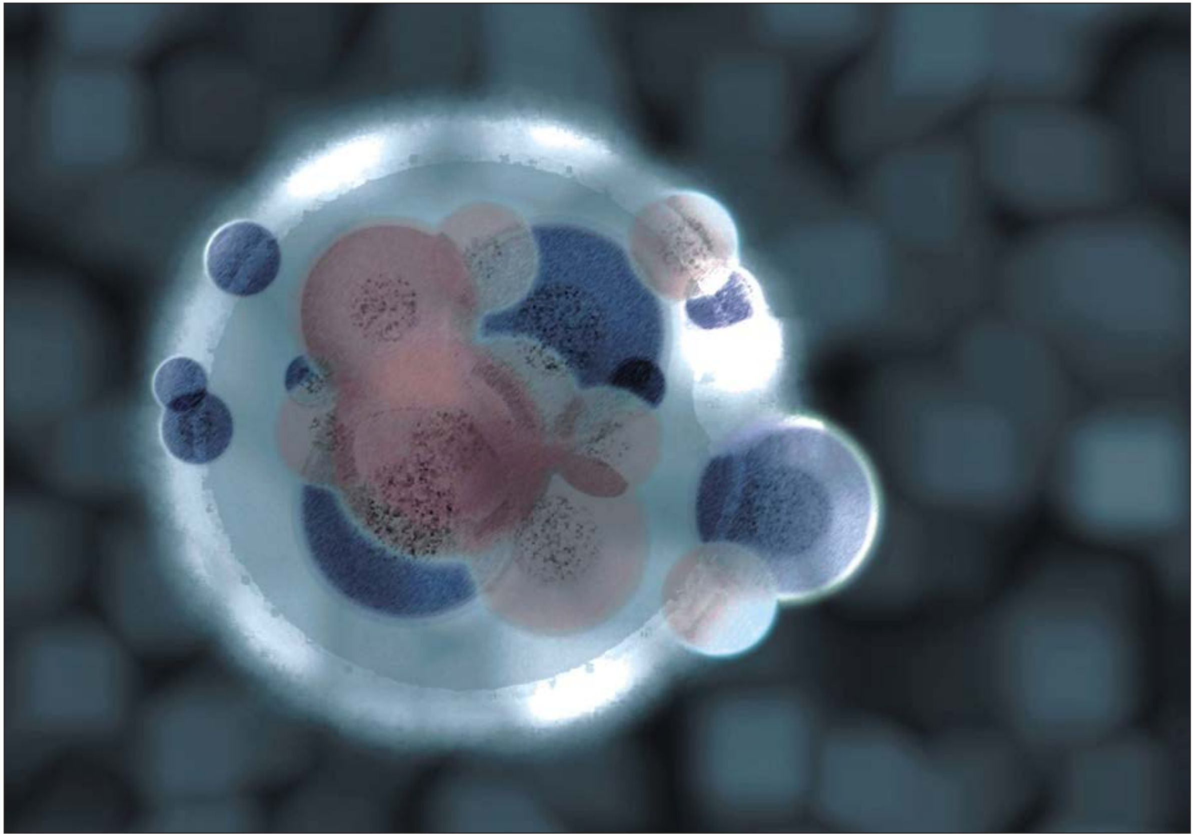

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Research Article
Development of Chewing Gum Containing Mulberry Leaf Extract with Anti-cariogenic Activity against *Streptococcus mutans*

