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Original Research Article

Xanthones from the Roots of *Garcinia rigida* Miq. and Their Cytotoxic Activity against HeLa Cervical Cancer Lines

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ABSTRACT

Garcinia rigida Miq. (Clusiaceae), a plant endemic to Borneo is a traditional medicine in Kalimantan, Indonesia. Xanthones are a promising metabolomic substance of *Garcinia* for cancer therapy. This research aimed to evaluate the cytotoxicity against HeLa cells of xanthones from the roots of *Garcinia rigida* Miq. Two xanthones, mangostanin (**1**) and 8-isoprenyl-1,6,7-trihydroxy-6',6'-dimethyl-pyrano(2',3':3,2)xanthone (**2**), were isolated from the roots of *G. rigida*. Xanthones **1-2** molecular structures were established and focused using ^{1}H, ^{13}C, HMQC, and HMBC spectra. Xanthones **1-2** were assayed regarding their cytotoxicity against HeLa cells using the MTT method. Compound **2** exhibited moderate cytotoxicity with an IC_{50} of 21.89 µM.

Keywords: *Garcinia rigida*, xanthones, cytotoxic, HeLa cells.

Introduction

Garcinia rigida Miq. (Clusiaceae) is one endemic plant in the Borneo Island (Indonesia). The genus *Garcinia* produces xanthones as the main metabolomics that exhibit antioxidant, anti-inflammatory, antimalaria, and cytotoxic activities.[1-8] Xanthones show functional groups such as hydroxy, methoxy, and terpenyl chains (isoprenyl, geranyl), which are important in molecular networking to inhibit or kill cancer cells.[6-8]. Previous studies have shown that xanthones from *G. cowa* and *G. bracteata* show significant activity against HeLa cells.[9-10]
Two known xanthones, mangostanin (**1**) and 8-isoprenyl-1,6,7-trihydroxy-6',6'-dimethyl-pyrano(2',3':3,2)xanthone (**2**) were found in the roots of *G. rigida*. The effect of hydroxy groups, isoprenyl chains, and pyrano rings in the structure of xanthones **1-2** against cervical cancer HeLa cells were also discussed.

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Materials and Methods

General experimental procedure

The stationary phases of column chromatography (CC) and planar radial chromatography used silica gel G_{60} and PF_{254}. The TLC plates control the separation process in the CC and planar radial chromatography. The structures of xanthones **1-2** using a UV spectrophotometer (Thermo Scientific™), an NMR spectrometer (JEOL ECA-400), and FTIR spectrophotometer performed the IR spectrum (FTIR with ATR, Perkin Elmer).

Plant material

The plant material (root) of *G. rigida* originates from Gandring Village (GPS coordinates: 0^{0}93'49" S, 114^{0}89'85" E), Teweh Baru District, South Barito, Central Kalimantan, Indonesia, in March 2023 with the specimen number TB-BJGR-IR5.

Extraction and isolation

For two days, the dried powder of *G. rigida* roots (2.2 kg) was macerated with 90% methanol. The solvents were eliminated using a rotavapor, producing a thick methanol extract weighing 90 grams. The MeOH extract was partitioned with ethyl acetate to produce an ethyl acetate fraction (17.9 g).[11-13]

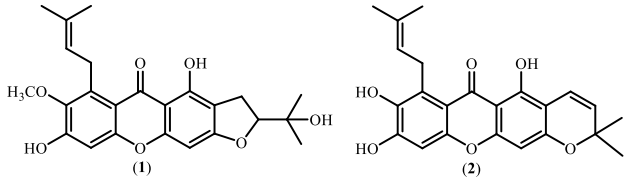

Figure 1: Xanthones of *G. rigida*

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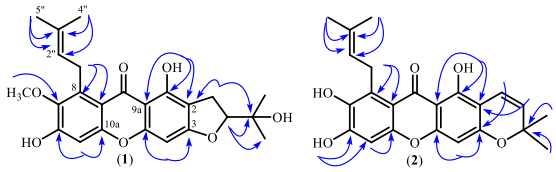

Figure 2: HMBC spectrum of xanthones 1-2

Silica gel CC was carried out to separate the EtOAc fraction with a gradient of *n*-hexane (C_6H_{14}): EtOAc (18:2 to 1:1 v/v) so that four fractions (A-D) were obtained. By radial chromatography, fraction B (3.3 g) was separated with C_6H_{14}: EtOAc (18:2 to 1:1 v/v) to obtain four subfractions (B_1-B_4). Re-separation of subfraction B_3 (194 mg) by radial chromatography with the eluents diisopropyl ether and diisopropyl ether: ethyl acetate (9:1 v/v) to obtain subfractions B_{31}-B_{34}. Fraction B_{34} (11.2 mg) was purified using the same method with eluents of *n*-hexane: chloroform (17:3 v/v), chloroform, and chloroform: EtOAc (9:1 v/v) to obtain mangostanin (**1,** 4.9 mg). Subfraction B_1 (25 mg) was separated using C_6H_{14}: chloroform (9:1 v/v) and chloroform to give subfractions B_{11}-B_{15}. Subfraction B_{14} (14.4 mg) was purified using radial chromatography with C_6H_{14}: acetone (19:1 to 9:1 v/v) eluent, which gave 8-isoprenyl-1,6,7-trihydroxy-6',6'-dimethyl-pyrano(2',3':3,2)xanthone (**2**, 7.2 mg).

