ORIGINAL ARTICLE

Role of Amaltas and Dandasa in Controlling Biofilm Formation of Streptococcus Sangius

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ABSTRACT:

Objective: To analyze the anti-biofilm activity of naturally occurring substances dandasa (juglans regia) and amaltas (Cassia fistula) against streptococcus sangius.

Materials and Methods: This experimental study was carried out at Bahria University Medical and Dental College(BUMDC) Karachi from December 2013 to March 2014.A total of fifty streptococcus sangius samples were taken from oral biofilm and identified using conventional, biochemical, cultural and molecular methods.Biofilm forming activity of these isolates was recorded and then exposed to dandasa and amaltas.

Results: Amaltas and Dandasa both in a concentration of 12.5mg/ml and 3.2mg/ml respectively showed good anti-biofilm forming activity against streptococcus sangius. Combination of dandasa with amaltas did not showed more effective inhibitory effect against biofilm formation suggesting an indifferent activity with anti-adhesive index of 0.75 against Streptococcus sangius

Conclusion Streptococcus sangius in oral biofilm exhibited biofilm formation which is the cause for antibiotic resistance and provides shelter to other organisms. Amaltas and dandasa provide a good antibiofilm activity individually against Streptococcus sangius.

Keywords: Streptococcus sangius, Amaltas, Dandasa, Biofilm.

INTRODUCTION:

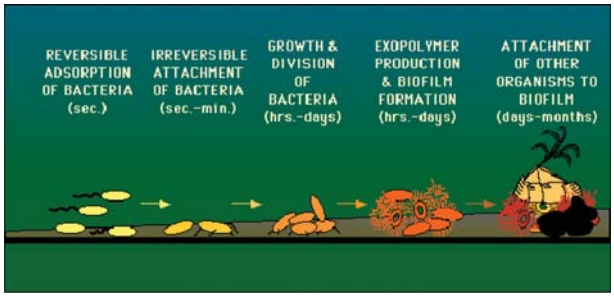
During the last decade increasing efforts have been done to replace or supplement mechanical and therapeutic measures with antiseptics or antibiotics. It has been observed that people are not usually bothered to maintain proper hygiene especially oral hygiene. They usually relate this reluctance to busy working schedule. Poor oral hygiene allows the oral bacteria a chance to form oral biofilm. This film in turn provides shelter to pathogenic microorganisms present within them. These biofilm forming organisms usually exhibit resistance to commonly used antibiotics. Exclusive and unnecessary use of antibiotics also play a role in creating resistant strains. Present study used naturally occurring products amaltas and dandasa for the control of biofilm formed by Streptococcus sangius.

Biofilms:

Biofilms are microbial communities that are formed irreversibly on different surfaces including teeth[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 microorganisms 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 accumulates on the teeth. In addition to the bacterial cells, plaque contains a small number of epithelial cells, leukocytes and macrophages. (Figure 1.)

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

Figure 1
Process of Biofilm Formation



Family Name: CAESALPINACEAE Botanical Name: CASSIA FISTULA 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 and called shower of cascading flowers.[7] 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

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long rod-like appearance (Figure 2a). On ripening the pods turn dark brown or black .This plant has antibacterial and antifungal activity[8,9,10,11,12].

Figure 2a
Cassia Fistula (Amaltas)

Walnut is a common temperate forest tree found throughout the world. The plant belongs to the family Juglandaceae. The dried bark of Juglansregia (Dandasa) is locally available in Pakistan (Figure 2b). This bark is used to improve oral hygiene traditionally. There are very few reports stating about side effects after their oral use but none has reported any severe toxicity outcome[14]. It has also been used for eczema, pruritus, blisters and as blood cleanser and laxative. The tree is rich in flavanoides including catechins, myricetin, naphthohydroquinone and Vitamin C.[13] Different other bioactivities have also been previously reported including antiaging, antiproliferative, antimutagenic, anti inflammatory and antinociceptive activities etc.[14]

Figure 2b
Juglans Regia (Dandasa)

MATERIALS AND METHODS:

Microscopic Examination: In this study 50 cases from dental clinics were selected and samples were obtained to culture for streptococcus sangius. Isolates were confirmed by grams staining, microscopy and biochemical tests.

All clinical isolates were identified at BUMDC and PNS-Shifa microbiology laboratory Karachi by standard biochemical methods .Study was conducted from December 2013 to February 2014

Collection and Preparation of Natural Compounds:

(a) Plant Collection:

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

(b) 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 was repeated thrice and final extractions were centrifuged. Supernatant were filtered through 0.2µm membrane, stored at -20°C and thawed before use. 50mg/ml of these products were used as initial concentration then further dilution was 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. In order to study the biofilm formation, culture was grown in Tryptone Soya Broth, matched with 0.5 McFarland. Culture was transferred in each well of microtitre plate. Along with the test, controls were also run having strep. mutans, pseudomonas aerogenosa, uninoculated broth and empty wells. Plates were made in duplicate, incubated and covered at 37ºC for 24h and 72h. Cell turbidity was monitored using a microtitre plate reader at an optical density (O.D) 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 100 µl 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 through ELISA reader and similarly another plate which was incubated for 72hrs was read for O.D determination.

