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Bio-Film Degradation Activity Of Microorganisms And Dental Plaque

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ABSTRACT
Biofilms are compositebacteriologicalcombinations encapsulated in a hydrated polymeric matrix of their individualmixture and cells stick to each other and often also to a surface.Current advances in research methodology have allowed researchers to study bacteria in their natural atmosphere.Dental plaque is the varied microbial community originates on the tooth surface rooted in a matrix of polymers of bacterial and salivary origin.When a tooth shallow is cleaned, anexercise film of proteins and glycoproteins is adsorbed quickly to the tooth surface.Dental biofilm forms through a well-arranged sequence of events, subsequent in structured and functionally planned species rich microbial community and modern molecular biological techniques have recognized about 1000 different bacterial species.There is a high level of attentionin the possessions of biofilms and microbial collectionssideways all sectors of industrial, environmental and medical microbiology.

Keywords: Biofilms, Dental plaque, Dental biofilm, proteins and glycoproteins

1. INTRODUCTION

Microbial bio-films are communities of bacteria, embedded in a self-producing matrix, forming on living and non-living solid surfaces. Bio-film associated cells adhere irreversibly on a wide variety of surfaces, including living tissues and indwelling medical devices as catheters, valves, prosthesis, and so forth.

They are considered an important virulence factor that causes persistent chronic and recurrent infections; they are highly resistant to antibiotics and host immune defences. Bacteria protected within bio-film exo-polysaccharides are up to 1,000 times more resistant to antibiotics than planktonic cells (free-floating), which generates serious consequences for therapy and severely complicates treatment options. An estimated 75% of bacterial infections involve bio-films that are protected by an extracellular matrix. Bio-film resistance is due to several reasons, like restricted diffusion of antibiotics into bio-film matrix, expression of multidrug efflux pumps, type IV secretion systems, decreased permeability, and the action of antibiotic-modifying enzymes. The increased bio-film resistance to conventional treatments enhances the need to develop new control strategies.

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Bio-film inhibition is considered as major drug target for the treatment of various bacterial and fungal infections, and pharmacological development of these drugs is now extensively studied. Several green nonlethal strategies for bio-film control developed, because anti bio-film agents is less susceptible to the emergence of resistance. Quinones complex irreversibly with nucleophilic aminoacids in proteins often leading to inactivation of the protein and loss of function. Probable targets in the microbial cell are surface exposed bound enzymes. It should not be surprising that they have been found in the activity probably due to their ability to complex with extracellular and soluble proteins .

Streptococcus mutans a grampositive coccus (round bacterium) is found in the oral cavity is a significant contributor to tooth decay (Loesche,1996). This bacterium, along with the closely related species *Streptococcus sobrinus*, can cohabit the mouth: The acidic environment created in the mouth by this process causes the highly mineralized tooth enamel to decay. *S. mutans* is one of a few specialized organisms equipped with receptors that improve adhesion to the surface of teeth using sucrose as a substrateto form a sticky polysaccharide (Ryan, 2004).However, other sugar like glucose, fructose, lactosecan also be digested by *S. mutans*, produces lactic acid as an end product. The plaque and acid leads to dental decay.
The microorganism is plays a vital role against oral pathogens. The poly herbal extracts has carried against different microorganisms. The phytochemicals present in this extract plays role in cure the dental plague. This phytoconstiuents contains secondary metabolite that performsthe activity in plague.

S. mutans causes tooth decayso attemptswere made to create a vaccine for the organism. So far, vaccines are not successful in humans. (Klein, 1988). *Streptococcus mutans* acquire the gene thatproduce biofilms through horizontal gene transfer with other lactic acid bacterial species, such as *Lactobacillus*.

Lactobacillusspp. plays a major role in the development of dental caries. Although effective methodsexists for the prevention and management of dental caries, it is still increasing amongst school-age children.*Streptococcus mutans* and *Lactobacillus spp*. arecommon pathogens isolated from human dental plaque and are considered as the major etiologic agents of caries. However, there is a vital role for *S. mutans* in the initiation and *Lactobacillus spp*. in the major progression of dental caries. In addition, risk factors such as host susceptibility, age, dietary habits socioeconomic and oral hygiene status have been associated with increased incidence of dental caries in human population.

