OPEN ACCESS
Journal of Multidisciplinary Applied Natural Science Vol. xx No. xx (xxxx) https://doi.org/10.47352/jmans.2774-3047.462
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Research Article

Prenylated Xanthones from *Calophyllum biflorum* Leaves: Isolation, Structural Characterization, and Evaluation of Antioxidant and Cytotoxic Activities

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Received : March 8, 2026	Revised : June 2, 2026	Accepted : July 9, 2026	Online : July 24, 2026

Abstract
The *Calophyllum* genus, part of the Calophyllaceae family, produces xanthones as dominant secondary metabolites. *Calophyllum biflorum* M.R. Hend. & Wyatt-Sm. is an endemic plant of Kalimantan; however, the potential of its xanthone content and biological activity has never been explored. In this study, three known prenylated xanthones: gartanin (**1**), ananixanthone (**2**), and α-mangostin (**3**), were isolated from *C. biflorum* leaves. Their chemical structures were determined by HRMS and 1D/2D NMR. The antioxidant activities of xanthones **1–3** (via DPPH radical) and their cytotoxicity toward 4T1 breast cancer cells were assessed. Xanthones **1–2** showed strong DPPH activity (IC50: 4.40 and 34.34 µM, respectively). Gartanin (**1**) also exhibited moderate activity against 4T1 cells (IC50 =16.70 µM). These results indicate the prenylated xanthones from *C. biflorum* as antioxidant agents.

Keywords: antioxidant, *Calophyllum biflorum*, cytotoxic, xanthone

1. INTRODUCTION

Plant biodiversity is very high in Indonesia, including the genus *Calophyllum* (Calophyllaceae), locally known as 'Bintangor'. Data from the World Flora Online (WFO) Plant List indicate that approximately 70% of *Calophyllum* species are distributed in Southeas Asia, including Indonesia. Traditionally, the roots, leaves, and bark of *Calophyllum* have been widely used for wound healing, pain relief, skin care, and the treatment of various inflammatory and infectious conditions [1]-[2]. Modern phytochemical studies have revealed that *Calophyllum* contains various secondary metabolites. These include coumarins [3]-[4], biflavonoids [5], triterpenes [6], chromanone acids [7]-[9], and xanthones [10]-[14]. Modern pharmacological studies have reported that secondary metabolites in *Calophyllum* exhibits diverse biological activities, such as antimalarial [7], antitumor [15], anti-inflammatory [16], antituberculosis [17], anticancer [12][13][18], anti-HIV [19], and antioxidant [20] activities. As a result, *Calophyllum* has the potential to be a source of biologically active natural products for the development of medical therapies.

Xanthones are the main group of phenolic derivatives from *Calophyllum*. They are characterized by a dibenzo-γ-pyrone core structure and exhibit a wide range of biological activities. Structural variations of *Calophyllum* xanthones, such as different substituents, including prenyl, hydroxyl, geranyl, furan, and pyran groups, are crucial for controlling the biological activities of xanthones [10]-[14]. Prenylated xanthones show better cytotoxic and antioxidant properties. One of the causes of cancer growth is related to oxidative stress factors from reactive oxygen species (ROS). These factors cause DNA mutations, lipid membrane damage, and protein modifications, which ultimately lead to the onset of various diseases, including cancer. Phenolic compounds such as xanthones can scavenge free radicals and exhibit cytostatic and cytotoxic effects against various cancer cells. Previous studies have shown that the number of hydroxyl substituents and prenyl chains in the xanthone structure increases its antioxidant activity and cytotoxic properties [20]. *Calophyllum biflorum* is an endemic plant of

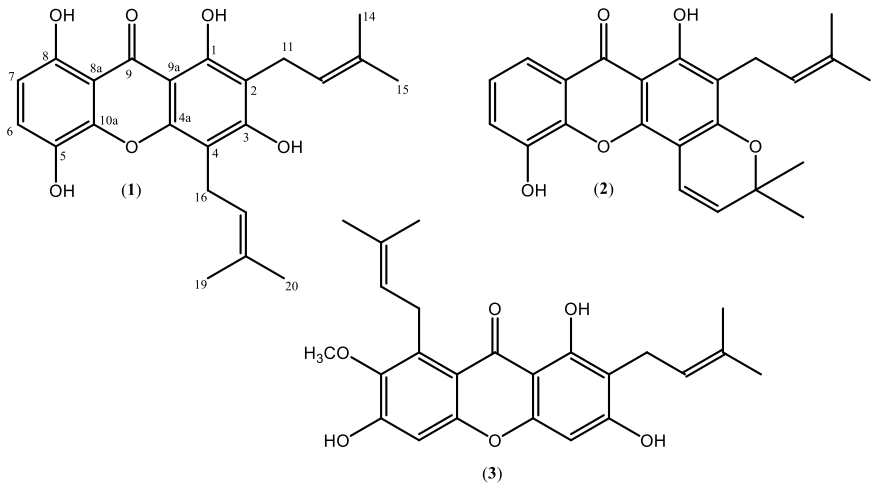

Figure 1. Xanthones **1–3** of *Calophyllum biflorum*.

Indonesia, and there have been no previous reports on xanthone phytochemistry [20]. Therefore, this study aimed to separate and characterize prenylated xanthones from *C. biflorum* leaves. Additionally, it aimed to assess their cytotoxicity against 4T1 cells and antioxidant activity against DPPH radicals.

2. MATERIALS AND METHODS

2.1. Materials

Calophyllum biflorum leaves were collected from Sikui Village, North Barito Regency, Central Kalimantan, Indonesia, in June 2024. After collection, they were identified at Herbarium Bogoriense, BRIN, Cibinong, West Java. The specimen (CB 20240603) was then stored at the Natural Product Chemistry Laboratory of Surabaya State University, Indonesia. The structures of xanthones **1–3** were analyzed using an NMR spectrometer (JEOL ECA 400) and a mass spectrometer (Waters HRESIMS). The maximum absorption of xanthone derivatives was measured using a UV spectrophotometer (Thermo Scientific™). Compounds **1–3** were separated by radial chromatography (silica gel PF_{254}, stationary phase) and column chromatography (silica gel PF_{254}, stationary phase), and thin-layer chromatography, silica gel GF_{254} used as the stationary phase.

2.2. Methods

2.2.1. Extraction and Isolation

The leaves of *Calophyllum biflorum* (1.1 kg) were extracted with 90% methanol (10 L) for 48 h [21]-[24]. Using a rotavapor, the solvent was removed to obtain a concentrated MeOH extract, which was then partitioned with *n*-hexane to remove non-polar compounds (three times) and chlorophyll, then partitioned with EtOAc to yield an ethyl acetate fraction (60 g). Using *n*-hexane: EtOAc (7:3 to 1:1 (v/v)), this fraction was separated using a column chromatograph, allowing for the three main fractions (A–C). Fraction B (7.70 g), by radial chromatography on silica gel, three subfractions (B1–B3) using *n*-hexane: Chloroform (9:1 to 1:1 (v/v)). B1 subfraction (53 mg) was further purified using *n*-hexane and diisopropyl ether (9:1 to 7:3 (v/v)), yielding ananixanthone (**2**) (13.6 mg). Subfraction B2 (204 mg) was purified using the same eluent system with ratios ranging from 7:3 to 1:1 (v/v), producing gartanin (**1**) (8 mg). Subfraction B3 (44.7 mg) was further purified using a mixture of n-hexane and EtOAc (19:1 to 8:2 (v/v)), yielding α-mangostin (**3**) (18.4 mg).

