Vietnam Journal of Chemistry, International Edition, **55**(5): 623-626, 2017
DOI: 10.15625/2525-2321.2017-00519

Anticaries activity of mangiferin isolated from *Mangifera indica* leaves in Vietnam

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Received 17 April 2017; Accepted for publication 20 October 2017

Abstract

Mangiferin (**1**) was isolated from Vietnamese *Mangifera indica* leaves. Its antimicrobial activities against the oral bacterium *Streptococcus mutans* GS-5 were evaluated in terms of inhibition of acid production and biofilm formation. The obtained results indicated that mangiferin at the concentration of 0.5 mM clearly inhibited acid production by this organism with a final pH value of 5.2 compared to 3.9 of the control. The reduction in biofilm biomass was found up to 92 % when it was treated with 1.0 mM mangiferin. Thus, mangiferin possesses moderate anticaries activity against *S. mutans* GS-5.

Keywords. *Mangifera indica*, mangiferin, *Streptococcus mutans*, anticaries activity.

1. INTRODUCTION

Streptococcus mutans is a gram-positive cocci shaped bacterium. The facultative anaerobe is commonly found in the human oral cavity, and is a major contributor of tooth decay. The result of decay can greatly affect the overall health of the individual [1]. *Streptococcus mutans* is the primary causative agent of human dental caries, one of the most popular diseases in human. *S. mutans* has been characterized as a strong biofilm producer.

Mangifera indica, commonly known as mango is a species of flowering plant in the family Anacardiaceae. It is found in the wild in Bangladesh, India and Pakistan where it is indigenous and cultivated varieties have been introduced to other warm regions of the world. The leaves of *Mangifera indica* have been claimed to be used to treat diarrhoea, dysentery, leucorrhoea, antioxidant [2], pains, malaria, diabetes, asthma, cough, antibacterial and throat affections [3] while the ashes from the burnt leaves have been used to manage burns, scald, toning up the gums and ulcer.

During our study on chemical constituent from *Mangifera indica* leaf, mangiferin compound was isolated and the structure was elucidated by 1D, 2D-NMR and MS. The anticaries activity of the compound against *S. mutans* was also evaluated.

2. MATERIALS AND METHODS

2.1. Plant material

Mangifera indica leaves were collected in Hanoi, Vietnam, on March 2017. A voucher specimen (No VHH.2017.2.05) was deposited at the Institute of Chemistry, VAST, Hanoi, Vietnam. Plant identification was performed by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature.

2.2. General experimental procedure

All NMR spectra were recorded on a Bruker Advance 500 FT-NMR spectrometer (500 MHz for ^{1}H, and 125 MHz for ^{13}C-NMR), and chemical shifts (δ) are reported in ppm using TMS as an internal standard. HR-ESI-MS spectra were recorded on Varian 910-FT-MS. Optical rotations were determined on a Jasco DIP-370 automatic polarimeter (Jasco, Tokyo, Japan). Column chromatography (CC) was performed on silica gel 230-400 mesh (0.040-0.063 mm, Merck) or YMC RP-18 resins (30-50 µm, Fujisilisa Chemical Ltd). Thin layer chromatography was performed on DC-Alufolien $60F_{254}$ (Merck 1.05715) or RP_{18} F_{254}, (Merck) plates. Compound was appeared by spraying with aqueous 10 % H_2SO_4 and heating for 5 minutes at 150 ^{o}C.

Antimicrobial assays: In the present study, the antimicrobial activity of mangiferin compound was tested against *Streptococcus mutans* GS-5.

