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Potential of antioxidants and cytotoxicity of prenylated xanthone from the twigs of *Garcinia xanthochymus*

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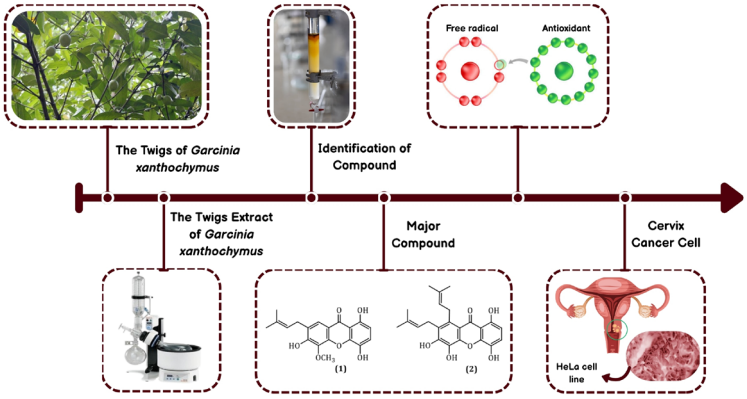
ABSTRACT: *Garcinia xanthochymus* Hook. F. ex T. Anderson (Clusiaceae) is a traditional herbalist remedy. Xanthones are the main phenolic compounds in *Garcinia* and exhibit diverse biological activities, including antioxidant and anticancer properties. These compounds can act as powerful free radical scavengers and mutant pathways of oxidative stress, as validated in earlier studies. In addition to their antioxidant activity, several xanthones have been shown to exhibit cytotoxicity in various cancer cell lines. This study was to determine the antioxidant and cytotoxic activities of xanthones isolated from *G. xanthochymus* twigs against cervical cancer (HeLa) cells. Two known xanthones, 1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone **(1)** and 1,4,5,6-tetrahydroxy-7,8-di-isoprenyl-xanthone **(2)**, were successfully isolated from *G. xanthochymus* twigs. The structures of these xanthones were elucidated by NMR spectroscopy (1D and 2D), and UV spectrophotometry. The cytotoxic compound **1** showed strong activity (IC_{50} = 2.00 µg/mL), while compound **2** was very weak. In the DPPH assay, compound **1** also showed a slightly greater antioxidant capacity (IC_{50} = 8.29 µg/mL) than compound **2** (IC_{50} = 9.09 µg/mL), indicating superior overall bioactivity.

GRAPHICAL ABSTRACT



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1. INTRODUCTION

Garcinia xanthochymus is a tropical plant species of the Clusiaceae family, which has been found widely in Southeast Asia as well as Indonesia, Malaysia, and Thailand (Joseph et al., 2016; Zou et al., 2024). Though elsewhere in the world it is known simply as Mangosteen, in Indonesia, it is known as "Manggis Kuning," which translates to "Yellow Mangosteen", and describes the rarer yellow color of its mature fruit compared to other species in this genus (Hassan et al., 2018). In addition to its ecological importance, *G. xanthochymus* is highly valued in traditional medicine. In ethnic medicine, the fruit, bark, and leaves are used to treat hyperthermia, diarrhea, and alimentary intoxication (Jin et al., 2021). These traditional uses suggest that bioactive secondary metabolites are responsible for their pharmacological activity and justify scientific investigation into their therapeutic potential. The metabolites identified in the *Garcinia* genus include xanthones, depsidones, flavonoids, biphenyls, and triterpenoids, which exhibit numerous pharmacological activities, such as anti-HIV (Corona et al., 2021), antimalarial (Likhitwitayawuid et al., 1998), antioxidant (Utami et al., 2018), anticancer (Assyifa et al., 2025; Kurniadewi et al., 2025; Zulfa et al., 2025), and anti-inflammatory (Xue et al., 2020). *Garcinia* contains a chemical compound known as xanthone, an aromatic polyphenolic compound with functional groups such as methoxy, hydroxyl, and prenyl that contribute to various bioactivities (Messi et al., 2014; Pailee et al., 2018).

The structure–activity relationships of xanthone derivatives indicate that the number, position, and type of functional groups on the xanthone skeleton are key determinants of their biological activity profiles. This substitutive tendency parallels antioxidant and anticancer activity. In particular, the hydroxyl groups and isoprenyl chains in the xanthone structure play an important role in neutralizing free radicals and are toxic to various cancer cells (Santo et al., 2020).

This study was performed to isolate and characterize the structures of xanthone derivatives from *G. xanthochymus* twigs and analyze their structure–activity relationships, regarding antioxidant and cervical cancer (HeLa) activities. Two xanthone compounds, specifically 1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone **(1)** and 1,4,5,6-tetrahydroxy-7,8-diisoprenylxanthone **(2)**, were successfully isolated from the twigs of *G. xanthochymus*. The biological activity of compounds **1**–**2**, particularly their antioxidant and cytotoxic effects against HeLa cells, has not been reported. This study evaluated the structure–activity relationships of compounds **1** and **2**, including their antioxidant and cytotoxic activities against HeLa cells.

2. EXPERIMENTAL SECTION

2.1. Materials and methods

2.1.1. General experimental procedure

The structure of the xanthone compound was elucidated using an NMR spectrometer (JEOL ECA-400) at 400 MHz (^{1}H-NMR) and 100 MHz (^{13}C-NMR), and a UV spectrophotometer (Shimadzu UV-1800). The chemical shift value (δ) is expressed in ppm units and the multiplicity in Hz. The reference solvent used is acetone-d_6. Si gel 60 (Merck 1.07734) was used as a stationary phase in column chromatography, Si gel 60 PF_{254} (Merck 1.05554) in radial planar chromatography, and Si gel TLC 60 GF_{254} (Merck 1.07730) in thin layer chromatography.

2.1.2. Plant materials

G. xanthochymus were collected from the Bogor Ecopark Garden in West Java, Indonesia, a conservation site dedicated to rare Indonesian plant species, in August 2025. Voucher specimens were deposited (GC-20252008-2025) and preserved at the Organic Chemistry Laboratory, Universitas Negeri Surabaya, Indonesia.