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Abstract
Background and Objective: *Streptococcus mutans* is a major causative pathogen of dental caries, which is a multifactorial oral infection resulting in progressive tooth destruction. Several medicinal plants, including *Morus alba* (mulberry tree) have been used in folk medicine for the treatment and prevention of dental caries. The objectives of this study were to evaluate the antibacterial and anti-cariogenic activities of the *M. alba* leaf extracts against *S. mutans* and to develop a chewing gum containing the extract. **Materials and Methods:** The MIC and MBC were determined by using broth dilution and drop plate methods, respectively. Biofilm formation and glycolytic pH drop assays were performed to evaluate the anti-cariogenic activity. High-performance liquid chromatography was used for phytochemical analysis of the extracts. The chewing gum containing the *M. alba* leaf ethanolic extract was prepared by varying different types and concentrations of ingredients. The freeze-thaw cycle testing was conducted to determine the stability of the developed chewing gum. **Results:** The ethanolic and aqueous *M. alba* leaf extracts possessed antibacterial activity against *S. mutans* with the MICs of 0.14 and 5 mg mL^{-1}, respectively. The ethanolic extract at the sub-MICs significantly inhibited both acid production and biofilm formation of *S. mutans.* The phytochemical analysis showed that the concentrations of chlorogenic acid and rutin in the ethanolic extract were significantly higher than those in the aqueous extract. Two satisfactory chewing gum formulas with good stability, gum A and B, were obtained. The gum B formula was found to have the better performance. **Conclusion:** The results from this study demonstrated the anti-cariogenic role of *M. alba* leaves even at the low concentration of sub-MICs. The prepared chewing gum B formula can potentially be further developed as a medicated chewing gum for the prevention of dental caries in the near future.

Key words: Cariogenic properties, *Streptococcus mutans*, *Morus alba*, chewing gum

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dental caries (tooth decay) is an important oral bacterial infection affecting on people at any age. It has been reported that 60-90% of schoolchildren and almost 100% of adults have experienced dental caries worldwide[1]. If dental caries is left untreated, it can result in a loss of the affected teeth, which causes both physical and mental difficulties. A primary causative agent of dental caries is *Streptococcus mutans*, the Gram-positive facultative anaerobic cocci, residing in human mouth[2]. In the presence of fermentable carbohydrate, especially sucrose, the bacteria produce acid via fermentation, leading to an imbalance between dental mineral demineralization and remineralization, which is a critical etiology of dental caries. Additionally, sucrose is also metabolized by *S. mutans* into extracellular polysaccharides (EPSs), which play an essential role in *S. mutans* adherence on the tooth surface and subsequent biofilm or dental plaque formation. The EPSs also provide reserve of energy substrate and act as diffusion barriers, which keep the bacterial produced-acid close to the tooth surface and prompt a loss of dental mineral content[3]. Thus, acid production (acidogenesis) and biofilm formation are the major virulence factors of *S. mutans*, which primarily involve in the pathogenesis of dental caries[4]. These cariogenic characteristics of *S. mutans* promote its colonization and further aggravate progression of tooth decay[5].

Several medicinal plants, including *Morus alba* (mulberry tree) have been used in folk medicine for the treatment and prevention of dental caries. Traditionally, the leaves of *M. alba* are chewed to reduce further destruction of the tooth during toothache caused by dental caries[6]. The crude ethanolic extract of *M. alba* leaves was reported to possess antibacterial activity against *S. mutans* with the MIC of 0.125 mg mL^{-1}. The inhibitory actions against biofilm formation and surface adherence of the ethanolic leaf extract of *M. alba* were also documented[6,7]. However, the effect of the extract on *S. mutans*-induced glycolytic pH drop, which represents the acid-producing capacity of the bacteria, has not been explored yet. Additionally, the anti-cariogenic activity against *S. mutans* of the aqueous leaf extract of *M. alba*, which resembles its traditional use for the treatment and prevention of dental caries has not been clearly studied.

Although, much has been achieved with respect to the prevention of dental caries, maintenance of an effective oral hygiene by tooth brushing with advanced toothpaste formulations and mouth rinses remains beyond reach for many people. Other mechanical aids, such as chewing gum, have been promoted for caries prevention[8]. Most commercially available chewing gums contain dietary polyols, xylitol and/or sorbitol, which sweeten the gum but cannot be metabolized into acid by the bacteria. The caries-reducing effect of chewing gum is primarily attributed to saliva stimulation through the chewing process, particularly when using after meals[9]. The development of a chewing gum containing natural products with anti-cariogenic activity may further enhance the caries-preventive action of the chewing gum. This study thus aimed to investigate the effects of *M. alba* ethanolic and aqueous leaf extracts on *S. mutans* growth and its cariogenic properties. Additionally, the chewing gum containing the *M. alba* leaf extract was developed to be used as a potential anticaries product.