Bacterial Adhesion Assay:

A 20ul of pre culture suspension matched with McFarland 0.5 was inoculated in glass tube containing 1 ml of Brain Heart Infusion broth plus 1 ml of tested compound of required concentration and then this tubewas left at an angle of 30 undisturbed for 18 hr at 37°C for culture 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 did vortexing for 30sec. It was then shifted to another culture tube (fraction B) .Finally 1 ml of Brain Heart Infusion broth and 1ml of tested compound added in the tube from which we put suspension in fraction B then sonication was done so that bacteria which is tightly adhered detached and we named this tube as (fraction C) Turbidity was checked in each fraction at OD 550 nm adjusted tube having 1ml

Brain Heart Infusion broth and 1ml compound only as OD 550 nm as 0.Percentage adhesion was calculated by putting values in following formula(C/A+B+C) * 100 ,WHERE A,B ,C considered as turbidity of fractions at OD 550nm[15].

RESULTS:

Out of 50 streptococcus sangius sample we had 35 samples which showed biofilm forming activity (figure 3a). It took 72 hrs for streptococcus sangius to form a proper biofilm(figure 3b). There was a drastic decrease in biofilm forming property of streptococcus sangius (figure 3c). Anti-adhesive activity of dandasa (3.2mg) was more effective in case of streptococcus sangius as compared to amaltas (Table 1) whereas both compound showed indifferent activity when used in combination (Table 2).

Figure 3a
Streptococcus Sangius Sample

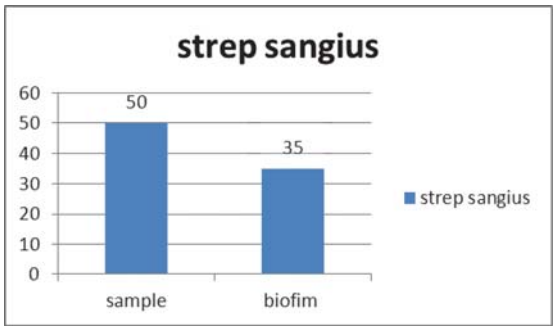
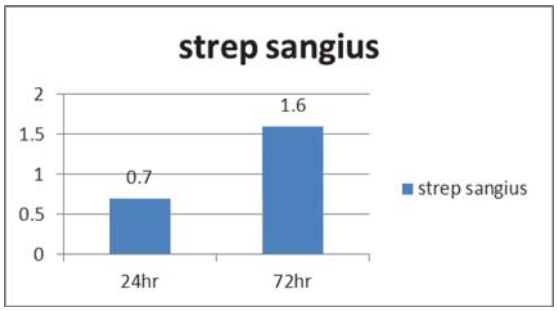


Figure 3b
Biofilm formation



Cut off value > 1.0 biofilm former

Figure 3c
Biofilm in the presence of Amaltas and Dandasa

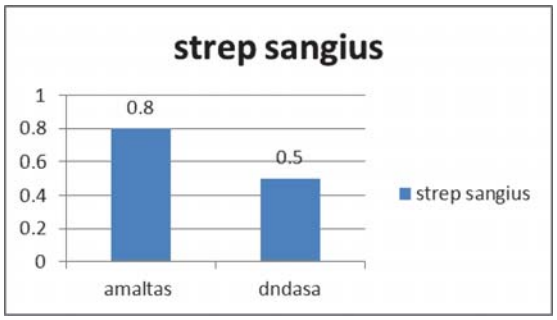


Table 1
Anti adhesive activity against Streptococcus Sangius

Streptococcus Sangius				
Amaltas	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%
Dandasa	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%

Table 2
Combine effect of Amaltas and Dandasa

	Dandasa (individually) (effective)	Combination	Amaltas (individually) (effective)	Combination	Antiadhesive index	Relation
Strep. sangius	3.2mg/ml	1.6 mg/ml	12.5 mg/ml	3.2 mg/ml	0.756	Indifferent

DISCUSSION:

People are getting undue exposure of pathogenic bacteria due to unhygienic conditions which may be avoided by using some preventive measures. Also as a result of ignorance and bad oral hygiene, bacteria form oral biofilm which provide protection to these bacteria. As a consequence there is a high incidence of infections and their complications. For the eradication of these infections people are using antibiotics but these antibiotics does not reach inside the biofilm and therefore bacteria remain viable inside the biofilms. Because of this reason antibiotics become resistant against these biofilm bacteria. Frequent use of different antibiotics to eradicate infection produce many side effects as well.[16,17] We used some natural products which are easily available as well as cheap in comparison to antibiotics. We also tried to find out anti-biofilm concentration of these natural products which came out to be much less in concentration in comparison to the concentration required for antimicrobial activity.

In our study 70% prevalence of streptococcus sangius was found in dental plaque samples. Isolated organisms from dental plaque formed firm invitro biofilm after 72 hr whereas when Dandasa and Amaltas were used no biofilm was formed even after 72 hrs. These results are favoured by other documented studies[18,19,20]. It is also observed in our study that anti-adhesive activity of

dandasa is more effective in case of streptococcus sangius. However when we combined both these natural products then none of the compound (amaltas and dandasa) showed synergistic or antagonistic activity and both compound showed indifferent activity[21,22,23]. This makes it evident that amaltas and dandasa are more effective when used separately. These findings are coinciding with the results of other studies[24,25]

CONCLUSION:

Our study concludes that it takes 72 hrs for an organism to develop biofilm. This is the actual or right time when antibacterial or anti-biofilm agent should be applied to prevent the formation of biofilm. Amaltas and Dandasa control the formation of biofilms and prevent infectious diseases. They are natural products with minimal side effects .Moreover they are cost effective and easily accessible to everyone.

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