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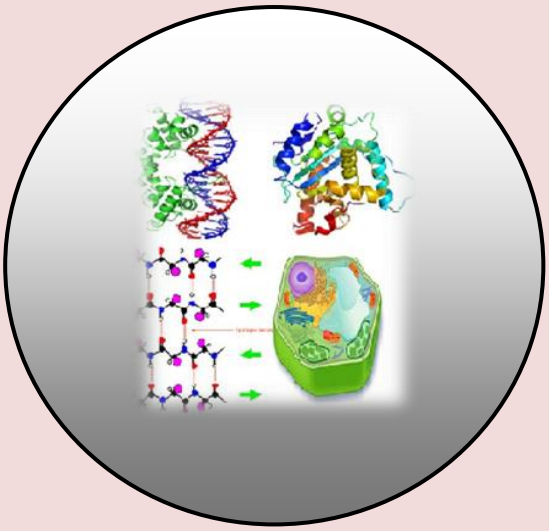

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RESEARCH PAPER

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An *in-vitro* Antibiofilm Activity of *Spirulina platensis*

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ABSTRACT

Biofilm is a complex assemblage of microbial community highly resistant to antibiotics. Biofilm inhibition is considered as a significant drug target to control bacterial infections. In this study, the effect of methanolic extract of freshwater filamentous algae Spirulina platensis was screened for its antibiofilm activity on the clinical isolates Pseudomonas aeruginosa and Staphylococcus aureus. Phytochemical screening in petroleum ether, dichloromethane, chloroform, ethyl acetate, methanolic, acetone extract of S. platensis showed the presence of phenolics, flavonoids, alkaloids, saponins and steroids. The minimum inhibitory concentration of S. platensis methanolic extract was found to be at 3 mg/ml for both clinical isolates. The methanolic extract (1.5 mg/ml) of S. platensis reduced the biofilm formation of P. aeruginosa and S. aureus to 86 and 83.5 % respectively.
Keywords: Antibiofilm, Quorum sensing, Spirulina platensis and Phytochemical.

INTRODUCTION

Several human infectious diseases are associated with biofilm-forming microorganisms. Biofilms are a complex assemblage of cells on abiotic or biotic surfaces encased in a self-synthesized exopolymeric matrix made of polysaccharides, DNA, proteins, lipids. The biofilm prevents the bacterial population from clearance by the immune system, and an antibiotic contributes to the pathogenesis of chronic infections (Ganesh *et al*., 2017). Further, the biofilm-forming bacteria colonize a variety of medical devices and lead to severe complications in the treatment, and sometimes the implanted device has to be removed or replaced. It has been found that bacteria's living in a biofilm mode of growth is 1000 times resistant to antibiotics than their planktonic cells (Packiavathy *et al*., 2012). Biofilm formation and its structure maintenance are related to quorum sensing (QS) (Wang *et al*., 2014). *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the opportunistic pathogen with QS mechanism to control the expression of several genes involved in the virulence factors and biofilm formation (Khan *et al*., 2009; Vasavi *et al*., 2017).

Several reports are available for antibiofilm agents from natural resources, particularly medicinal plants, marine sponges, algae and seaweeds (Nithya *et al*., 2010). But recently freshwater algae are gaining importance in pharmaceuticals because of their antibacterial activity. *Spirulina platensis* is a blue-green, edible, multicellular, filamentous, photoautotrophic algae belonging to Cyanophyta, known for its rich source of protein, vitamins, minerals (Jensen and Kneuster, 1993; Dillon *et al*., 1994; Mishima *et al*., 1998; Wang *et al*., 2007; Priyadharshini and Ratt, 2012, Desouki *et al*., 2015).