MATERIALS AND METHODS

Preparation of the *M. alba* ethanolic and aqueous leaf extracts: The leaves of *M. alba* (Buriram 60 variety) were collected from the plantation of the Silk Innovation Center, Mahasarakham University, Kamriang campus, Maha Sarakham, Thailand. The samples (leaf number 5-7 from the top) were authenticated by a specialist at the Silk Innovation Center. The leaves were cleaned with tap water and cut into small pieces. The samples were then dried in the hot-air oven at 45°C for 24 h and subsequently ground. For the aqueous leaf extract, 100 g of the ground sample were boiled in distilled water (1:10) for 3 h and filtered twice through a filter cloth. The filtrate were then filtered again through a filter paper (Whatman No. 1). The filtrate was further dried by using freeze dryer (ScanVac®, Denmark). For the ethanolic extract, 150 g of the ground samples were macerated with 95% (v/v) ethanol (500 mL) for 48 h and subsequently placed in a shaker incubator at 40°C, 150 rpm for further 48 h. The mixture was filtered through a filter paper (Whatman No. 1). The filtrate was concentrated by using rotary evaporator (Heidolph®, Germany) and then evaporated by using freeze dryer (ScanVac®, Denmark). The extracts were kept at -20°C before using in the experiment. Yields (%) of the aqueous and ethanolic extracts were 11.83 and 8.02%, respectively.

Identification and analysis of the phytochemicals in the *M. alba* ethanolic and aqueous leaf extracts: The analytical method was performed using HPLC technique from a previous study of Rana *et al.*[10] with slight modifications. The major compounds, chlorogenic acid and rutin in the ethanolic and

aqueous leaf extracts were analyzed by using Shimadzu LC-10AD Liquid chromatography (Shimadzu Corp., Kyoto, Japan). Identification and quantitation of the major compounds were performed by using Zorbax SB-C18 column, 250 mm×4.6 mm i.d., 5 µm (Agilent Technology, United States) coupled with photodiode array detector. The detection was monitored at 280 nm. Mobile phase consisted of acetonitrile (solvent A):0.05% Trifluoroacetic acid (TFA, solvent B) at flow rate1.0 mL min^{-1}. Gradient elution was set as followed; 10-20% (A), 0-12 min; 20- 25% (A), 12-20 min; 25-35% (A), 20-25 min; 35-40% (A), 25-27 min; 40-20% (A), 27-32 min; 20-10% (A), 32-37 min; 10% (A), 37-42 min. The amount of chlorogenic acid and rutin were finally reported as µg g^{-1} extract.

Determination of the MIC and MBC values: The Minimum Inhibitory Concentration (MIC) was determined by using a broth dilution method. *Streptococcus mutans* (DMST 1877) were inoculated into BHI broth media in test tubes and grown to a stationary phase up to 1.5×10^8 CFU mL^{-1} (McFarland No. 0.5) at 37°C with 5% CO_2. The bacteria suspension was further diluted with BHI broth to 1.5×10^6 CFU mL^{-1} (1 mL) and inoculated into fresh BHI (1 mL) containing various concentrations of the tested extracts. The MIC was recorded as the lowest concentration totally inhibiting visible bacterial growth in the test tube after 24 h of incubation. Chlorhexidine was used as the positive control whereas 10% DMSO (vehicle) was used as the negative control.

A drop plate method was used for the MBC value determination. The mixture (100 µL) from the test tube containing no visible bacterial growth from the earlier MIC determination experiment was spread onto the BHI agar and incubated at 37°C, 5% CO_2 for 24 h. The MBC was indicated by the lowest concentration of the extract resulting in no colony growth onto the plate. Three independent experiments were performed to obtain the mean MIC and MBC of the extracts.

Biofilm formation assay: The biofilm formation assay was performed following the method of Hasan *et al.*[11] with slight modifications. *S. mutans* (1.5×10^8 CFU mL^{-1}) were grown in 96 well microplate containing BHI (150 µL) with 5% (w/v) sucrose. The bacteria was incubated with the extracts at their sub-MICs or vehicle in 5% CO_2 incubator at 37°C for 6, 12, 20 or 24 h. After the incubation at the specific period, the media were decanted from the microplate to remove the planktonic cells. The microplate wells were washed gently with sterile deionized water to remove the remaining unattached cells. The adhered biofilm was fixed by adding formalin (37%, diluted 1:10) with 2% sodium acetate. The fixed biofilm was stained with 0.1% crystal violet (200 µL) and left for 15 min. The microplate wells were washed twice with sterile deionized water and then the biofilm-bound dye was removed by adding 150 µL of 95% ethanol. The microplate was shaken for 10 min and the volume of 100 µL was taken out to measure its optical density at 600 nm. Inhibition of biofilm formation was calculated as percentage from the following equation:

$$\text{Inhibition of biofilm formation (\%)} = \frac{OD_{600}\ \text{vehicle} - OD_{600}\ \text{extract}}{OD_{600}\ \text{vehicle}} \times 100$$

