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BIFLAVONOID COMPOUND FROM THE STEM BARK OF GAMBOGE (*Garcinia xanthochymus*)

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ABSTRACT

Garcinia xanthochymus (Guttiferae) commonly known as gamboge, is a perennial medicinal plant native to the north of Thailand and Myanmar. Gamboge is used in watercolors, as a yellow fabric dye and traditional medicine for treating diarrhea and dysentery, dispelling worms clearing away fire and removing food toxin. Phytochemical investigations on the methanol extract of the stem bark of Garcinia xanthochymus resulted in isolation of one biflavonoid compound (+)-morelloflavon. The structure of this compound was deduced on basis of spectroscopic data including ^{1}H NMR, ^{13}C NMR, HMQC and HMBC and comparison with the reported data. This compound has been reported from others species of Garcinia.

Keywords: *Garcinia xanthochymus, biflavonoid, (+)-morelloflavon*

INTRODUCTION

Garcinia xanthochymus (Guttiferae) commonly known as gamboge, is a tree endemic to india growing 8–10 m in height and is a perennial medicinal plant native to the north of Thailand and Myanmar [1]. Gamboge is used in watercolors and as a yellow fabric dye. Gamboge fruit are used in traditional medicine for treating diarrhea and dysentery; dispelling worms clearing away fire and removing food toxin .Plants in the guttiferae are rich sources of xanthones, biflavonoids and benzophenones [2]. These constituents have been reported to possess several biological activities, such as antibacterial activity, antimalarial activity, cytotoxicity analgetic, antioxidant, antiviral and neurotrophic activity and inhibition of cyclooxygenase. Previous phytochemical studies of *G. xanthochymus* have show the presence of 1,3,5,6-tetrahydroxy-4,7,8-(3-methyl-2-butenyl)xanthone, garciniaxanthone, 1,4,5,6-tetrahydro xy-7,8-di(3-methylbut-2-enyl)xanthone and 1,2,6-trihydro xy-5-methoxy-7-(3-methylbut-2-enyl)xanthone from the wood [3,4], xanthochymol, isoxanthochymol, volkensi flavone, 1,5-dihydroxyxanthone and 1,7-dihydroxyxan thone from the fruits [1], and 1,6-dihydroxy-4,5-dimethoxyxanthone and 1,5,6-trihydroxy-7,8-di(3-methyl-2-butenyl)- 6',6'-dimethylpyrano(2',3':3,4)xanthone from the bark of *G. xanthochymus* [5]. The $CHCl_3$ and EtOAc partitions from *G. xanthochymus* fruits displayed activity in the DPPH assay (IC50 = 32 and 105 ug/mL respectively and cytotoxicity against the SW-480 colon cancer cell line (IC_{50} = 15 and 50 µg/mL respectively

In our continuing phytochemical investigation of *G.xanthochymus* a biflavonoid compoud, (+)- morello flavone (**1**), was isolated from the methanol extract of the stem bark of *G.xanthochymus*. In this paper, we describe the isolation and structural elucidation of the isolated compounds. The compound was identified by their spectra data and comparison with the reported data.

EXPERIMENTAL SECTION

Plant material

The stem bark of *G.xanthochymus* were collected from Hutan Raya Bogor, West Java, Indonesia. This plant was identified and voucher specimen has been deposited in the Herbarium Bogoriensis Bogor.

Materials

Vacuum liquid chromatography (VLC) and column chromatography were carried using Merck silica gel 60 GF_{254} (230-400 mesh)), column chromatography using

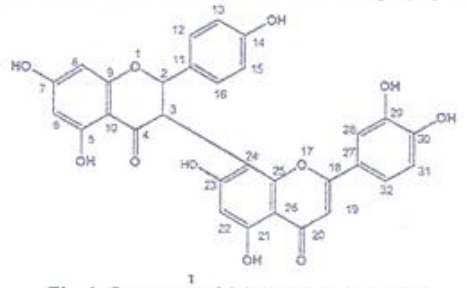

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Fig 1. Structure of (+)-morellofavone (1)

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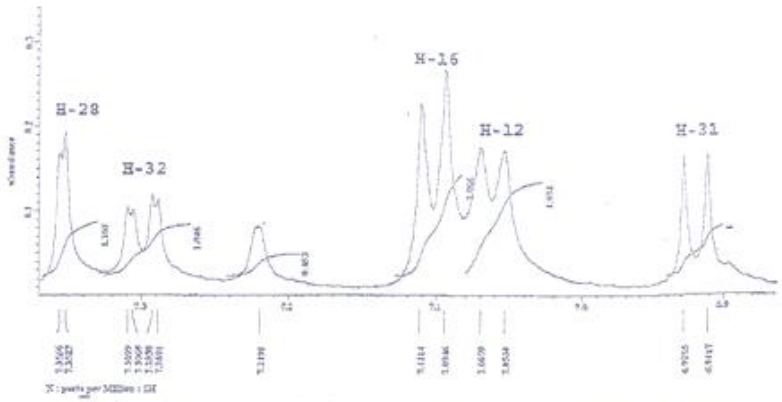