S. platensis extract shows therapeutic properties, such as the ability to prevent cancers, decrease blood cholesterol level, reduce nephrotoxicity of pharmaceuticals and toxic metals and provide protection against the harmful effect of radiation (Kumar *et al*., 2011). *S. platensis* is one of the most potential microalgae explored for antibacterial, antiviral, antibiofilm and anti-cancerous properties. The present work was carried out to analyse the antibiofilm activity of *S. platensis* against the clinical strains *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

MATERIAL AND METHODS

Preparation of *Spirulina platensis* extract

S. platensis powder was procured from E.I.D Parry (INDIA) limited, Tamilnadu**.** Ten gram of dried powder of *S. platensis* was weighed and extracted with 100 ml of solvents. The crude substance obtained after the evaporation was dissolved in DMSO and used for analysis.

Collection of microbial strains

The bacteria chosen for antibiofilm assay were the clinical isolates of *P. aeruginosa* and *S. aureus* from the Micro lab, Coimbatore. The stock cultures were maintained in nutrient agar slants at 4^{o}C.

Phytochemical analysis

The various phytochemical screening such as phenols, flavonoids, alkaloids, saponins and steroids present in the petroleum ether, dichloromethane, chloroform, ethyl acetate, methanol, and acetone solvent extracts of *S. platensis* were carried as per the standard procedure is given by Harborne, 1998.

Minimum inhibitory concentrations

Minimal Inhibitory Concentration (MIC) was considered as the lowest concentration in which there would be no visible growth of bacteria after 24 hours of incubation at 37°C (Wiegana *et al.,* 2008). The MIC of different extracts of *S. platensis* was performed against *P. aeruginosa* and *S. aureu*s by serial dilution method using a 96-well microtiter plate. Test inoculums of 100 µl of 18 h culture were added in 96 well microtitre plates containing various solvent extracts of *S. platensis* in 250 µl of nutrient broth media. Then the plates were incubated at 37°C for 24 hours. The lowest concentrations without visible growth were recorded as MIC.

Inhibition of Biofilm formation

The biofilm formation in a polystyrene microtiter plate was determined as described by O`Toole (2011). Three different concentrations (1.5, 0.75 and 0.37 mg/ml) of *S. platensis* extracts (100 µl) were pipetted into the wells of a microtiter plate. Then an overnight culture of *P. aeruginosa* and *S. aureus* was diluted to 1:100 in fresh medium and 100 µl of the dilution was added to each well and were incubated for 24 h at 37°C. Three controls were maintained: wells containing extract and growth medium (extract control); the wells containing the growth medium and the inoculum; and the wells containing only the growth medium (Saeidi et al., 2015).

The planktonic cells were removed by washing the wells three times with sterile water after incubation. To fix the adherent cells, 200 µl of absolute ethanol was added to each well for 15 min. Then 125µl crystal violet (0.1%) was added to all the wells and incubated for 30 minutes at room temperature. The excess stain was removed and washed with sterile distilled water to remove unbound crystal violet stain and the plates were air dried. Finally, the adhered biofilm bound crystal violet was eluted with 125 µl of 30 % glacial acetic acid per cell. The absorbance was measured using the ELISA reader at 595nm (Schillaci *et al.,* 2013*).*

MTT assay

MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide, a yellow tetrazole) assay is one of the most commonly method used for assessing cell metabolic activity/cytotoxicity/cell viability. Higher OD value indicates an increasing number of surviving organisms in the biofilm. Three different concentrations (1.5, 0.75 and 0.37 mg/ml) of *S. platensis* extracts (100 µl) were pipetted into the wells. A cell suspension was prepared as described in the MIC, and 100 µl were inoculated in 96 well microtiter plates. After incubation at 37°C for 24 h, supernatants were discarded and washed three times with sterile water. After those biofilms were incubated with MTT (0.5 mg /ml) at 37°C for overnight. The number of viable bacteria was determined by measuring the ability to reduce the yellow tetrazolium salt to purple formazan product. The absorbance was read at 570nm (Nemati *et al.,* 2013). Percentage inhibition was calculated using the equation

% Cell inhibition= 100 – [(At-Ab)/(Ac-Ab)] ×100

Motility assay

The effect of control and extracts on the swimming and swarming motility was determined by placing 2 µl of treated and untreated cultures in the center of swim and swarm agar plates. The plates were incubated at 37°C for 48h. The activity was determined by the decrease in the swimming and swarmed area radius (Wang et al., 2007).

