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## Anti-Cariogenic Properties of Malvidin-3,5-Diglucoside Isolated from *Alcea longipedicellata* Against Oral Bacteria

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**Abstract:** The aim of this study was to investigate the anti-cariogenic effects of the indigenous Iranian medicinal plant *Alcea longipedicellata* (Malvaceae), to inhibit the growth and acid production of *Streptococcus mutans* and other cariogenic bacteria involved in dental plaque. The growth inhibitory activity of the ethanol and chloroform extracts were tested against *S. mutans*, *S. salivarius*, *S. sobrinus* and *S. sanguis*. From an ethanol extract of *A. longipedicellata* flowers, malvidin-3,5-diglucoside (malvin) was identified as a principal constituents which was responsible for antibacterial activity of extract. The malvin showed bacteriocidal activity, while ethanol and chloroform extracts was bacteriostatic. The MIC value of the malvin was 0.16-0.22 mg mL<sup>-1</sup>. *In vitro* studies had shown that 0.1% malvin could inhibit strongly acid-producing ability of *S. mutans* and salivary glycolysis up to 2 h post rinsing and reduced total bacterial counts of saliva up to 40% 3 h post rinsing. 0.1% malvin was about 60% effective in inhibiting bacterial adherence, as shown by the low weight of accumulated *S. mutans* plaque to glass surface. In conclusion, the anti-acidogenic effect of *A. longipedicellata* suggests that this material could be a useful source for the development of promising anti-cariogenic agents and led to use for pharmaceutical preparations such as mouth rinse.

**Key words:** *Alcea longipedicellata*, anti-cariogenic activity, dental plaque, malvidin-3,5-diglucoside, malvin, mouth rinse

### INTRODUCTION

Dental caries involves demineralization, cavitations and breakdown of calcified dental tissue and is caused by microorganisms that ferment dietary carbohydrates, notably sucrose, to produce acids; these acids initiate dissolution of the tooth enamel. The main bacteria in this group are *Streptococcus mutans*, *S. salivarius*, *S. sobrinus*, *S. sanguis* and *S. mutans* is generally known to be the principle causative of dental caries. These bacteria metabolize carbohydrates and producing an adhesive polysaccharide such as dextran from the glucose moiety and lactic acid from the fructose moiety. The synthesis of sticky, insoluble glucan promotes the firm adherence of the organism to the tooth surface that contributes to the formation of dental plaque. Moreover, research indicates that the coexistence of *S. sobrinus* and

*S. mutans* is an important factor in the development of dental caries (So *et al.*, 2007; Yoo *et al.*, 2007).

To achieve sufficiently low levels of plaque to support a healthy tooth several mechanical and chemical oral hygiene aids are necessary in addition to the toothbrush. Mechanical methods usually used in dental care services to remove plaques are time consuming and never completely effective (Gonzalez Begne *et al.*, 2001). Chemical approaches including use of antimicrobials, pH modifiers and many others alone or in combination with toothbrush and dental floss provide the best and reliable method in caries prevention (Xiao *et al.*, 2006). Strongly active substances like chlorhexidine gluconate 0.2% mouth rinse (CHX) are capable of reducing the percentage of cultivable microorganisms in the dental biofilm to 0.002%. But it should not be forgotten that some side-effects are present when CHX is used for extended

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periods of time such as staining of the tongue and of the dental biofilm, perturbation of the taste, burning sensations or mucosal erosion (Sweetman, 2005).

A large proportion of the population now prefers natural products and the use of the products with ingredients of plant origin is increasingly being offered. Therefore, a rinsing solution with inhibitory effect on plaque formation with anti-microbial activity will be very useful. Different plant-derived phytopharmaceuticals with antimicrobial activity have been used for oral cavity hygiene or treating of diseases (Didry *et al.*, 1998). Some of these phytopharmaceuticals have been shown to be a good alternative to synthetic chemical substances for caries prevention.

