Antibacterial Activity of Prenylated Xanthones from Pericarp of *Garcinia mangostana* against Persistent Dental Infection Microorganism *Enterococcus faecalis*

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Abstract

As a part of ongoing research on antibacterial agents from botanical dietary supplements, Garcinia mangostana L. (commonly known as manggis in Indonesia) was selected for a detailed study. The dried and milled pericarp of G. mangostana was extracted by maceration with MeOH (3 × 5 L) at room temperature for 3 days. After filtration and evaporation of the solvent under reduced pressure, the combined crude methanolic extract (250 g) was suspended in H_2O (600 mL) to produce an aqueous solution, then partitioned in turn with n-hexane (3 × 500 mL), EtOAc (3 × 500 mL) and n-BuOH (3 × 500 mL) to afford dried n-hexane (32 g), EtOAc (40 g) and n-BuOH (60 g) extracts.

The EtOAc-soluble extract was found to have a significant antibacterial activity against Enterococcus faecalis ATCC 29212. Therefore, the EtOAc extract was selected for detailed purification. Repeated chromatography on silica gel of a EtOAc-soluble extract of pericarp led to isolation of four prenylated xanthones. The chemical structures of compounds 1-4 were identified as a-mangostin, b-mangostin, g-mangostin and garcinone-D on the basis of spectroscopic data and comparison to those related data previously reported.

The antibacterial activities of these compounds were evaluated against E. faecalis performed using broth micro dilution method. Among all compounds, compound 3 (g-mangostin) exhibit the most potent since it has a high inhibition diameter value in low concentration (10.93 d/mm in 100 mg/L).

Keywords: *Garcinia mangostana*, mangostin, antibacterial activity, *Enterococcus faecalis*.

Introduction

A successful pulp treatment depends on the technique and the status of radicular and coronal pulp tissues as well as on the type of medications used. In addition, infection control is an important factor that cannot be separated from successful primary dental pulp treatment.[1] Reducing or eliminating microorganism infection is the most important factor for this success through chemomechanic instrumentation process that can reduce most of the bacteria. However, microorganism retention in the dentin tubule usually causes permanent infection.[2]

The complexity of pulp chamber anatomy, as well as the microorganisms' ability to survive during starvation, leads to a situation where the microorganisms remain in the pulp chamber and root canal despite appropriate mechanical instrumentation and irrigation procedures.[3]

Therefore, pulp medications should be able to eliminate the residual microorganisms, neutralize the microorganism toxic products and prevent recurrent infection. The microorganism that is often isolated in the case of failed pulp treatment is *Enterococcus faecalis* anaerob facultative positive gram coccus. Although it is isolated in a small number in the primary tooth, this pathogen is able to proliferate in the root canal; therefore, it should be eliminated to recover and restore the tooth's normal function.[4] Pharmacological tests have shown that materials from nature are a source for potential bioactive compounds that may act as antibacterial agent. This antibacterial characteristic has led to a search for bioactive compounds that can be used as a primary tooth disinfection material especially against *E. faecalis*.[5]

Garcinia mangostana Linn. (mangosteen) belongs to Guttiferae family that has been used as in traditional medicine such as to cure skin infections, diarrhea, and chronic wound.[6] Mangosteen rind has been reported to contain a secondary metabolite that has a broad antibacterial biological activity including against *Enterococcus*.[7] Phytochemical study showed that the active component comes from the xanthone derivative group. Xanthone is a secondary metabolite that can be found in high-plants including mangosteen. Several studies show that xanthone extracted from mangosteen presents biological activities such as antioxidant, antitumor, anti-inflammation, antiallergic, antibacterial, antifungal, and antiviral activities.[8]

Although xanthone is already known as having a broad-spectrum of antibacterial activity, its use against *E. faecalis* has not been widely tested. The purpose of this study was to evaluate anti-bacterial activity of xanthone derivative compounds of mangosteen (*G. mangostana*) peel extract against *E. faecalis*.

Material and Methods

General Experimental Procedure: ^{1}H (500 MHz) and ^{13}C-NMR (125 MHz) spectra were measured on a JEOL JNM A-500 instrument and chemical shifts were given in d (ppm) values using TMS as an internal standard. Chromatographic separations were carried out on silica gel 60 (Merck) and silica gel GF_{254} for TLC preparative. TLC plates were precoated with silica gel GF_{254} (Merck, 0.25 mm) and detection was achieved on UV light at the wave length at 254 and 367 nm.