Statistical analysis

All the experiments were conducted in triplicates and the data are presented as a mean ± standard deviation.

RESULTS AND DISCUSSION

Phytochemical analysis

Phytochemicals are the natural bioactive compounds such as alkaloids, flavonoids, phenols, terpenoids and saponin present in plants. They have a great attraction because of their preventive role in various diseases. The various solvent extracts of *S. platensis* were screened for their phytochemical constituents and the results are shown in table 1. The petroleum ether, dichloromethane, chloroform, ethyl acetate, methanol and acetone extract of *S. platensis* showed the presence of phenols, flavonoids, alkaloids and saponins. Glycosides and terpenoids were absent in all the extracts. These results were in accordance with the data obtained by Ali et al. (2017), whereas, Mane and Chakraborty (2018) reported the presence of terpenoids and glycosides in the acetone and methanolic extracts of *S. platensis.* The presence of steroids was observed only in dichloromethane, chloroform and methanol extract. Thamilmaraiselvi and Steffi (2018) also reported the presence of steroids in the chloroform and methanolic extracts of *Spirulina.*

Table 1. Phytochemical screening of different extracts of *S.platensis.*

S. No	Phytochemical Constituents	Extracts of *Spirulina platensis*					
		PE	DI	CF	EA	MN	AE
1	Phenols	+	+	+	+	+	+
2	Flavonoids	+	+	+	+	+	+
3	Alkaloids	+	+	+	+	+	+
4	Glycosides	-	-	-	-	-	-
5	Saponins	+	+	+	+	+	+
6	Terpenoids	-	-	-	-	-	-
7	Steroids	-	+	+	-	+	-

"+" represents presence "-" represents absence

PE - Petroleum ether, DI - Dichloromethane, CF - Chloroform, EA - Ethyl acetate, MN – Methanol, AE- acetone

Table 2. MIC value of different solvent extracts of *S. platensis* against *P. aeruginosa* and *S. aureus.*

Extracts	*P. aeruginosa*							*S. aureus*						
	4.0	3.0	2.0	1.0	0.5	0.2	0.1	4.0	3.0	2.0	1.0	0.5	0.2	0.1
Methanol	-	-	+	+	+	+	+	-	-	+	+	+	+	+
Chloroform	-	+	+	+	+	+	+	-	-	+	+	+	+	+
Ethyl acetate	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Dichloromethane	-	-	+	+	+	+	+	-	-	-	+	+	+	+
Petroleum ether	-	+	+	+	+	+	+	+	-	+	+	+	+	+
Acetone	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ethanol	-	-	+	+	+	+	+	-	+	+	+	+	+	+

(-) indicates inhibition (+) indicates ineffectiveness

Minimum inhibitory concentration (MIC)

Biofilm-forming bacteria have shown 1000-fold resistance to antimicrobials and largely evade host immune system (Hoffman *et al*., 2005).

The effect of different solvent extracts of *S. platensis* on the growth of bacteria for *P. aeruginosa* and *S. aureus* was studied and the results obtained are shown in Table 2. The MIC value of the methanolic extract from the Table-1 it is evident that the methanolic extract at the concentration of 3 mg/ml was found to inhibit the growth of both the clinical isolates. Therefore, 3 mg/ml was considered as minimum inhibitory concentration and a concentration of 1.5 mg/ml, 0.75 mg/ml and 0.37 mg/ml was chosen for further analysis of antibiofilm activity.

