ISSN No:-2456-2165

Inhibition of *Pseudomonas aeruginosa* and *Staphylococcus aureus* Biofilm by *Prosopis juliflora* (Sw.) DC. Leaf Extract

Rahul R and Sathyabhama M*
Department of Biotechnology, PSG College of Arts & Science
Coimbatore, Tamilnadu, India

Abstract:- Bacterial biofilm is a complex microbial community which is highly resistant to antimicrobials. Biofilms cause health problems in food industries, hospitals and water environment. Many bacteria use the biofilm formation as a strategy to survive when exposed to antimicrobial compounds and to evade host immune responses. The biofilm formation in biotic and abiotic surface is associated with high rates of mortality in hospitalized patients. New alternative method to control the infection has been raised, now focusing on the therapeutic properties of medicinal plants and their phytochemical compounds with antimicrobial activity. In this study, *Prosopis juliflora* (Sw.) DC. leaf extract with three solvents (acetone, ethanol and water) was assessed for its biofilm inhibition activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The biofilm inhibition was observed in *P. juliflora* leaf extract. The maximum biofilm inhibition of *P. aeruginosa* was observed in the *P. juliflora* acetone leaf extract and for *S. aureus*, the maximum inhibition was observed in *P. juliflora* water leaf extract. This study suggests that plant extract of *P. juliflora* could be effectively used to inhibit the formation of *P. aeruginosa* and *S. aureus* biofilms.

Keywords:- *Bacteria, Biofilm, Prosopis juliflora, Pseudomonas aeruginosa, Staphylococcus aureus.*

I. INTRODUCTION

Biofilms are three dimensional microbial communities where a group of microorganisms are embedded in an extracellular complex matrix of polymeric substances. In nature, 99% of bacteria species can aggregate to form as biofilms, which are produced when bacteria extend their multiplication vertically and horizontally on a surface. The multicellular sessile colony produces a matrix which is composed of proteins, polysaccharides and extracellular DNA which allows them to form micro-niches and maintain steep chemical gradients. Many bacteria forms biofilm as a strategy to survive in an environmental with antimicrobial compounds and host immunity (Heidari et al., 2018).

Quorum sensing is a process of cell-cell communication, which allows bacteria to send information about cell density and adjust gene expression. It involves process such as detection, response and production to extracellular signaling molecules called auto-inducers (AIs). If the bacterial population density increases, auto-inducers accumulate increases and bacteria monitor this information to modify the changes in their cell numbers and decrease gene expression. Quorum sensing controls the process like biofilm formation, sporulation, antibiotic production, bioluminescence, virulence factor secretion and competence (Steven et al., 2012).

Pseudomonas aeruginosa is a gram negative bacteria belongs to *Pseudomonadales* family. It is rod shaped, monoflagellated and asporogenous bacterium. *Pseudomonas aeruginosa* is about 1-5µm long and 0.5 - 1.0µm wide. It is found in environments such as soil, water, humans, animals, plants, sewage and hospitals. *Pseudomonas aeruginosa* is a leading pathogen at most medical centers, carrying a 40 – 60% mortality rate (Lederberg et al., 2000). The functional iron serves as a signal for *P. aeruginosa* biofilm development. The study by Banin et al (2005) reveals the mechanism of iron signal in biofilm development is active transport of chelated iron or the level of internal iron *P. aeruginosa* produces at least 3 polysaccharides (alginate, Pel and Psl) for the stability of the biofilm structure (Ryder et al., 2007).

Staphylococcus aureus is a gram positive bacteria belongs to *Staphylococcaceae* family. It is spherical shaped, immobile and form grape like clusters. *Staphylococcus aureus* colonies are yellow in colour and grow large on a rich medium. *Staphylococcus aureus* is an antibiotic resistant pathogen. It is classified as Methicillin-resistant *Staphylococcus aureus* (MRSA). *Staphylococcus aureus* is facultative anaerobes which means they can grow by aerobic respiration or fermentation that produces lactic acid (Chan et al., 2006). *S. aureus* is the leading bacteria in causing nosocomial infections by forming biofilm (Reffuveille et al., 2017). The antimicrobial resistance of *S.aureus* is increased when it forms superbugs such as methicillin resistant *S. aureus* (MRSA) (Reffuveille et al., 2017).

