Bulletin of Pure and Applied Sciences
Vol.39A (Zoology), No.2,
July -December 2020: P.510-515
Original Research Paper

Print version ISSN 0970 0765
Online version ISSN 2320 3188
DOI 10.5958/2320-3188.2020.00060.1
Available online at www.bpasjournals.com

Silver Nanoparticles of *Atrocarpus heterophyllus* (Jack fruit) as Effective Antagonist of Bacterial Bio Films

[1]Mubeen Sultana*
[2]Aishwarya S.
[3]Lucky R.
[4]Asgari. S.M.

Author's Affiliation:
[1]Assistant Professor in Zoology, PG and Research department of Zoology, Justice Basheer Ahmed Sayeed College for Women (JBAS), SIET, Teynampet, Chennai, Tamil Nadu 600018, India
[2]Research Student, JBAS College, SIET, Teynampet Chennai, Tamil Nadu 600018, India
[3]Assistant Professor in Zoology, PG and Research department of Zoology, Justice Basheer Ahmed Sayeed College for Women (JBAS), SIET, Teynampet, Chennai, Tamil Nadu 600018 India
[4]Assistant Professor in Zoology, PG and Research department of Zoology, Justice Basheer Ahmed Sayeed College for Women (JBAS), SIET, Teynampet Chennai, Tamil Nadu 600018, India

***Corresponding author:**
Dr. Mubeen Sultana,
Assistant Professor in Zoology, PG and Research department of Zoology, Justice Basheer Ahmed Sayeed College for Women(JBAS), SIET, Teynampet, Chennai, Tamil Nadu 600018, India

E-mail:
mubeenjbas2020@gmail.com;
mubeensultana.d@jbascollege.edu.in

Received on 12.06.2020
Accepted on 21.11.2020

Abstract:
Membranes of older water filters, contain plethora of bacteria, which are difficult to be limited as they are known to form bacterial biofilms. In this regard, plant based nanoparticles, are reported to be effective, as antibacterial and antibiofilm agents. In the present study, leaf extract of *Artocarpus heterophyllus* was used to synthesize silver nanoparticles and its efficacy as antimicrobial and antibiofilm agent was studied. Biofilm inhibition assay using the extract was found to be8 2% and the Minimum Inhibitory concentration (MIC) value was found to be 62.5µgm, against the bacterial samples collected from the membrane filters. This study clearly suggests that leaf extract of *Artocarpus heterophyllus* when processed as silver nanoparticles, can be more efficient as antimicrobial and antibiofilm agents. This study clearly illustrates the antibacterial and antibiofilm properties of *Artocarpus heterophyllus* and may therefore be suggested as an ecofriendly and cost effective cleansing agent for the water filters. Membranes of older water filters, contain plethora of bacteria, which are difficult to be limited as they are known to form bacterial biofilms. In this regard, plant based nanoparticles, are reported to be effective, as antibacterial and antibiofilm agents. In the present study, leaf extract of *Artocarpus heterophyllus* was used to synthesize silver nanoparticles and its efficacy as antimicrobial and antibiofilm agent was studied. Biofilm inhibition assay using the extract was found to be82% and the Minimum Inhibitory concentration (MIC) value was found to be62.5µgm, against the bacterial samples collected from the membrane filters. This study clearly suggests that leaf extract of *Artocarpus heterophyllus* when processed as silver nanoparticles, can be more efficient as antimicrobial and antibiofilm agents. This study clearly illustrates the antibacterial and antibiofilm properties of *Artocarpus heterophyllus* and may therefore be suggested as an ecofriendly and cost effective cleansing agent for the water filters.