*Alcea longipedicellata* is a member of the Malvaceae family. *Alcea longipedicellata* used as traditional medicine for various diseases such as antiviral (Asres *et al.*, 2001), anti-inflammatory, astringent, demulcent, diuretic, emollient, febrifuge, blood circulation, for the treatment of constipation, dysmenorrhoea, haemorrhage (Tene *et al.*, 2007). In Iran, the role of *Alcea longipedicellata* in treatment of dyspepsia has been known for many years. *Alcea* species has attracted the attention of researchers because of their antimicrobial potential besides antioxidant, anti-inflammatory and cytotoxic activities, particularly due to flavonoids and other phenolic constituents. The blossom's liquid extract also exhibited anti-inflammatory activity and contains high concentrations of colorful flavonoids called anthocyanins (Farina *et al.*, 1995). Antibacterial effects of flavonoids have been observed previously against *B. cereus*, *B. subtilis*, *B. mycoides* and *S. aureus* (Marone *et al.*, 2001; Yari Kamrani *et al.*, 2007). It was therefore of interest, to examine the *A. longipedicellata* extract against dental plaque-forming bacteria.

## MATERIALS AND METHODS

**Plant material:** Flowers of *A. longipedicellata* were collected on August, 2006 from Ghotour valley, west Azarbaijan province, northern-west part of Iran, in an altitude of 1700 m. The plant was authenticated by Herbarium, Forests and Rangeland research institute of Iran and field identity was confirmed by Prof. V. Mozaffarian. Flowers were washed with distilled water and dried at room temperature to constant weight. The ethanol and chloroform extracts of *A. longipedicellata* flowers (85 g) were obtained by solvent-distillation method using a soxhelt apparatus for 3 h. The solvent was evaporated at reduced pressure to constant weight and the yields of the oily residue were 6.3 and 5.9 g, respectively.

**Bacteria:** The purely-isolated compound, chloroform and ethanol extracts were tested against 4 strains of bacteria (*S. mutans* ATCC 35668, *S. salivarius* 1448, *S. sobrinus* 1601 and *S. sanguis* 1449), which has been prepared from (PTCC or ATCC strains) and cultured in standard condition. These microorganisms were provided by Microbiology Laboratory, Razi Vaccine and Serum Institute, Tehran, Iran.

**Antimicrobial assay by agar diffusion method:** Antimicrobial activities of the ethanol (10%) and chloroform (10%) extracts of *A. longipedicellata* were determined using the agar diffusion method (Hirasawa *et al.*, 1999). Antibacterial activity was assayed by measuring the diameter of zone of inhibition against *Streptococcus* sp. seeded in inoculated Mitis salivarius (MS, Difco) agar plates. Non-alcoholic chlorhexidine gluconate 0.2% mouth rinse (CHX, Behvazan Co., Iran) was purchased from local market and used as a positive control.

**Flash column separation of ethanol extract:** The ethanol extract was deemed suitable for fractionation to recover the principal active constituent and was subjected to separation by normal phase flash column chromatography. The stationary phase consisted of approximately 200 mL of silica gel (40-63 m, 230-400 mesh). The ethanol extract (2 g) was loaded on the column and eluted using a continuous gradient of ethyl acetate to afford 44 fractions collected in 10-50 mL increments. Solvent was removed from each fraction by evaporation under reduced pressure. Column fractions were subjected to TLC separation and bioautography studies by spraying methyl thiazolyl tetrazolium chloride (MTT) reagent to monitor elution and relative purification of active constituents using standard method (Rohalison *et al.*, 1991).

**FT-IR and NMR spectroscopy:** The FT-IR spectrum was performed on a Nicolet 170SX spectrometer with a scanning range from 4000 to 400  $\text{cm}^{-1}$ . Proton and Carbon-13 NMR spectra were recorded on a Bruker DRX 500 spectrometer in  $\text{D}_2\text{O}$ .

