Original Article

Pathology

Role of Amaltas and Dandasa in Controlling Biofilm Formation of Streptococcus Mutans and Pseudomonas

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ABSTRACT

Background: People usually give least importance to their oral hyiegene as a consequence they are suffering from so many disease due to oral biofilm that form over the teeth. Also when patient is going for dental procedures they were exposed to water from the dental lines of this unit. Pseudomonas aeroginosa were the main culprit present in these water line and form biofilm there as well as in host causing infection .Biofilm forming property confers virulence characteristics thus making such bacteria highly resistant to commonly used antibiotics.

Objective: We analyzed the antibiofilm activity of natural occurring substances like dandasa (juglans regia) and amaltas(Cassia fistula).

Study Design: Experimental study

Place and Duration of Study: All clinical isolates were identified at BUMDC and PNS microbiology lab(Karachi) by standard biochemical methods. This Study was conducted between Nov 2012 to Feb 2013

Material & Methods: 40 streptococcus mutan and 32 pseudomonas aerogenosa samples were taken from oral biofilm and contaminated denal lines water respectively and characterized using conventional biochemical, cultural and molecular methods.Biofilm forming activity of these isolates were checked. Then I mixed the dandasa and amaltas and observe the antibiofilm forming activity.

Results: Both dandasa and amaltas in a conc. of 12.5mg/ml and 3.2mg/ml respectively show good antibiofilm forming activity against pseudomonas aerogenosa and in a conc 0.8mg/ml and 12.5 mg/ml respectively against streptococcus mutan.But combinations of dandasa with amaltas were found to be not more effective in inhibiting biofilm formation suggesting a indifferent activity with antiadhesive index of 1.0 and 0.75 against Streptococcus mutans,and pseudomonas aerogenosa respectively.

Conclusion: Streptococcus mutans in oral biofilm and pseudomonas in dental water lines exhibited biofilm formation which is the cause for antibiotic resistance and providing shelter to other organism .Amaltas and Dandasa provide a good antibiofilm activity individually .

Key Words: Streptococcus mutans, Pseudomonas, Amaltas and Dandasa, Biofilm

INTRODUCTION

It has been observed that people not usually bothered to have proper oral hygiene especially when they are involve in busy working schedule. Also it is seen that due to unnecessary dental procedures like scaling the normal smooth surface of teeth become uneven and the microorganism which are present in the normal flora of the oral cavity remain attached to these uneven surfaces and form the oral biofilm ,which provide shelter to these microorganisms. These biofilm forming organisms usually exhibit resistance to most commonly used antibiotics. Also at the same time these people getting exposure to water from these water lines

Biofilms: Biofilms are microbial communities that formed irreversibly on different surfaces[1,2] and can be difficult to control since they can form where cleaning is not performed properly. Biofilms can exist as a mass of microorganism with vertical and horizontal channels allowing liquid flow and dispersion of nutrients and waste components[3]. These biofilms provide pathogenic bacteria as a source of product contamination[4,5]. Oral biofilm (Dental plaque) is a soft deposit that accumulate on the teeth. In addition to the bacterial cell, plaque contain a small number of epithelial cell, leukocyte and macrophages.

Streptococcus mutans: Streptococcus mutans is a Gram-positive, found in the human oral cavity and is a significant contributor to tooth decay. S. mutans is one of a few specialized organisms equipped with receptors that improve adhesion to the surface of teeth. Sucrose is used by S. mutans to produce a sticky, extracellular, dextran-based polysaccharide that allows them to cohere to each otherand forming biofilm.[6]

Pseudomonas Aerugenosa: Pseudomonas aerugenosa is gram _ve rods. Pseudomonas aerugenosa able to grow in water with minimal nutrients .It contain 2 pigment pyocyanin and pyoverdin.It is primarily an opportunistic pathogen that causes infection in hospitalized patient. It can cause infection anywhere in the body but urinary tract infection ,pneumonia, upper respiratory infection, cystic fibrosis in lungs and

dermatological infection especially burn infection predominate .It can enter the blood and cause sepsis ,a severe otitis externa and skin lesion occur in those who swim in less chlorinated pools .Its ability to grow in aqueous solution contaminate the respiratory therapy and anesthesia given equipment ,intravenous fluid, dental water lines and even distilled water[7].