S. mutans GS-5 is a gift from Prof. Robert E. Marquis, University of Rochester, New York, US. It is proven virulent cariogenic strain selected for genomic sequencing [4]. The organism was grown in tryptic soy agar at 37 ^{o}C and subculture every week. For growth experiment, *S. mutans* GS-5 was statistically cultured in tryptone (3 %), yeast extract (0.5 %) and glucose (1 %) (TYG) at 37 ^{o}C. The cells are harvested and washed with the salt solution containing 50 mM KCl and 1 mM $MgCl_2$ for further experiment [5]. For glycolytic pH drop experiment, the cells were resuspended in dense suspension containing 2 mg (dry weight)/ml in the salt solution. The suspension was titrated with KOH to a pH just above 7.2. Glucose was added to the final concentration of 55.6 mM, and the fall in pH at room temperature was monitored with a Beckman 45 pH to detect acid production by *S. mutans* [6]. For biofilm experiment, the biofilms were grown in static batch cultures containing 3 % tryptone, 0.5 % yeast extract plus 1 % sucrose (TYS) broth in a 37 °C incubator for 24 h. Biofilms were formed in 96 wells plate. The amount of formed biofilms was detected by staining the wells with crystal violet solution (1 % w/v). The crystal violet was then dissolved in aqueous acetic acid (30 % v/v in distilled water) and the measured absorbance at λ = 595 nm (A_{595}) [7].

2.3. Extraction and isolation

Mangifera indica leaves (2.2 kg) were separated from the stems and oven dried at 40 ^{o}C for 72 h until a constant weight was obtained. Thereafter, the dried leaves were pulverized and extracted three times with hot MeOH and then evaporated under reduced pressure to give crude MeOH extract (187.0 g).This extract was suspended in water and then partitioned with *n*-hexane and butanol to give the *n*-hexane (MG1, 55.0 g), butanol (MG2, 85.0 g) and water residues (MG3, 36.0 g) after removal of the solvents *in vacuo*. The MG2 fraction (85.0 g) was chromatographed on a silica-gel column eluting with CH_2Cl_2–MeOH gradient (60:1 → 0:1, v/v) to yied four sub-fractions MG2A (12.0 g), MG2B (16.0 g), MG2C (25.0 g), and MG2D (23.0 g). The MG2C fraction was chromatographed on an RP-18 column, eluting with methanol- water (1:1, v/v) to give two smaller fractions MG2C1 (5.8 g), MG2C2 (7.4 g). The MG2C2 fraction was chromatographed on a RP-18 column, eluting with butanol-acetic acid-water (4:1:5, v/v) to yield **1** (95.0 mg).

Mangiferin (1): Yellow amorphous powder, mp: 269-270°, λ max: 205.6, 256.8, 238.4, 315.2, 367.2 nm. IR (KBr) cm^{-1}: 3366(O-H), 2937(C-H), ^{1}H-NMR (DMSO-*d*6, 500 MHz), ^{13}C-NMR (DMSO-*d*6, 125 MHz), see table 1; HR-ESI-MS found *m/z* 423.09264 $[M+H]^+$ (Calcd. for $C_{19}H_{19}O_{11}$, 423.09274).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow amorphous powder and its molecular formula was determined to be $C_{19}H_{18}O_{11}$ by HR-ESI-MS at *m/z* 423.09264 $[M+H]^+$ (Calcd. for $C_{19}H_{19}O_{11}$: 423.09274).