Antibiofilm activity

The antibiofilm activity of methanolic extract of *S. platensis* at the concentration of ½, ¼ and ⅛, of MIC was tested against *P. aeruginosa* and *S. aureus.* There was a significant reduction in biofilm formation was observed at a concentration of 1.5 mg/ml (Figure. 1). The percent inhibition of 70, 58 and 40 % for *P. aeruginosa* and 78, 64 and 42 % for *S. aureus* was observed. Lewis Oscar *et al*. reported that the methanolic extract of *S. platensis* at a concentration of 100 ng/ml showed 69 and 72% of inhibition against *P. aeruginosa* MTCC1934 and MTCC2453 respectively; 83 and 69% against *S. aureus* MTCC96 and 2940; 89 % for *C. violaceum*; and 90 % for *V. parahaemolyticus* (Lewis Oscar *et al.,* 2018). Maximum biofilm inhibition was observed for *S. aureus* compared to *P. aeruginosa.* The results obtained showed in line with Lewis Oscar *et al. (*2018).

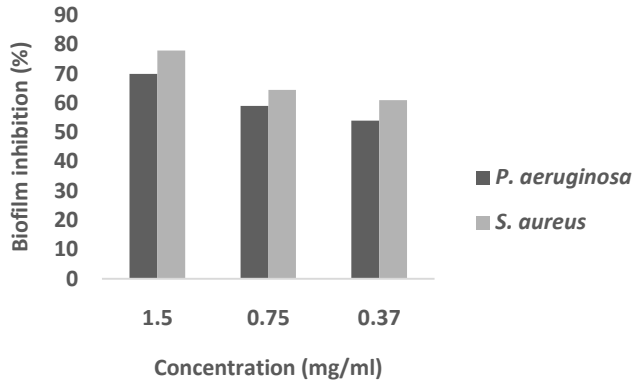

Figure 1. Biofilm inhibitory effect of *S. platensis* against *P. aeruginosa and S. aureus.*

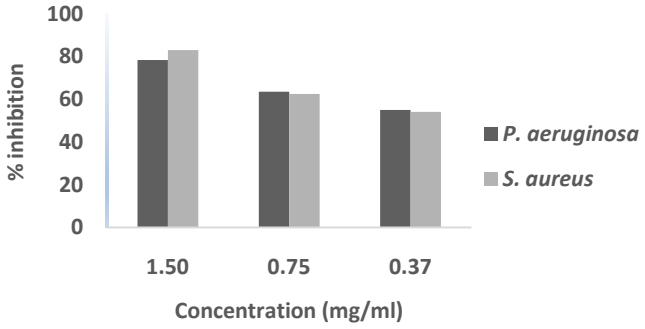

Figure 2. Effect of methanolic extract of *S. platensis* on the viability of bacteria.

MTT assay

The methanolic extract of *S. platensis* at different concentrations reduced the viability of *P. aeruginosa* and *S. aureus* significantly. The maximum reduction in the viability was found at 1.5 mg/ml concentration compared to other concentrations. Lewis Oscar reported that *S. platensis* methanolic extract showed higher biofilm inhibition at 0.1 µg/ml against *P. aeruginosa* (Lewis Oscar et al., 2018).

Swarming motility

The motility of bacteria plays a major role in biofilm formation. Flagella are involved in the swimming and swarming motility of the biofilm forming bacteria. Swarming motility is one of the virulence factors involved in biofilm development and dispersion (Butler *et al*., 2010). Therefore, inhibition of motility might limit the number of bacterial cells reaching the surface, therefore decreasing the biofilm formation. Lopex also reported that the swarming strains of *P. aeruginosa* showed an increased resistance to multiple antibiotics (Carson *et al*., 2002; Lopex, 2010). The results obtained in this study confirmed the antibiofilm activity of methanolic extract of *S. platensis* (Figure. 3).