Glycolytic pH drop assay: The glycolytic pH drop assay was carried out according to the method of Ban *et al.*[12]. *S. mutans* were grown in BHI broth at 37°C in 5% CO_2 incubator for 24 h. The bacteria was washed with a salt solution containing KCl (50 mM) and $MgCl_2$ (1 mM) and the bacterial cell suspension (7.5×10^8 CFU mL^{-1}) was then prepared in the same salt solution. The extracts (at sub-MICs) or vehicle was added into the bacterial cell suspension and pH of the suspension was adjusted to 7.2-7.4 by using KOH (0.2 M). A solution of glucose (final concentration of 0.5% w/v) was then added into the bacterial cell suspension. The pH of the bacterial cell suspension was continuously recorded every 1 min over a period of 60 min. The initiate rate of pH drop, which indicates the best measure of the bacterial acidogenesis was calculated from the change of pH in the first 10 min (0-10 min).

Preparation of the chewing gum containing the *M. alba* leaf extract: The chewing gum bases were formulated and later be used to develop chewing gum preparations containing *M. alba* leaf extract (0.28 mg per piece of 2 g gum). Phase A, a water soluble phase comprised of water, polyvinyl alcohol, acacia gum and Tween 60 was heated until dissolved. Phase B, a water insoluble phase comprised of beeswax, polybutene, gum rosin and Span 80 was heated until obtaining homogeneous mass. Phase A was added into Phase B and stirred vigorously. When the temperature decreased to about 40°C, sweetening agents, flavoring agent and the ethanolic extract of *M. alba* leaves were added and stirred vigorously. The gum was rolled on a plate and cooled down. Hereafter, *M. alba* chewing gums were cut into pieces of 2 g mass and dried in desiccator for 7 days. The preparations were kept at 4°C until use. Performance

evaluations including physical observation and mastication test were carried out by two researchers[13]. The physical properties including gum mass homogeneity, color, production duration and adhesion on container surface were recorded. The toughness, elasticity, sticking to teeth and fragmentation of the gum after chewing were determined from the mastication test.

Stability test of the chewing gum containing the *M. alba* leaf extract: The stability of the chewing gum containing the *M. alba* leaf extract was determined by using the freeze-thaw cycle method. The chewing gums were placed in the freezer at -20°C for 4 h and then left at room temperature (25°C) for further 4 h. This freeze-thaw cycle was repeated for 5 cycles. Weight variation and content uniformity were evaluated after the last 5th cycle. The Total Flavonoid Content (TFC) assay was used to determine the content uniformity of the chewing gum preparation. The TFC assay was conducted according to the method of Fatemeh *et al.*[14]. The chewing gum preparation (2 g) was suspended with 95% ethanol (2 mL) and the supernatant was used for the TFC assay. One milliliter of the sample mixture was mixed with 5 mL of deionized water and 0.3 mL of $NaNO_2$ (5% w/v). The mixture was incubated for 5 min and then 0.6 mL of $AlCl_3$ (10% w/v) was added and subsequently left for further 5 min. Two milliliter of NaOH (1 M) and 1.1 mL of deionized water were then added and mixed thoroughly. The OD of the mixture was measured at the wavelength of 510 nm. The total flavonoid content was calculated from the quercetin standard curve and expressed as µg of quercetin equivalent (QE)/piece of chewing gum.

Statistical analysis: The data were expressed as Mean±SD (phytochemical contents, initial rate of pH drop, weight variation, TFC) or Mean±SEM (inhibition of biofilm formation (%)). The statistical analysis was conducted by one-way analysis of variance (ANOVA) followed by Bonferroni *post-hoc* test or paired t-test. The data were considered as significant difference when p-value is less than 0.05.