P. juliflora belongs to *Fabaceae* family; Kingdom: Plantae; Order: Fabales. *P. juliflora* is known as mesquite which thrives in most soils including sandy, rocky, poor and saline soils with an altitude range of 300 – 1900m above sea level (Benson et al. 1941). Its deep tap roots help it to access sub surface waters. It takes over range lands and replaces native vegetation. *P. juliflora* have numerous negative effects which include complete loss of pasture and range lands for both domestic and wild ruminants

ISSN No:-2456-2165

(Henderson et al. 2001). It causes loss due to access to water and the destruction of fishing nets by the thorns. Even though the plant has much negative effects, in this work, we investigated its anti-biofilm activity. *P. juliflora* has been included in the Global Invasive Species Data (GISD 2010) (Singh et al., 2012).

In this study, we aimed to evaluate the biofilm inhibition activity of *P. juliflora* leaf extracts with three solvents against the biofilms formed by *P. aeruginosa* and *S. aureus* using an *in vitro* assay.

II. MATERIALS AND METHOD

A. Bacterial strains used

In our study, *P. aeruginosa* (Gram negative) and *S. aureus* (Gram positive) strains were used. Both the strains were sampled from human corneal infection, collected from the Department of Microbiology, Aravind Eye Hospital, Coimbatore, Tamil Nadu, India.

Luria Bertani broth was prepared by dissolving 1g of tryptone, 0.5g of yeast extract, 1g of sodium chloride in 100ml distilled water and sterilized in an autoclave for 15min, then cooled at room temperature. Both the bacterial strains were inoculated separately in tubes and kept for overnight incubation at 37°C in a rotary shaker.

B. Plant material used

The plant used in this work is *P. juliflora* leaves was collected from natural habitats in North Coimbatore region, Tamil Nadu, India (Figure 1a and b)

Fig 1:- Plant Used. a) *P. juliflora* plant b) *P. juliflora* plant leaves

C. Plant leaf extract preparation

Fresh leaves of *P. juliflora* were washed with a running tap water to remove all dust particles and rinsed in sterile distilled water. 5g of leaves were ground with three solvents (Acetone, Ethanol, Water). The leaf extract was filtered with Whatmann filter paper and stored in Samsung refrigerator at 4℃ (Rosewin et al., 2016).

D. Biofilm inhibition assay

Biofilm inhibition assay was performed in glass test tubes according to the modified protocol of Liziana et al., 2013. To 2ml of sterilized LB liquid media tubes, 40µl of overnight culture of *P. aeruginosa* and *S. aureus* was inoculated. Along with the inoculum, 20µl of *P. juliflora* extract was added to respective test tubes. 2ml of LB liquid media acts as blank. The control contains 2ml sterilized Lb broth and 20µl of distilled water instead of plant extract. The tubes were incubated in orbital shaker at 37℃ for overnight. After overnight incubation, the inoculum was discarded and the tubes were stained with 0.4% crystal violet (5ml) for 45 minutes and then washed off with distilled water to remove the excess stain and allowed to dry. After drying, the dye bound to the attached cells was solubilized with 70% ethanol (4ml) per tube. The colour intensity was measured in a spectrophotometer as absorbance at a wavelength of 550nm (A_{550}). The percentage of bacterial biofilm inhibition was determined for each extract by, subtracting control optical density (OD) minus experimental OD and dividing it by control OD.

III. RESULTS AND DISCUSSION

A. Biofilm Inhibition Assay

Effect of plant extracts on biofilm formation by *P. aeruginosa* and *S. aureus* was evaluated by modified protocol of Liziana et al., 2013.

The results are expressed as percentage of biofilm inhibition with respect to control (Table 1). Formation of *P. aeruginosa* biofilm on glass tubes in the presence of plant extracts of *P. juliflora* was shown in Figure 2. Among 3 plant extracts of *P. juliflora*, the maximum inhibition was seen in *P. juliflora* acetone leaf extract (Figure 4a), while the other extracts like *P. juliflora* leaf ethanol and leaf water extract shows 5.87% and 10.33% inhibition respectively (Table 1).