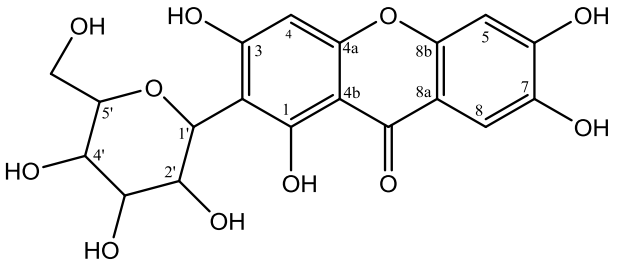

Figure 1: Structure of mangiferin

The ^{1}H-NMR spectrum of **1** (table 1) showed the following signals of three downfield aromatic singlet signals at δ_H 6.37 (s, 1H, H-4), 6.86 (s, 1H, H-5), and 7.38 (s, 1H, H-8). Furthermore, the characteristic chemical shifts of the sugar moiety were observed at δ_H 4.58 (1H, d, *J* = 10.0 Hz, H-1´), 4.04 (1H, m, H-2´), 3.16 (3H, m, H-3´, H-4´, H-5´), 3.41 (1H, dd, *J* = 11.0, 2.1 Hz, H-6´a), and 3.67 (1H, dd, *J* = 11.0, 4.6 Hz, H-6´b). The ^{13}C-NMR and DEPT spectra exhibited the presence of nineteen carbons, including nine quaternary carbons at δ_C 161.7 (C-1), 107.6 (C-2), 163.8 (C-3), 156.2 (C-4a), 101.3 (C-4b), 150.7 (C-6), 143.7 (C-7), 111.7 (C-8a), 154.6 (C-8b), one methylene at δ_C 61.5 (C-6'), eight methines at δ_C 93.3 (C-4), 102.6 (C-5), 107.6 (C-8), 73.1 (C-1'), 70.2 (C-2'), 78.9 (C-3'), 70.6 (C-4'), 81.5 (C-5'), and one carbonyl 179.1 (CO). The carbon signal at δ_C 73.1 (C-1´), suggested the C-*β*-D-glucoside moiety. In addition, the other carbons signals of the sugar appeared at δ_C 70.2 (C-2´), 78.9 (C-3´), 70.6 (C-4´), 81.5 (C-5´), and 61.5 (C-6´). Analysis of ^{1}H and ^{13}C-NMR spectroscopic data of **1** (table 1) showed that they were similar to those of mangiferin [8], a C-*β*-D-glucoside xanthone from the *Bersama engleriana*. The proton signals are assigned to the corresponding carbon signals directly on the basis of analyzing the interactions get on HSQC spectrum. The HMBC correlations of the anomeric proton between H-1' (δ_H 4.58) and C-2 (δ_C

107.6)/C-3 (δ_C 163.8)/C-1 (δ_C 161.7)/C-2' (δ_C 70.2)/ C-3' (δ_C 78.9) suggested the sugar moiety was at C-2. The location of the OH at C-1 was based on the correlations between proton at δ_H 13.74 and C-2 (δ_C 107.6)/C-1 (δ_C 161.7)/C-4b (δ_C 101.3), and hydrogen bonding with the carbonyl. The location of the aromatic protons at C-4, C-5, and C-8 was based on their HMBC correlations between H-4 (δ_H 6.37) and C-2 (δ_C 107.6)/C-3 (δ_C 163.8)/C-4a (δ_C 156.2)/C-4b (δ_C 101.3); between H-5 (δ_H 6.86) and C-8a (δ_C 111.7)/C-8b (δ_C 154.6)/C-6 (δ_C 150.7)/C-7 (δ_C 143.7); between H-8 (δ_H 7.38) and C-8a/C8b/C-6/C-7 (δ_C 32.36). From the above evidence and comparative data reported in the literature, the structure of **1** was determined as mangiferin as shown in figure 1.

Table 1: NMR data of compounds **1** and mangiferin [8]

C	Mangiferin [8]	δ_C [a,c]	δ_H [a,d] (mult., *J* = Hz)	HMBC
1	161.7	161.7	13.74 (1-OH)	C2, C-1, C-4b
2	107.5	107.6	-	
3	163.8	163.8	-	
4	93.3	93.3	6.37 (s)	C-3, C-2, C-4a, C-4b
4a	156.2	156.2	-	
4b	101.2	101.3	-	
5	102.4	102.6	6.86 (s)	C-8b, C-8a, C-6, C-7
6	150.9	150.7	-	
7	143.9	143.7	-	
8	107.8	107.6	7.38 (s)	C-8a, C-8b, C-6, C-7
8a	111.4	111.7	-	
8b	154.6	154.6	-	
CO	179.0	179.1	-	
1'	73.1	73.1	4.58 (d, 10.0)	C-1, C-2, C-3, C-2', C-3'
2'	70.3	70.2	4.04 (t, 10.0)	C-1', C-3'
3'	79.0	78.9	3.16 (m)	C-2', C-5'
4'	70.6	70.6	3.16 (m)	C-5'
5'	81.5	81.5	3.16 (m)	C-4', C-6'
6'	61.4	61.5	3.41 (dd, 11.0, 2.1), 3.67 (dd, 11.0, 4.6)	C-5'

[a]Measured in DMSO-*d*6, [c]125 MHz, [d]500 MHz, mangiferin [8] measured in DMSO-*d*6.