2.2.2. Gartanin (1)

UV (CH_3OH) λ_{max} (log ε) for yellow powder: 349 (3.68), 300 (3.50), 282 (3.95) 222 (4.04), 216 (4.03). HRMS *m/z* 396.1833 $[M]^+$, calculated mass for $C_{23}H_{24}O_6^+$ *m/z* 396.1830. ^{1}H-NMR data, δ_H ppm: 1.76 (3H, *s*, H-14), 1.79 (3H, *s*, H-19), 1.86 (6H, *s*, H-15/20), 3.47 (2H, *d*, J = 8.9 Hz, H-11), 3.52 (2H, *d*, J = 7.3 Hz, H-16), 5.24 (1H, *t*, J = 6.9 Hz, H-12), 5.29 (1H, *t*, J = 5.8 Hz, H-17), 6.67 (1H, *d*, J = 8.9 Hz, H-7), 7.23 (1H, *d*, J = 8.9 Hz, H-6), 11.27 (1H, *s*, 3-OH), 12.35 (1H, *s*, 8-OH), and 13.19 (1H, *s*, 1-OH). ^{13}C-NMR ($CDCl_3$, 100 MHz),

δ_C ppm: 18.1 (C-15/20), 21.7 (C-11), 22.1 (C-16), 25.8 (C-14), 26.0 (C- 19), 102.3 (C-9a), 105.9 (C-4), 107.3 (C-8a), 109.6 (C-2), 109.9 (C-6), 121.1 (C-17), 122.0 (C-12), 123.0 (C-7), 135.8 (C-5), 134.1 (C-18), 136.5 (C-13), 142.9 (C-10a), 153.9 (C-4a), 158.3 (C-8), 158.8 (C-1), 161.8 (C-3), and 184.8 (C-9).

2.2.3. Ananixanthone (2)

UV (CH_3OH) λ_{max} (log ε) for yellow powder: 334 (4.12), 270 (4.59), 236 (4.41), 2.16 (4.33), 206 (4.31). HRMS *m/z* 379.1550 $[M+H]^+$, calcd. mass for $C_{23}H_{23}O_5^+$ *m/z* 379.1545. ^{1}H-NMR data, δ_H ppm: 1.48 (6H, *s*, H-20/21), 1.70 (3H, *s*, H-14), 1.81 (3H, *s*, H-15), 3.24 (2H, *d*, J = 7.3 Hz, H-11), 5.23 (1H, t, J = 7.3 Hz, H-12), 5.61 (1H, *d, J* = 10.0 Hz, H-18), 6.76 (1H, *d, J* = 10.0 Hz, H-19), 7.21 (1H, *t, J* = 7.9 Hz, H-7), 7.27 (1H, *t, J* = 7.9 Hz, H-6), 7.75 (1H, *dd, J* = 7.9; 1.7 Hz, H-8), and 13.17 (1H, *s*, 1-OH). ^{13}C-NMR ($CDCl_3$, 100 MHz), δ_C ppm: 18.1 (C-15), 21.3 (C-11), 26.0 (C-14), 28.3 (C-20/21), 78.2 (C-17), 100.8 (C-4), 103.3 (C-9a), 112.4 (C-2), 115.1 (C-19), 117.2 (C-8), 120.3 (C-6), 121.3 (C-8a), 122.0 (C-2'), 124.1 (C-7), 127.3 (C-18), 131.8 (C-13), 144.2 (C-5), 144.4 (C-10a), 149.4 (C-4a), 158.8 (C-3), 160.6 (C-2), and 180.9 (C-9).

2.2.4. α-Mangostin (3)

UV (CH_3OH) λ_{max} (log ε) for yellow powder: 244 (4.60), 210 (4.31), 218 (4.41), 258 (4.50), 316 (4.44). HRMS *m/z* 409.1653 $[M-H]^-$, calculated mass for $C_{24}H_{25}O_6^-$ *m/z* 409.1651. ^{1}H-NMR data, δ_H ppm: 1.69 (3H, *s*, H-20), 1.76 (3H, *s*, H-15), 1.82 (3H, *s*, H-19), 1.84 (3H, *s*, H-14), 3.44 (2H, *d, J* = 7.2 Hz, H-11), 3.79 (3H, *s*, 7-OCH_3), 4.08 (2H, *d, J* = 6.4 Hz, H-16), 5.26 (1H, *t, J* = 6.8 Hz, H-17), 5.28 (1H, *t, J* = 6.8 Hz, H-12), 6.28 (1H, *s*, H-4), 6.80 (1H, *s*, H-5), and 13.77 (1H, *s*, 1-OH). ^{13}C-NMR ($CDCl_3$, 100 MHz), δ_C ppm: 17.1 (C-19), 17.5 (C-14), 21.1 (C-16), 25.0 (C-20), 25.1 (C-15),

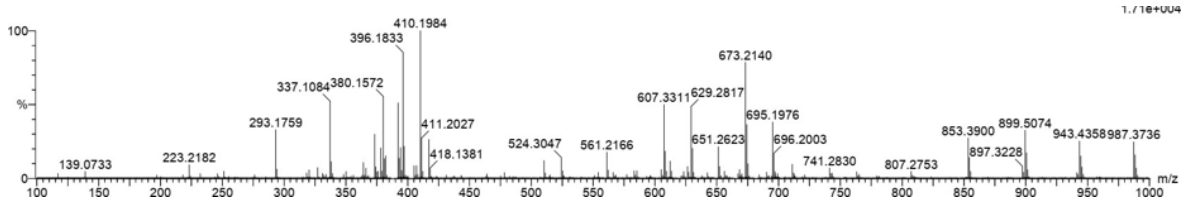

Figure 2. HRESIMS spectrum of gartanin (**1**).

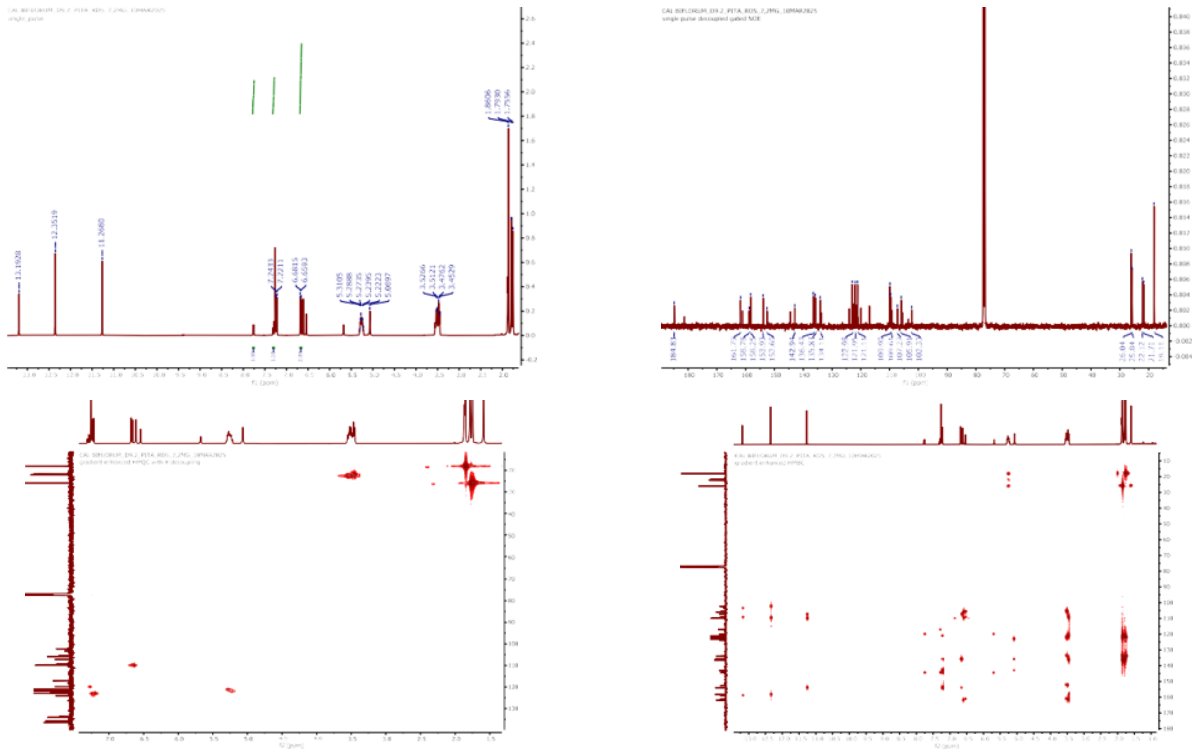

Figure 3. NMR data of gartanin (**1**).