RESULTS

Identification and analysis of the phytochemicals in the *M. alba* ethanolic and aqueous leaf extracts: The amounts of chlorogenic acid and rutin in the ethanolic extract and aqueous extract were analyzed by using HPLC. The concentrations of chlorogenic acid and rutin in the ethanolic extract were significantly higher than those in the aqueous extract (p<0.05) (Table 1).

Antibacterial activity of the *M. alba* leaf extracts against *S. mutans*: The ethanolic and aqueous extracts of *M. alba* leaves inhibited *S. mutans* growth with the MICs of 0.14 and 5.01 mg mL^{-1}, respectively. However, the exact MBCs of the extracts could not be determined at the highest concentration tested (1.05 and 10.00 mg mL^{-1}, respectively). The MIC and MBC of chlorhexidine (positive control) were 0.625 and 1.25 µg mL^{-1}, respectively.

Effects of *M. alba* leaf extracts on *S. mutans* biofilm formation: The ethanolic extract of *M. alba* leaves at every concentration tested (sub-MICs) significantly inhibited biofilm formation at every incubation period in a concentration-dependent manner (Fig. 1a). The maximal inhibitory action of

Table 1: Amounts of chlorogenic acid and rutin in the *M. alba* leaf extracts

Extracts	Chlorogenic acid (µg mg^{-1} extract)	Rutin (µg mg^{-1} extract)
Ethanolic extract	21.49±3.07*	9.90±0.19*
Aqueous extract	16.89±1.69	5.69±0.60

*p<0.05 when compared to the aqueous extract (paired t-test test, n = 6)

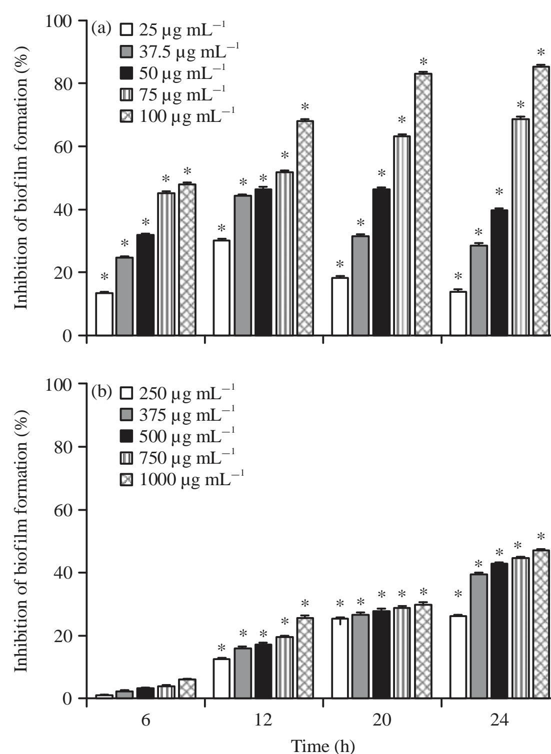

Fig. 1(a-b): Effects of the *M. alba* (a) Ethanolic leaf extract and (b) Aqueous leaf extract on biofilm formation of *S. mutans*

Data represented in Mean±SEM (n = 3), *p<0.05 when compared with negative control (One-way ANOVA followed by Bonferroni *post hoc* test)

84.79±0.98% was found at 24 h incubation with the highest concentration used of the ethanolic extract (100 μg mL^{-1}). On the other hand, the aqueous extract at every concentration tested (sub-MICs) produced a significant inhibitory action against biofilm formation at the incubation period of 12, 20 and 24 h but no inhibition was detected at 6 h incubation (Fig. 1b). At 12 and 24 h-incubation, the aqueous extract produced its inhibitory action against biofilm formation in a concentration dependent manner. However, at 20 h-incubation, the inhibitory action of the aqueous extract at any concentration used was quite similar.

