IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 4 Ver. VIII (Jul – Aug 2017), PP 12-16
www.iosrjournals.org

Effectiveness of Bay Leaves Aqueous Extract on *Streptococcus Mutans* In Comparision To Chlorhexidine Gluconate

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Abstract: *Daily use of an efficient anti-plaque compound can be very beneficial in plaque control. Some was assessed for its antibacterial activity Herbal extracts have received special attention because of being non-chemical and non-synthetic, and they have been long used in traditional medicine bay leaves aqueous extract was assessed for its antibacterial activity against Streptococcus mutans using agar diffusion technique and the adherence to tooth surface in comparison to chlorhexidine gluconate in vitro was also studied The results showed that bay leaves aqueous extract exhibit good antibacterial activity and this activity was found to be increased as the concentration of extract increased and the result also showed that the bay leaves aqueous extract was effective in inhibiting the adherence of Streptococcus mutans to tooth surface*
Keywords: *Bay leaves, streptococcus mutans, adherence*

Date of Submission: 31-07-2017 Date of acceptance: 28-08-2017

I. Introduction

Plaque associated oral disease affects a considerable portion of the population and is considered one of the major causes of tooth loss. In most cases, the chronic accumulation of dental plaque often leads to caries and periodontal disease), that may not only affect the patient's oral health, but may also contribute to a number of chronic systemic diseases [(1)]. *Streptococcus mutans* has long been associated with dental caries in man and is one of a few specialized organisms equipped with receptors that improve adhesion to the surface of teeth and its ability to form dental plaque and known to play a significant role in the etiology of dental caries.[(2)]

Dental caries is a dental biofilm-related oral disease associated with increased consumption of dietary sugar and fermentable carbohydrates. When dental biofilms remain on tooth surfaces, along with frequent exposure to sugars, acidogenic bacteria (members of dental biofilms) will metabolize the sugars to organic acids. Persistence of this acidic condition encourages the proliferation of acidogenic and aciduric bacteria as a result of their ability to survive at a low-pH environment. The low-pH environment in the biofilm matrix erodes the surface of the teeth and begins the "initiation" of the dental caries. If the adherence of *S. mutans* to the surface of teeth or the physiological ability (acidogenity and aciduricity) of *S. mutans* in dental biofilms can be reduced or eliminated, the acidification potential of dental biofilms and later cavity formations can be decrease [(3)]

Therefore there is a need of an antimicrobial agent to eradicate *Streptococcus mutans* in plaque and reduce caries risk in population in order that chlorhexidine is widely used to reduce dental plaque bacteria It thas potential antibacterial activity against gram negative and gram positive bacteria [(4)] In another hand natural products (plant extracts) have been proposed as novel therapeutic agents against dental caries, in order to minimize the adverse effects of synthetics (e.g., altered taste, mucosal desquamation and tooth staining) as well as to provide effective and safer alternatives for dental caries management. [(5)]

Bay leaf or *Eugenia polyantha Wight* has been known since long time ago as a species that can be used for therapy. The use of bay leaf has been developed medically, as an alternative medical plant. Bay leaves have a lot of chemical properties that are useful in medicine, even as basic materials in dentistry.

Eugenia polyantha Wight can be used in periodontics) chemically the chemical properties consist of tanines, flavonoid and essential oils (0.05%), including citric acid and eugenol.

Tanine is a liquid glycoside derived from polypeptide and ester polymer which can be hydrolyzed by the secretion of bile (3, 4, 5 trinidrokside benzoic acid) and glucose. Flavonoid is a genetic term used for aromatic heterocyclic oxygen compound which is derived from 2 phenilbenzopiran or its 2, 3 dehydro.Flavonoid is one of natural phenolic compound present in most plant.