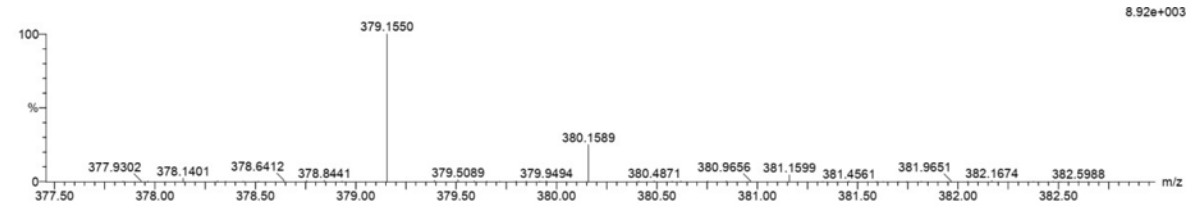

Figure 4. HRESIMS spectrum of ananixanthone (**2**).

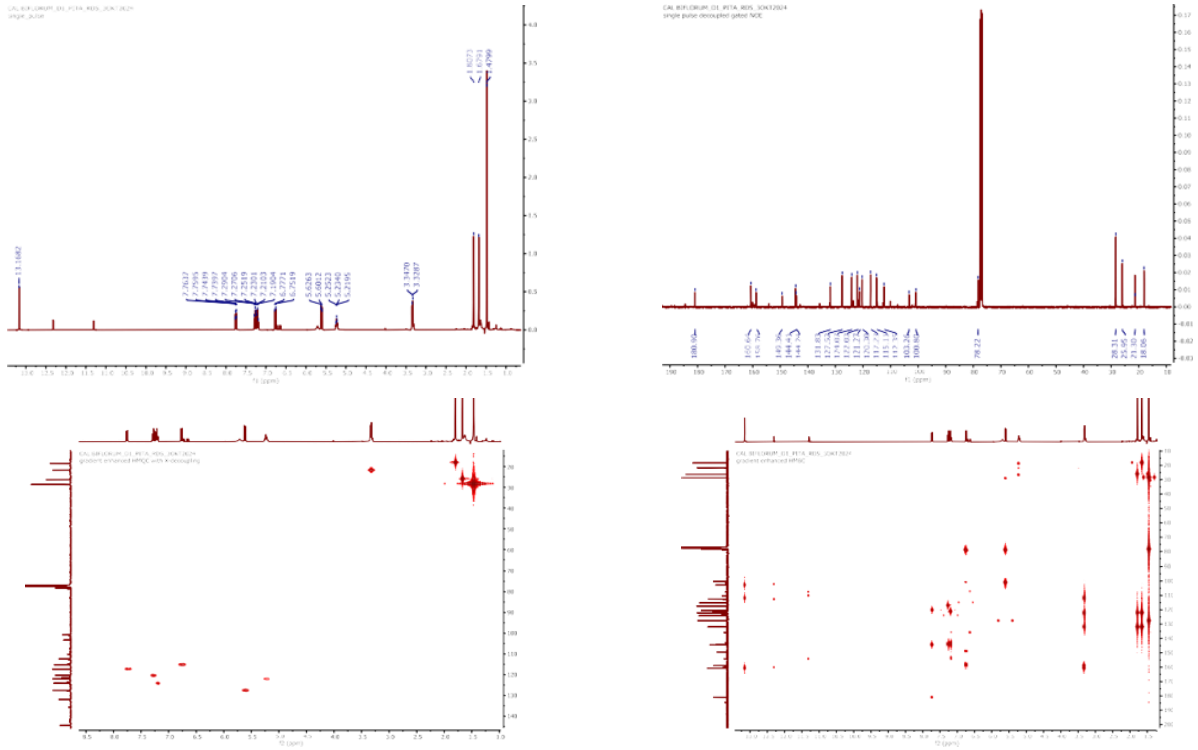

Figure 5. NMR data of ananixanthone (**2**).

26.1 (C-11),), 60.5 (7-OCH_3), 92.3 (C-4), 101.9 (C-5), 102.8 (C-9a), 111.1 (C-8a), 110.2 (C-2), 122.6 (C-17), 123.9 (C-12), 130.6 (C-13/18), 136.5 (C-13), 137.3 (C-8), 143.6 (C-7), 155.4 (C-6), 154.9 (C-3), 156.6 (C-10a), 160.9 (C-1), 162.1 (C-4a), and 182.0 (C-9).

2.2.5. Cytotoxic Activity

The cytotoxic potential of xanthones **13** toward 4T1 breast cancer cells, using doxorubicin and 1% DMSO as positive and negative controls, respectively. The 4T1 cell line was selected because it is a highly aggressive murine breast cancer model commonly used for evaluating potential anticancer agents. In RPMI-1640 medium, added 1% antibiotics (streptomycin-penicillin) and 10% FBS, 4T1 cells were cultured and incubated (5% CO_2, 37 °C, 24 h) in 96-well plates. 4T1 cells were harvested at a density of 1×10^5 cells/mL. Next, xanthones **1–3** were added at concentrations of 30, 10, 5, 1, and 0.1 in triplicate. Cell viability was determined at λ 540 nm. The IC_{50} values of xanthones **1–3** were calculated by regression analysis based on 50% growth inhibition [25]-[28].

2.2.6. Antioxidant Activity

To investigate the potential antioxidant activity of xanthones **1–3** against DPPH radicals, ascorbic acid (positive control) and ethanol (negative control) were used by UV spectrophotometry. The DPPH assay was selected because it is a rapid and widely used preliminary screening method for evaluating free radical scavenging activity of natural products. Xanthones **1–3** were each dissolved in ethanol in a 100 mmol/L acetate buffer and 5×10^{-4} mol/L DPPH in a 2:2:1 v/v ratio, and prepared at test concentrations of 100, 30, 10, 3, and 1 µM. All experiments were performed in triplicate. The IC_{50} values of

xanthones **1–3** were calculated from inhibition percentages recorded at λ 517 nm by regression analysis [29]-[31].

3. RESULTS AND DISCUSSIONS

Three prenylated xanthones (Figure 1): gartanin (**1**), ananixanthone (**2**), and α-mangostin (**3**), were found in *Calophyllum biflorum* leaves. Phytochemical investigations of this plant have not been previously conducted. Separation of xanthones **1–3** from ethyl acetate extract using silica open column chromatography and radial chromatography.