Cassia Fistula (Amaltas):

Family Name: Caesalpinaceae Botanical Name: Cassia Fistula Popular Name(s): Fistula, Laburnum, Purging Fistula, Golden Shower, Amaltas The Indian Laburnum, known also as Amaltas, is one of the most beautiful flowering trees. During the flowering season, the profusion of flowers hanging from its branches cover the tree so entirely that the tree, appearing bright yellow, can be spotted with ease even from a distance. Amaltas is classified as a tree of medium height. Leaf shedding proceeds the flowering season. When the tree is leafless, it is possible to see the long rod-like pods of the previous year. Young shoots and buds appear just after leaf shedding. The flowers are long hanging clusters a shower of cascading flowers[8]. The compound leaves of amaltas are dark green, except when young. The tender leaves are bright green or sometimes a beautiful rich copper color. What starts off as a thread like pod soon acquires the typical straight long rod-like appearance. On ripening the pods turn dark brown or black. This plant act as antibacterial and antifungal activity.[9,10,11,12,13]

Juglans regia(Dandasa): Walnut is a common temperate forest tree found throughout the world. The plant belongs to the family Juglandaceae. The dried bark of Juglans regia (Dandasa) is locally available in Pakistan. This bark is used to improve oral hygiene by traditional. There are very few reports stating about side effects after their oral use but none is reported any severe toxicity outcomes[15]. It also has been used for eczema, pruritus, blisters and as blood cleanser and laxative[16]. The tree is rich in flavanoides include catechins, myricetin, another compound naphthohydroquinone and Vitamin C[14]. Different other bioactivities have also been previously reported including antiaging, antiproliferative, antimutagenic, anti inflammatory and antinociceptive activities[15].

MATERIALS AND METHODS

Microscopic Examination: Oral biofilm sample were collected from 50 patient in different dental clinics to find out streptococcus mutans while 50 dental waterline sample were collected from different dental units to find out pseudomonas aerogenosa.

To check the purity of culture and to observe their morphological characteristics the isolates were observed by grams stain and biochemical test

All clinical isolates were identified at BUMDC and PNS microbiology lab (Karachi) by standard biochemical methods .Study conducted between Nov 2012-feb 2013

Collection and Preparation of Natural Compounds

Plant Collection

Dried amaltas available in the market and Juglans ragia (dandasa) which is the dried bark of Persian walnut tree which is purchased from local market.

Preparation of Aqueous Extracts: A solution of each dried plant material was prepared in sterile distilled water by taking 5gm/100ml and heating at 95° C in water bath for two minutes and cooling for two minutes. Procedure were repeated three times and final extractions were centrifuged. Supernatant were filtered through 0.2µm membrane stored at−20° C and thawed before used. Every time stored extract were used for not more then one week for different bioassays. 50mg/ml of these products were used as initial concentration then further dilution made accordingly.

Biofilm Forming Assay through Elisa Reader: We perform two methods using 96 well plates for determining the biofilm forming ability of all cariogenic bacterial isolates

A) In order to study the biofilm formation, culture was grown in Tryptone Soya Broth, matched with 0.5 McFarland and culture was transferred in each well of microtitre plate. Along with the test, controls were also run having strep mutan,pseudomonas aerogenosa, uninoculated broth and empty wells. Plates were made in duplicate, incubated and covered at 37º C for 24h and 72h. Cell turbidity was monitored using a microtitre plate reader at an optical density at 405 nm. After incubation medium was removed from wells and microtitre plate wells were washed with PBS to remove loosely associated cells, each well was stained with 100µl of 1% crystal violet solution for 45min and further washed 3 times with PBS over which 10% alcohol was added and O.D was recorded by. Measuring the absorbance thru ELISA reader similarly another plate which was incubated for 72hrs was read for O.D determination.