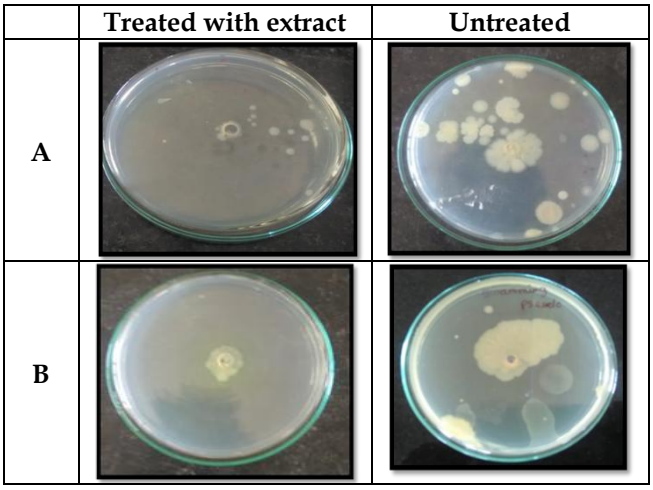

Figure 3. Swarming motility (A) *P. aeruginosa* (B) *S. aureus.*

CONCLUSION

The phytochemical analysis of various solvent extracts of *S. platensis* revealed the presence of phenols, flavonoids, alkaloids and saponins. They are all considered to have potential antibacterial, antioxidant and anticancer properties. The present study confirmed the antibiofilm potential of *S. platensis* against the clinically important pathogenic organism's *P. aeruginosa* and *S. aureus.*

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REFERENCES

Ali, H.E.A., Shanab, S.M.M., Abo-State, M.A.M. et al. (2014). Screening of Microalgae for Antioxidant activities, Carotenoids and Phenolic Contents. *Applied Mechanics and Materials*, 625, 156-159.

Butler, M.T., Wang, Q. and Harshey, R.M. (2010). Cell density and mobility protect swarming bacteria against antibiotics, *Proc. Natl. Acad. Sci. Unit. States Am.* 107 (8) 3776–3781.

Carson, C.F. Mee and B.J. Riley, T.V. (2002). Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphyloccocus aureus* determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy, Antimicrob. *Agents Chemother*. 46 (2002) 1914–1920.

Dillon, J.C., Phuc, A.P. and Dubacq, J.P. (1994). Nutritional value of the algae *Spirulina*. *World Rev Nutr and diet.* 77:32-46.

El-Desouki, N.I., Tabl, G.A., Abdel-Aziz, K.K., Salim, E.I., Nazeeh, N. (2015). Improvement in beta-islets of Langerhans in alloxan-induced diabetic rats by erythropoietin and *Spirulina*. *The Journal of Basic and Applied Zoology* 71: 20-31.

Vasavi, H.S., Sudeep, H.V., Lingaraju, H.B. and Shyam Prasad, H. (2017). Bioavailability-enhanced Resveramax™ modulates quorum sensing and inhibits biofilm formation in *Pseudomonas aeruginosa* PAO1. *Microbial Pathogenesis 104.*

Harborne, J.B. (1998). Phytochemical Methods. 3rd Edition, Chapman and Hall, London.

Hoffman, L. R., D'Argenio, D. A., MacCoss, M. J., Zhang, Z., Jones, R. A., and Miller, S. I. (2005). Aminoglycoside antibiotics induce bacterial biofilm formation. Nature 436, 1171–1175.

Issac Abraham Sybiya Vasantha Packiavathy, Palani Agiladeswari, Khar Syed Musthafa Shunmugiah Karutha Pandian and Arumugam Veera Ravi, (2012). Antibiofilm and quorum sensing inhibitory potential of *Cuminum cymium* and its secondary metabolite methyl eugenol against Gram negative bacterial pathogen, *Food Research International*, 45: 85-92.

Jensen, S. and G. Knutsen (1993). Influence of light and temperature on photoinhibition of photosynthesis in *Spirulina platensis*. *Journal of Applied Phycology*, 5: 495-504.