Gartanin (**1**) is the minor compound. Its chemical formula is $C_{23}H_{24}O_6$. The main ion peak appears at *m/z* 396.1833, matching the calculated mass in the HRESIMS data (Figure 2). Compound **1** exhibited absorption bands at λ_{max} 222, 282, 300, and 349 nm, characteristic of a xanthone skeleton in the UV spectrum [32]-[34]. The ^{1}H-NMR data of gartanin (**1**) shows a pair of *ortho* doublets (J = 8.9 Hz) of two aromatic signals showing at 6.67 (H-7) and 7.23 (H-6), characteristic of 1,2,3,4,5,8-hexasubstituted xanthone derivatives [34]. Gartanin (**1**) also exhibited three hydroxy groups were observed as sharp singlets at 13.19 (1-OH), 12.35 (8 -OH), and 11.27 (3-OH), as well as two prenyl chain consists of two vinylics [δH 5.29 (H-17), δH 5.24 (H-12)], two methylenes [δH 3.52 (H-16), δH 3.47 (H-11)], and four methyls [δH 1.86 (H-15/H-20), δH 1.79 (H-19), δH 1.76 (H-14)]. The ^{13}C NMR spectrum of gartanin displays 22 carbon signals consistent with prenylated xanthone derivatives. The carbon signals are divided into one carbonyl [δC 184.8 (C-9)], six oxy-aryls [δC 161.8 (C-3), 158.8 (C-1), 158.3 (C-8), 153.9 (C-4a), 142.9 (C-10a), 135.8 (C-5)], six quaternary carbons [δC 136.5 (C-13), 134.1 (C-18), 109.6 (C-2), 105.9 (C-4), 107.3 (C-8a), 102.3 (C-9a)], four methines [δC 123.0 (C-7), 122.0 (C-17), 121.1 (C-12), 109.9 (C-

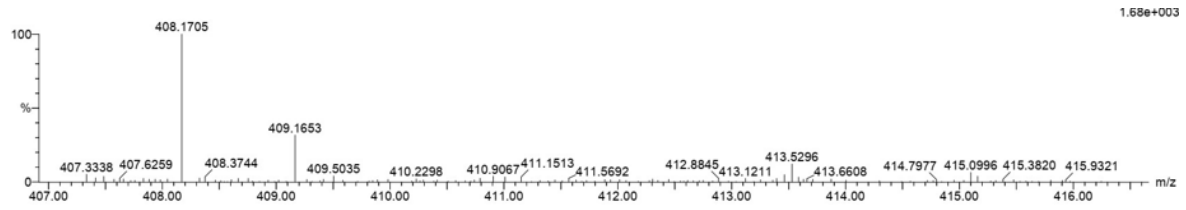

Figure 6. HRESIMS spectrum of α-mangostin (**3**).

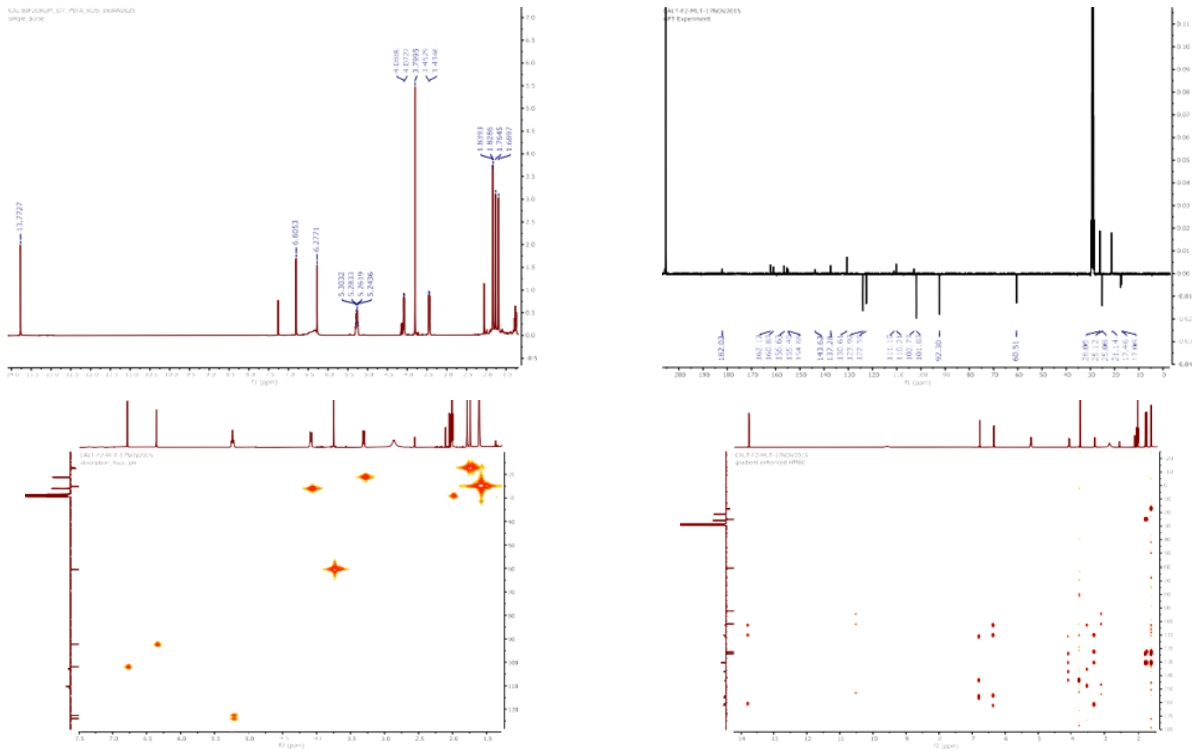

Figure 7. NMR data of α-mangostin (**3**).

Table 1. Antioxidant and cytotoxic activities of xanthones **1-3**.

Compound	IC50 (µM)		Statistical Data	
	DPPH	4T1	DPPH	4T1
1	4.44	16.70	0.9773	0.9286
2	34.34	40.21	0.8923	0.9667
3	168.01	43.94	0.9483	0.8359
Doxorubicin	-	0.40	-	-
Ascorbic acid	0.33	-	-	-

6)], two methylenes [*δC* 22.1 (C-16), 21.7 C-11)], one overlapping methyl [*δC* 18.1 (C-15/20)], two methyls [*δC* 26.0 (C-19), 25.8 C-14)]. 2D NMR has supported the location of the proton and carbon signals. The HMBC spectrum: a hydroxyl resonance in 1-OH (*δH* 13.19) gives correlations with the oxy-aryl carbon [C-1 (*δC* 158.8)] and two quaternary carbons [C-2 (*δC* 109.6), C-9a (*δC* 102.3)]. The other hydroxy proton in 3-OH (*δH* 11.27) correlated to C-2, one oxy-aryl [C-3 (*δC* 161.8)], and one quaternary carbon [C-4 (*δC* 105.9)]. The methylene proton at H-11 (*δH* 3.47) correlated to C-1, C-2, C-3, and the alkene carbon [C-13 (*δC* 136.5), C-12 (*δC* 121.1)], indicating a prenyl chain attached at C-2. The other methylene resonances at H-16 (*δH* 3.52), also related to C-2, C-3, C-4, and the alkene carbon [C-18 (*δC* 134.1), C-17 (*δC* 122.0)], proving a prenyl bound on C-4. The 8-OH proton (*δH* 12.35) showed connected to C-8 (*δC* 158.3), C-8a (*δC* 107.3), and C-7 (*δC* 123.0). The aromatic resonances at H-7 (*δH* 6.67) displayed associations with C-5 (*δC* 135.8), C-8, C-8a, and the other aromatic proton at H-6 (*δH* 7.23) associated with C-5, C-8, and C-10a (*δC* 142.9), supporting the substitution pattern of the aromatic ring. Based on the combined 1D and 2D NMR data (Figure 3), compound **1** is 2,4-diprenyl -1,3,5,8-tetrahydroxyxanthone, known as gartanin [35].