Cytotoxic activity

Xanthones **1-2** activity assay against cervical cancer (HeLa cells) was performed using the MTT method, and doxorubicin was used as a positive control. The Hela cells in DMSO without active compounds **1-2** are negative controls in the cytotoxic assay. Compounds **1-2** in various concentrations (1 to 300 µM) and then added to HeLa cell culture. The cells in 96 wells in RPMI-1640 media containing 1.5 g/L Na_2CO_3 were supplemented with 1% L-glutamate, 1% fetal bovine serum, and 1% antibiotic and antifungal formula. Incubation of HeLa cells was carried out in a 5% CO_2 incubator at 37ºC for 24 hours. Absorbance was measured using a microplate reader (Diatek DR-200Bc, ELISA, China) at λ_{max} 540 nm. [14-16]

Result and Discussion

Two xanthones (Figure 1), mangostanin (**1**) and 8-isoprenyl-1,6,7-trihydroxy-6',6'-dimethyl-pyrano(2',3':3,2)xanthone (**2**), were found from *G. rigida* roots. The NMR data of xanthones **1-2** have chemical shift (δ) values identic to those in the literature.[20]

Table 1: NMR spectra of compounds **1-2** in $CDCl_3$

No. C	1		2	
	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C
1	-	158.8	-	158.0
2	-	108.8	-	104.0
3	-	167.9	-	160.0
4	6.25 (*s*)	88.6	6.24 (*s*)	94.2
5	6.83 (*s*)	102.7	6.83 (*s*)	101.5
6	-	156.2	-	150.9
7	-	144.6	-	139.8
8	-	138.0	-	127.4
9	-	182.9	-	182.8
4a	-	157.5	-	156.4
8a	-	111.8	-	111.6
9a	-	102.7	-	104.4
10a	-	157.9	-	153.7
1'	-	-	-	-
2'	4.81 (*dd*, 7.3; 9.2)	92.8	-	78.0
3'	3.14 (*t*, 8.5)	27.0	6.72 (*d*, 9.8)	127.8
4'	-	71.4	5.56 (*d*, 9.8)	115.8
5'	1.28 (*s*)	25.9	1.47 (*s*)	28.4
6'	1.24 (*s*)	25.9	1.47 (*s*)	28.4
1"	4.11 (*d*, 7.4)	26.8	4.34 (*d*, 7.3)	26.1
2"	5.26 (*t*, 6.7)	124.7	5.31 (*t*, 7.4)	121.4
3"	-	131.4	-	136.0
4"	1.81 (*s*)	18.2	1.79 (*s*)	18.2
5"	1.64 (*s*)	25.5	1.89 (*s*)	25.9
1-OH	13.67 (*s*)	-	13.69 (*s*)	-
5-OH	-	-	5.43 (*s*)	-
7-OCH_3	3.79 (*s*)	61.2	-	-

Table 2: Cytotoxic activity against HeLa cells of xanthones 1-2.

Compound	µM
1	256.49±1.05
2	21.89±0.68
Doxorubicin	1.25±0.07

The UV (MeOH) spectrum of mangostanin (**1**), displaying λ_{max} (log ε): 220 (4.13); 246 (4.26); 278 (4.06); 318 (3.83), and IR (KBr) spectrum shows ν_{max} (cm^{-1}): 3311, 1657, 1573, and 1610.[17]

