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# Anti-bacterial Activity of Prenylated Xanthone from The Bark of *Garcinia lowa*

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**Abstract**

Bioactivity-guided fractionation of a ethyl acetate extract of *Garcinia lowa* bark has led to the isolation and identification of a known prenylated xanthone, mangosharin, (2,6-dihydroxy-8-methoxy-5-(3-methylbut-2-enyl)-xanthone (**1**, 15.4 mg) The structure of the compound was identified from analysis of their spectroscopic data and by comparison with previous studies.Compound **1** showed anti-bacterial activity against *Sreptococcus mutans* with MIC value of 7.25μg/mL.

***Keywords***: *Anti-bacterial, Garcinia lowa, mangosharin, prenylated xanthone.*

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## 1. INTRODUCTION

The genus *Garcinia* belong to Guttiferae family is widespread in the tropical rain forest of South East Asia and West Africa and well known to be rich in prenylated xanthones (Ampofo and Waterman, 1986; Bennet and Lee., 1989; Adegboye, 2008). Extensive research has shown that the *Garcinia* species exhibit a wide range of biological and pharmacological activities such as cytotoxic, antimicrobial, antimalarial and anti-HIV-1 ptotease inhibitory activity (Kosela *et al.,* 2000). During the course of our continuing search for novel antibacterial compounds from Indonesia *Garcinia* plants, the methanolic extract of the bark of *Garcinia lowa*, showed a significant antibacterial activity against *Sreptococcus mutans. Garcinia lowa*commonly known as "manggis hutan" in Indonesia, is a slow-growing tropical evergreen tree with leathery and glabrous leaves. The tree can attain 8-20 m in height and is mainly found in Indonesia and Malaysia (Martin, 1980). The edible fruit aril is white, soft, and juicy with a sweet, slightly acid taste and a pleasant aroma (Martin, 1980; Heyne, 1982). The pericarp of *G. lowa* has been used in Indonesian indigenous medicine for the treatment of skin, infection, wounds, and diarrhea (Heyne, 1982). In this paper we discribe the isolation, structure elucidation of known prenylated xanthone, mangosharin (**1**) along with its antibacterial activity against *Sreptococcus mutans.*

## 2. MATERIAL AND METHODS

### General Experimental Procedure

Ultra-violet spectra were recorded in methanol on Jasco UV-1575 spectrophotometer. The IR spectra were recorded on a Perkin-Elmer 1760X FT-IR in KBr. Mass spectra were obtained with a Water Qtof HR-MS XEV$^{otm}$ mass spectrometer. $^{1}$H- and $^{13}$C-NMR spectra were obtained with a JEOL JNM A-500 spectrometer using TMS as an internal standard. Chromatographic separations were carried out on silica gel 60 andocta desyl silane (ODS, Fuji Silysia). TLC plates were precoated with silica gel $GF_{254}$ (Merck, 0.25 mm), ODS, and detection was

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achieved by spraying with 10% $H_2SO_4$ in ethanol, followed by heating and under ultra violet-visible light on wavelenght 254 and 367 nm.

**Plant Material**

The stembark of *G. lowa*was collected in West Sumatera Province, Indonesia in April 2014. The plant was identified by the staff of the Bogoriense Herbarium, Bogor, Indonesia and a voucher specimen was deposited at the herbarium.