Keywords: *Artocarpus heterophyllus,* Bacterial Biofilm, Silver Nanoparticles, Antimicrobial Agent, Antibiofilmagent

INTRODUCTION

Nanotechnology is a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level. Biological synthesis of nanoparticles (NPs) has upsurge in the field of nanobiotechnology to create novel materials that are eco-friendly, cost effective, stable NPs with great importance for wider applications especially in the field of medicine, as antimicrobial agents. The major advantage of using plant extracts for SNP synthesis is that they are easily available, safe, and nontoxic in most cases, have a broad variety of metabolites in the synthesis. Traditional treatment processes against microbial infections depend on the usage of different components that can inhibit the growth or kill microorganisms. But most of the pathogenic microorganisms are able to develop protection against those particular compounds by the development of microbial biofilm. Biofilm is a community of microbial cells attached to the surface and is embedded in the extracellular polymeric substances (EPS) (Donlan, 2002).and once the organism forms biofilm, they often exhibit antibiotic resistance. Biofilm-mediated drug resistance can be attributed to the differential gene expression of biofilm cells.The presence of biofilms in food processing environments is a potential source of contamination that may lead to food spoilage and disease transmission (Hood and Zottola, 1995; Frank, and Chmielewski R2001). Quite a number of bacterial species, such as *Eschrerichia coli, S. epidermidis, E. cloacae, K. pneumoniae , Aeromonas veroni*, and *Staphylococcus aureus* are known to form biofilms. The effects of plant extracts to prevent biofilm formation and adherence have been showed in earlier studies by Quave*et al*., (2008); Sandasi*et al*., (2010). Antibiofilm effect of both the aqueous and chitosan coated extracts of *Azadirachta indica, Vitex negundo* and *Tridax procumbens* was evaluated against biofilm development of *E. coli* (Karthick Raja Namasivayam *et. al*, 2013). Silver has long been used for its antimicrobial properties as its toxicity to microorganisms is greater than many others metals while maintaining low toxicity to mammalian cells. (Zhao and Stevens 1998). It has been shown that Silver NP's are more efficient in mediating antimicrobial activity than silver ions all by itself (Lok *et al*., 2006 ; Rai *et al*., 2009) and as a result have been incorporated into wound dressings, medical devices, water purification system, linings of washing machines, dishwashers, refrigerators, toilet seats, and clothing. (Li *et al*., 2011). These studies, clearly illustrate the greater antimicrobial efficacy of silver nanoparticles, embedded with plant extracts specifically upon the biofilm forming bacterial species. Based upon these studies, it is quite evident that plant extract, coated with silver nanoparticles, seems to be better antibacterial and antibiofilm agents, and hence in the present study *Artocarpus heterophyllus* extract based silver nanoparticles, have been synthesized and their efficacy was tested against the microbial populations of water purifiers.

MATERIAL AND METHOD

Isolation of Microorganisms from the membrane

Spread Plate Method

Membrane sample collected from an aged water purifier was mixed properly in the first tube.1ml from the first tube was taken and added to the second tube that contains 9ml and mixed properly. 0.1ml was taken from the tubes of dilution 10^{-2}, 10^{-4} and 10^{-6} and were spread-plated on NA (Nutrient Agar) plates using a L-rod. An isolated colony was picked up from the agar plate culture and spread over the first quadrant (approximately ¼ of the plate) using close parallel streaks. The inoculating loop was immediately streaked very gently over a quarter of the plate using a back and forth motion. The loop was flamed and allowed to cool. A streak was extended into the third quarter of the plate. The loop was flamed again and allowed to cool. Finally, a streak into the center fourth of the plate. The loop was flamed once more. Streaked plates were incubated at 37°c for 24 hours. The colonies were examined and grown in the plate carefully. All colonies should have the same general appearance. If there is more than one type of colony, each type should be streaked again on a separate plate to obtain a pure culture

Biofilm Studies Using Crystal Violet Assay

Biofilm formation of the strains was assessed by Crystal Violet assay. 96 wells of microtitre plates were seeded with 180µl media and 20µl of culture in each well. The cells were incubated overnight at 37°C for 12 hrs. The nutrient broth was added in different dilutions. Culture medium was removed from the 96 well plate and the wells were washed with 200 µL of Phosphate Buffered Saline. Then, PBS was removed and the wells were stained with 1% Crystal violet solution to stain the polysaccharides of the Biofilm. It was incubated for 10 minutes at room temperature. The plate was washed in tap water and drained upside down on paper towels and 1% SDS was added to solubilize the stain. The plate was agitated on orbital shaker until color is uniform with no areas of dense coloration in bottom of wells. Absorbance of each well was measured at 570 nm. A graph was plotted for the absorbance value against the dilutions