Plant material: The pericarp of *Garcinia mangostana* Linn (GML) was collected in Puspahiang Plantation center, Tasikmalaya, West Java, Indonesia on April 2011 and identified by Mr. Joko Kusmoro (Universitas Padjadjaran). A voucher specimen has been deposited at the Herbarium of Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor, Sumedang, Indonesia.

Plant extraction: The dried pericarp (538.4 g) of GML was extracted with ethanol exhaustively (4 L × 3) at room temperature for 3 days. The extract was filtered and concentrated in a rotary evaporator to remove the ethanol to yield a reddish-brown extract (92.4 g). The extract (90 g) was subjected to silica gel vacuum liquid chromatography with *n*-hexane-ethyl acetate-methanol gradient to obtain 12 fractions combined according to TLC results. The 10% ethyl acetate eluate was re-chromatographed through a silica gel column with *n*-hexane:ethyl acetate (7:3) to obtain 12 fractions (A01-A12). Sub-fraction A06 was chromatographed on silica gel with *n*-hexane-ethyl acetate to yield 7 fractions (B01-B07). Sub-fraction B05 was further separated on preparative TLC on silica gel GF_{254} with *n*-hexane:acetone (4:1) to yield compound 1 (60 mg) and compound 2 (42 mg).

20% ethyl acetate eluate was chromatographed of silica gel, eluted successively with a gradient of *n*-hexane-ethyl acetate (20:1 to 1:2), to give seven sub-fractions (C01-C07). Sub-fraction C03 was chromatographed on a column of silica gel, eluted with *n*-hexane-acetone (1:1), to give compound 3 (50.8 mg). The 40% ethyl acetate eluate was chromatographed of silica gel, eluted successively with *n*-hexane-acetone (10:1 to 1:1) to give ten sub-fractions (D01-D10). Sub-fraction D05 was subjected to preparative TLC on silica gel GF_{254} eluted with *n*-hexane:EtOAc:acetone (3:2.5:0.5) and *n*-hexane:acetone (8:2), to give compound 4 (7 mg).

Spectroscopic data of compounds

a-mangostin (1) as a pale-yellow powder: UV l_{max} nm (log e): 246 (4.50), 318 (4.34), and 350 (4.05), IR ν_{max} cm^{-1}: 3480 (OH), 1670 (C=O), 1615 (C=C, Ar), 1456, 1270, and 1160. ^{1}H NMR (acetone-d_6, 500 MHz) d: 1.63, 1.64 (3H each, s, H-5' and H-5''), 1.78 (3H, s, H-4''), 1.83 (3H, s, H-4'), 3.36 (2H, d, *J*=6.9 Hz, H-1''), 3.79 (3H, s, 7-OMe), 4.12 (2H, d, *J*=6.4 Hz, H-1'), 5.30 (2H, m, H-2' and H-2''), 6.40 (1H, s, H-4), 6.80 (1H, s, H-5), 9.43, 9.55 (1H, each, brs, C-3-OH and C-7-OH), 13.80 (1H, s, C-1-OH). ^{13}C NMR (acetone-d_6, 125 MHz) d: 17.8 (C-4''), 18.4 (C-4'), 22.0 (C-1''), 25.9 (C-5'), 26.0 (C-5''), 26.9 (C-1'), 61.4 (7-OMe), 93.4 (C-4), 102.7 (C-5), 103.6 (C-1a), 111.3 (C-2), 112.2 (C-8a), 123.6 (C-2''), 125.0 (C-2'), 131.5 (C-3' and C-3''), 138.1 (C-8), 144.5 (C-7), 155.7 (C-3), 156.2 (C-6), 157.4 (C-5a), 161.7 (C-1), 170.0 (C-4a), 182.4 (C-10).