Fig 2. ^{1}H- NMR Spectrum of **1** (methanol-d_3, 500 MHz) at 6.9–7.37 ppm

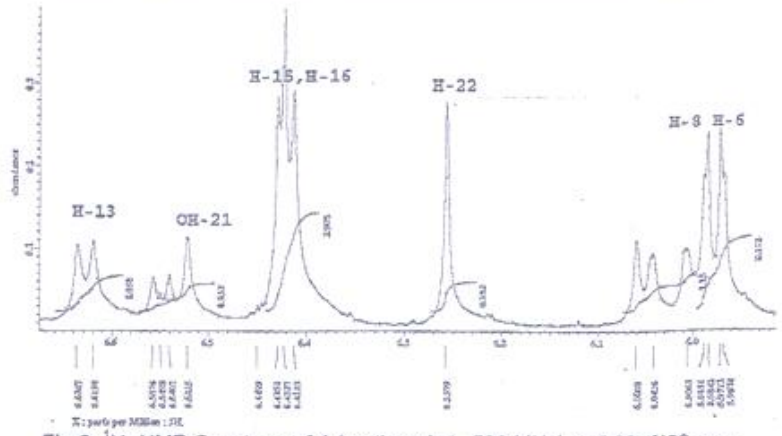

Fig 3. ^{1}H- NMR Spectrum of **1** (methanol-d_3, 500 MHz) at 5.02–6.66 ppm

Si gel 60 (70–230 mesh.). Analytical thin layer chromatography (TLC) was carried out using Merck (Art.5554) silica gel 60 F_{254}, pre-coated aluminium sheets solvents for chromatography were technical grade and distilled before use.

Instrumentation

Melting point was determined on a micromelting point apparatus NMR spectra were recorded at 500 MHz (^{1}H) and 125 MHz (^{13}C) on JEOL JNM ECA-500 spectrometer. Organic materials were detected by first viewing the plate under UV light at 254 nm and 365 nm. The extracts were organic mixture samples were applied in the pre-adsorbed form on silica gel 60 (70–230 mesh.)

Procedure

Extraction and isolation

The dried powdered of the steam bark of *G.xanthochymus* (2 kg) was macerated sequentially with n-hexane, EtOAc, and methanol at room temperature. Concentrated in vacuo to evaporate and give n-hexane (30 g), EtOAc (40 g), and methanol (35 g). The methanol extract (30 g) was further fractionated by vacuum liquid chromatography (VLC) with gradient elution, using n-hexane–EtOAc (8:2 – 2:8) *and EtOAc*. The based of thin layer chromatography (TLC) analyze afforded 4 fractions F1-F4. Fraction F3 (3 g) obtained was purification using column chromatography on silica gel eluted with n-hexana-EtOAc (8: 2 – 2:8) to give four subfractions F3.1–F.3.4. Fraction F3.3, further purification by recrystallization with EtOAc afforded yellow crystal (18 mg).

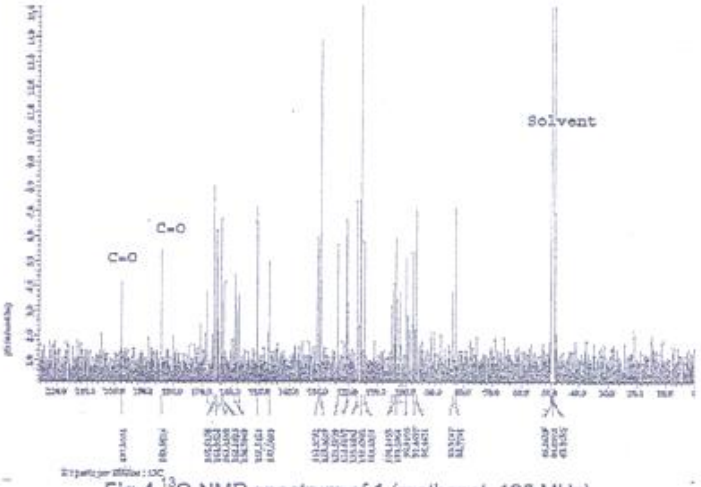

Fig 4. ^{13}C-NMR spectrum of **1** (methanol, 125 MHz)