2.1.3. Extraction and isolation

G. xanthochymus twigs powder (2.1 kg) was macerated using 90% methanol. The solvent was removed with a rotavap, yielding a thick MeOH extract (60 g). The MeOH extract was partitioned with EtOAc, producing the EtOAc extract fraction (9.7 g) (Marliana et al., 2018; Tanjung et al., 2018; Tjahjandarie et al., 2022). The EtOAc fraction was separated by column chromatography (Si gel) with C_6H_{14}: EtOAc (8:2 to 1:1 v/v), to yield three primary fractions (A-C). Fraction B (1.9 g) was isolated via radial planar chromatography with C_6H_{14}: $CHCl_3$ (7:3 v/v) and $CHCl_3$: EtOAc (8:2 v/v), yielding four subfractions (B1 – B4). Subfraction B2 was purified with C_6H_{14}: diisopropyl ether (7:3 v/v), producing 1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone (**1**, 66.3 mg). Subfraction B3 was purified with C_6H_{14}: diisopropyl ether (7:3 to 3:7 v/v), to yield 1,4,5,6-tetrahydroxy-7,8-diisoprenylxanthone (**2**, 4.1 mg).

2.1.4. 1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone (1)

Yellow powder. UV (MeOH) λmax (log ε): 235 (3.65), 250 (3.60), 284 (3.41), and 318 (3.24) nm. HRESIMS *m/z* 343.2258 $[M+H]^+$, calculated mass for $C_{23}H_{24}O_6$ *m/z* 343.2256. NMR (^{1}H, ^{13}C) spectroscopic data for compound **1** are summarized in Table 1.

Table 1
NMR data of xanthones **1–2**.

No.	(1) δ_H (mult, *J* in Hz)	δ_C	(2) δ_H (mult, *J* in Hz)	δ_C
1	-	154.8	-	155.1
2	6.58 (*d*, *J* = 8.6)	109.7	6.61 (*d*, *J* = 8.8)	110.1
3	7.26 (*d*, *J* = 8.6)	124.0	7.21 (*d*, *J* = 8.8)	123.2
4	-	138.0	-	137.2
5	-	135.0	-	135.0
6	-	155.3	-	150.4
7	-	127.4	-	126.6
8	7.69 (*s*)	120.6	-	135.0
9	-	182.0	-	184.4
4a	-	144.9	-	143.1
8a	-	114.2	-	112.3
9a	-	109.4	-	109.7
10a		149.8		146.9
1'	3.39 (*d*, *J* = 7.3)	28.7	3.48 (*d*, *J* = 6.6)	25.2
2'	5.30 (*t*, *J* = 8.0)	122.4	5.10 (*t*, *J* = 6.6)	123.7
3'	-	133.8	-	131.1
4'	1.73 (*s*)	17.8	1.80 (*s*)	18.3
5'	1.75 (*s*)	25.9	1.67 (*s*)	25.9
1"	-	-	4.10 (*d*, *J* = 6.0)	29.1
2"	-	-	5.09 (*t*, *J* = 6.1)	125.2
3"	-	-	-	130.9
4"	-	-	1.79 (*s*)	18.2
5"	-	-	1.67 (*s*)	25.9
1-OH	12.19 (*s*)	-	12.56 (*s*)	-
5-OCH_3	4.06 (*s*)	61.9	-	-

2.1.5. 1,4,5,6-tetrahydroxy-7,8-diisoprenylxanthone (2)

Yellow powder. UV (MeOH) λmax (log ε): 228 (4.32), 256 (4.29), 283 (4.01), and 340 (3.94) nm. HRESIMS *m/z* 396.1833 $[M]^+$, calculated mass for $C_{23}H_{24}O_6$ *m/z* 396.1830. NMR (^1H, ^{13}C) spectroscopic data for compound **2** are summarized in Table 1.

2.1.6. Antioxidant activity

The antioxidant activity of xanthones **1–2** was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay at λ 517 nm using a UV spectrophotometer. Gallic acid was used as a positive control, and the DPPH solution without test compounds served as the negative control. The isolated xanthone derivatives were prepared at various concentrations and added to the DPPH solution, which was incubated at room temperature for 30 minutes (Arif et al., 2025; Tanjung et al., 2016). The capacity of xanthone **1–2** to scavenge DPPH radicals was obtained from the relationship between concentration and the percentage of radical scavenging to obtain the IC_{50} value based on linear regression.

2.1.7. Anticancer activity

Cytotoxicity of xanthone **1–2** against HeLa cervical cancer cells, using doxorubicin as a positive control and 1% DMSO as a negative control. HeLa cells were cultured in 96-well plates containing RPMI-1640 medium with fetal bovine serum (FBS), Na_2CO_3, antibiotics, and incubated under maintained conditions (5% CO_2, 37°C, 24 hours). Cells (a density of 3×10^4 cells/cm^3) were harvested at 3×10^4 cells/ml for the cytotoxicity assay. The cells were then treated with xanthones **1–2** at various concentrations under the same conditions for 24 h. The resulting formazan was dissolved in 1% DMSO and measured using a microplate reader at λ 540 nm. Finally, the cytotoxicity of xanthones **1** and **2** against HeLa cells was measured using this method. The IC_{50} values for **1–2** were obtained from a linear regression program (Saputri et al., 2023; Tanjung et al., 2025; Tjahjandarie et al., 2021a).

3. RESULT AND DISCUSSION

3.1. Result

From *G. xanthochymus* twigs, two known xanthones (Figure 1), 1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone **(1)** and 1,4,5,6-tetrahydroxy-7,8-diisoprenylxanthone **(2)**, were successfully isolated. The chemical shifts (δ) in the NMR data for compounds **1–2** are similar to previously reported values (Chanmahasathien et al., 2003; Han et al., 2007).

1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone **(1)** and 1,4,5,6-tetrahydroxy-7,8-diisoprenylxanthone **(2)** were presented as a yellow powder. The NMR data of **1–2** are presented in Table 1. The antioxidant and cytotoxicity data for compounds **1–2** are shown in Table 2.