b-mangostin (2) as a pale-yellow powder: UV l_{max} nm (log e): 250 (4.55), 316 (4.30), and 348 (4.08), IR ν_{max} cm^{-1}: 3475 (OH), 1672 (C=O), 1615 (C=C, Ar), 1450, 1270, and 1165. ^{1}H NMR (acetone-d_6, 500 MHz) d: 1.61 (3H, s, H-4''), 1.62 (3H, s, H-4'), 1.72 (3H, s, H-5''), 1.75 (3H, s, H-5'), 3.28 (2H, d, *J*=7.0 Hz, H-1'), 4.00 (2H, d, *J*=7.0 Hz, H-1''), 5.17 (1H, t, *J*=7.0 Hz, H-2'), 5.18 (1H, t, *J*=7.0 Hz, H-2''), 3.73 (3H, s, 3-OMe), 3.82 (3H, s, 7-OMe), 6.24 (1H, s, H-4), 6.74 (1H, s, H-5), 9.32 (1H, brs, C-6-OH), 13.35 (1H, s, C-1-OH). ^{13}C NMR (acetone-d_6, 125 MHz) d: 17.8 (C-5''), 18.3 (C-5'), 21.3 (C-1'), 25.8 (C-4'), 29.7 (C-4''), 31.2 (C-1''), 55.8 (3-OMe), 62.0 (7-OMe), 88.9 (C-4), 101.5 (C-5), 103.8 (C-1a), 111.5 (C-2), 112.3 (C-8a), 122.3 (C-2'), 123.4 (C-2''), 131.7 (C-3''), 132.0 (C-3'), 137.0 (C-8), 142.5 (C-7), 154.5 (C-4a), 155.3 (C-5a), 155.7 (C-6), 160.1 (C-1), 163.4 (C-3), 182.0 (C-10).

g-mangostin (3) as a pale-yellow powder: UV l_{max} nm (log e): 248 (4.56), 320 (4.36), and 352 (4.10), IR ν_{max} cm^{-1}: 3500 (OH), 1665 (C=O), 1620 (C=C, Ar), 1460, 1280, and 1170. ^{1}H NMR (acetone-d_6, 500 MHz) d: 1.62 (6H, s, H-5' and H-5''), 1.78 (3H, s, H-4''), 1.80 (3H, s, H-4'), 3.34 (2H, d, *J*=6.8 Hz, H-1'), 4.18 (2H, d, *J*=6.4 Hz, H-1''), 5.27 (2H, m, H-2' and H-2''), 6.36 (1H, s, H-4), 6.80 (1H, s, H-5), 7.60, 9.40, 9.51 (1H, each, brs, C-3-OH, C-6-OH, and C-6-OH), 13.85 (1H, s, C-1-OH). ^{13}C NMR (acetone-d_6, 125 MHz) d: 17.5 (C-4''), 18.1 (C-4'), 22.1 (C-1''), 25.9 (C-5'), 26.0 (C-5''), 26.3 (C-1'), 93.0 (C-4), 101.3 (C-5), 103.9 (C-1a), 110.4 (C-2), 112.1 (C-8a), 123.6 (C-2''), 124.6 (C-2'), 129.4 (C-8), 131.5 (C-3' and C-3''), 141.7 (C-7), 152.3 (C-5a), 153.4 (C-6), 155.9 (C-3), 161.8 (C-1), 161.9 (C-4a), 183.5 (C-10).

Garcinone D (4) as a yellow powder: UV l_{max} nm (log e): 248 (4.47), 322 (4.25), and 348 (4.10), IR ν_{max} cm^{-1}: 3480 (OH), 1660 (C=O), 1610 (C=C, Ar), 1440, 1265, and 1150; ^{1}H NMR (acetone-d_6, 500 MHz) d:1.65 (3H, s, H-4''), 1.66 (3H, s, H-4'), 1.79 (3H, s, H-5'), 1.84 (3H, s, H-5''), 3.40 (2H, d, *J*=7.0 Hz, H-1'), 3.62 (2H, d, *J*=7.0 Hz, H-1''), 3.85 (3H, s, 3-OMe), 5.27 (1H, t, *J*=7.0 Hz, H-2'), 5.35 (1H, t, *J*=7.0 Hz, H-2''), 7.30 (1H, t, *J*=7.8 Hz, H-7), 7.40 (1H, dd, *J*=7.8, 1.6 Hz, H-6), 7.71 (1H, dd, *J*=7.8, 1.6 Hz, H-8), 13.20 (1H, s, C-1-OH). ^{13}C NMR (acetone-d_6, 125 MHz) d: 18.0 (C-4''), 23.0 (C-1''), 23.2 (C-1'), 25.2 (C-4'), 25.8 (C-5''), 25.9 (C-5'), 62.2 (3-OMe), 106.5 (C-1a), 114.4 (C-4), 116.4 (C-8),117.8 (C-2), 121.6 (C-6), 122.1 (C-8a), 123.6 (C-2''), 123.9 (C-2'),124.7 (C-7), 131.9 (C-3''), 132.0 (C-3'), 146.6 (C-5a), 147.3 (C-5), 153.7 (C-4a), 159.8 (C-1), 164.8 (C-3), 182.9 (C-10).