Effects of *M. alba* leaf extracts on *S. mutans* glycolytic pH drop: The pH drop caused by glycolytic acid production of *S. mutans* was significantly inhibited by the ethanolic extract of *M. alba* leaves at every concentration tested (sub-MICs). The onset pH of 7.23±0.03 was decreased to 4.11±0.02 after 60 min-incubation in the control group, whereas the initial pH of 7.25±0.02 was decreased to 4.63±0.05 after 60 min incubation with the *M. alba* leaf ethanolic extract at the concentration of 100 μg mL^{-1} (Fig. 2). The ethanolic extract of *M. alba* leaves at every concentration tested (sub-MICs) significantly reduced the initial rate of pH drop with the lowest rate of 0.0797±0.0074 unit min^{-1} found at the concentration of 100 μg mL^{-1} ($p<0.05$, n = 3) (Table 2). Although, the aqueous extracts also decreased the initial rate of pH drop, these changes did not attain a statistically significant difference when compared to the negative control.

Preparation of the chewing gum containing the *M. alba* leaf extract: Since the ethanolic leaf extract of *M. alba* possessed the better antibacterial and anti-cariogenic activities than the aqueous extract, the *M. alba* ethanolic leaf extract was selected to be used in the chewing gum preparation. The gum bases were prepared by selection and optimization of an individual ingredient. Gum base B13 and B14 formulations, which comprised of the same excipients in different amount showed more desired performance than other gum base formulations (Table 3, 4). Therefore, the two chewing gum formulations containing *M. alba* ethanolic leaf extract (0.28 mg/piece), gum A and B were developed from the gum base B13 and B14. The performance evaluation of gum A and gum B was shown in Table 5. The results indicated that gum B had a better performance than gum A.

Stability test of the chewing gum containing the *M. alba* leaf extract: After the freeze-thaw cycle, weight variation and content uniformity of gum A and gum B were not different

Table 2: Effects of *M. alba* leaf extracts on initial rate of glycolytic pH drop

Extract	Concentration (mg mL^{-1})	Initial rate of glycolytic pH drop (pH unit/min)
Ethanolic extract	0	0.1750±0.0105
	25	0.1437±0.0042*
	37.5	0.1090±0.0035*
	50	0.1013±0.0035*
	75	0.0940±0.0026*
	100	0.0797±0.0074*
Aqueous extract	0	0.2033±0.0837
	250	0.0950±0.0527
	375	0.0933±0.0506
	500	0.0847±0.0506
	750	0.0697±0.0505
	1,000	0.0653±0.0454

*$p<0.05$ when compared with negative control (One-way ANOVA followed by Bonferroni *post hoc* test, n = 3)

Table 3: Ingredients of chewing gum base formulation (% w/w)

Ingredients	B13	B14
Gum base		
Polybutene	12	10
Polyvinyl alcohol	4.47	8.47
Gum rosin	8	8
Beeswax	20	20
Acacia gum	8.48	7
Emulsifiers	8	8
Sweetening agents	0.59	0.59
Flavoring agent	0.09	0.09
Purified water	38.37	37.85

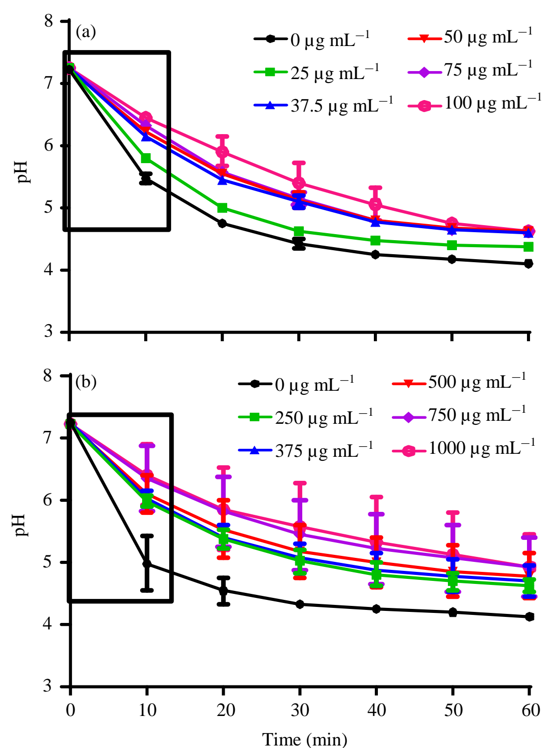

Fig. 2(a-b): Effects of *M. alba* (a) Ethanolic leaf extract and (b) Aqueous leaf extract on glycolytic pH drop
Values enclosed in box corresponds to the initial rate of the pH drop), data represent in Mean±SD (n = 3)