**Determination of MIC values:** MIC values for chloroform, ethanol extracts and the isolated active compound (Malvin) from *A. longipedicellata* ethanol extract tested against *S. mutans*, *S. salivarius*, *S. sobrinus*, *S. sanguis* were measured by the liquid serial dilution method using 10 mL of sterile 5% Sucrose-Trypticase Soy Broth (TSB). The chloroform extract was diluted with chloroform and

mixed to medium with aid of sonication (50 w, 10 s). The ethanol extract was diluted with distilled water. Bacteria ( $1 \times 10^6$  cfu mL<sup>-1</sup>) were added to each culture tube containing serially diluted test extract or control and incubated for 24 h at 37°C under anaerobic condition. The lowest concentration of the extracts that inhibited growth was noted.

**Determination of bactericidal or bacteriostatic activity:**

To estimate whether inhibition of growth was bactericidal or bacteriostatic, 10 times MIC of the *A. longipedicellata* extracts were used in the experiment. Samples were collected over an extended period. The stocks concentration used were 1 mg mL<sup>-1</sup> malvin and 100 mg mL<sup>-1</sup> of ethanol extract for *Streptococcus* species. These samples were diluted and inoculated on to a plate at each appropriate time. After incubation, the number of colonies was counted. Further, to investigate the effect of the extracts and malvin on non-multiplying bacterial cells, resting bacterial cells were prepared. The growing cells were harvested, washed three times with 50 mM tris-HCL buffer (pH 7.3) and used for determination (Nguefack *et al.*, 2004).

**Inhibition of glycolysis:** The effects of the malvin on acid production by *S. mutans* was studied by a reported method (Corner *et al.*, 1990; Bae *et al.*, 2006) with slight modification. Two milliliter of fresh clarified human saliva, was added 0.1 mL of 5% glucose and 0.1 mL of various concentrations of the ethanol extracts of *A. longipedicellata* extract (6 and 10%) and 0.1% malvin. Control consisted of the saliva/glucose mixes containing 0.1 mL of PEG 300 (20% v/v). The pH of the samples were recorded immediately after preparation and at 10, 15, 30, 45, 60, 90, 120 min and 24 h.

**Effect on sucrose-dependent adherence to smooth glass surface:**

To assess the adherence of growing cells of *S. mutans* ATCC 35668 to a glass surface, organism was grown at 37°C at an angle of 30° for 18 h in test-tubes. Individual 18-24 h colonies from brain-heart infusion agar plates were suspended in 5.0 mL of sterile 145 mM NaCl and the suspension adjusted to 0.5 on McFarland scale. A portion of the suspension was mixed with TSB (1:100 dilution, v/v) containing 29 mM sucrose and then 2.48 mL were transferred to a test-tube. Subsequently, 20 µL of 2 fold dilution series of malvin, ethanol extracts (6 and 10%), CHX (0.2%) and water as a control were inoculated, gently stirred and then incubated. After incubation, the adherence cells were

washed and suspended (Bae *et al.*, 2006). The amount of adherent cells was measured at 550 nm by spectroscopy method. Five replicate were made for each concentration of the tested extract.

**Study in vivo:** Three young men, between the ages 22-30 years, with 20 or more natural teeth free of dental caries were recruited for this study. The protocol for study was approved by the local ethical review committee. The procedure, possible discomfort, or risk was fully explained to the volunteers and their written concede obtained. Subjects kept on their usual oral hygiene routines and no attempts were made to change or standardize of eating habits. We preferred to collect salivary rather than plaque samples, because salivary microbial counts are more consistent than plaque counts (Mundorff *et al.*, 1990; Ofek *et al.*, 2003).

Non-stimulated saliva samples (2 mL) were collected prior to study and served as the control and were placed immediately on ice. Mouth rinse was prepared by suspending 1 mg mL<sup>-1</sup> malvin isolated from *A. longipedicellata* in PEG 300 (20% v/v) and distilled water. The subjects were asked to rinse their mouths with 10 mL of mouth rinse for 1 min and do not rinsed their mouths with water afterwards. Further saliva samples were collected after 10 min, 1 and 3 h post-rinsing. Subjects were not allowed to eat or drink between sample collections. Placebo rinses was prepared using PEG 300 (20% v/v) in distilled water. Each saliva sample was immediately diluted 10<sup>3</sup> times with sterile saline and streaked on BHI Agar plates to determine the total saliva bacteria. Mitis salivarius agar (Difco) supplemented with sucrose (200 g L<sup>-1</sup>) and bacitracin (0.2 units mL<sup>-1</sup>) for the count of oral *Streptococci*. The plates were incubated into jars at 37°C for 24 h. After incubation, the colonies were counted using a stereomicroscope. Among colonies grown on mitis salivarius agar supplemented with sucrose, only those with the morphology of mutans *Streptococci* were considered.

