



Original Article

## Development of Guava Extract Chewable Tablets for Anticariogenic Activity against *Streptococcus mutans*

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**Abstract** *Streptococcus mutans* is a normal flora bacteria found in human oral cavity. This bacterium has been known to cause dental caries and bad breath odor. This study was conducted to develop the guava extract chewable tablets against *S. mutans* ATCC 25175. The guava leaves obtained from *Psidium guajava* L. were extracted with water by boiling 15 minutes and lyophilized to be the powder. The reconstituted crude extract was varied in concentration over the approximate 8×, 16×, 32× MIC and investigated for antibacterial activity. The minimal inhibitory concentration (MIC) against *S. mutans* of crude extract was 5 mg/ml which was equal to minimum bactericidal concentration (MBC). Crude extract was also tested for antimicrobial susceptibility compared with standard antibiotic kanamycin. The largest clear zone of  $12.50 \pm 0.71$  mm in diameter was observed in crude 32× MIC comparing to 13 mm in kanamycin. The negative control gave no clear zone. The guava chewable tablets were formulated as complied with commercial chewable tablets. The tablets were tested for their antimicrobial susceptibility test and largest clear zone of  $11.50 \pm 0.71$  mm in diameter was observed in the 32× MIC tablet. Moreover, measurement of bactericidal activity (time kill study) of 32× MIC tablet demonstrated the killing effect against *S. mutans*, the results showed that colony counts of tested bacteria were declined to zero within 2-3 hours. Although, those results support the traditional use of *P. guava* for the treatment of dental caries, further study is required for this medicinal plant. ©All right reserved.

**Keywords:** chewable tablet; dental caries; *Psidium guajava*; *Streptococcus mutans*

### INTRODUCTION

Dental infections such as dental caries is an infectious disease in which the oral bacterium *Streptococcus mutans* has been implicated as a principal etiological agent, although other oral bacterial species probably contribute to this disease. These bacteria are known to cause bad breath odor. Among the various oral micro-organisms, *S. mutans* has been identified as a plaque-forming bacterium capable of producing dental caries in experimental animals and in humans.<sup>1</sup>

The ability of *S. mutans* to adhere firmly to tooth surfaces in the presence of sucrose and to release acids by fermenting various dietary

sugars has been associated with its caries-inducing potential.

Many attempts have been made to eliminate *S. mutans* from the oral flora. Antibiotics such as chlorhexidine, penicillin, ampicillin, tetracycline, erythromycin and vancomycin are very effective in preventing dental caries *in vivo* and *in vitro*. However, their excessive use can result in alterations of the oral and intestinal flora and cause undesirable side effects such as the development of bacterial tolerance, vomiting, diarrhea and teeth stains. These problems necessitate further search for natural antimicrobial agents that are safe for humans and specific for oral pathogens.<sup>2</sup>

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Many natural products have been used in traditional medicine and shown to be a good alternative to synthetic chemical substances for caries prevention. Antibacterial compounds have been isolated from a large number of plant species throughout the world and it has been reported that high level of antibacterial activity was detected in guava leaves.<sup>2</sup>

Among plants recommended for dental caries, the toothpaste prepared from tender leaves of *P. guajava* commonly known as guava has been used in folklore practices to maintain the oral hygiene. Leaf extracts of guava have some pharmacological activities, such as anti-inflammatory, antidiarrheal, antioxidant, antimutagenic, besides antimicrobial activities. Their aqueous extracts have *in vitro* antibacterial effect on the growth of plaque bacteria.<sup>3</sup> It is then suggested that the extracts may have potential use as anti-plaque agents. Therefore, the objective of this study is to develop the guava extract chewable tablets for anticariogenic activity against *S. mutans*.

## MATERIALS AND METHODS

### Preparation of Material and Extract

Leaves of *Psidium guajava* L. (Myrteceae) were cleaned and dried in the oven at 60°C for 48 hours. The dried leaves were pulverized and then extracted by adding 1 g of plant powder to 10 ml of deionized water and brewed as tea by boiling for 15 minutes. The extract was allowed to cool and centrifuged. The supernatant was lyophilized and kept in refrigerator.