ISSN No:-2456-2165

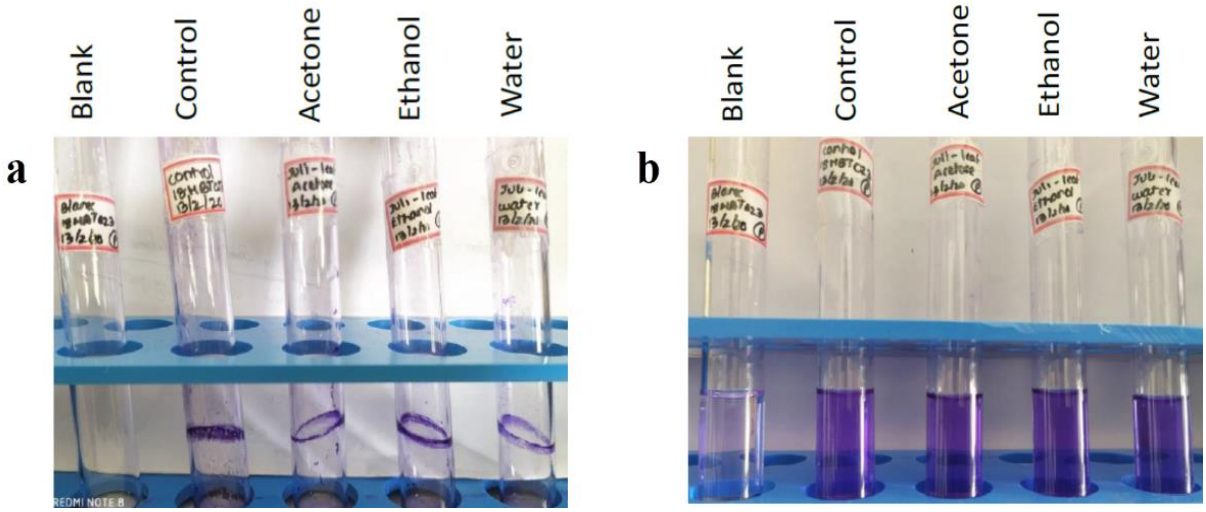

Fig 2:- Biofilm inhibition assay a) Dry *P. aeruginosa* biofilm stained with 4ml of crystal violet. b) Dye bound to the attached cells was solubilized with 70% ethanol

The results are expressed as percentage of biofilm inhibition with respect to untreated control (Table 2). Formation of *S. aureus* biofilm on glass tubes in the presence of different plant extracts of *P. juliflora* was shown in Figure 3. Among 3 plant extracts of *P. juliflora*, the maximum inhibition was seen in *P. juliflora* water leaf extract (51.89%) (Figure 4b), while the other extracts like *P. juliflora* leaf acetone and leaf ethanol extract shows 39.17% and 44.92% inhibition respectively (Table 2).

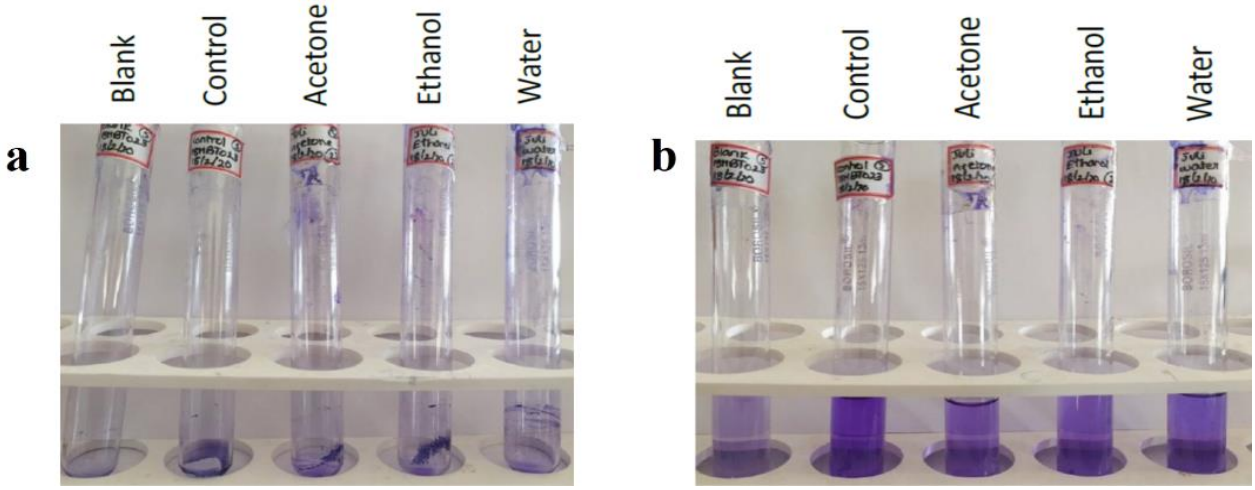

Fig 3:- Biofilm inhibition assay a) Dry *S. aureus* biofilm stained with 4ml of crystal violet. b) Dye bound to the attached cells was solubilized with 70% ethanol