Synthesis of Silver Nano Particles Using Herbal Extracts

Fresh and healthy leaves of *Artocarpus heterophyllus* were collected and thoroughly washed with distilled water. 30g of leaf sample was weighed, ground with motor and pestle, and boiled with 500ml of distilled water for 15 minutes. The suspension was cooled and filtered.SNP synthesis was carried out by mixing 400ml of filtrate with 100ml of aqueous solution of 1mM $AgNO_3$. The mixture was incubated until a color change was obtained. Then, the reaction mixture was centrifuged at 1000rpm for 20 minutes. The supernatant was discarded and the pellet obtained was washed repeatedly with sterile distilled water, dried and finely powdered for characterization of the yield obtained was 45mg of Silver nanoparticles. The aqueous silver ions were reduced to SNP's when added to natural leaf extract of *Artocarpus heterophyllus.* It was observed that the colour of the solution turned from pale yellow to dark brown after 24 hours of incubation which indicated the formation of SPN's. The formation and stability of the reduced SNP's in the colloidal solution was monitored by UV-*vis* Spectrophotometer analysis.

Preparation of Isolated Cultures in Nutrient Broth

One loop full of isolated microorganisms from the slant was inoculated onto a sterile nutrient broth. The broth was kept for incubation at 37^0C for 24 to 48hrs.

Biofilm Inhibition Studies Using Crystal Violet Assay

100ml of nutrient broth was prepared and sterilized at 121^0C for 15 minutes in 15 lbs. To 3ml of nutrient broth 100µl of 24 to 48 hours old cultures maintained in the broth was added. A stock solution of Silver nano particles of 10mg/ml was prepared using distilled water and dissolved completely. Different concentrations of SNP's such as 20,40,60,80 and 100µl was added to the tubes with isolated cultures. The tubes were mixed properly and kept for incubation at 37^0C for 24 to 48 hours. After incubation, biofilm formation was observed as ring around the top or at surface of the tube and also sometimes at the bottom. Then the media was discarded carefully without disrupting biofilm and 3.5ml of 0.1% of crystal violet solution was added. It was allowed to stay for 10-15 minutes and crystal violet solution was pipetted out and 3ml of 30% of Glacial acetic acid was added and absorbance was read at 570nm.

Minimum Inhibitory Concentration (MIC)

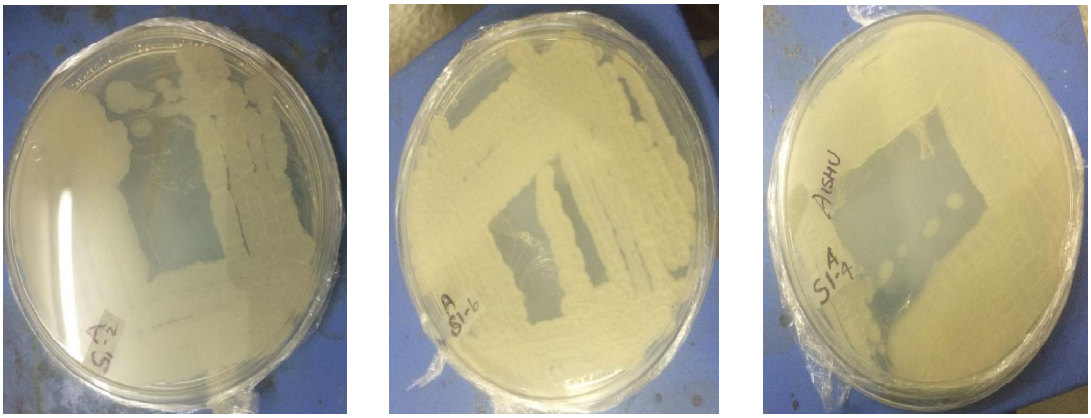
The samples were subjected to antibacterial activity by micro dilution method against S1, S2 and S3 cultures. Luria broth (Himedia, Mumbai) was prepared and sterilized by autoclaving at 121°C, 15 lbs. for 15 minutes. 100µl of broth was added to the 96 well microtitre plates. The 100 µl of the given sample was added in the first well and then serially diluted till the eighth well. The 10µl of log phase culture was introduced into the respective wells. Similarly tetracycline (100µl from10mg/ml) was added to 100µl of broth and serially diluted. Then 10µl of log phase culture was added. This served as the positive control. Broth and culture was taken as Negative control. Sterile broth serves as a control. The plates were incubated at 37°C for 24 h. MIC was determined as the complete growth inhibition at the lowest concentration of the sample.

