African Journal of Basic & Applied Sciences 8 (4): 208-211, 2016
ISSN 2079-2034

DOI: 10.5829/idosi.ajbas.2016.208.211

Evaluation of Plant Extracts on Biofilm

[1]P. Ruban and [2]K. Gajalaksmi

[1]Department of Biotechnology, SNMV College of Arts and Science,
Malumachampatti Coimbatore-50 Tamil Nadu, India
[2]Department of Plant Biology and Plant Biotechnology,
PSGR Krishnammal College for Women, Peelamedu, Coimbatore, Tamil Nadu, India

Abstract: A biofilm is any group of microorganisms in which cells stick to each other on a surface and plant derived products or drugs have been used since time immemorial in health care for treatments of a variety of infectious diseases [1]. In this study, aqueous extract from different plants (*Azadirachta indica, atharanthus roseus, Tectona grandis*) were investigated for antimicrobial activity. The microorganism tested were *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa.* All species showed some antimicrobial activity against *S. aureus, Pseudomonas aeruginosa* in 50 µl concentration, zone of inhibition (MIC) such as 28.2, 31, 35 mm. This study showed that these plant species could be useful sources for new antimicrobials.

Key words: Biofilm · Azadirachta indica · Atharanthus roseus · Tectona grandis · Antimicrobial

INTRODUCTION

Bacterial biofilm are formed when unicellular organisms come together to form a community that is attached to a solid surface and encased in an exopolysaccharide matrix. Biofilms can be made up of single or multiple bacterial species. For example, it has been estimated that dental biofilms contain >500 different bacterial taxa [2]. The presence of biofilms is common in food industry. Biofilms can exist on all types of surfaces in food plants ranging from plastic, glass, metal, wood to food products [3]. The attachment of the bacteria to the food product or the product contact surfaces leads to serious hygienic problems and economic losses due to food spoilage. In addition to that, a number of reports have appeared on the persistence of several food borne pathogens on food contact surfaces. For these reasons, it is considered that the presence of biofilms in the food systems is a serious public health risk.

Medicinal plant-derived compounds have increased widespread interest in the search of alternative antibacterial agents because of the perception that they are safe and have a long history of use in folk medicine for the treatment of infectious diseases [4]. These derived products or drugs have been used since time immemorial in health care for treatments of a variety of infectious diseases [5]. In oral health care, use of plant twigs or leaves was in practice globally [6]. The introduction of allopathic or chemical based drugs caused the gradual decline in the use of herbal medicine. In this study, we evaluated antimicrobial effects of extracts from different plant species against biofilm bacteria.

MATERIALS AND METHODS

Sample Collection: Biofilm were collected from waste water treatment plant which receive wastewater from urban households and the samples were inoculate nutrient agar and samples (microorganisms) were screened and identified by biochemical test the identified organisms are were *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*.

Standard Test Tube Method: Biofilm formation may be determined in several different ways, but most frequently it is demonstrated with the standard tube (ST) test. The qualitative assay for Biofilm formation was performed according to the method described by Christensen *et al*. 1985 [7]. A loop full organism from a single colony in pure culture on blood agar was inoculated onto 5ml of trypticase soy broth. The inoculated tubes were incubated at 37°C.After 24 hours;

Corresponding Author: P. Ruban, Department of Biotechnology, SNMV College of Arts and Science, Malumachampatti Coimbatore-50 Tamil Nadu, India.

Table 1: Minimal Inhibitory Concentrations (µg/mL) of aqueous extracts different plants against a *B.Subtillus, P. aeruginosa* and *Staphylococcus aureus*

Name of the plant extract	Concentration (µg/mL)	*B.Subtillus* (MIC)	*P. aeruginosa* (MIC)	*S. aureus* (MIC)
Azadirachta indica	30µl	8.5 mm	5 mm	9 mm
	40µl	10 mm	2.3 mm	9.3 mm
	50µl	13mm	17.5 mm	29.3 mm
Catharanthus roseus	30µl	15 mm	7.4 mm	11 mm
	40µl	20.9 mm	13.1 mm	19 mm
	50µl	23.7 mm	28.6 mm	27.2 mm
Tectona grandis	30µl	19 mm	21.8 mm	14 mm
	40µl	17 mm	26 mm	23 mm
	50µl	28.2 mm	31 mm	35 mm

the contents were decanted. The tubes were stained with 1%safranine for 7 min. A positive result was indicated by the presence of an adherent film of stained material on the inner surface of the tube. Presence of stained material at the liquid-air interface alone was not regarded as indicative of slime production.