S.No	***Plant Leaf Extract***	***Solvent***	***P. aeruginosa***		***S. aureus***	
			OD Reading at 550nm	***Percentage of Biofilm inhibition (%)***	***OD Reading at 550nm***	***Percentage of Biofilm inhibition(%)***
1	*Blank*	*-*	*0.00*	*-*	*0.00*	*-*
2	*Control*	*-*	*0.919*	*-*	*0.975*	*-*
3	*P. juliflora*	*Acetone*	*0.737*	*19.80*	*0.469*	*39.17*
4		*Ethanol*	*0.865*	*5.87*	*0.537*	*44.92*
5		*Water*	*0.824*	*10.33*	*0.593*	*51.89*

Table 1:- Biofilm inhibition percentage of plant extracts of *P. juliflora* against *P. aeruginosa* and *S. aureus*

ISSN No:-2456-2165

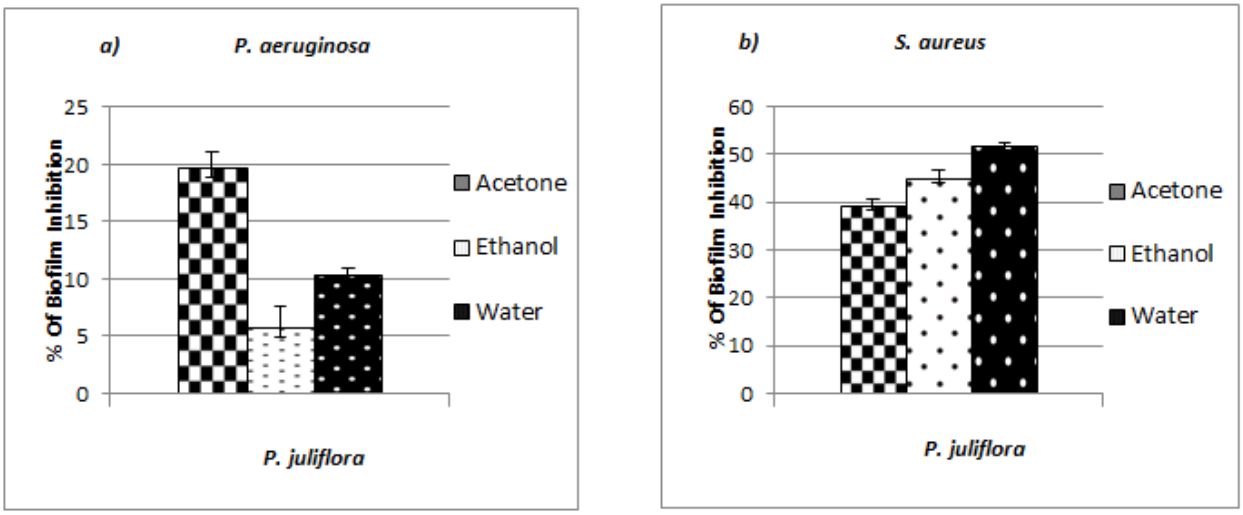

Fig 4:- Percentage of biofilm inhibition a) Effect of different plant extracts of *P. juliflora* on *P. aeruginosa* biofilm b) Effect of different plant extracts of *P. juliflora* on *S. aureus* biofilm

IV. CONCLUSION

In our study, *P. aeruginosa* and *S. aureus* biofilm formations were examined. Both the strains were isolated from human corneal infection. Three extracts of *P. juliflora* leaves were used. The highest percentage of biofilm inhibition against *P. aeruginosa* and *S. aureus* was noticed in *P. juliflora* leaf acetone extract and *P. juliflora* leaf water extract, respectively. The finding demonstrated that *P. juliflora* leaf extract can be good candidate for future studies as biofilm inhibiting agents, due to its ability to inhibit the biofilms of *P. aeruginosa* and *S. aureus*. It can be concluded that *P. juliflora* is a rich source of novel and biologically active metabolites. Metabolites produced by this plant of *Fabaceae* may be of great interest for the pharmaceutical industry and medicinal research. So this plant *P. juliflora* can be recommended as a plant of pharmaceutical importance. Further metabolite analysis will pave way to identify the pharmacological importance of this plant.

REFERENCES

[1]. Saddiq, Amna Ali and Huda Alghamdi. "Aloe vera extract: A novel antimicrobial and antibiofilm against methicillin resistant Staphylococcus aureus strains." Pakistan journal of pharmaceutical sciences 31 5(Supplementary) (2018): 2123-2130.