3.2. Discussion

The UV spectrum of compound **1** exhibits absorption at λmax (log ε): 235 (3.65), 250 (3.60), 284 (3.41), and 318 (3.24) nm, which is a distinctive characteristic of the xanthone skeleton (Saputri et al., 2024; Setiawan et al., 2025; Tjahjandarie et al., 2021b). The ^1H-NMR data indicated that compound **1** displays resonances of two aromatic units,

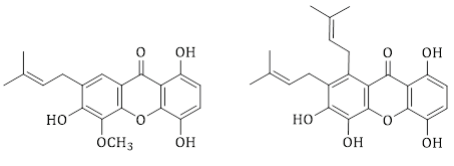

Figure 1. Xanthones of *G. xanthochymus*.

including one set of *ortho*-doublet (J = 8.6 Hz) at H-2 (δ_H 6.58) and H-3 (δ_H 7.26), and one isolated aromatic at H-8 (δ_H 7.69). The isolate also exhibits resonances from one hydrogen bond from the hydroxyl group at 1-OH (δ_H 12.19), one methoxy at 5-OCH_3 (δ_H 4.06), and one 3-methyl-2-butenyl chain distributed from one sp^3 methylene [δ_H 3.39 (*d*, 7.3 Hz, H-1')], one sp^2 vinylic [δ_H 5.30 (*t*, 8.0 Hz, H-2')], and two sp^3 methyl [δ_H 1.73 (*s*, H-4'), 1.75 (*s*, H-5')]. The ^{13}C NMR data revealed the resonance of 19 carbon atoms, including 12 sp^2 carbon aromatics, one sp^2 carbonyl, one sp^3 methylene, three sp^3 methyl, and one sp^2 vinylic. The HMBC correlations showed that the resonance of 1-OH correlated with two sp^2 carbon atoms of the aromatic ring [C-1 (δ_C 154.8), C-9a (δ_C 109.4)] and one sp^2 methine of the aromatic ring [C-2 (δ_C 109.7)]. The resonance of H-2 correlated to C-1 and two sp^2 oxy-aryls [C-4 (δ_C 138.0), C-4a (δ_C 144.9)], supporting two oxy-aryls at C-1 and C-4 on the xanthone structure. The resonance of aromatic on H-8 showed three bond correlation with two sp^2 of oxy-aryls [C-6 (δ_C 155.3), C-10a (δ_C 149.8)], one sp^2 of carbonyl [C-9 (δ_C 182.0)], and one sp^3 of methylene [[C-1' (δ_C 28.7)]] of 3-methyl-2-butenyl that assigns the hydroxy group attached at C-6, and 3-methyl-2-butenyl chain at C-7. The resonance of methylene at H-1' correlated to C-6, C-7, and the double bond [C-2' (δ_C 122.4), C-3' (δ_C 133.8)]. The results of this correlation emphasize the methoxy bound at C-5. The detected interatomic correlations further substantiate that compound **1** possesses a structural framework consistent with that of 1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone (Han et al., 2007).

The UV spectrum of compound **2** shows absorption at λmax (log ε): 228 (4.32), 256 (4.29), 283 (4.01), and 340 (3.94) nm, identical to compound **1.** The ^1H NMR data of 1,4,5,6-tetrahydroxy-7,8-diisoprenylxanthone (Table 1) revealed resonances of one set of *ortho*-doublet of aromatic on ring A (J = 8.8 Hz) at H-2 and H-3 [δ_H 6.61 and 7.21], one hydroxyl proton (δ_H 12.56, 1-OH). The isolated also displayed two 3-methyl-2-butenyl chains distributed from two sp^3 methylene [δ_H 3.48 (*d*, J = 6.6 Hz, H-1'), 4.10 (*d*, J = 6.0 Hz, H-1")], two sp^2 vinylic [δ_H 5.10 (*t*, 6.6 Hz, H-2', 5.09 (*t*, 6.1 Hz, H-2")], and four sp^3 methyl [δ_H 1.80 (*s*, H-4'), 1.67 (*s*, H-5', 1.79 (*s*, H-4"), 1.67 (*s*, H-5"). The fundamental difference in the resonance of the isolate with 1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone revealed the presence of a 3-methyl-2-butenyl chain at C-8 and a hydroxyl group at C-5, supported by HMBC correlation data. The ^{13}C NMR data of 1,4,5,6-tetrahydroxy-7,8-diisoprenylxanthone showed resonances for 22 carbon atoms. The signals of 1-OH and H-2 showed the same correlations with 1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone, indicating two hydroxy groups at C-1 and C-4 in the HMBC spectrum. The resonance of methylene at H-1' (δ_H 3.48) shows a correlation with three sp^2 carbons of aromatic in ring B (C-6, C-7, and C-8) and two sp^2 carbons of the double bond (C-2' and C-3'). Meanwhile, the proton of methylene at H-1" (δ_H 4.10) correlates with C-6, C-7, one sp^2 carbon of aromatic (C-8a), two sp^2 of double bond (C-2" and C-3"), which emphasizes two chains of 3-methyl-2-butenyl bonded at C-7 and C-8. Therefore, the NMR spectrum confirms the structure of 1,4,5,6-tetrahydroxy-7,8-diisoprenylxanthone (Chanmahasathien et al., 2003).

Table 2.
Cytotoxic and antioxidant activities of xanthones **1–2.**

Compound	HeLa (µg/mL)	DPPH (µg/mL)
1,4,6-Trihydroxy-5-methoxy-7-isoprenylxanthone **(1)**	2.00	8.29
1,4,5,6-Tetrahydroxy-7,8-diisoprenylxanthone **(2)**	13.99	9.09
Doxorubicin*	0.24	-
Gallic Acid*	-	4.21

* Positive control.