Congo red Agar Method: The CRA method technique is based on culturing staphylococcal strains on a solid agar medium supplemented with Congo red dye. The method developed by (Freeman *et al*.).The composition of medium was brain heart infusion broth (BHIB) 37g/l, sucrose 50g/l,agar 10g/l,congo red 0.8g/l. The Congo Red stain, was prepared as a concentrated aqueous solution and autoclaved separately at 121°C for 15 min and was added when the agar was cooled at 55°C. Plates were inoculated and incubated aerobically at 37°C for 24 hours. Isolates that produced black colonies with dry crystalline consistency were regarded as slime positive; where as those showing pink colonies were slime negative [8].

Plant Extract: Leaves from different plants such as Azadirachta indica, atharanthus roseus, Tectona grandis were collect and dried in a circulating-air oven at 40°C and then ground. Subsequently it was soaked in 90/10% (v/v) ethanol-water for 48 h at 25°C, protected from light. Extracts were obtained by vacuum evaporation, resulting in two residues, referred to as the aqueous extract which was lyophilized and the hydro alcoholic extract, which was taken with ethyl acetate and left at room temperature until complete evaporation of the solvent. The extracts were stored at - 10°C.

Antimicrobial Assay: In different concentration the aqueous were collected like 10µl-50µl.In Nutrient agar medium, the organism swabbed and the discs were placed with control. The minimum inhibitory zones were measured. The values are shown in the Table 1. The same concentration of the aqueous was allowed to the tubes during the well developed colony of *S.aureus*. There would be expected that the colony disappeared, instead of that, the colony grew well in the test tubes.

RESULTS AND DISCUSSION

Biofilm producing bacteria are responsible for many recalcitrant infections and are notoriously difficult to eradicate. They exhibit resistance to antibiotics by various methods like restricted penetration of antibiotic into biofilms, decreased growth rate and expression of resistance genes [9]. There are various methods for biofilm detection [7]. In this study we evaluated 3 isolates by two screening methods for their ability to form biofilms (Figure 1 and Table 1).

Biofilm prevents the penetration of antimicrobials and enables the microbial cells to be drug resistant. In addition to this, decreased growth rate and expression of possible resistant genes make antimicrobials ineffective to biofilm microbial cells [10]. Thus organized structure and mechanisms of biofilm are responsible for the emergence of drug resistant bacteria. The present allopathic formulations which used in oral care contain antibiotics, antimicrobial agents, surfactants and alcohol, are not efficient in eradicating oral pathogens completely; on the contrary they were found to be Cytotoxic [11,12]. So the plants derived products are of choice against oral pathogens. Hence, in this study aqueous extract of commonly available plant species in India, Azadirachta indica, Catharanthus roseus, Tectona grandis leaves were evaluated for their biofilm suppression activity. The leaf parts of plants were chosen because leaves contain more secondary metabolites which are responsible for antimicrobial property [13].

The zone of inhibition of three medicinal plants aqueous extract is presented in Table 1 in this Tectona grandis shows maximum zone of inhibition 35 mm against *S. aureus* and 31 mm against *P. aeruginosa.* Azadirachta indica leaves were found to solubilize the *P. aeruginosa*

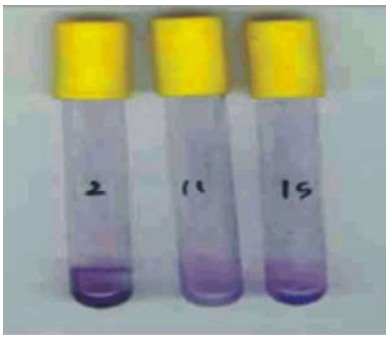

Fig. 1: Standard test tube method
The ST method confirmed
which give the positive results gave the positive results.
for the slime production.

2) Congo Red Agar method (CRA)
S.aureus was streaked in the medium

and S. aureus at 50µl. To substantiate our results that characterize of inhibitory mechanism in T. grandis Linn. Leaf and bark to determine its effectiveness against Listeria monocytogenes and methicillin resistant *Staphylococcus aureus* (MRSA) by employing disc diffusion method. The study also investigated the antibacterial compound is 5-hydroxy-1,4-naphthalenedione (Juglone) [14] by gas chromatography-mass spectrometry and 1H and C NMR analyses [15, 16]. Later, another research study investigated that, Juglone has been found to be inhibitory to oral pathogens, notably *Streptococcus mutans*, *Streptococcus sanguis*, Porphyromonas gingivalis and Prevotella inter media [17].