[2]. Jabalameli, Fereshteh et al. "Evaluation of biofilm production and characterization of genes encoding type III secretion system among Pseudomonas aeruginosa isolated from burn patients." Burns : journal of the International Society for Burn Injuries vol. 38,8 (2012): 1192-7. doi:10.1016/j.burns.2012.07.030

[3]. Heidari, H et al. "Characterization of virulence factors, antimicrobial resistance patterns and biofilm formation of Pseudomonas aeruginosa and Staphylococcus spp. strains isolated from corneal infection." Journal francais d'ophtalmologie vol. 41,9 (2018): 823-829. doi:10.1016/j.jfo.2018.01.012

[4]. Yuyama, Kamila Tomoko et al. "Bioactive Compounds Produced by Hypoxylon fragiforme against Staphylococcus aureus Biofilms." Microorganisms vol. 5,4 80. 12 Dec. 2017, doi:10.3390/microorganisms5040080

[5]. Bazargani, Mitra Mohammadi, and Jens Rohloff. "Antibiofilm Activity of Essential Oils and Plant Extracts Against Staphylococcus Aureus and Escherichia Coli Biofilms." Food Control 61 (2016): 156–164. Crossref. Web.

[6]. El-Ganiny, Amira M. et al. "Prevention of Bacterial Biofilm Formation on Soft Contact Lenses Using Natural Compounds." Journal of Ophthalmic Inflammation and Infection 7.1 (2017): n. pag. Crossref. Web.

[7]. Vazirani, Jayesh, Siva Wurity, and Md Hasnat Ali. "Multidrug-Resistant Pseudomonas Aeruginosa Keratitis." Ophthalmology 122.10 (2015): 2110–2114. Crossref. Web.

[8]. Khan, Shamila et al. "Identification of Pathogenic Factors Potentially Involved in Staphylococcus Aureus Keratitis Using Proteomics." Experimental Eye Research 151 (2016): 171–178. Crossref. Web.

[9]. Banar, Maryam et al. "Evaluation of Mannosidase and Trypsin Enzymes Effects on Biofilm Production of Pseudomonas Aeruginosa Isolated from Burn Wound Infections." Ed. Abdelwahab Omri. PLOS ONE 11.10 (2016): e0164622. Crossref. Web.

[10]. Sadighian, Hooman et al. "Genetic Characterization of Pseudomonas Aeruginosa-Resistant Isolates at the University Teaching Hospital in Iran." Advanced Biomedical Research 4.1 (2015): 156. Crossref. Web.

[11]. Lederberg, Joshua et al. "Pseudomonas Encyclopedia of Microbiology" Second Edition. Volume 3. San Diego, 2000. p. 876-891.

[12]. Costerton, W., and Anwar, H. Pseudomonas aeruginosa: The Microbe and Pathogen. Pseudomonas aeruginosa Infections and Treatment. 1994. p.1-17.

[13]. Botzenhardt, K., and Doring, G. Ecology and epidemiology of Pseudomonas aeruginosa. Pseudomonas aeruginosa as an Opportunistic Pathogen. 1993. p. 1-7.
[14]. Fick, R. Pseudomonas aeruginosa—the Microbial Hyena and Its Role in Disease: An Introduciton. Pseudomonas aeruginosa: The Opportunist. 1993. p. 1-6.
[15]. Gillaspy, Worrell, Orvis, et al in Gram-positive Pathogens (eds Fischetti, V., Novick, R., Ferretti, J., Portnoy, D. & Rood, J.) 381-410 (ASM Press, Washington, D.C, 2006).
[16]. Banin, E., M. L. Vasil, and E. P. Greenberg. "From The Cover: Iron and Pseudomonas Aeruginosa Biofilm Formation." Proceedings of the National Academy of Sciences 102.31 (2005): 11076–11081. Crossref. Web.
[17]. Ryder, Cynthia, Matthew Byrd, and Daniel J Wozniak. "Role of Polysaccharides in Pseudomonas Aeruginosa Biofilm Development." Current Opinion in Microbiology 10.6 (2007): 644–648. Crossref. Web.
[18]. Reffuveille, Fany et al. "Bone Environment Influences Irreversible Adhesion of a Methicillin-Susceptible Staphylococcus Aureus Strain." Frontiers in Microbiology 9 (2018): n. pag. Crossref. Web.
[19]. Henderson, L. "Alien weeds and invasive plants. A complete guide to declared weeds and invaders in South Africa". Plant Protection Research Institute Handbook No. 12, 300pp (2001). PPR, ARC South Africa.