Ananixanthone (**2**) has the chemical formula C23H22O5 as determined by the HRESIMS (Figure 4). The ion peak [M+H]+: m/z 379.1550 (calcd. mass 379.1545 for the formula The proton NMR spectrum exhibited aromatic signals at δH 7.75 (H-8), 7.27 (H- 6), and 7.21 (H-7), showing that 1,2,3,4,5 - pentasubstituted xanthone derivatives. Ananixanthone 2 also showed a hydroxy proton appeared as a sharp singlet at 13.17 (1-OH), and two methyls [δH 1.81 (H-15), 1.70 (H-14)], a vinyl [δH 5.23 (H-12)], and one methylene [δH 3.24 (H-11)] were recognized as signals corresponding to the prenyl chain. In addition, two cis vinylic protons at [δH 6.76 (H-19), 5.61 (H-18)], and a gem-dimethyl [δH 1.48 (H-20/21), suggesting the existence of a 2,2-dimethyl pyran system. The ^{13}C NMR data of ananixanthone displayed 22 carbon signals, distributing into a carbonyl [*δC* 180.9 (C-9)], five oxy-aryls [*δC* 160.1 (C-1), 158.8 (C-3), 149.4 (C-4a), 144.4 (C-10a), 144.2 (C-5)], five quaternary carbons [*δC* 131.8 (C-13), 121.3 (C-8a), 112.4 (C-2), 103.3 (C-9a), 100.8 (C-4)], six methines [*δC* 127.3 (C-18), 124.1 (C-7), 122.0 (C-12), 120.3 (C-6), 117.2 (C-8), 115.1 (C-19)], one methylenes [*δC* 21.3 (C-11)], an oxy-carbon [*δC* 78.2 (C-17)], a *gem*-dimethyl [*δC* 28.3 (C-20/21)], two methyls [*δC* 26.0 (C-14), 18.1 C-15)]. The HMBC correlation of 2, a hydroxyl proton in 1-OH (*δH* 13.17), connected to C-1, C-2, and C-9a. The methylene proton at H-11 (*δH* 3.24) correlated to C-1, C-2, C-3, C-12, and C-13, supporting a 3-methyl-2-butenyl attached at C-2. A proton of gem-dimethyl at H-20/21 (*δH* 1.48) showed associations with an oxy-carbon (*δC* 78.2, C-17), one double bond (*δC* 127.3, C-18), and one cis vinylic at H-20 (*δH* 6.76) correlated to C-3, C-4, C -4a, and C-17, confirming the 2,2-dimethylpyrano attached on C-3 and C-4. An aromatic resonance at H-8 (*δH* 7.75) relates with a carbonyl [*δC* 180.9, (C -9)], an oxy-aryl [*δC* 144.4, (C-10a)], a methine of the aromatic [*δC* 120.3, (C-6)], indicating a bound hydroxy group at C-5. The HMBC spectrum of two other aromatic resonances at H-7 (*δH* 7.21) and H-6 (*δH* 7.25) also supports the presence of such a hydroxy group. Based on the NMR data (Figure 5),

compound **2** is ananixanthone [12].

The α-mangostin (**3**) exhibits the molecular formula $C_{24}H_{26}O_6$ in the ion peak $[M-H]^-$ at *m/z* 409.1653 $C_{24}H_{25}O_6^-$ [(calcd. mass [M-H]- 409.1651, DBE 12.5, Δ 0.5)]. The ^{1}H NMR spectrum shows aromatic resonances [*δH* 6.28 (H-4), 6.80 (H-5)], a methoxy [*δH* 3.79 (7-OCH_3) and a hydroxy signal [*δH* 13.77 (1-OH)]. α-Mangostin also exhibits two prenyl groups contributed by two vinyls [*δH* 5.28 (H-2'), 5.26 (H-2")], two methylene [*δH* 4.08 (H-16), 3.44 (H-11], and four methyl [*δH* 1.84 (H-14), 1.82 (H-19), 1.76 (H-15), 1.69 (H-20)] protons. The carbon signal of the xanthone nucleus consists of 13 carbon signals: *δC* 160.9 (C-1), 110.2 (C-2), 134.9 (C-3), 92.3 (C-4), 162.1 (C-4a), 101.9 (C-5), 155.4 (C-6), 143.6 (C-7), 137.3 (C-8), 111.1 (C-8a), 182.0 (C-9), 102.8 (C-9a), and 156.6 (C-10a) in the ^{13}C NMR data. The carbon resonances of the 3-methyl-2-butenyl chain are distributed over *δC* 26.1 (C-11), 123.9 (C-12), 130.6 (C-13/C-18), 17.5 (C-14), 25.1 (C-15), 21.1 (C-16), 122.6 (C-17'), 17.1 (C-18), 25.0 (C-20), and one methoxy carbon at *δC* 60.7 (7-OCH_3). The HMBC correlation of the hydroxyl resonances at 1-OH (*δH* 13.77) and the methylene proton at H-11 (*δH* 3.44) is similar to that of compounds **1** and **2**, favoring a prenyl attached at C -2, and two hydroxy [C-1 and C-3]. The HMBC correlation of an aromatic resonance at H-4 (*δH* 6.28) also confirms the location of the prenyl and the two hydroxy groups. Another aromatic proton at H-5 (*δH* 6.80) shows connections with two oxy-aryl [*δC* 155.4 (C-6), 143.6 (C-7)] and one quaternary carbon [*δC* 111.1 (C-8a)]. The methylene signal at H-16 (*δH* 4.08) relates to C-7, C-8a, a quaternary carbon [*δC* 137.3, (C-8)], and the alkene carbon [*δC* 130.6 (C-18), *δC* 122.6 (C-17)], indicating a prenyl attached at C-8 and a methoxy at C-7. From HRESIMS (Figure 6) and NMR spectra (Figure 7), the structure of xanthone **3** is α-mangostin [36].

The antioxidant potential of xanthones **1–3** against DPPH radical scavenging using UV spectrophotometry was determined at a maximum absorbance of λ = 517 nm using the UV spectrophotometry method. The potential antioxidant activity of xanthones **1–3** against DPPH radical scavenging using a UV spectrophotometry was determined at a maximum absorbance of 517 nm using UV spectrophotometry. As shown in Table 1 and Figure 8, gartanin (**1**) and ananixanthone (**2**) exhibited high antioxidant activity with IC_{50} values of 4.44 and 34.34 µM, respectively, while α-mangostin was inactive. Based on the xanthone structure, gartanin (**1**) has four hydroxyls at C-1, C-3, C-5, and C-8, as well as two terpenyl chains at C-2 and C-4. Ananixanthone (**2**) shows hydroxyl groups at C-1, C-3, C-5, and C-8, one terpenyl at C-2, and a pyran ring connected at C-3 and C-4. The hydroxyl group at C-8 of compound **1** may enhance electron delocalization and hydrogen-donating ability, thereby stabilizing radical species and contributing to antioxidant activity. A methoxy group at C-7 in α-mangostin (**3**) reduces electron delocalization, resulting in inactive activity [37]-[39]. In general, the antioxidant activity of xanthone derivatives is strongly influenced by the presence of phenolic hydroxyl groups within the xanthone framework. These hydroxyl groups can donate hydrogen atoms to neutralize free radicals, thereby stabilizing the radical species. Consequently, xanthone derivatives containing a greater number of hydroxyl substituents generally exhibit stronger radical

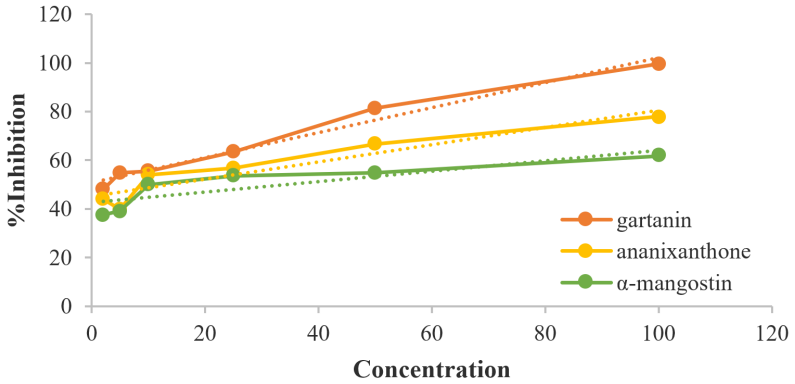

Figure 8. Antioxidant activity of xanthones **1–3** against DPPH radical.