**Plant Extraction**

Dried ground stembark of *G. lowa* (0.5 kg) was extracted with methanol in room temperatur. Evaporation of the methanol extract in reduced pressure to produce the dark brown residue (52 g). The dark brown residue dissolved in water (1:1) and successively partitioned with *n*-hexane, EtOAc, and MeOH. Evaporation resulted in the crude extracts of *n*-hexane (16.5 g), EtOAc (12.4 g), and MeOH (10.6 g), respectively. The ethyl acetate extracts exhibited strongestantibacterial activity against *Sreptococcus mutans*with MIC values of 10.9 μg/mL. The EtOAc extract (16.5 g) was subjected to vacuum liquid chromatography over silica gel using a gradient elution mixture of *n*-hexane-EtOAc (10:0-0:10) as eluting solvents to afford 8 fractions (A-H). Fraction E (2.2 g) was subjected to column chromatography over silica gel using a mixture of $CHCl_3$:EtOAc (9:1) as eluting solvents to afford 5 fractions (E01-E07). Fraction E04 (80.1 mg) was subjected to column chromatography over silica gel using a mixture of $CHCl_3$:$Me_2CO$ (7:3) to give **1** (6.8 mg). The purification of compounds were analyzed by thin layer chromatography (TLC) on silica gel and ODS with several solvents system and showed a single spot (> 95% pure).

**Antibacterial Activity**

**Bacterial Strains**

A Gram-positive strains, *Sreptococcu mutans* ATCC 25175, was used for the antibacterial assays. The bacteria was cultured individually on Tryptic Soy Broth (TSB) at 37 $^oC$ for 18 h, before inoculation for assay. The broth culture (100 μL), which contained 108 bacteria per mL (theinoculum size of each test strain was standarized at 108 cfu $mL^{-1}$) using a McFarland Nephelometer standard). Sterile Tryptic Soy Agar (TSA) plates were seeded with test bacterial strains and allowed to stand at 37 $^oC$ for 24 h (Adegboye *et al.,* 2008).

**Agar-well Diffusion Method**

The bacterial isolates were first grownin nutrient broth for 24-48 h before use. The isolates were then subcultured on Tryptic Soy Agar plates. The wells were bored into the agar medium using a sterile 6 mm cork borer. The wells were filled with solution of the extract and intense care was taken not to allow the solution to spill on the surface of the medium. The plates were allowed to stand on the laboratory bench for 1-2 h to allow proper inflow of the solution into the medium. Subsequently, the plates were placed in an incubator at 37 $^oC$ for 24 h. The plates were later observed for their zones of inhibition. The effects of the extract on bacterial isolates were compared with those of standard antibiotic chlorhexidine of 5 μg/mL. The experiment was carried out in triplicate (Adegboye *et al.,* 2008; Eloff, 1998).

**Determination of Minimum Inhibitory Concentration (MIC)**

Minimum inhibitory concentration was determined by the brothmicro-dilution assay technique in 96 well micro-titer plates.Overnight broth cultures of the each test organism (90 uL) wereseeded into the wells and the isolated compounds (10 uL) wereadded to each well at decreasing concentration starting from 1000-75 ug $mL^{-1}$. The plates were incubated for 24 h at 35 ± 1$^o$C, and 1%chlorohexidine solution was used as the microbial growth indicator.MIC was determined as the least concentration of the isolatedcompound that inhibited the growth of the test organisms (Eloff, 1998).

**Determination of Mangosharin (1).**

UV (EtOH) $\lambda_{max}$ (nm): 243, 262, 306, 374; IR $\nu_{max}$ ($cm^{-1}$): 3350, 2960, 1648 and 1606; HR-TOFMS (*m/z*): 326.1157 (calcd. for $C_{19}H_{18}O_5$ 326.1152); $^1$H NMR (500 MHz, $CD_3OD$) $\delta_H$ (ppm): 7.37 (1H, d, *J*=4.0 Hz, H-1), 7.20 (1H, d, *J*=9.1 Hz, H-3), 7.12 (1H, dd, *J*=4.0, 9.1 Hz, H-4), 6.30 (1H, s, H-7), 5.08 (1H, t, *J*=7.4 Hz, H-12), 3.80 (3H, s, 8-OMe), 3.22 (2H, d, *J*=7.4 Hz, H-11), 1.67 (3H, s, H-14), 1.55 (3H, s, H-15). $^{13}$C NMR (125 MHz, $CD_3OD$) $\delta_C$ (ppm): 180.6 (C-9), 164.3 (C-8), 158.6 (C-10a), 156.2 (C-6), 153.3 (C-2), 149.7

(C-4a), 131.6 (C-13), 124.1 (C-3), 121.8 (C-12), 120.6 (C-9a), 118.5 (C-4), 111.2 (C-8a), 108.1 (C-1), 103.1 (C-5), 89.5 (C-7), 66.4 (8-OMe), 24.5 (C-15), 21.4 (C-11), 17.6 (C-14).