### In Vitro Antibacterial Sensitivity of Crude Extract against *S. mutans*

MICs were determined by the microdilution method recommended by the National Committee for Clinical Laboratory Standards with the Brain Heart Infusion (BHI) broth (Difco Laboratories, Detroit, Mich.). For MIC determinations, suspensions with a turbidity equivalent to that of a 0.5 MacFarland standard were prepared by suspending growth from the Brain Heart Infusion (BHI) agar (Difco Laboratories, Detroit, Mich.) plates in 2 ml of sterile broth.

Suspensions were further diluted to obtain a final inoculum of  $5 \times 10^5$  CFU per well. The controls included inoculated growth medium without plant extract sample. Sample blanks contained uninoculated medium only. Trays were incubated in the CO<sub>2</sub> incubator (5% CO<sub>2</sub>) at 37°C and the MICs were recorded after 24 hours of incubation. The MIC was defined as the lowest concentration of compounds at which the microorganism tested did not demonstrate visible growth. Minimum bactericidal concentration (MBC) was defined as the lowest concentration yielding negative subcultures or only one colony.

### Agar Diffusion Method

The BHI agar plates were swabbed with inoculum which a turbidity equal to a 0.5 MacFarland standard and cylinder cups were placed on plate with at least 15 mm from the edge of the plate and 24 mm apart from the other cups. The concentration of crude extract was varied into three concentrations which are 8, 16, 32 fold of the MIC. An amount of 300 µl of each concentration of crude extract was put in 6 mm diameter cup. All plates were incubated in the CO<sub>2</sub> incubator (5% CO<sub>2</sub>) at 37°C for 24 hours. The diameter of inhibition zone for each concentration after incubation was measured and compared to the standard antibiotic (kanamycin) and the negative control (water).

### Formulation of Guava Chewable Tablets

Guava chewable tablets were formulated as complied with commercial chewable tablets. Each tablet, total weight of 1 g, contained crude extract, PVP K30 (as 10 % solution), mannitol, aerosil, magnesium stearate, peppermint and menthol.

### Evaluation of Physical Properties of Guava Chewable Tablets

Guava chewable tablets were evaluated their physical properties as follows:

- Weight (g) was measured by an analytical balance with 4 digits.
- Hardness (kg) was determined by Tablet hardness tester (Monsanto type).
- % Friability was determined by Tablet friability apparatus (Drum).

### *In Vitro* Antibacterial Sensitivity of Guava Chewable Tablet against *S. mutans*

Antibacterial sensitivity test was tested using agar diffusion method as described above. The inhibition zone of each tablet was measured and compared to the standard antibiotic (kanamycin) and the negative control (placebo tablet).

### Measurement of Bactericidal Activity of Guava Chewable Tablets

Time kill assays provide information of the rates at which organisms are killed. Cell suspensions of *S. mutans* were prepared by growing in BHI broth and adjusted to match the turbidity of 0.5 McFarland standard. At the indicated time of incubation, sample of 1 ml was removed at 0, 30 seconds, 5, 15, 30 minutes, 1, 2 and 3 hours; made serial dilutions in 0.9 % NSS and 100  $\mu$ l aliquots were inoculated by spread plate method on BHI agar plates. The numbers of viable colonies were counted after incubated in CO<sub>2</sub> incubator (5% CO<sub>2</sub>) at 37°C overnight. Viable colonies were calculated to determine colony-forming unit (CFU) per ml and a graph of time against the logarithm of the viable count was plotted. Cultivation of bacteria in NSS was used as control.

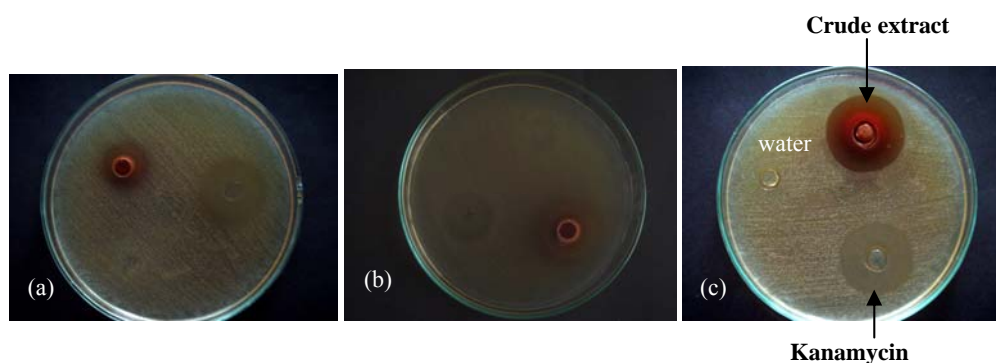
## RESULTS AND DISCUSSION

This study was designated to evaluate the antibacterial activities of aqueous extracts from leaves of *P. guajava*. The antibacterial activity of the extracts against *S. mutans* was