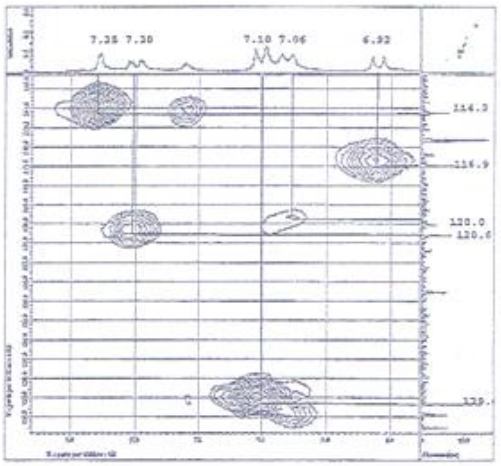

Fig 5. HMQC spectrum of **1**: H-31, H-16, H-18, H-32, H-28 (methanol-d_3, 500 MHz)

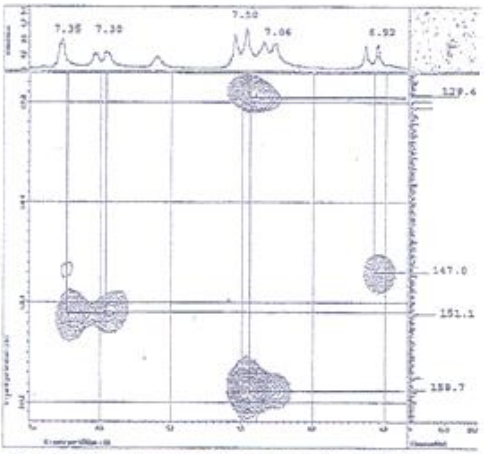

Fig 6. HMBC spectrum of **1**: correlation of H-31, H-16, H-18, H-32, H-28 with carbon at δ_C129 -160 ppm (methanol-d_3, 500 MHz)

RESULT AND DISCUSSION

The methanol extract from the dried stem bark of *G. xanthochymus* has been isolated a. bilfavonoid as (+)-morelloflavon (**1**) as yellow crystal, m.p 300–301 °C. The H-NMR spectrum (Table 1) of **1** showed the presence of there aromatic proton at δ_H 6.92 (H-31) (1H, d, J = 8.45 Hz), δ_H 7.30 (H-32) (1H, d, J = 1.95; 8.45) and 7.35 (H-28) (1H, d, J = 1.95). Characteristic for 1,3,4-trisusbtituted benzene (Fig. 2).

One signal singlet also observed in this spectrum at δ_H 6.26 (H-22) (1H, *s*). In the ^{1}H NMR spectrum the presence of signal- signal proton aromatic at δ_H 5.97 (H-6) (1H, *d*, J = 1.9) and δ_H 5.98 (H-8) (1H, *d*, J = 1.9) showed coupling metha, two pairs proton coupling ortho at δ_H 7.06 (H-12) (1H, *d*, J = 8.4), δ_H 6.62 (H-13) (1H, *d*, J = 8.4) and δ_H 6.42 (H-15) (1H, *d*, J = 8.45) and δ_H 7.10 (H-16) (1H, *d*, J = 8.45). Signal doublet at δ_H δ_H 5.76 (1H, *d*, J = 12.3 Hz) showed coupling proton trans axial-axial at C SP^3 (Fig. 3). Coupling proton trans axial-axial at C SP^3 usually to had constanta coupling (J) 8-14 Hz [6].

Support for the structure **1** was obtained from ^{13}C NMR, HMQC and HMBC data (Table 1). ^{13}C NMR

Table 1. ^{1}H NMR (500 Mhz), ^{13}C NMR (125 MHz), HMQC and HMBC data for **1** in methanol-d_3 and comparison morelloflavon* has been reported [7].

No C	δ_C (ppm) Morelloflanone*	δ_C (ppm) **1**	δ_H (ppm), integration, multiplicity, J (Hz) **1**	HMBC **1**
2	81.0	82.8	5.75 (1H, *d*, 12.3)	C-16
3	48.8	48.8	4.61(1H, *s*)	
4	195.6	197.9		
5	163.5	163.4		
6	96.2	96.5	5.97(1H, *d*, 1.9)	C-10
7	166.3	168.3		
8	95.2	97.5	5.98 (1H, *d*, 1.9)	C-10
9	162.5	164.9		
10	101.5	103.5		
11	128.0	130.6		
12	128.1	120.0	7.06 (1H, *d*, 8.4)	C-2, C-13, C-14, C-16
13	114.4	115.7	6.62 (1H, *d*, 8.4)	C-14, C-15, C-16
14	157.1	158.7		
15	114.4	115.6	6.42 (1H, *d*, 8.5)	C-13,C-14, C-16
16	128.1	129.4	7.10 (1H, *d*, 8.5)	C-2, C-14
18	163.2	165.9		
19	102.4	103.3	6.42 (1H, *s*)	C18, C-26, C-27
20	181.4	183.9		
21	160.3	157.5		
22	98.6	99.9	6.26 (1H, *s*)	C-23, C-24, C-26
23	161.4	162.7		
24	10.5	102.1		
25	155.0	151.0		
26	103.2	105.1		
27	121.2	123.5		
28	113.1	114.2	7.35 (1H, *d*, 1.95)	C-30, C-32
29	145.4	147.0		
30	149.4	151.1		
31	116.1	116.9	6.92 (1H, *d*, 8.45)	C-27, C-29
32	119.0	120.6	7.3 0 (1H, *d d*, 1.95; 8.45)	C-28 , C-30