**Statistics analysis:** Data obtained for acid production and inhibition of bacterial growth were analyzed. Data due to the mouth rinse were analyzed, for the inhibition of bacterial growth by comparing the prerinsing values with the post-rinsing values using paired t-test. For *in vitro* glycolysis, the data from the test at various time intervals were compared with those obtained for control at the same times interval using t-paired test. All statistical analysis was done in SPSS version 10.0. All values were considered significant with p<0.05.

## RESULTS

**Antimicrobial activity assay by agar diffusion method:**

Antibacterial activity was determined by measuring the diameter zone inhibition. The ethanol extract showed more inhibitory effect than chloroform extract on all test bacteria and used for rest of this study (Table 1). CHX always have highest inhibitory zone.

**Flash column chromatography and identification of active compounds:**

The ethanol extract was selected for separation by flash column chromatography for recovery of a principal active constituent as TLC had shown enrichment of the main compound and was deemed to be relatively free of other compounds which may have impeded recovery. The major compound eluted as an yellow oil with 450-650 mL of 100% ethyl acetate. TLC separation of fractions containing this compound resulted in migration of a compound of  $R_f$  0.63 (Fraction number-12), colored orange against a pink background prior to spraying and producing a purple spot upon derivatisation with the MTT reagent. Duplicate bioautography resulted in a large zone of inhibition of the same  $R_f$ .

The IR spectrum of isolated flavonoid showed a broad intensive band at  $3388\text{ cm}^{-1}$ , which is characteristic of the hydrogen bond, according to its position and structure. A sharp and relatively intensive, band at  $1640\text{ cm}^{-1}$  which corresponds to the bending vibrations of the OH group. Data for malvidin-3,5-diglucoside (malvin):  $^1\text{H NMR}$  (500 MHz,  $\text{D}_2\text{O}$ ,  $25^\circ\text{C}$ ) Aglycon: 8.70 (br s, H-4), 6.92 (d, 1.8 Hz, H-6), 7.26 (d, 1.8 Hz, H-8), 7.87 (d, 1.8 Hz, H-2',6'), 3.80 (bs,  $\text{OCH}_3$ ); Glucoside A and B: 5.35 (d, 7.8 Hz, H-1), 3.80 (t, 9.6 Hz, H-2), 3.64 (t, 9.6 Hz, H-3), 3.53 (t, 9.6 Hz, H-4), 3.67 (m, H-5), 4.62 (dd, 6.6, 12.2 Hz, H-6), 4.41 (dd, 6.6, 12.2 Hz, H-6), and  $^{13}\text{C NMR}$  ( $\text{D}_2\text{O}$ ,  $25^\circ\text{C}$ ) Aglycon: 167.1 (C2), 147.5 (C3), 138.9 (C4), 114.6 (C4a), 156.5 (C5), 103.7 (C6), 170.2 (C7), 113.5 (C8), 160.3 (C8a), 119.0 (C1'), 110.5 (C2'), 153.2 (C3'), 114.1 (C4'), 152.9 (C5'), 110.5 (C6'), 56.2 ( $\text{OCH}_3$ ), Glucoside: 103.2 (C1), 74.2 (C2), 77.4 (C3), 70.8 (C4), 78.4 (C5), 62.5 (C6). The structure of malvin is shown in Fig. 1.

**Minimum inhibitory concentration:** The results show that malvin had the most inhibitory effect rather than chloroform and ethanol extracts on the tested *Streptococcus* species (Table 2).