Test Solutions: The test solutions consisted of xanthone derivatives ranging from 100 to 10000 ppm concentration in methanol solvent (Tables 1 to 4). The positive control treatment was chlorhexidine gluconate in 2000 ppm and 600 ppm concentration (0.2%) while the acetone was also used as negative control.

Bacteria Revitalization: The bacteria used in this study were *E. faecalis* ATCC 51299. The bacteria were revitalized by growing the bacteria in Muller Hinton Broth liquid media for 18-24 hours with a mixing speed of 150 rpm at 37°C. If the turbidity of the liquid media had already met the Mc Farland standard, which was evident through the bacteria turbidity concentration of equals to 10^8 CFU/mL, the re-vitalization can be performed. The grown bacteria in the liquid media were retrieved using a loop that was scratched on a blood agar solid media. The bacteria were grown in the incubator with 5% CO_2 for 18-24 hours.

Antibacterial Activity Test: The antibacterial activity test was performed using the paper disc diffusion method (Kirby-Bauer Test). In this method mangosteen peel extract xanthone derivatives were defined for its bacteria activities through diffusion with the gelatin plate that were already inoculated by the test bacteria i.e. *E. faecalis*. The paper disc containing antibacterial agent was placed on the gelatin media planted with the test bacteria that would be diffused on the gelatin media. The concentrations selected were ranging from 100 to 2000 ppm in methanol solvent. The positive control treatment used was Chlorhexidine gluconate in 2000 and 600 ppm concentration and the acetone negative control was also used as solvent. The Petri dish was then incubated in 37°C for 24 hours and a caliper (mm) was used to measure the inhibition zone formed around the hole.

The observation was basically to see whether there is a growth inhibition zone for the tested bacteria or not. The inhibition zone is a clear area formed around the antibacterial agent. The clear area indicates that there is an inhibition of growth of the tested bacteria that is caused by the antibacterial agent on gelatin surface.

Results and Discussion

Repeated chromatography on silica gel of a EtOAC-soluble extract of pericarp led to isolation of four prenylated xanthones 1-4. The known compounds were identified using NMR and by comparison with published data for a-mangostin (1),[9] b-mangostin (2),[10] g-mangostin (3)[9] and Garcinone D (4)[11] (Figure 1).

The antibacterial activities of these compounds were evaluated against *E. faecalis* which were performed using disk diffusion testing (two repetitions). Compounds 1, 2, 3 and 4 exhibited antibacterial activity against *E. faecalis* with minimum inhibitory concentration (MIC) as follows: 2500, 250, 300, and 100 mg/L respectively (Table 1, 2, 3 and 4). From the mangosteen peels, 4 extracts were successfully extracted i.e. ethylacetate extract, methanol, *n*-butanol, and *n*-hexane. These four extracts were tested for their antibacterial activity against *E. faecalis* to get the most active minimum inhibition concentration of the extract for further isolation to get the most potent single compound.

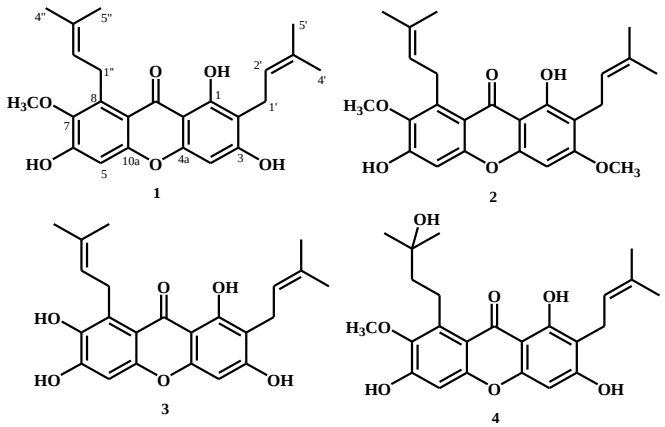

Figure 1: Chemical structure of prenylated xanthones.[14]

The antibacterial testing method applied was the disk diffusion testing (two repetitions). The ethyl acetate extract and methanol extract from the mangosteen extract in 5000 ppm, 10000 ppm and 20000 ppm concentrations present activities by forming the inhibition zone against *E. faecalis*.