Azadirachta indica showed maximum zone of inhibition (29.3) at 50µl concentration this result were correlated with Mohammed Omer *et al*. [18]. Aqueous extract of Neem leaf extract has a good therapeutic potential, extract of Neem plant was very effective against *Staphylococcus aureus* and E.*coli*. They found that an extract concentration of 0.5 mg/ml had significantly reduced *Staphylococcus aureus* inoculum after 24hrs, while extracts with increasing concentrations completely wiped out viable bacteria in a lesser time.

CONCLUSION

This evaluation of plants extracts for their anti-biofilm activity against oral pathogens. Our study results highlight the scientific evidence for the use of plants in oral care and treat the emergence of multidrug resistant microorganisms and potential side effects of allopathic health care products. Further this study result requires support by the evaluation of antimicrobial activity against drug resistant clinical isolates and cytotoxicity on human gingival fibroblast cells.

REFERENCES

1. Hall Stoodley, L., J.W. Costerton and P. Stoodley, 2004. Bacterial biofilms: from the natural environment to infectious diseases. Nature Reviews Microbiology, 2(2): 95-108.
2. Whittaker, C.J., *et al*., 1996. Mechanisms of adhesion by oral bacteria. Annu. Rev. Microbiol., 50: 513-552.
3. Flemingson Pamela, E., Ambalavanan, T. Ramakrishna and R. Vijayalakshmi, 2008. Effect of three commercial mouth rinses on cultured human gingival fibroblast: An in vitro study. Indian J. Dent. Res., 19(1): 29-35.
4. Guarrera, P.M., 2005. Traditional phytotherapy in central Italy, Fitoterapia, 76: 1-25.
5. Rios, J.I. and M.C. Recio, 2005. Medicinal plants and Antimicrobial activity. J. Ethnopharmacol., 100: 80-84.
6. Wu, C.D., I.A. Dardut and N. Skauq, 2001. Chewing Sticks: Timeless natural toothbrushes for oral cleansing. J. Periodontal Res., 36(5): 275-84.
7. Christensen, G.D., W.A. Simpson, J.J. Yonger, L.M. Baddor, F.F. Barrett, D.M. Melton and E.H. Beachey, 1985. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J. Clin. Microbiol., 22: 996-1006
8. Freeman J, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. J Clin Pathol 1989; 42:872-4.
9. Kim, L., 2001. Riddle of biofilm resistance. Antimic Ag Chemother, 45(4): 999-1007.

10. Lewis, K., 2001. Riddles of Biofilm Resistance. Antimicrobials agents and Chemotherapy, 45(4): 999-1007.
11. Walker CB. Microbiological effects of mouth rinses containing antimicrobials. J. Clin. Periodontol. 1998,15(8):499-505.
12. Flemingson Pamela, E., Ambalavanan, T. Ramakrishna and R. Vijayalakshmi, 2008. Effect of three commercial mouth rinses on cultured human gingival fibroblast: An in vitro study. Indian J. Dent. Res., 19(1): 29-35.
13. Maji, S., P. Dandapat, D. Ojha, C. Maity, S.K. Halder, P.K.D. Mohapatra, T.K. Pathak, B.R. Pati, A. Samanta and K.C. Mondal, 2010. *In vitro* antimicrobial potentialities of different solvent extracts of ethnomedicinal plants against clinically isolated human pathogens. Journal of Phytology, 2: 57-64.
14. Rafullah, M.K. and M.M. Suleiman, 1999. 5-Hydroxylapachol: a cytotoxic agent from Tectona grandis, Phytochem., 50: 439-442.
15. Nahida Ansari, S.H., A.N. Siddiqui and Pistacia Lentiscus, 2012. A Review On Phytochemistry And Pharmacological Properties, Int. J. Pharm and Pharm. Sci., 4(4): 16-20.
16. Neamatallah, A., L. Yan, S.J. Dewar and B. Austin, 2005. An extract from teak (Tectona grandis) bark inhibited Listeria monocytogenes and methicillin resistant Staphylococcus *aureus*, Lett in Applied Microbio., 41: 94-96.
17. Didry, N., L. Dubreuil and M. Pinkas, 1994. Activity of anthraquinonic and naphthoquinonic compounds on oral bacteria. Pharmazie, 49: 681-683.
18. Mohammed Omer, Helena Khatoon and Fatima, XXXX. Antibacterial Activity of Azadirachta indica (Neem) Leaf Extract against pathogenic organism. The American journal of of Biochemistry and Biotechnology, 3(5): 317-324.