Bacterial Adhesion Assay: A 20ul of pre culture suspension match with McFarland 0.5 inoculated in glass tube containing 1 ml of Brain Heart Infusion broth plus 1 ml of tested compound of required concentration and then left this tube at an angle of 30 undisturbed for 18 hr at 37oC for culturing and adhesion. After that suspension transfer into new culture tube(fraction A).Add 1 ml Brain Heart Infusion broth and 1ml compound tested in empty tube from which we put in fraction A then do vortexing for 30sec then shift this to another culture tube (fraction B) .Finally 1 ml of Brain Heart Infusion broth and 1ml of tested compound add in the tube from which we put suspension in fraction B then sonication done so that bacteria which is tightly adhered detached and name this tube as (fraction C) .Then check turbidity of each fraction at OD 550 nm adjusted tube having 1ml Brain

Heart Infusion broth and 1ml compound only as OD 550 nm as 0 .Then calculate the %adhesion by putting values in following formula(C/A+B+C)* 100, where A, B ,C considered as turbidity of fractions at OD 550nm [16].

RESULTS

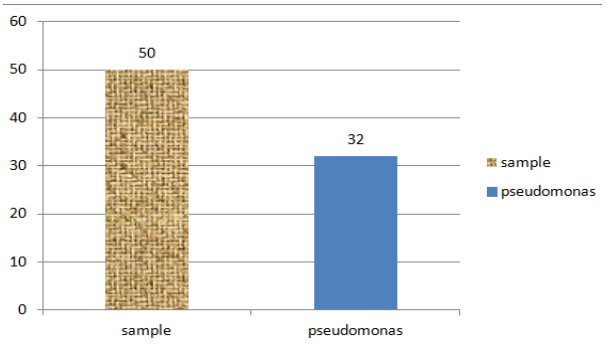

Figure No.1: Pseudomonas sample

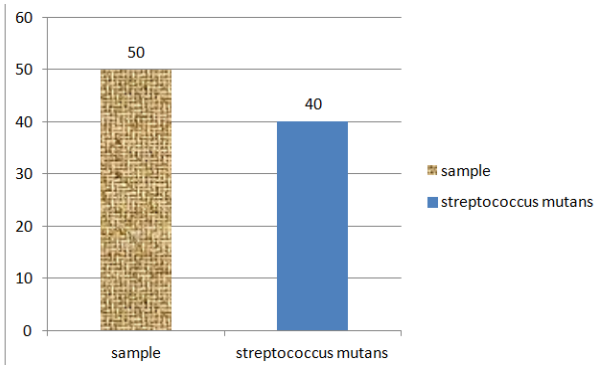

Figure No.2: Streptococcus mutans sample

Table No.1: Anti adhesive activity against pseudomonas

Pseudomonas				
Dandasa	A	B	C	%of adhesion
12.5mg/ml	2.5 ± 0.244	1.3 ± 0.294	.6 ± 0.163	14% (effective)
6.2mg/ml	2	1	1	25%
Amaltas	A	B	C	%of adhesion
3.2mg/ml	2 ± 0.163	1.6 ± 0.141	.5 ± 0.141	12.1% (effective)
1.6mg/ml	2.5 ± 0.294	1.5± 0.355	1± 0.141	20%

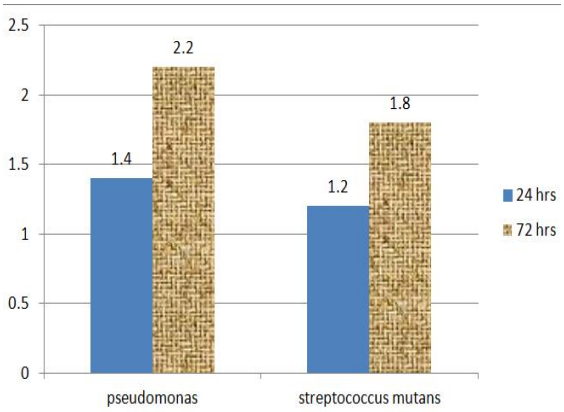

Figure No.3: Biofilm formation in 24 hrs and 72 hrs Cutoff value > 1.0 biofilm former