**Minimum bactericidal concentration:** Table 3 shows the antibacterial effects of *A. longipedicellata* extracts and isolated flavonoid on growing bacterial cells and resting bacterial cells of *S. mutans* ATCC 35668. The growth or survival of both the growing and resting cells treated with malvin decreased gradually with the incubation time.

Table 1: Mean area of the zone of microbial growth inhibition provided by ethanol and chloroform extracts of *A. longipedicellata* and chlorhexidine

Microorganisms	*Diameter of inhibition zone (mm)		
	Ethanol extract (10%)	Chloroform extract (10%)	Chlorhexidine (0.2%)
<i>S. mutans</i>	18±0.2	14±0.7	24±0.4
<i>S. salvarious</i>	20±0.4	14±0.7	26±0.5
<i>S. sanguis</i>	18±0.4	14±0.8	26±0.7
<i>S. sorbinus</i>	18±0.3	12±0.5	20±0.6

\*: The antimicrobial test was done using the agar-well diffusion method on BHI agar and incubated for 24 h at  $37^\circ\text{C}$ ; values are given in mm and expressed as mean±SD (n = 5)

Table 2: Minimal Inhibitory Concentration (MIC) of chloroform, ethanol extracts of *A. longipedicellata* and malvin against tested microorganisms

Microorganisms	Treatments ( $\text{mg mL}^{-1}$ )		
	Chloroform	Ethanol	Malvin (0.1%)
<i>S. mutans</i>	≥8	3.75	0.16
<i>S. salvarious</i>	6	3.75	0.28
<i>S. sanguis</i>	6	3.50	0.22
<i>S. sorbinus</i>	≥8	3.00	0.22

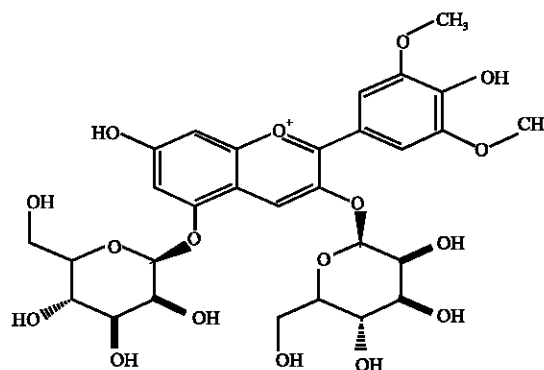


Fig. 1: Malvin (malvidin-3,5-diglucoside)

**Effect on acid production by salivary bacteria:** The result of *in vitro* salivary glycolytic assay revealed that at concentrations of 6% of the ethanol extract had no significant inhibition on acid production ( $p > 0.05$ ) when compared to vehicle (PEG 200 v/v and water). In glycolytic reaction, the pH values did not changed significantly ( $p < 0.05$ ) at higher concentrations of extract (10%) over a period of 120 min. The isolated flavonoid showed the good inhibitory results on acid production which is comparable to 10% ethanol extract ( $p < 0.05$ ). The significantly lower pH values of all the samples as compared with the control values are due to the low pH of the extract itself (Table 4).

**Inhibition of adherence of *S. mutans*:** As seen in Table 5 inhibition of *in vitro* adherence of *S. mutans* to glass

Table 3: Antibacterial effect of *A. longipedicellata* chloroform and ethanol extracts and malvin against *S. mutans* ATCC 35668 at growing stage

Tested materials	Time (h)				
	0	2.5	5	10	24
Chloroform extract	1×10 <sup>6</sup> *	9.5×10 <sup>5</sup>	9.0×10 <sup>5</sup>	8.3×10 <sup>5</sup>	7.1×10 <sup>5</sup>
Ethanol extract	1×10 <sup>6</sup>	8.0×10 <sup>5</sup>	6.8×10 <sup>5</sup>	6.2×10 <sup>5</sup>	5.3×10 <sup>5</sup>
Malvin	1×10 <sup>6</sup>	7.0×10 <sup>4</sup>	6.5×10 <sup>3</sup>	3.3×10 <sup>3</sup>	1.5×10 <sup>3</sup>
Distilled water	1×10 <sup>6</sup>	9.0×10 <sup>6</sup>	1.0×10 <sup>7</sup>	5.0×10 <sup>7</sup>	1.0×10 <sup>8</sup>