RESULTS

In the present study, the bacterial cultures where collected and isolated from the old membranes of the water purifiers, and were cultured and treated with silver nanoparticles of leaf extract of *Artocarpus heterophyllus*

Culture Isolation

From the serial dilution technique, three morphologically different colonies, namely S1, S2 and S3, were isolated from the NA plates with 10^{-8} dilution. The single colonies were isolated by quadrant streaking method and colony obtained at the tail end was isolated and maintained in the slants for further use.



Sample 1 (S1) **Sample 2 (S2)** **Sample 3 (S3)**

Figure 1: Streak plates with bacterial cultures isolated from the membranes

Study of Biofilm Forming Ability of the Isolated Cultures

The three isolated strains namely S1, S2 and S3 were subjected for crystal violet assay to study the biofilm forming ability. Known biofilm forming strain *Pseudomonas aeruginosa* was used as a positive control. The absorbances obtained were tabulated in the form of graph which showed that optical density of S1, S2 and S3 were closer to that of the control. This confirms the biofilm forming ability of the isolated strains (Table 1).

Table 1: Biofilm Forming Ability of the Isolated Cultures

Sample Name	Absorbance
S1	0.195
S2	0.2379
S3	0.2049
Control	0.4237

The three isolated strains namely S1, S2 and S3 were subjected for crystal violet assay. The absorbance obtained was tabulated.

Characterization of Silver nanoparticles(SNP'S)

The UV-vis spectra showed maximum absorbance at 410nm, which varied with different concentrations of silver nitrates. The absorption spectrum of the synthesized SNP showed a well-defined plasmon band at 410nm, characteristic of nanosized silver.

Biofilm Inhibition Assay

% inhibition was calculated using the formula and tabulated. S1 culture shows 80.69% of inhibition, S2 culture shows 81.33% of inhibition, S3 culture shows 82.10% of inhibition while positive control shows 93.27% of inhibition.

Table 2: Biofilm Inhibition Assay

	Percentage (%)			
Conc (µg)	**S1**	**S2**	**S3**	**Positive Control**
200	64.65	69.36	69.69	81.50
400	77.31	76.81	76.04	83.99
600	78.77	78.75	79.12	86.99
800	79.82	79.10	80.34	88.28
1000	80.69	81.33	82.10	93.27

Minimum Inhibitory Concentration

MIC is theleast concentration of a chemical or a compound that is required to inhibit the growth of microorganism. This is determined by serial dilution method on a 96well plate. After 24hours of incubation, turbidity on the well indicated the growth of microbial culture. The concentration at which no turbidity was observed is noted down and the least concentration in that is taken as the MIC value. Inhibitory concentration of silver nanoparticles was found to be 125µg for S1, 62.5µg for S2 and 62.5µg for S3.

Table 3: MIC value of the Silver nanoparticles against the culture

Name of the sample	**Name of the microorganism**	MIC value (µg)	
		Sample	**Positive control**
Silver nanoparticles	S1 culture	125	15.6
	S2 culture	62.5	15.6
	S3 culture	62.5	31.25

Antibacterial activity of the SNPs against the three isolated cultures by using broth micro dilution method, tetracycline was used as a positive control

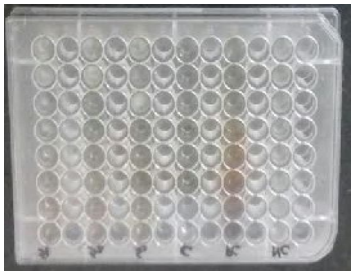


Figure 2:
Concentrations: A-1000µg; **B**-500µg; **C**- 250µg; **D**- 125µg; **E**-62.5µg; **F**- 31.25µg; **G**-15.62µg; **H**- 7.81µg
(1)- S1 culture (3) - S2 culture (5)- S3 culture (7) Control – Broth (9) Positive control – Broth, tetracycline and culture(11) Negative control – Broth and culture