The cytotoxicity (Table 2) of 1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone (**1**) has very high activity against HeLa cells (IC_{50} = 2.00 µg/mL), while compound **2** is very weak (Aldin et al., 2021; Saputri et al., 2021; Tanjung et al., 2022). Cytotoxic data from xanthones **1-2** also show the same thing against human leukemia cells HL-60 (Niu et al., 2012; Ruan et al., 2017). The cytotoxic activity results showed that the isoprenyl group at C-8 caused a decrease in its cytotoxicity. Based on the cytotoxicity data of compound **1**, it is recommended that it be tested at the in vivo and molecular biology levels to move towards pre-clinical testing. Meanwhile, the antioxidant activity of xanthones **1** and **2** shows potential activity against DPPH radicals (Azmin et al., 2025; Tanjung et al., 2014; Tjahjandarie et al., 2025). Compound **2** has more isoprenyl chains, which increase hydrophobicity and reduce biological activity by increasing steric effects that limit its interaction effectiveness in biological systems.

4. CONCLUSION

Two known xanthones, 1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone (**1**) and 1,4,5,6-tetrahydroxy-7,8-diisoprenylxanthone (**2**), were successfully isolated from *G. xanthochymus* twigs. Compound **1** showed significant activity against HeLa cervical cancer cells. In the antioxidant activity, compounds **1** and **2** were classified as active, thereby strengthening the potential of xanthones from G. *xanthochymus* as promising bioactive candidates.

MANDATORY DISCLOSURE ON USE OF ARTIFICIAL INTELLIGENCE

The authors did not use artificial intelligence in the preparation of this manuscript.

AUTHORS CONTRIBUTIONS

Leny Nur Fitriani performed the experimental investigation, analyzed the data, and prepared the original draft of the manuscript. Harum Margasari, Fitri Nurvita Sari, Muhammad Ulul 'Azmi, and Novian Ilham Ramadhan contributed to data acquisition and validation. Mulyadi Tanjung and Tjitjik Srie Tjahjandarie provided critical supervision, methodological input, and scientific oversight. Ratih Dewi Saputri conceived and designed the study, supervised the project, and acted as the corresponding author. All authors critically revised the manuscript and approved the final version.

CONFLICTS OF INTEREST

All authors declare that there is no conflict of interest.

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SUPPLEMENTARY

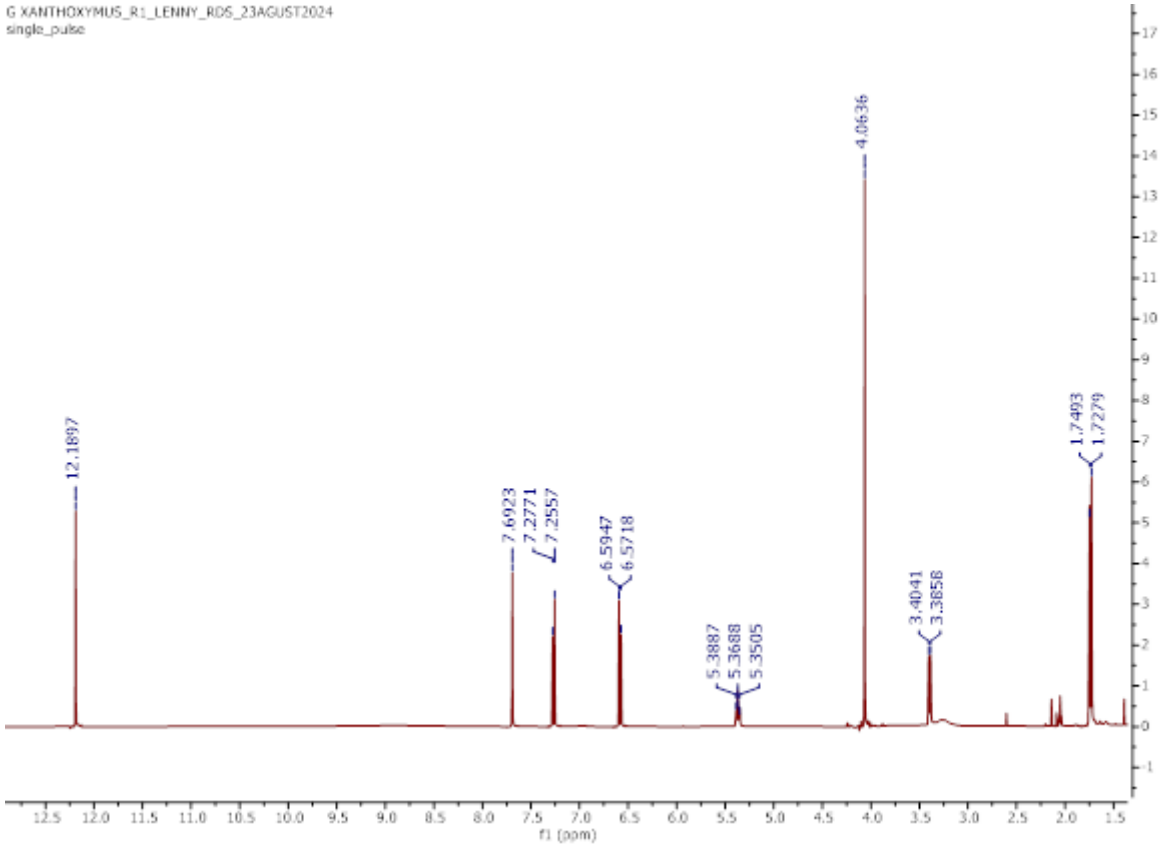

Figure S1. ^{1}H NMR spectrum of 1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone **(1)**.