Among prenylated xanthone derivatives from mangosteen extract, α-mangostin has been shown by several studies to exert the most potent antibacterial activity.[10,12] From a previous study of a-mangostin isolated from the stem bark of *G. mangostana* L., it was found to be active against vancomycin resistant Enterococci (VRE) and methicillin resistant *Staphylococcus au*reus (MRSA) with MIC values of 6.25 and 6.25 to 12.5 mg/mL respectively. These findings suggested that α-mangostin alone or in combination with GM against VRE and in combination with VCM against MRSA might be useful in controlling VRE and MRSA infections.

Crude extract of GML pericarp on cariogenic bacteria such as *S. mutans* with MIC and MBC was similar to the result from Torrungruang et al[13] study in 2007. The strong bactericide activity from this extract shows that GML is a candidate for further development of antiplaque material and caries. Extract test of mixed GML pericarp and papain as irrigation solution of permanent tooth root canal showed an effective result against *Streptococcus gordonii* (MIC 11.25) and *E. faecalis* (MIC 0.66).[14]

The use of chlorhexidine as disinfecting materials deciduous dental pulp replacing sodium hypochlorite has been commonly used. Research by Zamany et al[15] concluded that chlorhexidine was quite effectively used as a disinfectant dental pulp. In this study, the MIC chlorhexidine is still higher than a-mangostin, b-mangostin, g-mangostin, and garcinone D, but it appears that chlorhexidine was using 2-fold concentration (2000 ppm) while the concentrations of a-mangostin and b-mangostin is 1000 ppm.

Table 1
Analysis of compound 1 against *E. faecalis*

S.N.	Concentration (ppm)	Inhibition diameter (d/mm)		Mean (d/mm)	Result
1	10000	7.80	8.00	7.90	Active
2	5000	7.60	7.90	7.75	Active
3	2500	8.10	7.60	7.85	Active
4	Acetone negative control	6.30	6.25	6.28	Active
5	Positive control: *Chlorhexidine* (2.000)	15.80	16.15	15.98	*Susceptible*

Table 2
Analysis of compound 2 against *E. faecalis*

S.N.	Concentration (ppm)	Inhibition diameter (d/mm)		Mean (d/mm)	Result
1	1000	10.20	10.25	10.23	Active
2	500	8.90	7.75	8.30	Active
3	250	8.00	7.75	7.88	Active
4	Acetone negative control	6.65	6.30	6.48	Active
5	Positive control: *Chlorhexidine* (2.000)	15.70	15.10	15.40	*Susceptible*

Table 3
Analysis of compound 3 against *E. faecalis*

S.N.	Concentration (ppm)	Inhibition diameter (d/mm)		Mean (d/mm)	Result
1	600	13.40	12.20	12.80	Active
2	300	13.40	11.30	13.35	Active
3	100	11.00	10.85	10.93	Active
4	Acetone negative control	-	-	-	Inactive
5	Positive control: *Chlorhexidine* (600)	19.40	18.40	18.90	*Susceptible*

Table 4
Analysis of compound 4 against *E. faecalis*

S.N.	Concentration (ppm)	Inhibition diameter (d/mm)		Mean (d/mm)	Result
1	600	11.60	10.20	10.90	Active
2	300	10.00	9.60	9.80	Active
3	100	-	-	-	Inactive
4	Acetone negative control	-	-	-	Inactive
5	Positive control: *Chlorhexidine* (600)	19.25	19.80	19.53	*Susceptible*

Conclusion

Four prenylated xanthones, a-mangostin, b-mangostin, g-mangostin and garcinone-D, have been isolated from pericarp of *G. mangostana*. The antibacterial activities of these compounds have been evaluated against *E. faecalis* using broth micro dilution method. Among all compounds, compound 3 (g-mangostin) exhibits the most potent antibacterial activity.

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