tested by using broth microdilution assay according to NCCLS. The MIC is defined as the lowest concentration of the compound at which the microorganism tested does not demonstrate visible growth and minimum bactericidal concentration (MBC) is defined as the lowest concentration yielding negative subcultures or only one colony. The MIC against *S. mutans* of crude extract was 5 mg/ml which was equal to the MBC. Other studies about antibacterial activities of *P. guajava* reported similar results.<sup>4</sup>

The antibacterial sensitivity was determined by agar diffusion method as described before, the largest clear zone of  $12.50 \pm 0.71$  mm was obtained in crude 32 $\times$  MIC, whereas the positive control, kanamycin, produced a clear zone of 13 mm. The negative control (water) gave no clear zone (Figure 1). Our result indicated that the crude extract at the concentration of 32 $\times$  MIC provided the highest inhibitory activity against the microorganisms, followed by 16 $\times$  MIC and 8 $\times$  MIC concentrations, respectively. On the basis of results obtained from this study, it is evident that the bioactive compound(s) such as flavonoids, tannins in the guava leaf extract is involved in bacteriostatic activity against *S. mutans* 25175.<sup>5</sup>

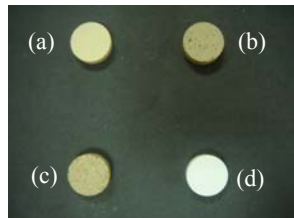
In preparing guava extract chewable tablets, the classical wet granulation method was used.<sup>6</sup> The ingredients of guava chewable tablets were indicated in Table 1. Physical appearances of the tablets were smooth, absence of cracks, uniform, not mottled and no physical imperfections (Figure 2).



**Figure 1.** The inhibition zones of crude extract and kanamycin against *S. mutans*: (a), crude extract at 8 $\times$  MIC; (b) crude extract at 16 $\times$  MIC and (c) crude extract at 32 $\times$  MIC.

**Table 1.** Ingredients of guava chewable tablets

Ingredients	Quantity in mg per 1 g tablet		
	8 MIC tablet	16 MIC tablet	32 MIC tablet
Crude extract	40	80	160
PVP K30 (as 10% solution)	qs	qs	qs
Mannitol	930	890	810
Aerosil	2	2	2
Magnesium stearate	20	20	20
Peppermint	3	3	3
Menthol	0.3	0.3	0.3

**Figure 2.** Photographs of guava chewable tablets: (a) 8× MIC tablet, (b) 16× MIC tablet, (c) 32× MIC tablet and (d) placebo tablet.

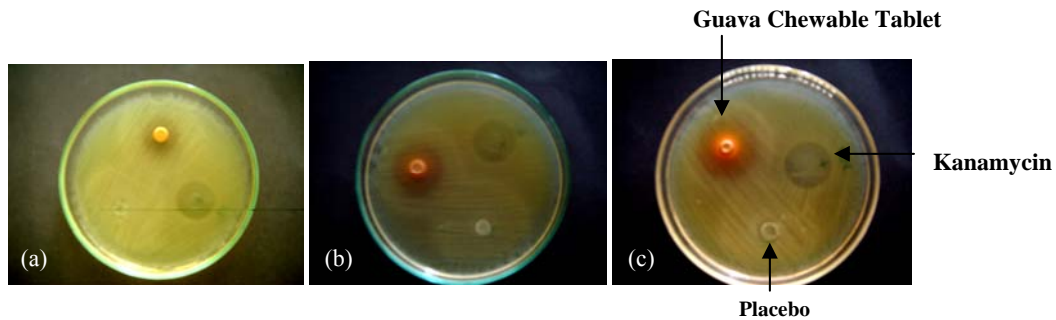
Many pharmacopoeias defined standards to which tablets should conform for factors such as uniformity of weight (weight variation) and friability. Other test which there was as yet no official requirements includes hardness. The uniformity of weight demonstrated by weight variation but in this study could not be demonstrated by this method because the requirements of this test which was defined certainly active ingredient in the product. Therefore, weight average was used to evaluate the physical properties of guava leaf chewable tablets. From evaluation of tablets at concentrations of 8×, 16× and 32× MIC found that the average weight (g) of 8×, 16× and 32× MIC tablets were 1.0230, 1.067 and 1.0470, respectively. As a result, weight average (g) of all three formulas of tablets was within range of 1 g. The hardness test was performed to provide a measure of tablet strength by using Monsanto Hardness Tester. Tablets should be hard enough for packaging and shipping but not so hard as to create difficulty upon chewing. The hardness (kg) was 8.2, 9.9 and 11.4, respectively. It was displayed that the hardness of 32× MIC tablet was higher than the others formula which the hardness of all three formulas were within standard range between 7-14 kg.<sup>7</sup> Furthermore,