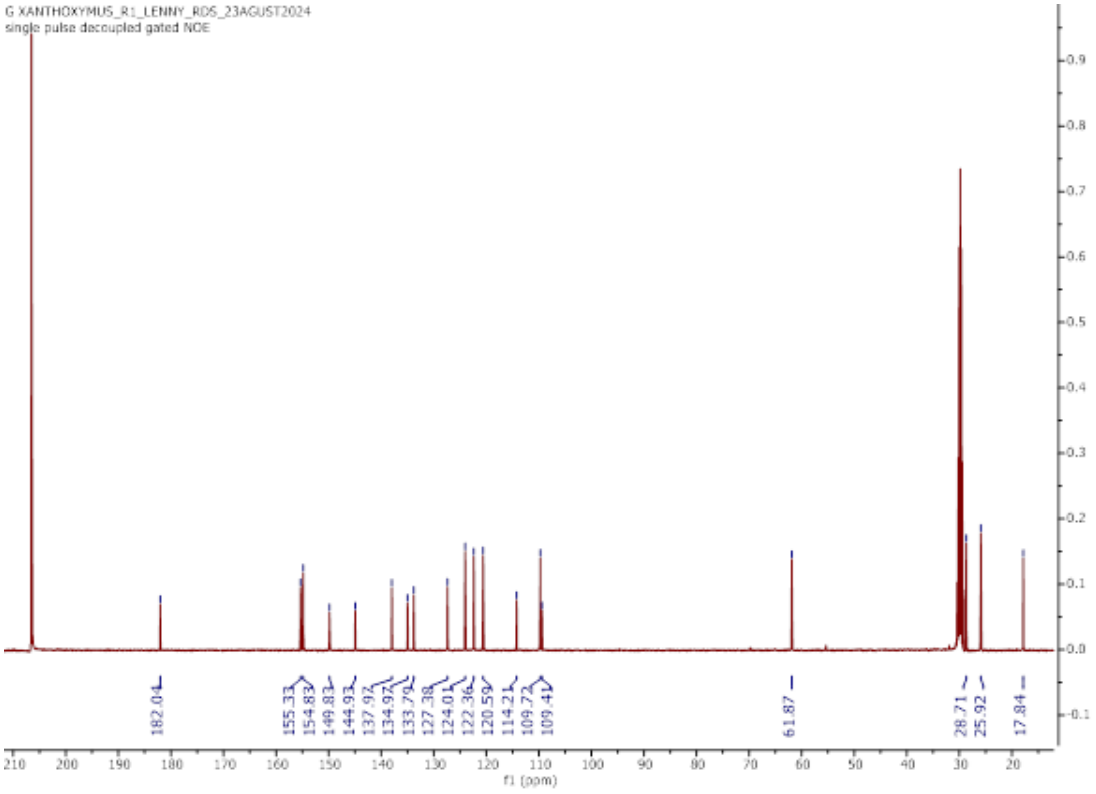

Figure S2. ^{13}C NMR spectrum of 1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone **(1)**.

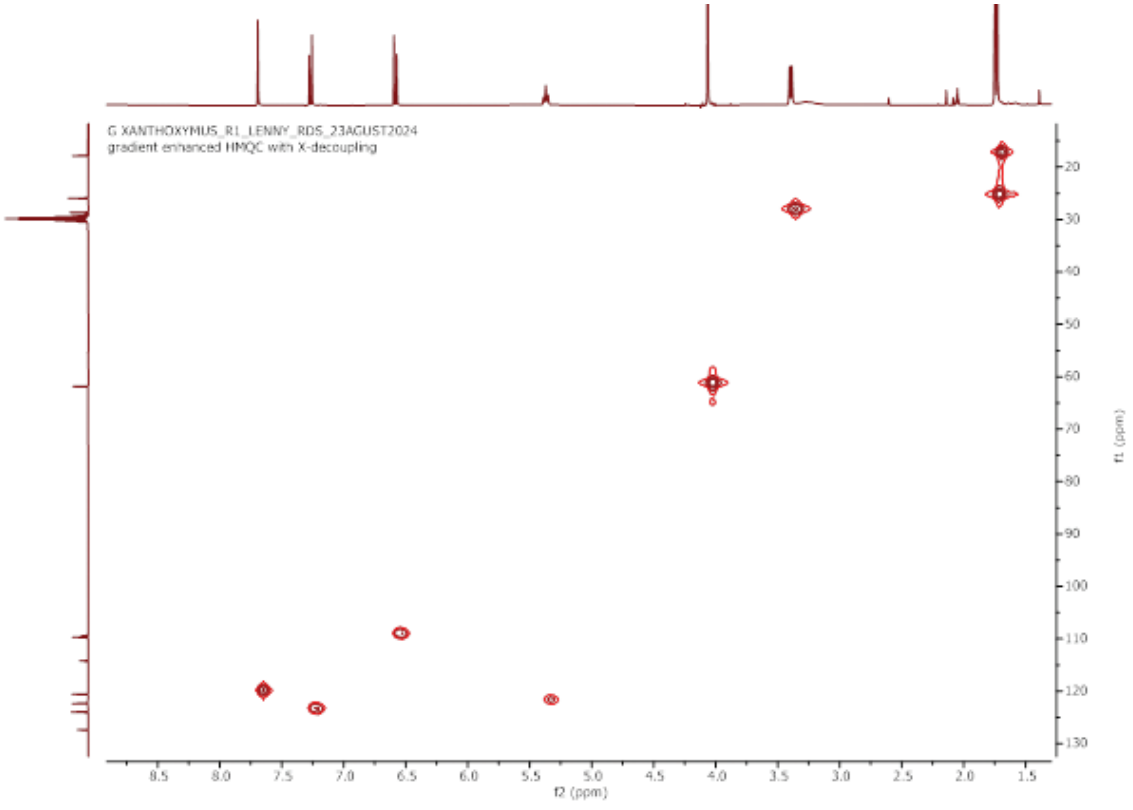

Figure S3. HMQC spectrum of 1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone **(1)**.