Khan, M.S.A., Zahin, M., Hasan, S. Husain, F.M. and Ahmad, I. (2009). Inhibition of quorum sensing regulated bacterial functions by plant essential oils with special reference to clove oil. *The Society for Applied Microbiology, Letters in Applied Microbiology*, 354–360.

Kumar, A. and Ting, Y.P. (2013). Effect of sub-inhibitory antibacterial stress on bacterial surface properties and biofilm formation. Colloids and Surfaces B-Biointerfaces 111: 747–754. *Lopex*, 2010.

Lopez, D. Vlamakis. H . and Kotler, R. (2010). Biofilms. Cold Spring Harbor Perspect. Biol. 2(7): 1-11.

Mane, R.S and Chakraborty, B. (2018). Phytochemical screening of Spirulina platensis extracts from Rankala Lake. J. Algal Biomass Utln. 9(4): 38-4

Mishima, T., J. Murata, M. Toyoshima, H. Fujii, M. Nakajima, T. Hayashi, T. Kato and I. Saiki, (1998). "Inhibition of tumor invasion and metastasis by *calcium spirulina* (Ca-SP), a novel sulfated was polysaccharide derived from blue-green alga, *Spirulina platensis*. *Clin. Exp. Metastasis*, 16(6): 541-50.

Nemati, F. Dehpouri, A. A. Eslami, B. Mahdavi, V. Mirzanejad, S. (2013). Cytotoxic Properties of Some Medicinal Plant Extracts from Mazandaran, Iran, *Iranian Red Crescent Medical Journal*, 15 (11): e8871

Nithya, C., Aravindraja, C. and Pandian, S.K. (2010). *Bacillus pumilus* of Palk Bay origin inhibits quorum sensing mediated virulence factors in Gram negative bacteria, *Research in Microbiology*, 60, 454-460.

O'Toole, G.A. (2011). Microtiter dish biofilm assay. JoVE. 47.

Priyadarshani, I. and Rath, B., (2012). Commercial and industrial applications of microalgae -A Review. *Journal of Algal Biomass Utilization* 3(4): 89-100.

Saeidi, N., Wong, C.K., Lo, T.M., Nguyen, H.X., Ling, H. et al. (2015). Engineering microbes to sense and eradicate *Pseudomonas aeruginosa*, a human pathogen. *Molecular Systems Biology* 7: 521. pmid: 21847113.

Ganesh, P.S., and Rai, V.R.S. (2017). Attenuation of quorum-sensing-dependent virulence factors and biofilm formation by medicinal plants against antibiotic resistant *Pseudomonas aeruginosa. J Tradit Complement Med* 8(1):170-177.

Schillaci, D., Cusimano, M.G., Cunsolo, V., Saletti, R., Russo, D., Vazzana, M., Vitale, M. and Arizza, V. (2013). Immune mediators of sea-cucumber *Holothuria tubulosa* (Echinodermata) as source of novel antimicrobial and anti-staphylococcal biofilm agents. *AMB Express*. Jun 24; 3 (1): 35.

Thamilmaraiselvi B, Steffi P F. (2018). Investigation of phytochemical constituents in Spirulina fusiformis for antibacterial activity. *Natl* **J** *Physiol Pharm Pharmacol* 8: (11):1491-1495.

Wang, S., Yu S. and Zhang, Z. (2014). Coordination of swarming motility, biosurfactant synthesis, and biofilm matrix exopolysaccharide production *in Pseudomonas aeruginosa*, *Appl Environ Microbio* 80: 6724-6732.

Wang, L., Pan, B., Sheng, J., Xu, J. and Hu, Q. (2007). Antioxidant activity of *Spirulina platensis* extracts by supercritical carbon dioxide extraction. *Food Chemistry* 105: 36-41.

Wiegana I, Hilpert, Hancock RE. (2008). Agar and broth dilution method to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. W. Nat. Protoc 3: 163-175.

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