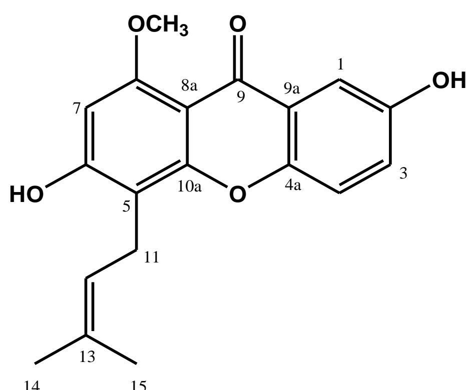


**Figure 1.** Chemical structure of mangosharin (**1**)

## 3. RESULTS AND DISCUSSION

Mangosharin (**1**) was obtained as a yellowish powder. The molecular formula of **1** was established to be $C_{19}H_{18}O_5$ based on HR-TOFMS spectral data (*m/z*): 326.1157 (calcd. for $C_{19}H_{18}O_5$ 326.1152 and NMR data (Table 1), thus requiring eleven degrees of unsaturation. The UV absorptions at 243, 262, 306 and 374 nm indicated that **1** to be a hydroxylated xanthone. The IR spectrum showed strong absorption bands at 3350 and 1648 $cm^{-1}$ which were due to a phenolic hydroxyl and a chelated carbonyl group.

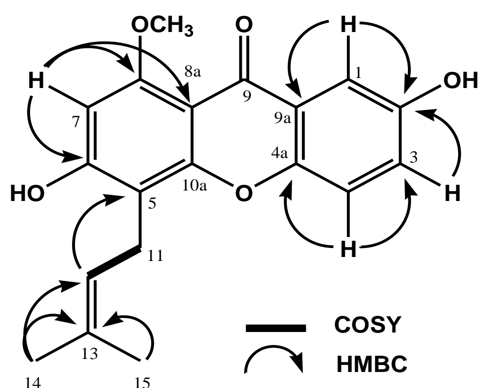


**Figure 2.** Selected HMBC and COSY correlations of mangosharin (**1**).

The $^1$H NMR spectrum of **1** indicated the presence of ABX signals at $\delta_H$ 7.37 (1H, d, *J*=4.0 Hz), 7.20 (1H, d, *J*=9.1 Hz) and 7.12 (1H, dd, *J*=4.0, 9.1 Hz) which were assigned to H-1, H-3 and H-4, respectively. The occurrence of the doublet of doublet at $\delta_H$ 7.12 was due to *ortho*-coupling with the doublet at $\delta_H$ 7.20 (*J*=9.1 Hz) and *meta*-coupling with $\delta_H$ 7.37 (*J*=4.0 Hz). It was noted that H-1 was at a downfield as it was deshielded by the carbonyl group at C-9. The remaining one proton singlet at $\delta_H$ 6.30 was clearly assigned to an isolated aromatic proton at H-7.