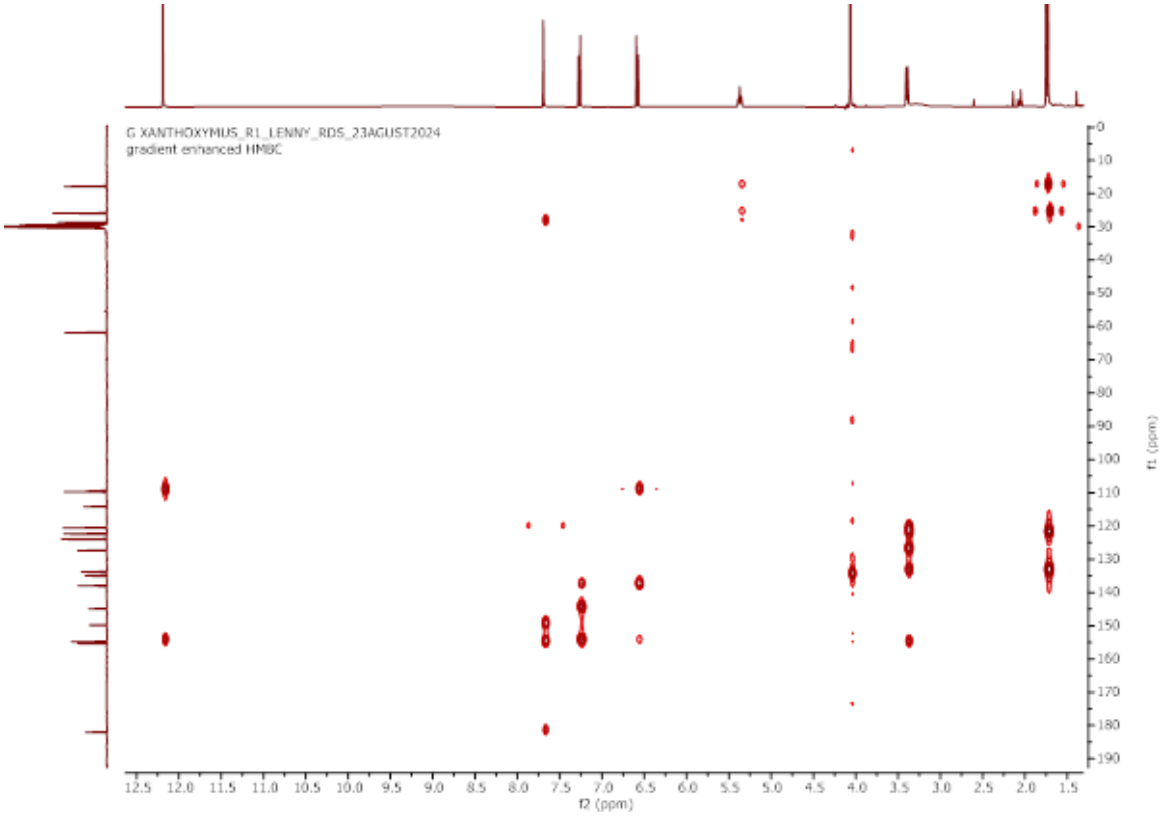

Figure S4. HMBC spectrum of 1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone **(1)**.

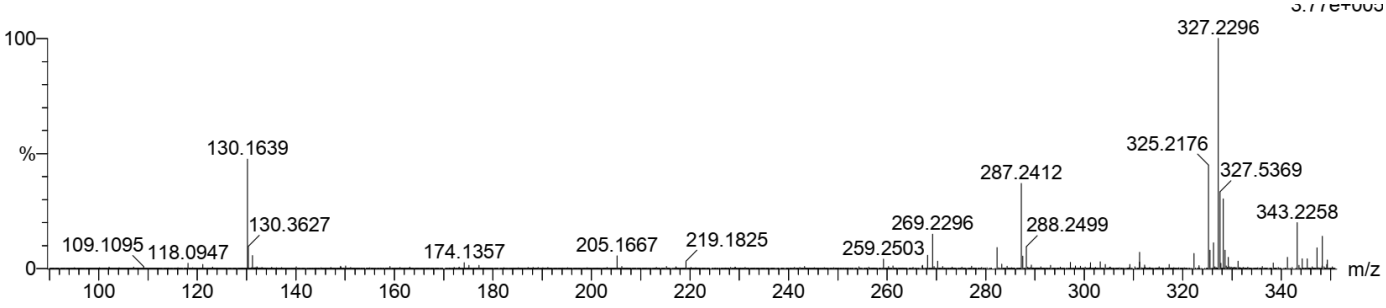

Figure S5. HRESIMS spectrum of 1,4,6-trihydroxy-5-methoxy-7-isoprenylxanthone **(1)**.