Essential oil is mainly consists of terpenoid compound with atomic carbon framework of five [(6)]

II. Methods

1- Sample collection: samples of saliva were obtained from patients aged 18 – 25 years old with no medical history attending dental clinic under standard conditions. Each individual was instructed to chew a piece of Arabic chewing gum (0.4-0.5gm) for five minutes to stimulate salivary flow as much as possible (Al-Bazaz 2010) [7] and (Abd-awn *et al* 2012) [8] saliva was collected in sterilized screw capped bottles.

2- Isolation and identification of bacteria: isolation and identification of Streptococcus mutans was done according to the method described by Holbrook & Beighton (1986) [9] and Finegold & Baron (1986)[10]. Saliva was collected in sterilized screw capped bottles.

The collected saliva was homogenized by vortex mixer for two minutes. Ten-fold serial dilutions were prepared using sterile normal saline. Two dilutions were selected and inoculated on Mitis-Salivarius Bacitracin Agar (MSB Agar), the selective media for Streptococcus mutans, which was prepared according to the manufacturer's instructions. 0.1ml was withdrawn from dilutions of 10-1 and 10-2 using adjustable micropipette with disposable tips and then spread in duplicate by using sterile microbiological glass spreader on the plates of MSB agar. The plates were then incubated anaerobically by using a gas pack supplied in an anaerobic jar for 48 hours at 37°C followed by aerobic incubation for 24 hours at 37°C. A single colony from Streptococcus mutans was transferred to 10 ml sterile Brain Heart Infusion Broth (BHI-B) and then incubated aerobically for 24 hours at 37°C to activate the inoculums. The purity of the isolates was checked by inoculation of 0.1 ml of the isolates from BHIB suspensions on media by spreader as mentioned before, and then a selective colony was transferred to 10 ml of sterile BHI-B and incubated aerobically for 24 hours at 37°C.

3- Identification: Identification of *Streptococcus mutans* was done according to the following: -

a) Colony morphology.
b) Bacterial cell Morphology.
c) Biochemical test.
d) Identification system for *Streptococcus mutans* of Analytic Profile Index (API) 20 strep.

4- Antibacterial activity: The antibacterial activity of the Bay leaves aqueous extract was assessed in this study using the agar diffusion test. Four different concentrations were prepared from the stock of bay leaves aqueous extract.

The concentrations prepared were: 20 mg/ml, 30 mg/ml, 40 mg/ml, and 60 mg/ml..

The surface of sterile Muller- Hinton agar plates were inoculated with 0.2ml of a 24 hrs broth culture (10^5cfu/ml of test organisms and evenly spread using bent sterile glass rod) wells of 6mm in diameter were aseptically punched on each agar plate using a sterile cork bore. From stock solutions of extract four concentrations (20, 30, 40 and 60) mg/ml were prepared. 50µl of each concentration of each extract were loaded into wells separately. The inoculated plates were incubated at 37c^o for 24hrs and the antibacterial activity was evaluated by measuring the diameter (mm) of inhibition zones around the wells. All the tests were performed in triplicates.

5- The minimum bactericidal concentration determination (MBC): To determine The minimum bactericidal concentration (MBC) of the bay leaves aqueous extract, all the concentrations of the bay leaves aqueous extract that revealed inhibition zones were mixed with BHI-A to get 25ml of agar and extract then poured into Petri dishes and allowed to harden and inoculated with 0.1ml from the activated isolates of *Streptococcus mutans*. All these Petri dishes were incubated for 24 hours at 37°C including the control plates (negative control which contained BHI-A with microbial inoculums without the addition of the extract and the positive control plates which contained BHI-A and different concentrations of the aqueous extract without microbial inoculums). Each Petri dish was checked and examined for microbial growth. The minimum bactericidal concentration (MBC) was determined as the lowest concentration of the extract that killed the microorganisms.

6- Adherence: To study the effect of the Bay leaves aqueous extract on the adherence of Streptococcus mutans to tooth surface, three concentrations were used: the minimum bactericidal concentration (MBC) and two concentrations just lower than the MBC. These three concentrations were compared with 0.2% chlorhexidine gluconate, control positive (broth and bacteria without agent), and control negative (broth and agent without bacteria). A stainless steel wire was threaded from one end in the root of a previously cleaned, polished and sterilized first premolar.