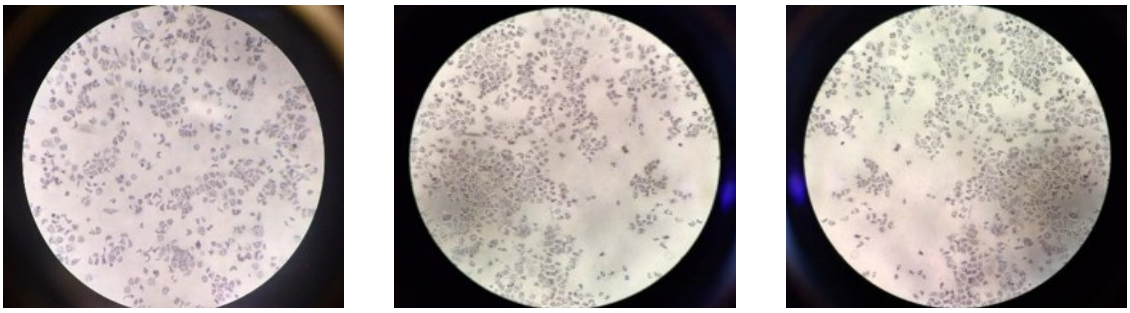

Figure 9. Cytotoxic activity of xanthones **1-3**.

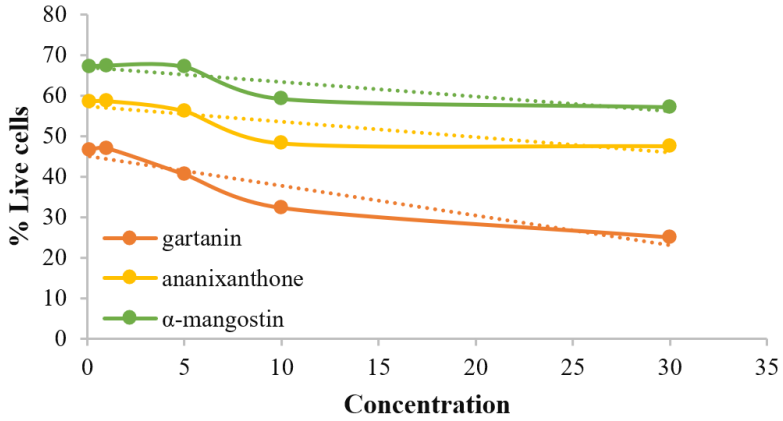

Figure 10. Cytotoxic activities of xanthones **1–3** against 4T1 cells.

scavenging activity in DPPH assays. Consistent with this structure–activity relationship, several studies have reported that hydroxylated xanthones isolated from *Calophyllum* species exhibit significant antioxidant activity. For example, calothorexanthone from *Calophyllum* have been reported to display DPPH radical scavenging activity with IC_{50} values 17.46 µM [40]. The antioxidant activity observed for gartanin (**1**) in the present study (IC_{50} = 4.44 µM) is therefore comparable to those previously reported values, further supporting the important role of hydroxyl substituents in enchancing radical scavenging activity.

Figures 9 and 10 demonstrate that gartanin (**1**) exhibited moderate cytotoxicity against 4T1 breast cancer cells, with an IC_{50} of 16.7 µM, whereas compounds **2** and **3** were inactive under identical conditions. This variation in cytotoxicity suggests that differences in the chemical structures of these xanthone derivatives significantly influence their biological effects on cancer cells. The cytotoxic profiles of compounds **1–3** provide valuable insights into the structure-activity relationships (SAR) within this group. The hydroxyl group at the C-8 position in compound **1** likely contributes to its cytotoxic activity. This hydroxyl group may affect intermolecular interactions such as hydrogen bonding, influencing the biological effects of xanthone derivatives. Previous research indicates that hydroxyl and prenyl groups can modify the biological properties of xanthones by changing their physicochemical characteristics and SAR [41]. In contrast, compounds **2** and **3**, which lack this hydroxyl group, did not show cytotoxicity under identical conditions. Thus, the activity observed in compound **1** may result from the combined effects of hydroxyl and prenyl groups rather than a single feature, though further mechanistic studies are needed for confirmation. While compound **1** showed moderate cytotoxicity against 4T1 cells, its potency was less than doxorubicin, the positive control in the same tests. Nonetheless, natural products can offer diverse structural scaffolds that serve as promising starting points for further optimization and biological research.

Another structural feature that may contribute to the cytotoxic activity of these compounds is the

presence of prenyl substituents. Prenyl groups increase the lipophilicity of natural products, facilitating their interaction with biological membranes and enhancing cellular uptake. As a result, prenylated xanthones often exhibit stronger cytotoxic activity than non- prenylated analogues due to their improved ability to penetrate cancer cells. In addition to prenyl substituents, hydroxyl groups are also known to influence the biological activity of xanthone derivatives. For example, cytotoxic caloxanthone B isolated from *Calophyllum depressinervosum* has been reported to display cytotoxic activity against K562 cells with an IC_{50} value of 1.23 µg/mL [20], highlighting the importance of hydroxyl and prenyl substituents in determining the biological activity of xanthone derivatives. Conversely, compounds **2** and **3** showed no cytotoxicity under the same experimental conditions. This may be due to differences in the number and placement of hydroxyl groups or variations in substituent patterns on the xanthone skeleton. Such structural differences could affect their biological activity by changing their physicochemical properties and structure–activity relationships. The lack of activity in α-mangostin (**3**) was somewhat surprising, as prior studies have documented its cytotoxic effects on various cancer cell lines. The discrepancy might result from variations in experimental conditions, cell line specificity, or differences in compound concentration or purity. The antioxidant and cytotoxic activities of xanthones **1–3** provide clear to SAR data. The presence of a hydroxyl group at C -8 in compound **1** may influence hydrogen bonding interactions and could contribute to the observed antioxidant and cytotoxic activities [20][42]-[43].

In summary, the findings of this study show that prenylated xanthones derived from *Calophyllum biflorum* possess unique biological activities, which correlate well with their structural characteristics. The presence of hydroxyl groups in conjunction with prenyl substituents seems to contribute significantly for both, antioxidant and cytotoxic activities. These observations contribute to the growing body of evidence supporting prenylated xanthones as excellent natural product scaffolds in drug discovery due to their pharmacological potential. Nevertheless, several limitations should be acknowledged. The biological evaluation in this study was limited to a single antioxidant assay (DPPH) and one cancer cell line (4T1). Therefore, further investigations, including additional antioxidant assays, testing on a broader range of cancer cell lines, and mechanistic studies, are necessary to better understand the therapeutic potential of these compounds.

4. CONCLUSIONS

Three prenylated xanthones: gartanin (**1**), ananixanthone (**2**), and α-mangostin (**3**), were successfully isolated from the leaves of *Calophyllum biflorum*. Based on biological evaluation, gartanin (**1**) was identified as the major bioactive constituent, showing high antioxidant activity and moderate activity against 4T1 breast cancer cells. Ananixanthone (**2**) and α-mangostin (**3**) also showed measurable antioxidant and cytotoxic activities; however, their overall biological profiles were less prominent compared to compound (**1**). Overall, these findings indicate that gartanin (**1**) demonstrates notable antioxidant and moderate cytotoxic activities, suggesting its potential biological relevance. Nevertheless, further comprehensive biological evaluation and mechanistic studies are required to confirm its pharmacological potential.