Table 4: Performance evaluation of the chewing gum bases

Performance evaluation	B13	B14
Physical properties		
Gum mass homogeneity	Homogeneous	Homogeneous
Color	White	White
Production duration	14 days	10 days
Adhesion on container surface	Partly adhere on container surface	Not adhere on container surface
Mastication test		
Toughness and elasticity	Fair	Fair
Sticking to teeth and gum fragmentation	Slightly stick to teeth Fragmentation detected within 3 min of chewing	No sticking to teeth and fragmentation observed within 3 min of chewing, some fragmentations detected after 3 min of chewing

Table 5: Performance evaluation of gum A and B formulations

Performance evaluation	Gum A	Gum B
Physical properties		
Gum mass homogeneity	Homogeneous	Homogeneous
Color	Bright green	Dull green
Mastication test		
Toughness and elasticity	Fair	Fair
Sticking to teeth and gum fragmentation	Slightly stick to teeth Fragmentation detected within 3 min of chewing	No sticking to teeth and fragmentation observed within 3 min of chewing, some fragmentations detected after 3 min of chewing
Flavor	Good, peppermint odor, no rancidity	Good, peppermint odor, no rancidity

Table 6: Freeze-thaw stability testing of the chewing gum containing the *M. alba* leaf extract

Gum	Freeze-thaw cycle	Weight variation (g) (n = 20)	Content uniformity (TFC) (µg QE/piece) (n = 10)
Gum A	Before	1.7279±0.2501	19.4779±3.9064
	After	1.7328±0.2506	21.3837±7.0272
Gum B	Before	1.8814±0.2003	16.6804±5.4859
	After	1.8268±0.2815	20.1591±2.0580

from before the freeze-thaw cycle (Table 6). This indicated the acceptable stability of the prepared chewing gum containing the *M. alba* leaf extract.

DISCUSSION

The ethanolic extract of *M. alba* leaves showed a more potent antibacterial activity against *S. mutans* than its aqueous counterpart with the MICs of 0.14 and 5.01 mg mL^{-1}, respectively. Nonetheless, the MBCs of both extracts could not be determined at the highest concentration tested. The MIC value of the ethanolic extract found in this study is thus in agreement with the results of Islam *et al.*[7] in which the MIC of 0.125 mg mL^{-1} was reported.

The ethanolic extract of *M. alba* leaves significantly inhibited the biofilm formation of *S. mutans* at every incubation period in a concentration-dependent manner, whereas the aqueous extract exhibited its inhibition at 12, 20 and 24 h-incubations. These indicated that the ethanolic extract affected on various phases of *S. mutans* biofilm. However, the aqueous extract did not influence on the 6 h adherent phase of biofilm formation. The inhibitory actions of the ethanolic *M. alba* leaf extract against *S. mutans* biofilm formation and glass surface adherence were also reported earlier by Islam *et al.*[7]. The EPSs produced by glucosyltransferase enzyme contribute to bulk and structural integrity of *S. mutans* biofilm[15]. Thus, the extracts possibly disrupted the biofilm formation by inhibiting against glucosyltransferase enzyme. Lokegaonkar and Nabar[6] reported that the ethanolic extract of *M. alba* leaf extract at the high concentration of 20 mg mL^{-1} potently inhibited the expression and function of *S. mutans* glucosyltransferase. However, it is not known whether the extracts at the sub-MICs produce those effects.

Acid-producing property of *S. mutans* is another crucial step in pathology of dental caries. The acidogenesis of *S. mutans* was significantly depleted in the presence of the ethanolic extract of *M. alba* leaves at the sub-MICs. This is the first report of the inhibitory action of the *M. alba* ethanolic leaf extract at the concentrations of sub-MICs against acid-producing property of *S. mutans*. Although the aqueous extract at the sub-MICs also decreased the initial rate of glycolytic pH drop, the inhibition did not reach a statistically significant level.