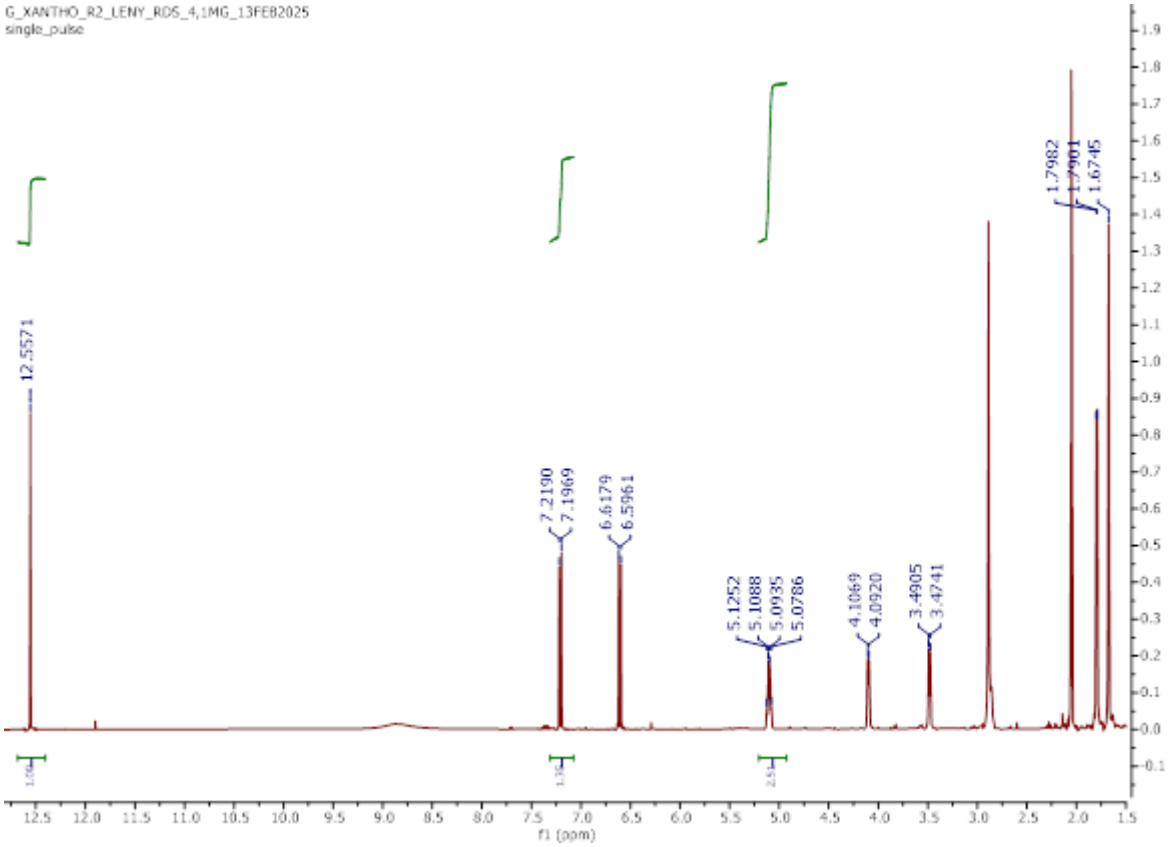

Figure S6. [1]H NMR spectrum of 1,4,5,6-tetrahydroxy-7,8-diisoprenylxanthone **(2)**.

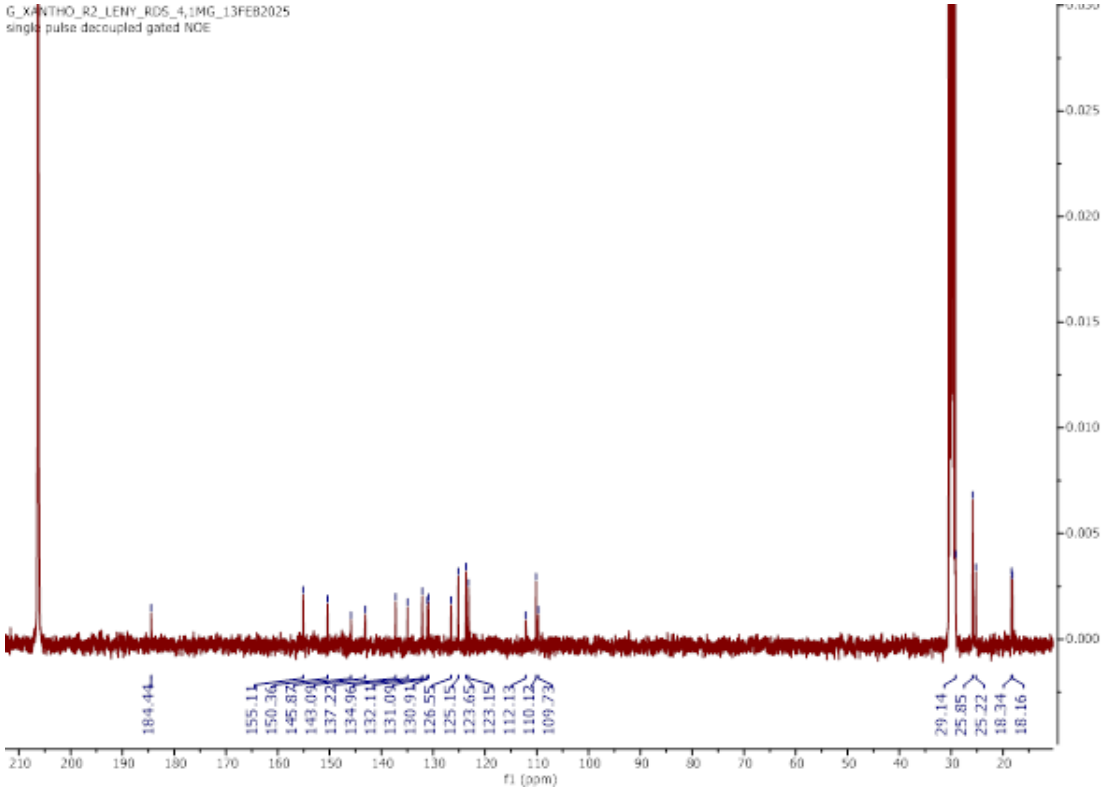

Figure S7. [13]C NMR spectrum of 1,4,5,6-tetrahydroxy-7,8-diisoprenylxanthone **(2)**.