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Conflicts of Interest

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

This research has been funded by the Basic Research Project, Surabaya State University, 2023 [1117/UN38/HK/PP/2023].

DECLARATION OF GENERATIVE AI

Not applicable.

REFERENCES

[1] S. Ferdosh. (2024). "The Extraction of Bioactive Agents from Calophyllum inophyllum L., and Their Pharmacological Properties". *Scientia Pharmaceutica.* **92** (1): 1-13. 10.3390/scipharm92010006.

[2] J. Singh, V. Meshram, and M. Gupta. (2020)."Bioactive Natural Products in Drug Discovery". Springer, Singapore. 10.1007/978-981-15-1394-7.

[3] C. Ito, M. Itoigawa, Y. Mishina, V. C. Filho, F. Enjo, H. Tokuda, H. Nishino, and H. Furukawa. (2003). "Chemical Constituents of Calophyllum brasiliense. II. Structure of Three New Coumarins and Chemopreventive Activity of 4-Substituted Coumarins". *Journal of Natural Products.* **66** (3): 368-371. 10.1021/np0203640.

[4] C. H. Ma, B. Chen, H. Y. Qi, B. G. Li, and G. L. Zhang. (2004). "Two Pyranocoumarins from the Seeds of Calophyllum polyanthum". *Journal of Natural Products.* **67** (9): 1598-1600. 10.1021/np0499158.

[5] S. G. Cao, K. Y. Sim, and S. H. Goh. (1997). "Biflavonoids of Calophyllum venulosum". *Journal of Natural Products.* **60** (12): 1245-1250. 10.1021/np970303f.

[6] C. Y. Ragasa, V. Ebajo, M. M. De Los Reyes, E. H. Mandia, R. Brkljača, and S. Urban. (2015). "Triterpenes from Calophyllum inophyllum Linn". *International Journal of Pharmacognosy and Phytochemical Research.* **7** (4): 718-722.

[7] T. S. Tjahjandarie, W. A. S. Nugroho, H. Palgunadi, R. D. Saputri, and M. Tanjung. (2023). "Two New Chromanone Acids from the Stem Bark of Calophyllum peekelii". *Natural Product Research.* **37** (19): 3214-3219. 10.1080/14786419.2022.2062754.

[8] U. Hasanah, T. S. Tjahjandarie, and M. Tanjung. (2019). "Chromanone Acid Derivatives from the Stem Bark of Calophyllum incrassatum". *IOP Conference Series: Earth and Environmental Science*. **217** : 012010. 10.1088/1755-1315/217/1/012010

[9] N. N. Arif, N. Ahmat, R. D. Saputri, T. S. Tjahjandarie, and M. Tanjung. (2025). "Cytotoxic and Anti-Plasmodial Activities of Chromanone Acids from Calophyllum wallichianum Planch. & Triana". *Advanced Journal of Chemistry A.* **8** (9): 1468-1476. 10.48309/ajca.2025.499062.1764.

[10] R. D. Saputri, Tukiran, Suyatno, F. A. Wati,

S. A. Dzulkarnain, M. Zakiyah, T. S. Tjahjandarie, and M. Tanjung. (2024). "Xanthine Oxidase Inhibitory Activity of Xanthones from Calophyllum pseudomole P. F. Stevens". *Tropical Journal of Natural Product Research.* **8** (1): 5932-5935. 10.26538/tjnpr/v8i1.31.

[11] T. S. Tjahjandarie, M. Tanjung, D. F. Rahmania, C. I. Rhidoma, and R. D. Saputri. (2021). "Calodioscurins A and B, Two New Isoprenylated Xanthones from the Stem Bark of Calophyllum dioscurii P. F. Stevens". *Natural Product Research.* **35** (7): 1153-1158. 10.1080/14786419.2019.1643864.

[12] M. Tanjung, F. Rachmadiarti, A. Prameswari, U. W. Agyani, R. D. Saputri, T. S. Tjahjandarie, and Y. M. Syah. (2018). "Airlanggins A-B, Two New Isoprenylated Benzofuran-3-Ones from the Stem Bark of Calophyllum soulattri". *Natural Product Research.* **32** (13): 1493-1498. 10.1080/14786419.2017.1380027.

[13] M. Tanjung, T. S. Tjahjandarie, R. D. Saputri, B. D. Kurnia, M. F. Rachman, and Y. M. Syah. (2021). "Calotetrapterins A-C, Three New Pyranoxanthones and Their Cytotoxicity from the Stem Bark of Calophyllum tetrapterum Miq". *Natural Product Research.* **35** (3): 407-412. 10.1080/14786419.2019.1634714.

[14] M. Tanjung, T. S. Tjahjandarie, R. D. Saputri, M. F. Aldin, and H. Purnobasuki. (2022). "Two New Pyranoxanthones from the Stem Bark of Calophyllum pseudomole P. F. Stevens". *Natural Product Research.* **36** (3): 822-827. 10.1080/14786419.2020.1808638.

[15] T. Liu, Z. Q. Zhang, X. Xiao, and X. Q. Li. (2022). "Bioassay-Guided Isolation of Antitumor Polyprenylphloroglucinols from Calophyllum polyanthum and Their Primary Mechanism". *Biomedicine & Pharmacotherapy.* **151** : 113129. 10.1016/j.biopha.2022.113129.

[16] S. C. Tsai, Y. H. Liang, J. H. Chiang, F. C. Liu, W. H. Lin, S. J. Chang, W. Y. Lin, C. H. Wu, and J. R. Weng. (2012). "Anti-Inflammatory Effects of Calophyllum inophyllum L. in RAW264.7 Cells". *Oncology Reports.* **28** : 1096-1102. 10.3892/or.2012.1873.

[17] C. T. A. Pires, R. B. L. Scondro, M. A. Brenzan, D. A. G. Cortez, V. L. D. G. Lopes, V. L. Siqueira, L. Cardoso-Filho, R. M. Goncalves, K. R. Caleffi-Ferracioli, and R. F. Cardoso. (2016). "Anti-Mycobacterium tuberculosis Activity of Calophyllum brasiliense Extracts Obtained by Supercritical Fluid Extraction and Conventional Techniques". *Current Pharmaceutical Biotechnology.* **17** (6): 532-539. 10.2174/1389201017666160114094723.

[18] T. Karunakaran, N. S. Firouz, R. Santhanam, and V. Y. M. Jong. (2022). "Phytochemicals from Calophyllum macrocarpum Hook. f. and Its Cytotoxic Activities". *Natural Product Research.* **36** (2): 654-659. 10.1080/14786419.2020.1795658.

[19] C. Spino, M. Dodier, and S. Satheeswaran. (1998). "Anti-HIV Coumarins from Calophyllum Seed Oil". *Bioorganic and Medicinal Chemistry Letters.* **8** (24): 3475-3478. 10.1016/S0960-894X(98)00628-3.

[20] N. H. Zamakshshari, G. C. L. Ee, I. S. Ismail, Z. Ibrahim, and S. H. Mah. (2019). "Cytotoxic Xanthones Isolated from Calophyllum depressinervosum and Calophyllum buxifolium with Antioxidant and Cytotoxic Activities". *Food and Chemical Toxicology.* **133** : 110800. 10.1016/j.fct.2019.110800.

[21] R. D. Saputri, R. Retnowati, U. Supratman, T. S. Tjahjandarie, and M. Tanjung. (2023). "Three Novel Quinolinone Alkaloids from the Leaves of Melicope denhamii". *Natural Product Research.* **37** (2): 197-203. 10.1080/14786419.2021.1960524.