2. MATERIALS AND METHODS

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The present investigations, focused on the efficacy of *Areca catechu, Acalypha indica,* and *Piper betel* against the oral pathogens, *Streptococcus mutans*and *Lactobacillus* in prevention of dental caries.

Collection of plant material

The *Areca catechu, Acalypha indica, Piper betel were brought from* Tambarammarket. The plant materials were identified and authenticated by a botanist in the Department of Botany, Pachaiyappa'sCollege,. The specimens were deposited at Department of Zoology, Pachaiyappa's College, Chennai – 600 030.

Extraction of various parts of *Areca catechu, Acalypha indica, piper betel*

The *Areca catechu, Acalypha indica, piper betel* was cleaned shade dried and coarsely powdered. Successive solvent extraction was done by cold percolation method (Harborne, 1998) by soaking in hexane, chloroform, ethyl acetate, ethanol and methanol successively in an aspirator bottle for 48 h. Aqueous extracts of all the plants were also prepared. After 48 h, the extracts were filtered by Whatman Filter paper No.1. By distillation the solvent was removed the extracts were concentrated and dried in Lyodel Freeze Dryer.

Minimum Bactericidal Concentrations (MBC) on *Streptococcus mutans*and *Lactobacillus*

Dilutions and inoculations were prepared in the same manner as described for the determination of MIC. The control tube without plant extract is immediately sub cultured (Before incubation) along with the tubes with plant extract at 37ºC overnight. The MIC of the control organism was read to check that the drug concentrations are correct. The growth was compared with the original inoculum. The same number of colonies presents indicate bacteriostatic only. Reduced number of colonies-indicate slow bactericidal activity and if no growth was observed the whole inoculum is killed.The highest dilutionshowing at least 99% inhibition is taken as Minimum Bactericidal Concentrations (MBC). (Dinesh *et al*, 2016).

Biofilm degradation activity test

The method used is micro-dilution method. Biofilms formed by synthetic saliva (Mc Dougall solution) is put in a 24 well plate together with TSB medium, 3% glucose and bacterial inoculant. The mixture was incubated for 24 h at 37 ºC. After the biofilm is formed, the remaining medium was discarded. Extracts are added at a concentration of 500 µg/mL and then incubated 24 hours at temperature of 37 ºC. Biofilms attached to the wall of the wells is washed using phosphate buffer. Crystal violet 1% was added to the wells and left for 15 minutes. Well rinsed with sterile water three times and 95% ethanol was added and suspension incubated for 45 minutes and the solution was transferred to a new micro-plate. Suspension absorbance of each well was measured using a microplate reader at a wavelength of 595 nm to determine the %

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degradation. Chlorhexidine was used as positive control and 20% DMSO as a negative control.(Irmanida*et al,* 2016).

3. RESULTS AND DISCUSSION

In case of *Lactobacillus* the extract of PAA (Poy herbal compounds) extracts showed a higher activity. From this investigation it was observedthat PAA (Poly herbal compounds) extracts at a concentration of 1000 µl/mL inhibited.Both groups of bacterial strains.The phenolic nature *Areca catechu* extract may be responsible for inhibiting bacterial growth.

The Minimum Bactericidal Concentration for*Lactobacillus* by *Areca catechu* was 62.5 µg/mL,*Acalypha indica was 15.6* µg/mL , *Piper betel* was 125 µg/mL and for the polyherbal combination (PAA) was 7.2 µg/mL respectively. respectively.

In this study, the bioflim inhibition potential of *Areca catechu, Acalypha indica, Piper betel* and their combined extract (PAA) against *S. Mutans*and *Lactobacillus sp.* was evaluated by using micro-dilution method. Reports states that the biofilms acquire resistances to inhibitors under nutrient limited or depleted conditions in contrast to their susceptibility conditions. *Areca catechu, Acalypha indica, Piper betel* and their combined extract (PAA) shown to have anti-biofilm efficacy under both nutrient repleted and nutrient depleted conditions,which indicate the presence of bio active agents against *Streptococcus mutans*and *Lactobacillus sp*. of the extracts.