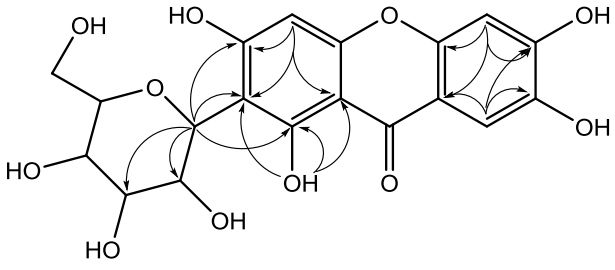

Figure 2: Important HMBC correlations of compound **1**

Inhibition of acid production of *Streptococcus mutans* by compound 1

Cariogenicity of *S. mutans* can be related directly to its capacity to produce acids from glycolysis at low pH values. As shown by the data in Fig. 3, glycolysis by cells of *S. mutans* in suspensions was clearly inhibited by mangiferin at concentration of 0.25 mM.

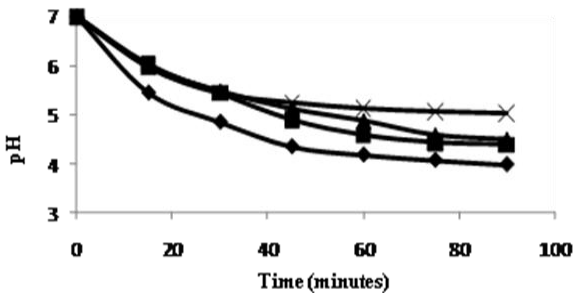

Figure 3: Effect of MG2 on acid production by *S. mutans.* (◆) Control; (■) 0.1 mM; (▲) 0.25 mM; (×) 0.5 mM

At the concentration as high as 0.5 mM, the final pH was 5.2 compared to 3.9 of the control. In the assay, the organisms were given excess glucose (0.5 % m/v or ca. 28 mmol/l) so that, as shown in the figure, glycolysis was not limited by catabolite supply. The inhibition was dose-dependent. The results also suggest that the proton-translocating membrane F-ATPases located on the membrane and enzymes of the glycolytic pathway in the cytoplasm may be sensitive to mangiferin.

Inhibition of biofilm formation by *Streptococcus mutans*

The effect of mangiferin on the ability of *S. mutans* to form biofilms was measured in a plastic microplate. The results presented in Fig. 4A showed a reduction in biofilm biomass up to 67 % was found at the concentrations of 0.5 mM and 92 % at the concentration of 1.0 mM compared to the untreated biofilms. The same result was found with biofilms formed in eppendorf tubes and stained with crystal violet (Fig. 4B).

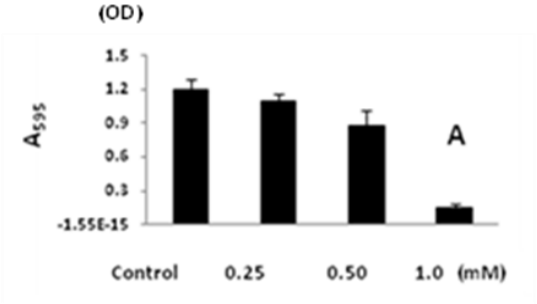

Figure 4: Effect of mangiferin on biofilm formation by *S. mutans*

In general, mangiferin inhibits acid production and biofilm formation by *S. mutans*, a major causative agent of dental caries in human. This suggests that the compound possesses an anti-caries activity. Further examinations on action mechanism of mangiferin on *S. mutans* are under examination.

Acknowledgments. *The authors would like to thank Institute of Chemistry, VAST. This work was financially supported by project VHH.2017.2.05.*

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