**Table 1.** NMR data for compound **1**(500 MHz for $^1$H and 125 MHz for $^{13}$C in $CD_3OD$)

| PositionC | $\delta_H$ [(ΣH, mult., *J* (Hz)] | $\delta_C$ (mult.) |
|---|---|---|
| 1 | 7.37 (1H, d, 4.0) | 108.1 (d) |
| 2 | - | 153.3 (s) |
| 3 | 7.20 (1H, d, 9.1) | 124.1 (d) |
| 4 | 7.12 (1H, d, 4.0, 9.1) | 118.5 (d) |
| 4a | - | 149.7 (s) |
| 5 | - | 103.1 (s) |
| 6 | - | 156.2 (s) |
| 7 | 6.30 (1H, s) | 89.4 (d) |
| 8 | - | 164.3 (s) |
| 8a | - | 111.2 (s) |
| 9 | - | 180.6 (s) |
| 9a | - | 120.6 (s) |
| 10a | - | 158.6 (s) |
| 11 | 3.22 (2H, d, 7.4) | 21.0 (t) |
| 12 | 5.08 (1H, t, 7.4) | 121.8 (d) |
| 13 | - | 131.6 (s) |
| 14 | 1.67 (3H, s) | 17.3 (q) |
| 15 | 1.55 (3H, s) | 25.4 (q) |
| 8-Ome | 3.80 (3H, s) | 55.6 (q) |

The presence of 3-methylbut-2-enyl substituent was indicated by the $^1$H NMR signals at $\delta_H$ 5.08 (1H, t, *J*=7.4 Hz, H-12), 3.22 (2H, d, *J*=7.4 Hz, H-11), 1.67 (3H, s, Me-14) and 1.55 (3H, s, Me-15). In the COSY spectrum, the nature of the allylic and homoallylic coupling system within the prenyl moiety was clearly demonstrated in structure of **1**. It showed correlations between the olefinic proton C-12 and benzylic proton of C-11 and the geminal dimethyl group of C-14 and C-15, respectively. The $^{13}$C-NMR spectrum showed nineteen carbon resonances, which were classified by their chemical shifts and the HMQC spectrum as one methoxy, two methyls, one sp$^3$ methylene, five sp$^2$ methines, nine sp$^2$ quartenary carbons and one carbonyl.

These functionalities accounted for eight out of the total eleven degrees of unsaturation. The remaining three degrees of unsaturation were consistent with xanthone structureand prenyl moiety (Chin *et al.,* 2008; Mahabusarakam, 2005). In order to clarify the position of functional group in structure of **1**, HMBC experiment was carried out and the results was shown in Figure 2. HMBC correlations between proton at H-7 ($\delta_H$ 6.30) to C-8 ($\delta_C$164.3) and C-8a ($\delta_C$ 111.2), indicated that methoxyl group was located at C-8. A correlation between the methine signal at $\delta_H$ 5.08 (H-12) with C-5 ($\delta_C$ 103.1), suggested that the 3,3-dimethylallyl moiety is attached to C-5. The isolated aromatic proton at $\delta_H$ 6.30 (H-7) showed four crosspeaks with the aromatic carbon signals at C-5 ($\delta_C$103.1), C-6 ($\delta_C$ 156.2 ), C-8 ($\delta_C$ 164.3) and C-8a ($\delta_C$111.2), whereas, the proton signals at $\delta_H$ 7.20 (H-4) and $\delta_H$ 7.12 (H-3) gave correlation peaks with C-2 ($\delta_C$ 153.3) and C-4a ($\delta_C$ 149.7), confirmed that two hydroxyl groups were located at C-6 and C-2, respectively. A detailed comparison of spectral data of **1** with those of previously reported, mangosharin (Daud *et al.,* 2006), revealed that both compounds showed very similar, consequently compound **1** was identified as mangosharinand was shown for the first time in this species.Mangosharin(**1**) showed strong anti-bacterial activity against *Sreptococcus mutans* with MIC value of 7.25µg/mL. These results suggested that prenylated xanthones are potential compound for antibacterial drugs.

## 4. CONCLUSION

A prenylated xanthone, mangosharin (**1**) has been isolated from the bark of *Garcini lowa* (Guttiferae). Its chemical structure was identified on the basis of spectroscopic datas and by comparison with previous data reported previously. Mangosharin (**1**), was the first time isolated from *Garcinia lowa* and showed strong antibacterial activity against *Sreptococcus mutans.*

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