DISCUSSION

The present studies, has yielded positive results, where in leaves of *Artocarpus heterophyllus*, have been used for the synthesis of silver based nanoparticles and their antibacterial and anti-biofilm properties were studied. Different concentrations of the nanoparticles were used and biofilm inhibiting capacity and minimum inhibitory concentration (MIC) value of the leaf extract was calculated. These results showed greater antibacterial andantibiofilm properties against bacteria,

lining the filter membranes. This data clearly suggests that the leaf extract of *Artocarpus heterophyllus* can be effective as an antibiofilm agent and its efficacy can been enhanced when it was processed as silver nanoparticles. These observations, coincide with that of Haleemkhan *et al*., (2015) where silver nanoparticles of leaf extract of *Murraya koenigii* (Indian curry leaf tree) was synthesized and was tested for antibacterial activity against three human pathogenic bacteria *(Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa) and* the bactericidal activity significantly increased in the presence of AgNPs against pathogenic bacteria. A similar kind of study reported by Logeshwari *et al*, (2012), revealed, highest antimicrobial activity of Ag NPs synthesized using plant sources such as *Citrus sinensis* and *C. asiatica*, and there was highly efficient antimicrobial activity against pathogenic bacteria such as *Pseudomonas aeruginosa.* On similar lines, this study also illustrates and reveals the antibacterial and antibiofilm properties of leaf extract of *Artocarpus heterophyllus*. The crystal violet assay for biofilm inhibition and the MIC value of the silver nanoparticles of *Artocarpus heterophyllus* showed an enhanced antibacterial and anti biofilm effect upon bacterial biofilms of water filter membranes, and therefore may be suggested to be used as cleansing agents.

CONCLUSION

The present study revealed the antibacterial and antibiofilm properties of *Artocarpus heterophyllus.* It also illustrates that these properties were greatly enhanced when the leaves were synthesized as silver nanoparticles, and so may be used as cleansing agents for water purifiers as they are ecofriendly and costeffective.

Conflict of Interest

All authors contributed equally for the Project and authors declare there is no conflict of interest

REFERENCES

1. Donlan, R.M., Costeroton, J.W. (2002). Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev.* 15: 167-193.
2. Frank J, Chmielewski R. (2001). Influence of surface finish on the cleanability on stainless steel. *J Food Prot 68(8):1178-82*
3. Haleemkhan A.A., Naseem, Vidya Vardhini B. (2015). Synthesis of Nanoparticles from Plant Extracts ; *International Journal of Modern Chemistry and Applied Science,* 2(3): 195-203
4. Hood, S.K., Zottola, E.A, (1995). Biofilms in food processing. *Food Control,* 6: 9-18.
5. S. Karthick Raja Namasivayam and E.Allen Roy, (2013). Anti biofilm effect of medical plant extract against clinical isolates of biofim of Escherichia coli, *International Journal of Pharmacy and Pharmaceutrical sciences*, 15
6. Li WR, Xie XB, Shi QS, Duan SS, Ouyang YS, Chen YB (2011). Antibacterial effect of silver nanoparticles on *Staphylococcus aureus*. *Biometals,* 24: 135–141.
7. Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, Tam PK, Chiu JF, Che CM, (2006). Proteomic analysis of the mode of antibacterial action of silver nanoparticles. *Journal of Proteome Research,* 5: 916–924.
8. Logeswari P, Silambarasan S, Abraham J (2012). Synthesis of silver nanoparticles using the plants extract and analysis of their antimicrobial property. *J Saudi Chem Soc.* 48(3).
9. Quave CL, Pieroni A, Bennett BC. (2008). Dermatological remedies in the traditional pharmacopoeia of Vulture-Alto Bradano, inland southern Italy. *Journal of Ethnobiology and Ethnomedicine. 4*
10. Rai, M., Yadav, A. and Grade, A. (2009). Silver nanoparticles as a new generation of antiomicrobials". *Biotech. Adv.,* 27: 76-83
11. Sandasi M, Leonard CM, Viljoen AM. (2010). The in vitro antibiofilm activity of selected culinary herbs and medicinal plants against Listeria monocytogenes. *Lett Appl Microbiol,* 50: 30–35
12. Zhao GJ, Stevens SE, (1998). Multiple parameters for the comprehensive evaluation of the susceptibility of Escherichia coli to the silver ion. *Biometals,* 11: 27–32.