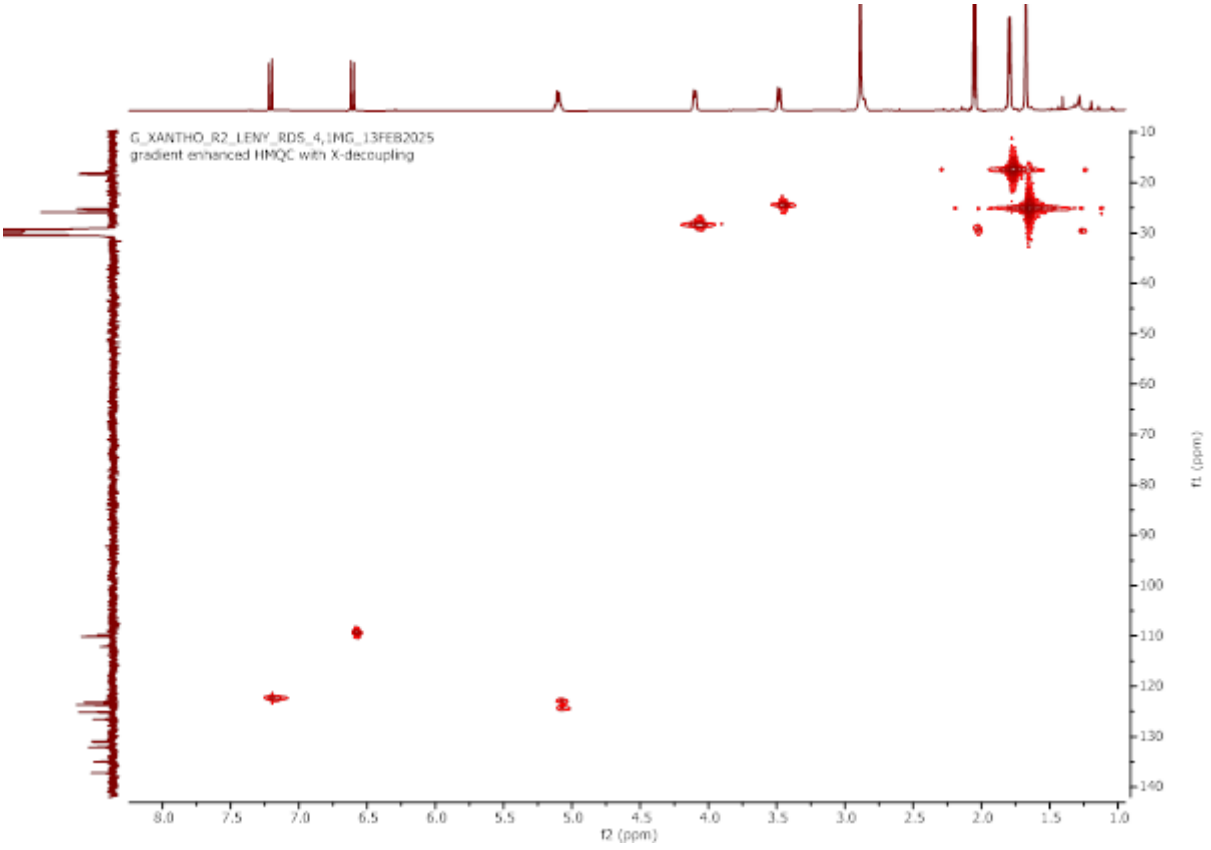

Figure S8. HMQC spectrum of 1,4,5,6-tetrahydroxy-7,8-diisoprenylxanthone **(2)**.

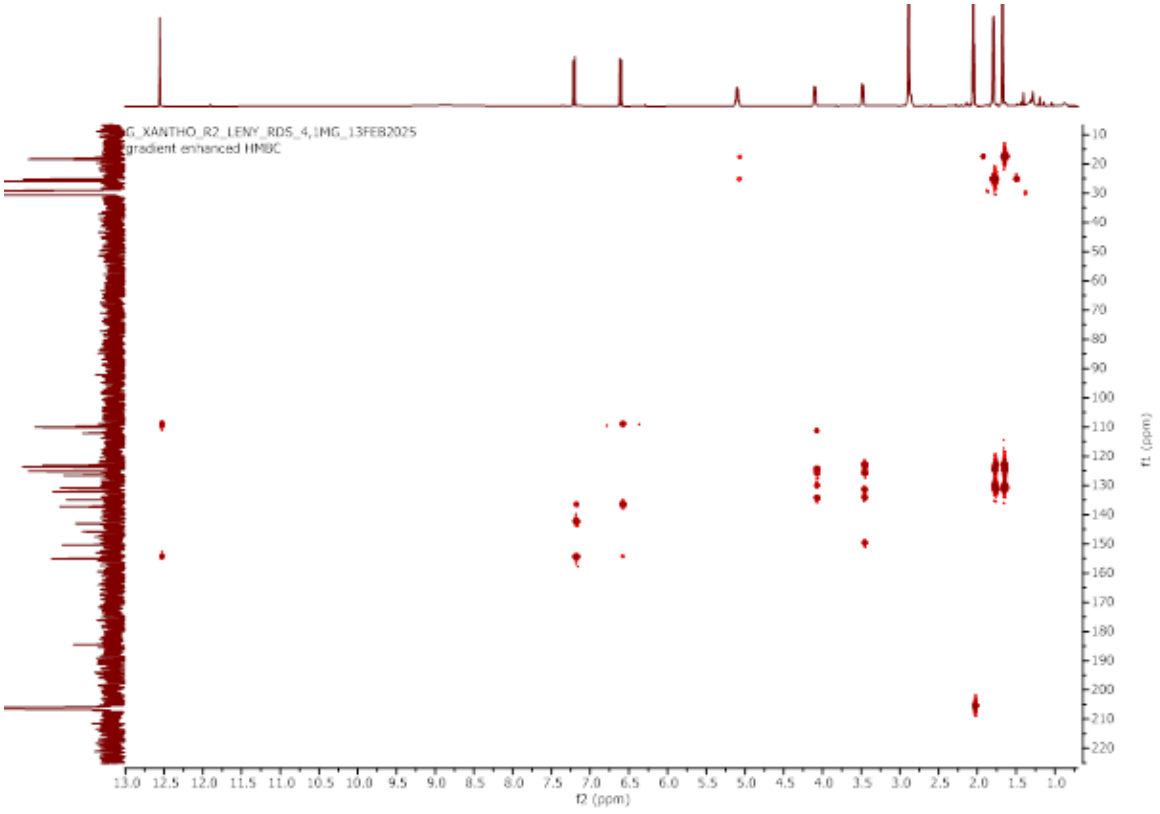

Figure S9. HMBC spectrum of 1,4,5,6-tetrahydroxy-7,8-diisoprenylxanthone **(2)**.

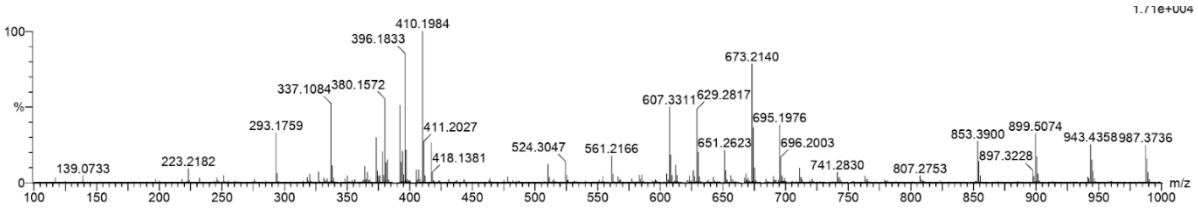

Figure S10. HRESIMS spectrum of 1,4,5,6-tetrahydroxy-7,8-diisoprenylxanthone **(2)**.