\*: Colony forming units (cfu mL<sup>-1</sup>)

Table 4: Temporal effectiveness of 6 and 10% ethanol extracts of *A. longipedicellata* and 0.1% malvin mouth rinse on *in vitro* salivary glycolysis inhibition pH of the before and post-rinsing saliva samples collected at various time intervals (min)

Extract concentration	0	10	15	30	45	60	90	120	1440
Saliva sample + Glucose	6.24±0.02	6.21±0.01	5.76±0.03	5.49±0.03	5.22±0.05	5.07±0.07	5.03±0.08	5.02±0.09	5.50±0.05
6% ETEX* + Glucose	6.14±0.03	6.09±0.01	5.83±0.04	5.56±0.10	5.37±0.04	5.28±0.04	5.19±0.05	5.00±0.10	5.39±0.03
10% ETEX + Glucose	5.69±0.02	5.68±0.02	5.59±0.06	5.44±0.03	5.32±0.04	5.25±0.01	5.17±0.04	5.01±0.03	5.15±0.07
0.1% Malvin + Glucose	5.74±0.05	5.73±0.03	5.82±0.04	5.88±0.03	5.92±0.03	5.91±0.03	5.94±0.01	5.92±0.01	5.63±0.02
PEG + water + Glucose	6.25±0.02	6.20±0.07	5.84±0.05	5.53±0.07	5.43±0.05	5.29±0.04	5.27±0.05	5.12±0.05	5.52±0.06

\*: Ethanol extract

Table 5: Temporal effectiveness of mouth rinsing with malvin (0.1%) from *A. longipedicellata* on total salivary bacterial and oral streptococci counts

Time (min)	Reduction of total salivary bacterial count after mouth-rinsing (%)*	Reduction of oral Streptococci count after mouth-rinsing (%)*
10	55.34±6.94	51.44±3.45
60	44.25±7.70	42.74±6.65
180	37.10±8.37	36.14±5.39

\*: Mean±SD (n = 5)

Table 6: Effect of various concentrations of ethanol extract (6 and 10%) of *A. longipedicellata* and 0.1% malvin on the inhibition of adherence of growing *S. mutans* PTCC-1601 to glass surface

Tested material	Ethanol Ex. (6%)	Ethanol Ex. (10%)	Malvin (0.1%)	CHX (0.2%)	Water
Inhibition of adherence (%)	39±0.57	45±1.52	60±0.57	94±1.52	36±1.15*

\*: Mean±SD (n = 5)

was evident when the cells were grown in BHI broth containing sucrose, 0.1% malvin and various concentrations of the ethanol extract of *A. longipedicellata*. At a concentration of 0.1% malvin showed about 55% inhibition of adherence while CHX (0.2%) produced an inhibition greater than 94%.

**Study in vivo efficacy of mouth rinses:** Mouth rinsing with 0.1% malvin isolated from *A. longipedicellata* showed a significant reduction in total Streptococcal count at 10 min, 1st and 3rd h of post rinsing (p<0.05). Rinsing with the vehicle showed no significant difference in the salivary bacterial count at any time interval (Table 6).

## DISCUSSION

Dental caries is still one of the health problem all over the world. The main bacterial species associated with this disease are *S. mutans* and *S. sobrinus*. Since the attachment and proliferation are key steps in successful colonization in the dental plaque biofilm and pathogenesis of these microorganisms, different strategies such as inhibition of bacterial growth and prevention of microbial colonization were developed to protect the dental caries. An ideal antimicrobial agent would inhibit both of these

activities. Antimicrobial susceptibility of oral has been tested to a number of plant extracts and natural substances (Xiao *et al.*, 2006; Didry *et al.*, 1998). *Alcea* species have a wide range of uses in herbal medicine in treatment of some diseases such as sore throats, laryngitis and tonsillitis, coughs, dryness of the lungs and digestive upsets (Schmidgall *et al.*, 2000). The blossoms of *Alcea* species contain: Mucilage, which on hydrolysis affords arabinose, glucose, rhamnose, galactose and galacturonic acid. The leaves also contain a small amount of tannins and noteworthy flavonoid sulfates (Farina *et al.*, 1995).