Chlorogenic acid and rutin are the two major phytochemicals which have been documented to be found in *M. alba* leaves[16,17]. It was shown from the phytochemical analysis that the ethanolic extract of *M. alba* leaves contained significantly higher amounts of both chlorogenic acid and rutin than that observed in the aqueous extract. The antibacterial and anti-cariogenic activities against *S. mutans* of the *M. alba* leaf extracts found in this study was thus likely to be linked with the amounts of both chlorogenic acid and rutin. Chlorogenic acid was reported to play a role in antibacterial activity against *S. mutans* of the coffee bean extract with the MIC of 0.8 mg mL^{-1} [18]. The MIC against *S. mutans* of rutin, isolated from *Vitis vinifera* were reported at 250 µM[19]. Islam *et al.*[7] also reported the potent inhibitory action of 1-deoxynojirimycin, isolated from the ethanolic extract of *M. alba* leaves with the MIC of 0.0156 mg mL^{-1}. Thus, 1-deoxynojirimycin may also act as an active antibacterial agent in the ethanolic extract of *M. alba* leaves.

Chlorogenic acid and rutin (500 µM), isolated from *Ilex paraguariensis*, significantly inhibited *S. mutans* glucosyltransferase activity with the inhibition (%) of approximately 35 and 70%, respectively[20]. Thus, suppression of glucosyltransferase by these two phytochemicals may also contribute the inhibition against biofilm formation of the *M. alba* leaf extracts. The evidence of purified plant based compounds acting against *S. mutans* acid production is still sparse. Gregoire *et al.*[21] showed that chlorogenic acid isolated from cranberry fruit (*Vaccinium macrocarpon*) had no effect on acid production of *S. mutans*. Meanwhile, the effect of rutin on *S. mutans* glycolytic pH drop has not been documented yet. However, it was reported that synthetic deoxynojirimycin and quercetrin, the phytochemicals which can be identified in *M. alba* leaves, significantly inhibited the glycolytic pH drop produced by *S. mutans*[5]. Thus, deoxynojirimycin and quercetrin possibly play a role in the anti-acid producing action of the *M. alba* leaf extracts.

A chewing gum provides oral health benefits including clearance of food debris, reduction in oral dryness, increase of biofilm pH and remineralization of tooth enamel. These basic effects of chewing gum are attributed to increased mastication and salivation[22]. An incorporation of the *M. alba* ethanolic leaf extract in the chewing gum may further amplify the above-mentioned benefits by reducing the number of harmful bacteria, specifically *S. mutans*, in saliva, which results in the lower amount of oral biofilm. Moreover, the ethanolic extract also had a significant inhibition against crucial cariogenic properties of *S. mutans* including biofilm formation and acid-production as shown in this study.

The gum base B13 and B14 were chosen to be used for the subsequent formulation of chewing gum containing the *M. alba* leaf extract since less fragmentation were observed after chewing when compared to other gum bases. Nonetheless, small fragments of gum base should not be detected after chewing in an ideally desirable chewing gum. Thus, further improvement is still needed for the formulation of the chewing gum base by adjusting the type and quantity of natural rubber, resin and plasticizer[23]. The normal salivary flow rate in human is 0.3-0.4 mL min^{-1}. During chewing, the salivary flow rate would accelerate twice into 0.7 mL min^{-1} [24]. The extract at the concentration of 0.28 mg/piece was used in the formulation of the chewing gum. Thus, in 2 min chewing, the extract concentration of approximately 0.2 mg mL^{-1} would be obtained in the saliva. At this concentration of above the MIC, the extract is expected to effectively exhibit its antibacterial and anti-cariogenic activities against *S. mutans*. However, further clinical experiments should be performed to confirm this suggestion.

CONCLUSION

The *M. alba* ethanolic leaf extract exhibited the antibacterial and anti-cariogenic activities against *S. mutans*. The extract was subsequently used in the formulation of a chewing gum with acceptable physical performance and stability. The prepared chewing gum containing *M. alba* leaf ethanolic extract can be further developed as a medicated chewing gum for the prevention of dental caries.

SIGNIFICANCE STATEMENT

This study demonstrated that the ethanolic leaf extract of *M. alba* possessed more pronounced activities against *S. mutans* growth and cariogenic properties than its aqueous counterpart. The chewing gum containing the *M. alba* ethanolic leaf extract with acceptable characteristics and stability was also successfully developed. This novel *M. alba* chewing gum formulation can be used as an oral care product model for further clinical and commercial applications in the near future.

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