[22] M. Tanjung, T. S. Tjahjandarie, M. F. Aldin, S. Mardhiyyah, R. D. Saputri, Y. M. Syah, and N. Ahmat. (2025). "Two New Flavonols from Macaranga inermis Pax & K. Hoffm". *Natural Product Research.* **39** (3): 498-505. 10.1080/14786419.2023.2272783.

[23] T. S. Tjahjandarie, M. F. Aldin, R. D. Saputri, and M. Tanjung. (2025). "Dihydrostilbenes from Macaranga javanica Müll. Arg. and Their Antiplasmodial Activity". *Natural Product Research.* **39** (19): 5437-5443.

10.1080/14786419.2024.2342555.

[24] E. Marliana, R. Hairani, T. S. Tjahjandarie, and M. Tanjung. (2018). "Antiplasmodial Activity of Flavonoids from Macaranga tanarius Leaves". *IOP Conference Series: Earth and Environmental Science*. **144** : 012011. 10.1088/1755-1315/144/1/012011

[25] M. Tanjung, I. Nurmalasari, A. K. Wilujeng, R. D. Saputri, F. Rachmadiarti, and T. S. Tjahjandarie. (2018). "Acronyculatin P, A New Isoprenylated Acetophenone from the Stem Bark of Acronychia pedunculata". *Natural Product Sciences.* **24** (4): 284-287. 10.20307/nps.2018.24.4.284.

[26] M. Tanjung, M. F. Aldin, N. N. Arif, R. D. Saputri, N. Ahmat, and T. S. Tjahjandarie. (2025). "4-Propyl- and 4-Phenylcoumarins from Mesua lepidota T. Anderson and Their Cytotoxic Activity". *Natural Product Research.* **39** (20): 5874-5882. 10.1080/14786419.2024.2358526.

[27] R. D. Saputri, T. S. Tjahjandarie, and M. Tanjung. (2021). "Two Novel Coumarins and an Acetophenone Derivative from the Leaves of Melicope quercifolia". *Natural Product Research.* **35** (8): 1256-1261. 10.1080/14786419.2019.1644634.

[28] T. S. Tjahjandarie, M. Tanjung, R. D. Saputri, D. O. Rahayu, A. N. I. Gunawan, and M. F. Aldin. (2021). "Two New 2-Arylbenzofurans from Sesbania grandiflora and Their Cytotoxicity toward Cancer Cells". *Natural Product Research.* **35** (24): 5637-5642. 10.1080/14786419.2020.1821016.

[29] M. Tanjung, R. D. Saputri, and T. S. Tjahjandarie. (2014). "Antioxidant Activity of Two Isomeric Benzoxepins from the Stem Bark of Bauhinia aculeata". *Journal of Chemical and Pharmaceutical Research.* **6** (1): 705-708.

[30] N. F. N. Azmin, N. Ahmat, A. S. Kamarozaman, N. S. Sulaiman, J. Jalil, and M. Tanjung. (2025). "Flavonoids of Cynometra cauliflora, Their Plausible Biosynthetic Pathway and SAR Study Against DPPH Radicals". *Malaysian Journal of Chemistry.* **27** (1): 102-109. 10.55373/mjchem.v27i1.102.

[31] T. S. Tjahjandarie, A. R. Setiawan, A. H. Zulfa, R. D. Saputri, N. Ahmat, and M. Tanjung. (2025). "Bioassay-Guided Isolation of Flavonoids from Flemingia macrophylla and Their Cytotoxic and Antioxidant Activities". *Advanced Journal of Chemistry A.* **8** (10): 1631-1639. 10.48309/ajca.2025.510870.1800.

[32] S. Mardhiyyah, M. Zakiyah, E. D. Renata, T. S. Tjahjandarie, U. Supratman, R. Retnowati, R. D. Saputri, and M. Tanjung. (2023). "Cytotoxic Activity of Xanthones from the Stem Bark of Cratoxylum sumatranum". *Tropical Journal of Natural Product Research.* **7** (9): 3908-3910. 10.26538/tjnpr/v7i9.9.

[33] B. T. D. Trinh, T. T. T. Quach, D. N. Bui, D. Staerk, L. D. Nguyen, and A. K. Jäger. (2017). "Xanthones from the Twigs of Garcinia oblongifolia and Their Antidiabetic Activity". *Fitoterapia.* **118** : 126-131. 10.1016/j.fitote.2017.03.003.

[34] Q. Xue, Y. Chen, H. Yin, H. Teng, R. Qin, H. Liu, Q. Li, Z. Mei, and G. Yang. (2020). "Prenylated Xanthones and Benzophenones from the Fruits of Garcinia bracteata and Their Potential Antiproliferative and Anti-Inflammatory Activities". *Bioorganic Chemistry.* **104** : 104339. 10.1016/j.bioorg.2020.104339.

[35] R. A. Oetari, H. Hasriyani, A. Prayitno, and S. Sahidin. (2019). "Gartanin Compounds from Ethanolic Extract of Garcinia mangostana Pericarp". *Open Access Macedonian Journal of Medical Sciences.* **7** (22): 3891-3895. 10.3889/oamjms.2019.527.

[36] F. Kurniadewi, L. M. Fallah, I. R. Kartika, M. Nurjayadi, E. Hermawati, and A. Danova. (2025). "Isolation, Characterization, and Evaluation of Xanthone Derivatives from Garcinia mangostana Twigs as Tyrosine Kinase Inhibitors with Potential EGFR Selectivity". *Advanced Journal of Chemistry A.* **8** (6): 1024-1042. 10.48309/ajca.2025.481129.1699.

[37] A. H. Zulfa, S. Assyifa, A. R. Setiawan, M. Tanjung, T. S. Tjahjandarie, and R. D. Saputri. (2025). "Xanthones from the Roots of Garcinia rigida Miq. and Their Cytotoxic Activity Against HeLa Cervical Cancer Cell

Lines". *Tropical Journal of Natural Product Research.* **9** (4): 1833-1836. 10.26538/tjnpr/v9i4.57.

[38] S. Kaennakam, K. Mudsing, K. Rassamee, P. Siripong, and S. Tip-Pyang. (2019). "Two New Xanthones and Cytotoxicity from the Bark of Garcinia schomburgkiana". *Journal of Natural Medicines.* **73** : 257-261. 10.1007/s11418-018-1240-8.

[39] L. C. Klen-Junior, A. Campos, R. Niero, Y. Van der Heyden, and V. C. Filho. (2020). "Xanthones and Cancer: From Natural Resources to Mechanisms of Action". *Chemistry & Biodiversity.* **17** (2): e1900499. 10.1002/cbdv.201900499.

[40] L. T. T. Nguyen, D. M. Nguyen, and L. H. D. Nguyen. (2013). "A New Xanthone from the Bark of Calophyllum thorelii". *Natural Product Research.* **27** (6): 563-567. 10.1080/14786419.2012.682992.

[41] I. Miladiyah, Jumina, S. M. Haryana, and Mustofa. (2018). "Biological Activity, Quantitative Structure-Activity Relationship Analysis, and Molecular Docking of Xanthone Derivatives as Anticancer Drugs". *Drug Design, Development and Therapy.* **12** : 149-158. 10.2147/DDDT.S149973.

[42] X. Li. (2018). "Comparative Study of 1,1-Diphenyl-2-picryl-hydrazyl Radical (DPPH) Scavenging Capacity of the Antioxidant Xanthone Family". *ChemistrySelect.* **3** (46): 13081-13086. 10.1002/slct.201803362.

[43] Y. Chen, H. Fan, G. Z. Yang, Y. Jiang, F. F. Zhong, and W. He. (2010). "Prenylated Xanthones from the Bark of Garcinia xanthochymus and Their 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activities". *Molecules.* **15** (10): 7438-7449. 10.3390/molecules15107438.