isolate of *E.coli* and *P.aeruginosa* were obtained from Madurai medical college hospital, Tamil Nadu, India. Bacterial strain was maintained on slope of nutrient agar slant.(Hi media, Mumbai).Nutrient broth was used for inocula preparation. Cultures was inoculated from fresh slopes and incubated with shaking at 37°C for 24 hours. Cells were collected by centrifugation and the collected cell debris washed twice in phosphate buffer saline and suspended to OD520 prior to use in biofilm experiments.

Biofilm inhibition assay

Coating of silver nanoparticles incorporated plant extracts on the catheter

Coating of the silver nanoparticles incorporated plant extracts was carried out by the modified method of Karthick Raja Namasivayam et al [12]. Catheter was obtained from local medical shop (romo10) the catheter was cut in to 1x1 urface and the cut pieces (5 nos) were transferred to a beaker containing 20mL of silver nanoparticles suspension (10µg concentration) and 2.5 ml of respective plant extracts kept in ultrasonicator for three hours at room temperature, Coating of nanoparticles was confirmed by color change of the catheter surface fine dispersion of particle by scanning electron microscopy and Fontier transform infra red spectroscopy (FTIR) these pieces were used for biofilm inhibition study.

Biofilm Inhibition Study

The cut pieces was transferred to a test tube containing 5mL of 24 hour culture, the inoculated tubes were kept in C for 3 days (72 hrs) after the incubation period the whole content was aspirated and 5mL of 1% crystal violet was added and incubated at room temperature for 10mins. Crystal violet was removed and successive washing was made using sterile phosphate buffer saline to remove unbound cells or free plantonic cells. After washing, 5mL of ethanol was added kept at room temperature for 15 minutes the reaction mixture was read at 590 nm

Evaluation of biochemical composition

Isolation of biofilm matrix material from the microtitre plate and catheter was carried out by standard method [13].Adherent biofilms were transferred to screw cap bottles containing 10 ml distilled water. The bottles were sonicated for 5 min in an ultrasonic water bath and vortexed vigorously for 1 min to disrupt the biofilms. Cell suspensions were then pooled and centrifuged. The collected supernatant used as source for studying biochemical composition mainly protein and carbohydrates

RESULTS AND DISCUSSION

Biogenesis of silver nanoparticle from leaf extract broth of *Azadiracta indica* was primarily confirmed by colour change of the reaction mixture from green to brown, plasmon absorption maxima at 420nm by U.V spectrophotometer (Figure 1). Particles morphology was studied by Scanning electron microscopy (SEM).SEM images were recorded by using a Carlzeiss Supra 55 field emission scanning electron microscope equipped with an energy-dispersive spectrum (EDAX, oxford instruments) capability. In a SEM setup, the nanoparticulate sample, coated to be conductive (e.g. gold, palladium), is scanned in a high vacuum chamber with a focused electron beam. The scanning electron microscopy study reveals uniform spherical particles with the size of 50-60nm(Figure 2a) and the presence of silver in the reaction mixture was further confirmed by EDX (Figure 2b).