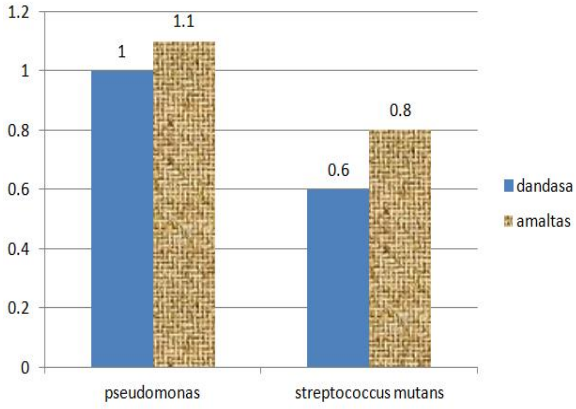

Figure No.4: Biofilm in the presence of amaltas and dandasa

Table No.2: Anti adhesive activity against streptococcus mutans

Streptococcus mutans				
Dandasa	A	B	C	%of adhesion
0.8mg/ml	2.0 ± 0.244	1 ± 0.141	.5 ± 0.141	14% (effective)
0.4mg/ml	2.0	1.5	1.5	30%
Amaltas	A	B	C	%of adhesion
12.5mg/ml	2 ± 0.163	1.6 ± 0.141	.5 ± 0.141	12.1% (effective)
6.2mg/ml	3	1.5	1.5	25%

Table No.3: Combine effect of amaltas and dandasa

	amaltas (effective)	Combination	Dandasa (effective)	Combination	Anti-adhesive index	relation
Pseudo-monas	3.2mg/ml	1.6 mg/ml	12.5 mg/ml	3.2 mg/ml	0.756	Indifferent
Strep mutan	12.5 mg/ml	6.2 mg/ml	0.8 mg/ml	0.4 mg/ml	1.0	indifferent

DISCUSSION

Now a days people are getting undue exposure of pathogenic bacteria which may be avoided by using some preventive measures. Peoples were involve in so many interventional procedures that is not required like when exposed to dental procedure if it is not required like scaling people were exposed to water from these dental water lines which might contain pseudomonas that form biofilm and continuous source of

contamination. Also a result of ignorance bad oral hyiegene bacteria form oral biofilm which provide protection to these bacteria a consequences there is a high incidence of infection and their complication.

In this study we analyzed the antimicrobial and antibiofilm activity of natural compounds like dandasa and amaltas .It is seen from the above results that there is 80%prevalence of streptococcus mutans in dental plaque sample as shown in fig 2 and there is 64%prevalence of pseudomonas aerogenosa in different dental units waterlines as shown in fig 1 Above results shows that isolated organism from dental plaque and dental water lines form firm invitro biofilm after 72 hr as in fig 3 but when we were using dandasa and amaltas as anti biofilm former then there is no biofilm form even after 72 hrs as shown in fig 4. It is also observe in our study that in case of pseudomonas antiadhesive activity of amaltas is more effective as it shows 12.1% effectiveness even at 3.2 mg/dl concentration as compare to 12.5mg/dl concentration of dandasa gives the same results as shown in table 1. whereas antiadhesive activity of dandasa is more effective in case of streptococcus mutans as shown in table 2. Later in our study it is observe that when we combine both these natural product then none of the compound(amaltas and dandasa) show synergistic or antagonist activity and both compound shows indifferent activity as shown in table 3. So from the above mention results it is shown that amaltas and dandasa more effective when use separately although amaltas more effective antibiofilm activity against pseudomonas aerogenosa whereas dandasa having good activity against streptococcus mutans .Our study results also similar to previous studies on natural compound antibiofilm forming activity[17,18,19,20].

CONCLUSION

It is concluded from the above observation that it take 72 hrs for an organism to develops biofilm and it is the time when organism usually firm their attachment and it is the actual or right time when antibacterial or antibiofilm agent may be applied to prevent the formation because once biofilm forms it provide shelter to the micro organism against antibacterial agents .Amaltas and Dandasa play a vital role in controlling these biofilms and prevent us from several infectious diseases.

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