The teeth were then immersed in 10 ml of the agent for 2 minutes except for control positive. The wires and the teeth were then washed with sterilized deionized water and dried, immersed in 10ml Brain Heart Infusion Broth containing 5% sucrose (pH=7). The study and control tubes were incubated with 2% of bacterial isolates and incubated aerobically at 370C for seven days. A positive score was given to the microbial growth on wire, teeth and bottle indicating a non-effective treatment and vice versa. This method was described by Al Bazaz 2010 [7] and aldhaher *etal.*2015.[11]

III. Results

Table (1) Antibacterial activity of different concentrations of bay leaves aqueous extract against streptococcus mutans

extract	Concentration (%)	Mean diameter of inhibition zone (mm)
Bay leaves aqueous extract	20	13.06
	30	14.88
	40	16.98
	60	20.4
chlorhexidine	0.2	16

F value = 436.12, P value = 0.000 (highly significant)

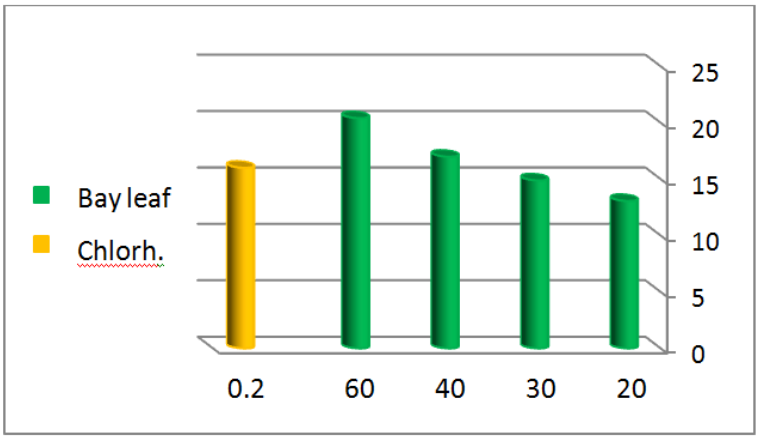

Figure 1: Bar Chart Graph showing the mean diameter of inhibition zones of the different concentrations of the Bay leaves aqueous extract and Chlorhexidine gluconate against *Streptococcus mutans*

The diameter of inhibition zone of the four concentrations of Bay leaves aqueous extract used in this study against S.mutans was demonstrated in the table (1) these diameters increased when the concentration of the extract increased this was demonstrated in figure (1)

Table 2: t-test between each concentration of bay leaves aqueous extract and chlorhexidine (CHX)

Concentration of extract	CHX	t-test
20	0.2	11.81*
30	0.2	11.43*
40	0.2	-9.77*
60	0.2	-25.19*

Comparison between each concentration used in this study and chlorhexidine was done using T-test Statistically highly significant difference (p 0,001) was shown and the antibacterial activity of Bay leaves aqueous extract against *S.mutans* was higher at the concentrations (40 mg/ml, 60mg/ml) than the antibacterial activity of chlorhexidine. Results revealed that 30 mg/ml is the MBC of Bay leaves aqueous extract for *S.mutans*. While bacterial growth is still shown when 20mg/ml of Bay leaves aqueous extract was used against *S.mutans* this concentration inhibit the growth of *S.mutans* but is not bactericidal for *S.mutans*.

Table 3: The effect of different concentrations of bay leaves aqueous extract on the adherence of *Streptococcus mutans*

Agents	Adherence
Bay leaves aqueous extract 30mg/ml (MBC)	Negative
Bay leaves aqueous extract 20mg/ml	Positive
Bay leaves aqueous extract 15mg/ml	Positive
Control positive	Positive
Control negative	Negative
Chlorhexidine 0.2%	Negative

The results of this study also revealed that bay leaves aqueous extract is very effective to prevent adherence of *Streptococcus mutans* to tooth surface at the concentration of 30mg/ml as shown in table (3)

IV. Discussion

Dental plaque is a significant risk factor for the development of dental and periodontal disease [(12)]. Recent evidence indicates that rather than being an accumulation of bacteria, it is a complex biofilm consisting of a variety of bacteria embedded in a polysaccharide matrix. This structure allows for bacteria to exhibit pathogenic characteristics [(13)].