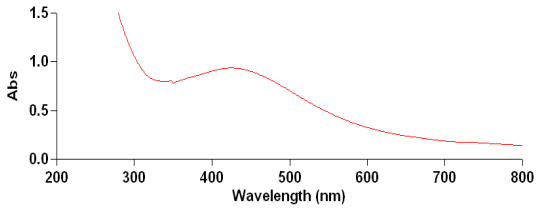

Fig. 1: UV absorption spectra of silver nanoparticles (AgNPs

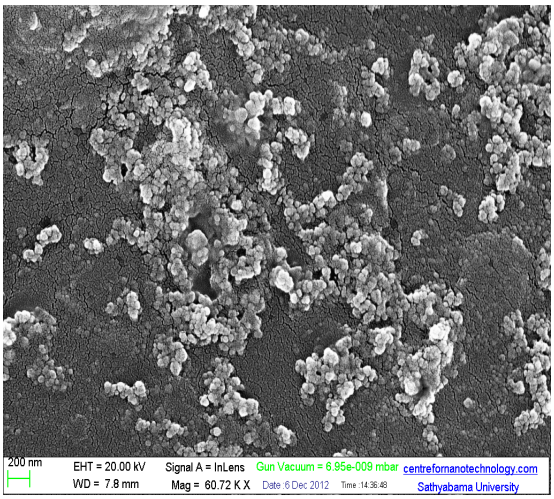

Fig. 2a: SEM image of synthesized silver nanoparticles

Coating of biogenic silver nanoparticle was easily identified by color change of catheter (Figure 4)dispersion of nanoparticle on the catheter surface was confirmed by scanning electron microscope which reveals the uniform spherical particles were embedded on the catheter surface with the size of 50 to 60nm. Biofilm inhibition study clearly revealed nanoparticles incorporated both the plant extracts inhibited biofilm of both the tested strains. .Results were represented as inhibition percentage of biofilm development(Table 1).In the case of *E.coli*, silver nanoparticles with *V.negundo* recorded maximum anti biofilm effect with 84.0.Free silver nnaoparticles and free *V.negundo* extract recorded 67 and 45.0%. (Table 1).75.0 % of biofilm inhibition has been inferred from silver nanoparticles incorporated *A.sativum* whereas free A.sativum revealed 40.0 % of biofilm inhibition. In the case of *P.aeruginosa*, silver nanoparticles incorporated *V.negundo* brought about 81.0% maximum biofilm inhibition.Silver nanoparticles with *A.sativum* showed 80.0 %. 60.0,63.0 and 55.0 % of inhibition was recorded in free silver nanoparticles,*V.negundo* and *A.sativum*. Effect of nanoparticles incorporated plant extracts on the biochemical composition of biofilm matrix revealed total carbohydrate and total protein of both the tested organism were highly reduced (Table 2).

Table 1: shows

S. No.	Treatment	Biofilm inhibition (%)	
		E.coli	***P.aeruginosa***
1	F-AgNp	67.0	71.0
2	*V.negundo*	45.0	63.0
3	AgNp- *V.negundo*	84.0	81.0
4	*A.sativum*	*40*.0	55.0
5	AgNp-*A.sativum*	75.0	80.0

Table 2: shows

S. No.	Concentration	Biofilm inhibition(%)			
		E.coli		***P.aeruginosa***	
		AgNp- AS **TP**	**AgNp-VN TC**	***AgNp- AS*** **TP**	**AgNp-VN TC**
1	10	80.0	78.0	95.0	110.0
2	25	60.1	64.3	70.0	82.1
3	50	56.2	58.1	68.4	67.0
4	75	49.4	49.2	59.2	58.0
5	100	15.0	19.0	29.0	19.0

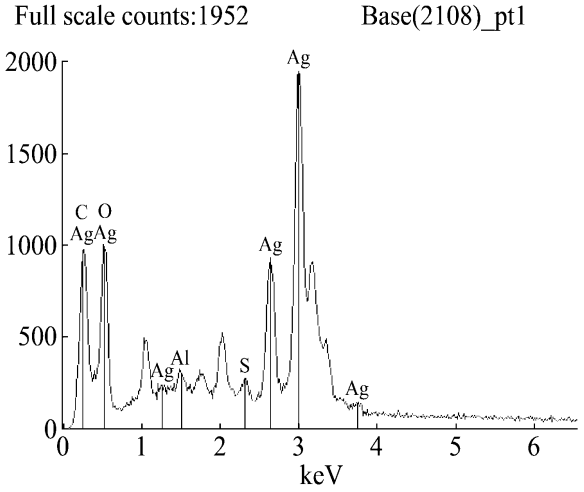

Fig. 2b: EDX spectra of synthesized silver nanoparticles

Due to increasing tolerance of the biofilm community to antibiotics, biocides and mechanical stress, it has become just as difficult to completely eradicate mature biofilms. Several studies have examined the effect of various types of antimicrobial treatment in controlling biofilm formation on various solid surfaces including medical devices The vast majority of the chemical agents currently available for biofilm control are broad-spectrum non-specific micro biocide agents. Chloro hexidine, triclosan, and essential oils (*e.g*., Listerine) are the most commonly used and clinically tested antimicrobials. In order to control biofilm formation on medical devices and all costs associated, a large number of new strategies and approaches have been developed in the last few years, including: antimicrobial locks (in the case of catheters).surface modification of biomaterials with antimicrobial. Plant extracts and other biologically active compounds isolated from plants have gained widespread interest in this regard as they have been known to cure diseases and illnesses since ancient times [1]. Anti biofilm effect of various metallic nanoparticles against clinical isolates of pathogenic microorganism has been reported [14,15,16]. Plant extracts mediated biofilm inhibition has already studied [17,18,19].Bioactive metabolites from the plants known to cause anti biofilm effect by the inhibition of biofilm formation or interfere with the biofilm formation [17].Karthick Raja. Namasivayam et al [14] have reported the synergistic effect of biogenic silver nanoparticles and plant products and also with antibiotics on the biofilm of clinical isolates of *Staphylococcus aureus* and *Candida tropicalis*. Biochemical composition of biofilm matrix total carbohydrate and total protein was also highly reduced. Reduction of biochemical composition was observed in all the tested concentration of silver nanoparticles incorporated both the plant extracts against both the tested organism .

The matrix is one of the most distinctive features of a microbial biofilm. It forms a three dimensional, gel- like, highly hydrated and locally charged environment in which the microorganisms are largely immobilized. Matrix-enclosed micro colonies, sometimes described as stacks or towers, are separated by water channels which provide a mechanism for nutrient circulation within the biofilm the composition of the matrix varies according to the nature of the organism and reduction of the biochemical composition of the biofilm matrix leads to weakening of the biofilm thus facilitate entry of the drugs [21]. The present study demonstrated that silver nanoparticles incorporated plant extracts was found to exhibit enhanced antibiofilm activity. This study can further be used to prevent or minimize bacterial infections leading to the development of new generation of antimicrobial agents.

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