Minimum Inhibition Concentration (MIC) was performed to arise the lowest concentration of an antimicrobial ingredient and to evaluate the antimicrobial efficacy of various compound by measuring the effect of decreasing concentration of plant extracts of *Arecacatechu, Acalypha indica, Piper betel* and PAA which inhibits the microbial strain of *Streptococcus mutans* and *Lactobacillus* growth.

The strain of *Streptococcus mutans* as an MIC of 15.6µg/mL for *Areca catechu* and an MIC of 15.6 µg/mL for *Acalypha indica* and that of 15.6 µg/mL for *Piper betel* and that of 7.2 µg/mL for PAA.

The strain of *Lactobacillus* has an MIC of 62.5µg/mL for *Areca catechu* and an MIC of 15.6 µg/mL for *Acalypha indica* and that 125 µg/mL for *Piper betel* and that of 7.2 µg/mL PAA.

As Minimum Bactericidal Concentration (MBC) of an antibacterial agent is necessary for screening a drug efficiency the methanolic exractsof *Areca catechu, Acalypha indica,Piper betel* and PAA were subjected to minimum bactericidal concentration as it a tool to simultaneously evaluate multiple antimicrobial agent for potency.

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The MBC of *Areca catechu,Acalypha indica,Piper betel* were 15.6 µg/mLand PAA were 7.2 µg/mL and various concentration such as ,62.5 µg/mL, 31.2 µg/mL, 15.6 µg/mL and 7.2 µg/mL respectively against *Streptococcus mutans* and *Lactobacillus* was 62.5 µg/mL,15.6 µg/mL,125 µg/mL and 7.2 µg/mL respectively .

The *Streptococcus mutans*and *Lactobacillus* was found to be susceptible for PAA extract.

Bioflim inhibition is considered as a major drug target for the treatment of various bacterial and fungal infection the creates bioflim.Plants biomolecules with prominent biological effects that would have paved the way to inhibit DNA and RNA synthesis and possible inhibition of extra cellular microbial enzymes and causing bacterial death.

The antibacterial mechanism of medicinal plants against these oral pathogens *Streptococcus mutans* and*Lactobacillus* might be due to inhibition of cell wall synthesis (Cowan,1999.,Marcucci et al.,2001) causing energy depletion (onner,1993) also due to cell permeability increase which might constitute cellular loss membrane disruption and function of cellular constituents that might have lead to the mutation ,cell damage and cell death (Kim et al ., 1995).

4. CONCLUSION

Dental biofilm is a mounting publichealth, psychological, and social threat that is connectedto the food we eat, microbes we harbor and general healthstatus. Therefore, the aim of this study is to provideabasic outlook of oral biofilms and its impact on health, psychological and social interactions.

Figure1: Biofilm degradation activity of methanol extract of *Areca catechu, Acalypha indica,* and *Piper betel* and their combination (PAA) against *Streptococcus mutans*.

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Table1: Biofilm degradation activity of methanol extract of *Areca catechu, Acalypha indica,* and *Piper betel* and their combination (PAA) against *Streptococcus mutans.*

Concentrations in µg/ml	*Areca catechu*	*Acalypha indica*	*Piper betel*	*PAA*	Antibiotic
1000	79.16±0.23	82.5±0.21	80.83±0.15	74.16±0.29	70±0.12
500	84.16±0.17	85.83±0.15	82.5±0.13	75.83±0.24	73.33±0.13
250	86.66±0.15	88.33±0.18	83.33±0.11	78.33±0.27	75±0.17
125	87.5±0.12	89.16±0.16	85±0.1	80±0.22	76.66±0.19
62.5	88.33±0.11	89.16±0.13	85.83±0.1	81.66±0.2	80.83±0.15

Figure2: Biofilm degradation activity of methanol extract of *Areca catechu, Acalypha indica,* and *Piper betel* and their combination (PAA) against *Streptococcus mutans.*