One of the most commonly used anti-plaque chemical agents is chlorhexidine gluconate [(14)]. Even though this agent has well documented antimicrobial efficacy against bacteria associated with dental plaque, Zaura-Arite *et al* [(15)] showed that when chlorhexidine was applied to a 48-hour old plaque biofilm, it was only effective on the outer layer of the structure, whereas most of the middle and core layers were resistant to its effects. Chlorhexidine therefore requires multiple applications in order to be clinically effective. The chronic use of chlorhexidine has also been associated with oral epithelial desquamation, altered taste sensation and staining of the teeth and oral prosthesis [(14)].

These limitations together with that of other chemical plaque controlling agents, has prompted researchers to search for alternative antimicrobial sources . One of these includes the wide variety of herbs and plant-derived products that are currently marketed for therapeutic use [(1)]

Bay leaves extract has proven antimicrobial effects against a range of oral bacteria, including the periodontal pathogens. Therefore the antibacterial activity of Bay leaf aqueous extract against *Streptococcus mutans* was evaluated in this study and, the MBC was determined for the bay leaves aqueous extract but not for chlorhexidine gluconate since the latter is traditionally used in a concentration of 0.2% in the mouth rinses containing chlorhexidine gluconate

The results of this present study demonstrate that bay leaves aqueous extract has good antibacterial activity against streptococcus mutans and these results were in agreement with the results of Sumono and Wulan (2008) [(6)] who reported that *E. polyantha* (bay leaves) solution has the ability to reduce the numbers of *Streptococcus* spp.who, showed that *Eugenia polyantha Wight* can reduce *Streptococcus sp* colony in samples who rinsed with 100%, 75% and 50% *Eugenia polyantha Wight* solution. because it contains tanine, flovonoid and essential oil, that has antibacterial effect. In addition, Setiawan (2002) [16] also reported that bay leaves leaves are able to inhibit the growth of both gram positive and gram negative bacteria.
Several study also found that bay leaves extract has good antibacterial activity.[17, 18]

The antibacterial activity of Bay leaves may be attributed to tanine [17] Whereas, tanine is one of active matters of *Eugenia polyantha Wight* and part of phenol group,that that can inhibit the growth of bacteria by precipitation and denaturation of bacterial protein. Flavonoid also has antibacterial properties because it has the ability to interact directly with the DNA of the bacteria. The basic structure of the DNA itself has an important role in the transcription and duplication process, therefore, every compound that has the ability to disturb the stability of the double helix DNA structure will be able to affect all the growth process and metabolism of the bacteria. Those interaction will result in the damage of the permeability of the bacteria cell wall, microsome and lysosome. In addition, flavonoid is also capable to produce transduction energy that will affect the cytoplasm of the bacteria and slow down its motility. It is known that the hydroxyl ion that can inhibit the growth of bacteria by precipitation and denaturation of bacteria protein.[6]

V. Conclusion

dental caries remains the most prevalent and oral infectious disease worldwide antimicrobial mouthrinses are most frequently recommended to patients whose mechanical oral hygiene procedures are not adequate for the control of dental caries herbal extracts have significant antimicrobial effects against plaque bacteria and therefore they may be used as a natural mouth washes .this study proved that bay leaves is very effective against *Streptococcus mutans* and may be used as a natural mouth wash to prevent dental caries

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Zainab A. Aldhaher. "Effectiveness of Bay Leaves Aqueous Extract on Streptococcus Mutans In Comparision To Chlorhexidine Gluconate." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS), vol. 12, no. 4, 2017, pp. 12–16.