a tablet may be compressed to a satisfactory hardness and yet show considerable powdering after normal handling, resulting in roughened edges and unsatisfactory appearance which was tested by the friability test and % friability of all three formulas were 0.752, 0.720 and 0.693, respectively which were not more than 1.0%, whereas 32× MIC tablet showed the lowest % friability. Therefore, the physical properties of guava chewable tablets corresponding to standard USP 29 which defined % friability of tablet sample not more than 1.0% were considered acceptable for chewable tablet products.

Three formulas of guava chewable tablets were tested for their antibacterial activity against *S. mutans* which carried out by Agar diffusion method compared with standard antibiotic, kanamycin. Water was used as negative control. The largest zone of inhibition was observed in 32× MIC tablet of  $11.50 \pm 0.71$  mm in diameter (Figure 3). It was indicated that 32× MIC tablet provided the best inhibition activity against this micro-organisms while 16× MIC tablet provided the lower inhibition activity than 32× MIC tablet but higher than 8× MIC tablet. Based on the observed, it would be possible that guava leaf chewable tablet contains constituents that are responsible for antibacterial activity.<sup>8-11</sup>

In this *in vitro* study, the measurement of bactericidal activity of guava chewable tablets was determined by time kill assay which normal saline solution was used to represent saliva in the oral cavity. Time kill curve of *S. mutans* indicated that 32× MIC tablet showed bacteriostatic activity after 15 minutes while, 8× MIC tablet and 16× MIC tablet showed bacteriostatic activity after 30





**Figure 3.** The inhibition zone of each concentration of tablets and kanamycin against *S. mutans*: (a) 8× MIC tablet, (b) 16× MIC tablet and (c) 32× MIC tablet.

minutes but showed no bactericidal activity as employed period of time of this study as shown in Figure 4. Limsong *et al.* (2004) also observed the similar results with guava extract on *S. mutans* ATCC 25175.

### CONCLUSION

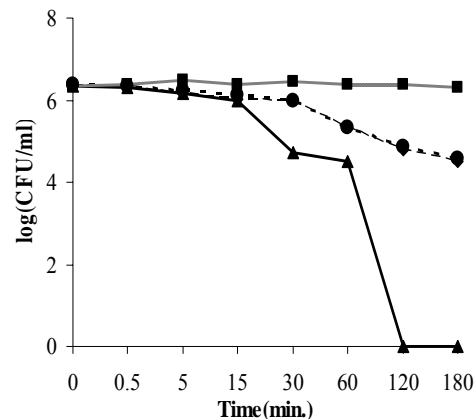
From this study, these observations indicated that 32× MIC tablet had highest growth inhibitory efficacy against *S. mutans* which suitable to apply and develop as antiplaque agent for the treatment of dental caries. It can be formulated as oral cavity consumer herbal products such as chewing gums, toothpastes, mouthwashes and dental floss. However, the formulation should have better taste because 32× MIC tablet produces the bitter taste and nonpleasant mouth-feel resulted from the stimulation of bitter taste buds by many organic medicinal compounds containing in the preparation.

### ACKNOWLEDGEMENTS

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**Figure 4.** Time kill assays of guava leaf chewable tablets against *S. mutans* ATCC 25175 and cultivation of bacteria in NSS: 8× MIC tablet (●), 16× MIC tablet (◆), 32× MIC tablet (▲) and normal saline solution (■).

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