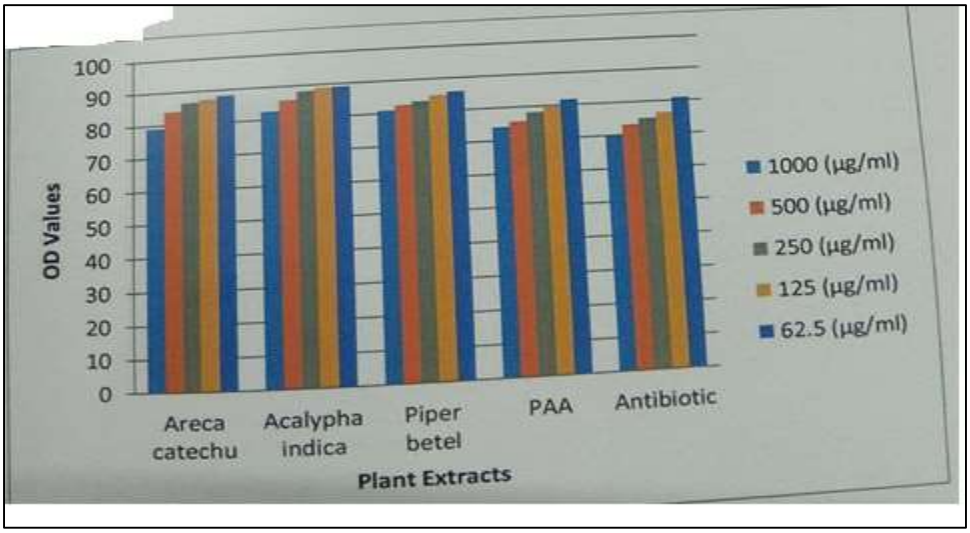

Figure 3: Biofilm degradation activity of methanol extract of *Areca catechu, Acalypha indica,* and *Piper betel* and their combination (PAA) against *Lactobacillus sp.*

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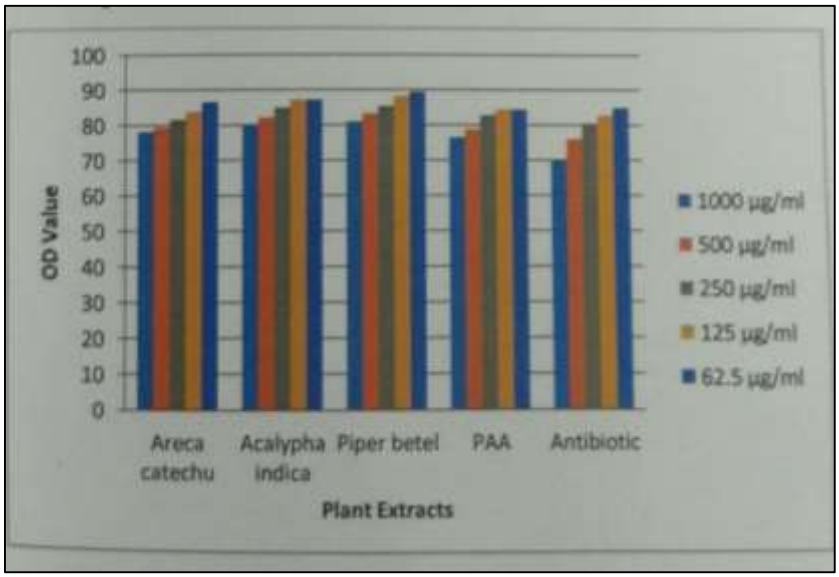
Table 2: Biofilm degradation activity of methanol extract of *Areca catechu, Acalypha indica,* and *Piper betel* and their combination (PAA) against *Lactobacillus sp.*

Concentrations in µg/ml	*Areca catechu*	*Acalypha indica*	*Piper betel*	PAA	Antibiotic
1000	78.3±0.13	80±0.12	81.11±0.2	76.66±0.14	70±0.1
500	79.44±0.15	82.2±0.13	83.33±0.22	78.88±0.16	76.11±0.14
250	81.66±0.11	85±0.13	85.55±0.21	82.77±0.17	80.55±0.1
125	83.88±0.12	87.22±0.21	88.33±0.24	84.44±0.18	82.77±0.13
62.5	86.66±0.14	87.22±0.21	89.44±0.24	84.44±0.18	85±0.15

Figure4: Biofilm degradation activity of methanol extract of *Areca catechu, Acalypha indica,* and *Piper betel* and their combination (PAA) against *Lactobacillus sp.*



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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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