The ^{1}H NMR of mangostanin displayed 11 total protons of aromatic, chelated hydroxy, methoxy, dihydrofuran rings, and isoprenyl chains (Table 1). At ring A, a singlet aromatic proton exhibits at δ_H 6.25 (H-4) and the same signal at δ_H 6.83 (H-5) (ring B). Two protons of hydroxy and methoxy groups were detected at δ_H 13.67 (1-OH) and δ_H 3.79 (7-OCH_3). The proton of the 2′-(1-hydroxy-1-methylethyl)-dihydrofuran chain consists of a methylene proton [δ_H 3.14 (H-3')], an oxy-methine [δ_H 4.81 (H-2')], and two methyls [δ_H 1.24 (H-6′); δ_H 1.28 (H-5′)]. The proton of the isoprenyl chain consists of a methylene [δ_H 4.11 (H-1")], a vinyl [δ_H 5.26 (H-2")], and two methyls [δ_H 1.64 (3H, *s*, H-5″), δ_H 1.81 (3H, *s*, H-4″)].[18-19] The placement of two aromatic units, hydroxy, methoxy, isoprenyl chain, and 2′-(1-hydroxy-1-methylethyl)-dihydrofuran ring, was detected by HMBC spectrum. The ^{13}C-NMR (Table 1) spectrum of mangostanin (**1**) showed 23 perfectly separated carbon atoms supported by the 2D-NMR spectrum. A hydroxy at 1-OH (δ_H 13.67) showed a correlation to an oxyaryl at C-1 (δ_C 158.8), and two quartenary carbon [C-2 (δ_C 108.8), C-9a (δ_C 102.7)]. A methylene proton at H-3′ (δ_H 3.14) shows cross-peak to C-2 and an oxy-carbon [C-4' (δ_C 71.4)]. An aromatic at H-4 (δ_H 6.25) linked to C-2, C-9a, and two oxyaryls [C-3 (δ_C 167.9), C-4a (δ_C 157.5)], indicating on ring A the 2′-(1-hydroxy-1-methylethyl)-dihydrofuran fused at C-2 and C-3. An aromatic at H-5 (δ_H 6.83) correlate with three oxyaryls [C-6 (δ_C 156.2), C-7 (δ_C 144.6), C-10a (δ_C 157.9)], C-8a (δ_C 111.8, quaternary carbon), 7-OCH_3 (δ_H 3.79) linked to C-7, and a methylene proton of the isoprenyl chain at δ_H 4.11 (H-1") correlated to C-7, C-8a, two quaternary carbons [C-8 (δ_C 138.0), C-3" (δ_C 131.4)], and a methine carbon at C-2" (δ_C 124.7) highlighting the isoprenyl chain at C-8 and the methoxy group at C-7. The HMBC correlation shows that ring B is 3-isoprenyl-1-hydroxy-2-methoxybenzene (Figure 2). The xanthone structure of **1** was identified as mangostanin.[20]

8-Isoprenyl-1,6,7-trihydroxy-6',6'-dimethyl-pyrano(2',3':3,2)xanthone (**2**) has a yellow solid, and the UV spectrum (λ_{max}: 222, 268, 290, and 334 nm) similar to mangostanin. The NMR data of **2** also display the same chemical shift with mangostanin, especially in the two aromatic units [δ_H 6.24 (H-4), δ_C 94.2 (C-4), δ_H 6.83 (H-5), δ_C 101.5 (C-5)], an isoprenyl at C-8 [δ_H 4.34 (H-1″), δ_C 26.1 (C-1″), δ_H 5.31 (H-2″), δ_C 121.4 (C-2″), δ_C 136.0 (C-3″), δ_H 1.79 (H-4″), δ_C 18.2 (C-4″), δ_H 1.89 (H-5″), δ_C 25.9 (C-5″)], and the chelated hydroxy at C-1 [δ_H 13.69 (1-OH)].

The main difference in compound **2** is that a 2,2-dimethylpyrano ring is linked at C-2 and C-3 [δ_C 78.0 (C-2′), δ_H 6.72 (1H, J = 9.8 Hz, H-3′), δ_C 127.8 (C-3′), δ_H 5.56 (1H, J = 9.8 Hz, H-4′), δ_C 115.8 (C-4′), δ_H 1.47 (6H, *s*, H-5′/H-6′), δ_C 28.4 (C-5′/C-6′)], and demethylation at C-7. Twenty-two carbon atom signals in compound **2** were seen in the ^{13}C-NMR spectrum and were perfectly separated (Table 1). The HMBC spectrum of **2** also shows a similar correlation with **1**, especially in two aromatic units, an isoprenyl at C-8 and the hydroxy group at C-1. The hydroxy proton at 1-OH correlated to C-1 (δ_C 158.0, oxyaryl), C-2, and C-9a (δ_C 104.0, δ_C 104.4, quaternary carbon). A vinylic proton at H-4′ (δ_H 5.56) correlates with C-2′, and C-3 emphasizes the pyrano ring linked at C-2 and C-3. Based on the NMR spectrum, the structure **2** is as shown in Figure 1.[20]

Xanthones **1**-**2** showed IC_{50} values of 256.49 and 21.89 µM, respectively (Table 2) against HeLa cells. Compound **2** described moderate activity, and mangostanin was inactive.[21-22] From the structure, compound **2** has a 2,3-dimetylpyrano ring compared to compound **1**, which shows a dihydrofuran ring connected at C-2 and C-3, and demethylation at C-7 increases activity. The planarity of the 2,3-dimetylpyrano ring can penetrate and damage HeLa cells so that its activity increases.

Conclusion

Two known xanthones, mangostanin (**1**) and 8-isoprenyl-1,6,7-trihydroxy-6',6'-dimethyl-pyrano(2',3':3,2)xanthone (**2**), were found in the roots of *G. rigida* Miq. Compound **1** was categorized as inactive against HeLa cells, and compound **2** had moderate activity.

Conflict of Interest

The author and all members of the research team declare that there is no conflict of interest.

Author's Declaration

The author and research team state that the research is original, and the content of this article is the author's responsibility.

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