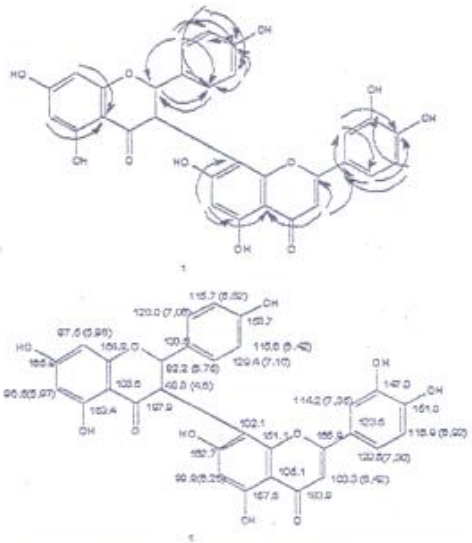

Fig 7. Selected HMBC correlation and δ-assignment of **1**

spectrum revealed the presences of 32 carbon resonances due to two carbonyl carbon at δ_C 183.9 and δ_C 197.9, 28 Aromatic carbon, and two carbon SP^3.signal at δ_C 82.8 and δ_C 48.8 ppm (Fig. 4).

The position of the proton was assigned from HMQC and HMBC spectrum (Fig. 5 and 6). Aromatic proton at δ_H 7.06 (d, J = 8.4) in spectrum HMBC showed four correlation [^2J : C-13 (δ_C 115.7); ^3J : C-14 (δ_C 158.7), C-16 (δ_C 129.4) and C-2 (δ_C 82.8) which placement at C-12. Proton at δ_C6.62 (1H, *d*, J = 8.4) be coupled with proton at δ_H 7.06 as meta coupling so, it can be placed at C-13 position and at HMBC spectrum showed correlation {^2J : C-14 (δ_C 158.7); ^3J : C-15 (δ_C 11.6); and ^4J : C-16 (δ_C 129.4). Three aromatic proton at δ_H 7.06 (1H, *dd*, J = 1.95; 8.45), δ_H 7.35 (1H, *d*, J = 1.95) and δ_H 6.92 (1H, *d*, J = 8.45) be place at C-32, C-28 and C-31 respectively with ortho and metha position in HMBC spectrum showed correlation with carbon [^3J : C-28 (δ_C 114.2), C-30 (δ_C 151.1)]; [^3J : C-30 (δ_C 151.1), C-32 (δ_C 120.6)]; and [^3J : C-27 (δ_C 123.5), C-29 (δ_C 147.0)] respectively. Two proton at δ_H 5.97 (1H, *d*, J = 1.9) and δ_H 5.98 (1H, *d*, J = 1.9) showed correlation ^2J at carbon C-10 (δ_C 103.5) which

indicated the placed of C-6 and C-8. Two sharp singlet of two proton each at δ_H 6.42 and δ_H 6.26 showed correlation [^2J : C-18 (δ_C 165.9), ^3J C-26 (δ_C 105.1), C-27 (δ_C 123.5) and [^2J : C-23 (δ_C 165.9), ^3J : C-24 (δ_C 102.1), C-26 (δ_C 105.1) respectively be placed as aromatic proton at C-19 and C-22. Signal doublet at δ_H 5.75 (J = 12.3) showed correlation at carbon [^3J : C-16)] and indicated the place at C-2 (δ_C 82.8). Two proton aromatic each at δ_H 6.42 (d, J = 8.5) and δ_H 7.10 (d, J = 8.5) so be coupling metha and HMBC spectrum showed correlation with carbon at C-13 (^3J); C-14, C-16 (^2J) and C-2, C-14 (^3J) respectively which indicated the placed of C-15, and C-16. HMBC spectrum showed at Fig. 5 and 6.

According to analyzation of spectroscopy data above this compound **1** is (+)-morelloflavone. This compound had been reported from alcoholic extract of leaves of *G. nervosa* [7], the fruit of *G. dulcis* [8] and from methanol extract the bark of *G. multiflora*. This compound showed radical scavenging and antibacterial activities [9]. Selected HMBC correlation and δ-assignment of **1** showed Fig. 7.

CONCLUSION

A biflavonoid, (+)- morelloflavon had been isolated from methanol extract of the stem bark of *Garcinia xanthochymus*. This compound adds kinds of phytochemistry studies from the stem bark of *G. xanthochymus*.

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