In this study, we assessed the antibacterial activity of *A. longipedicellata* extract on oral streptococci with the aim of preventing dental caries. Malvin has been demonstrated to manifest good antimicrobial activity, as evidenced by the MICs of obtained against oral Streptococci. But no significant difference was seen between the inhibitory growth of *S. mutans*, *S. sanguis*, *S. sobrinus* and *S. salivarius*. Either the malvin or CHX showed better antibacterial effect on *S. salivarius*, probably due to exhibition of the same antibacterial mechanisms.

Malvin killed more than 40% of both growing and resting cells of *S. mutans* ATCC 35668 within 1 h. With respect to *S. mutans*, the action of malvin was bactericidal.

Our data in agreement with other reports proposing that *A. longipedicellata* have anti-bacterial activity (Asres *et al.*, 2001). Malvin is one of the more stable anthocyanin derivatives. The acyl and glycosidic substituents of malvin, besides stabilizing the molecule, also give it good alcohol solubility.

The inhibition of acid production in the saliva samples after applying 0.1% malvin may be due to the antibacterial effect of malvin on the salivary bacteria *in situ* at that concentration. At concentration of 6% of ethanol extract, the pH values change significantly throughout the glycolytic reaction showing absence of anti glycolytic effect. The mechanism of the observed long term effect upon the acidogenicity of the saliva on mouthrinsing with the malvin could therefore be related to the malvin bactericidal or bacteriostatic effect. On the other hands, malvin had an immediate effect on the salivary bacteria and this effect continued up to 2 h. The importance of hydroxylation at the 2 position for antibacterial activity of malvin is supported by earlier work (Sato *et al.*, 1995). Methoxy groups were reported to drastically decrease the antibacterial activity of flavonoids while one or two additional hydroxyl groups at the 2 and 5' positions inhibited the growth of *S. mutans*.

In the sucrose-dependent adherence study, glass surface was used to represent the hard surface of the tooth (Fathilah and Rahim, 2003). Bacterial adherence to glass surface is the model system chosen because the adherence is mediated by glucan as well as the *in vivo* situation and the glass adherence assay is still used in some recent studies (Koo *et al.*, 2000; Nostro *et al.*, 2004). The adherence of *S. mutans* to the glass surface was markedly inhibited by sub-MIC concentration of isolated flavonoid from *A. longipedicellata*. Flavonoids are known to have anti-GTase activity (Prabu *et al.*, 2006). This enzyme is responsible for the conversion of sucrose to sticky insoluble glucan, which promotes the firm adherence of *S. mutans* to the surface of the tooth. This inhibition of bacterial attachment prevents adherence of *S. mutans* to glass surface by a mechanism thought to involve dextran formation from sucrose. Thus, the isolated flavonoid could successfully prevent plaque formation on the surface of the tooth, as it inhibited the sucrose-induced adherence, this process that foster the colonization of the organism on the surface of the tooth. At concentrations of 0.1% malvin had an immediate inhibitory effect on the salivary bacteria and this effect was retained for 3 h. The sustained antibacterial and antiglycolytic effect observed in the oral cavity on mouth rinsing with the isolated flavonoid from *A. longipedicellata* suggested that malvin had a good

substantively in the oral cavity. Based on results obtained from the present study, it is evident that malvin and other phytoconstituents present in the ethanol extract of *A. longipedicellata* are involved in the bactericidal and bacteriostatic activity against *S. mutans* at different concentrations. Thus, when considering the overall effect of malvin on *S. mutans*, it can be said that the use of malvin as a mouth rinse will have an effect on the growth, adherence and acid-producing ability of *S. mutans*.

We now have a scientific evidence to justify the use of *A. longipedicellata* as a mouth rinse which could maintain the oral hygiene and prevent dental carries.

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