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Original Research Article

Cytotoxic Activity of Xanthones from the Stem Bark of *Cratoxylum sumatranum*

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ABSTRACT

Two xanthones, gerontoxanthone I and macluraxanthone, were isolated from the stem bark of *Cratoxylum sumatranum*. Their structures were determined based on UV, HRESI-MS, 1D, and 2D NMR spectral data. Compounds **1** and **2** showed high activity against T47D cells with an IC_{50} of 1.10 and 1.60 µg/mL.

Keywords: *Cratoxylum sumatranum*, xanthone, gerontoxanthone I, macluraxanthone, T47D cells, cancer

Introduction

Cratoxylum sumatranum (Jack) Blume (Hypericaceae) is a pioneer plant found in the secondary forest of Kalimantan Island, Indonesia. The bark and leaves of the plant have medicinal uses, including stomachache, ulcer, fever, cough, itching, and food poisoning.[1] Xanthones are chemotaxonomic marker compounds in the genus *Cratoxylum*, and they exhibit interesting biological properties such as anticancer, antioxidant, antibiotic, and antimalarial activities.[2-6] In the present study, phytochemical analysis of the stem bark of *C. sumatranum* collected in Central Kalimantan, Indonesia, has led to the isolation of gerontoxanthone I (1) and macluraxanthone (2). The cytotoxic activity of compounds 1 and 2 was assayed using the breast cancer (T47D cells) by MTT assay.

Materials and Methods

General experimental procedures
The UV maxima (λ_{max}) of xanthones 1 and 2 in methanol were determined using a UV-1900 Shimadzu spectrophotometer. The 1D and 2D NMR in acetone-d_6 were measured using a JEOL JNM FTNMR spectrometer. The chemical formula and molecular mass of the xanthones in acetone were measured using LCT Premier™ XE (Waters) mass spectrometer. Column chromatography (CC) used silica gel and Sephadex LH-20. The visualization of xanthone spots on TLC was by a UV lamp and cerium sulphate reagent.

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Plant material
The stem bark of *C. sumatranum* was collected from Sungai Jaya Village, South Barito, Central Kalimantan, Indonesia, in December 2020. Dr. Ismail Rachman of the Herbarium Bogorienses, Bogor, Indonesia, identified the plant material, and a deposited voucher specimen was issued with the number CS-BJCK-IR2.

Extraction and isolation
The xanthones were extracted from the stem bark of *C. sumatranum* (2.2 kg) with methanol 96% at room temperature for a week by maceration. A rotary evaporator was used to concentrate the extract to yield a thick methanol extract (125 g). The methanol extract was partitioned by hexane and ethyl acetate to produce a hexane fraction (38.4 g) and an ethyl acetate fraction (10.0 g). The separation of the EtOAc fraction (9.0 g) by silica gel CC, eluting with hexane-acetone (19:1 to 4:1 v/v) afforded three column fractions A, B, and C. Separation of fraction C (4.0 g) with CC using Sephadex LH-20 with methanol as the mobile phase produced C_1 and C_2 subfractions. The separation of the C_2 subfraction also by Sephadex LH-20 produces C_{21}, C_{22}, and C_{23} subfractions. The purification of subfraction C_{23} (1.0 g) by radial chromatography using a mixture of hexane : acetone (19:1, 17:3, and 3:1 v/v) yielded geronthoxanthone I, **1** (9.0 mg) and macluraxanthone **2**, (60 mg).

Cytotoxic activity
The cytotoxic activity of the xanthones against breast cancer (T47D cells) was carried out using the MTT assay by the colorimetric method. The T47D cells were cultivated in RPMI 1640 medium, 1 mL fetal bovine serum (FBS) containing antibiotic (100 µg/mL streptomycin, and 100 µg/mL penicillin), was added at room temperature for 48 hours in a 5% CO_2 incubator.[7-9] Xanthones 1 and 2 in the concentration of (100, 50, 10, 1, and 0.1 µg/mL) were added to the T47D cells and then incubated for 24 hours at room temperature. The microplate reader spectrophotometer measured the active compounds 1 and 2 capability to kill cells at λ = 590 nm. Doxorubicin was used as the positive control.[11-13]

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Result and Discussion

Two xanthones, gerontoxanthone I (1) and macluraxanthone (2), were isolated from the *C. sumatranum* stem barks. The HR-ESI-MS, UV, 1D, and 2D NMR spectra established the structures of xanthones.

Characterization of fraction C as Gerontoxanthone I

Gerontoxanthone I (1) was obtained in the form of a yellow oil and exhibits a maximum absorption at λ_{max} (log ε): 253 (4.28), 284 (3.81), and 327 (3.95) nm in the UV spectrum, which is characteristic of the chromophore.of xanthones.[12] The molecular ion $[M+H]^+$ of 1 at *m/z* 397.1523 (calculated 397.1525) corresponds to a molecular formula $C_{23}H_{24}O_6$. The ^{1}H-NMR spectrum of 1 showed the presence of phenolic groups, 3-methyl-2-butenyl, and 3-methyl-1-butenyl proton signals were confirmed with HMQC and HMBC spectra. Two aromatic proton doublets with ortho coupled multiplicity at δ_H 7.00 (1H, *J* = 8.7 Hz, H-7) and δ_H 7.61 (1H, *J* = 8.7 Hz, H-8) were characteristic of a 1,2,3,4,5,6-hexasubstituted xanthone derivatives.[14] Two singlet protons of the H-bonded hydroxy group were observed at δ_H 13.85 (1H, *s*, 1-OH) and the other hydroxyl group at δ_H 7.75 (1H, *s*, 3-OH). The 3-methyl-2-butenyl chain was deduced from the olefinic proton at δ_H 5.21 (1H, *t*, *J* = 7.1 Hz, H-2')], one methylene proton at δ_H 3.35 (2H, *d*, *J* = 7.1 Hz, H-1') and two methyl protons at δ_H 1.76 (3H, *s*, H-4'), δ_H 1.63 (3H, *s*, H-5'). The proton signals of the 3-methyl-1-butenyl chain consist of one olefinic proton (δ_H 6.58 (*dd*, *J* = 10.6; 17.8 Hz, H-2")), two separate terminal olefinic protons (δ_H 5.45 (1H, *dd*, *J* = 0.8; 17.8 Hz, H-1a")); δ_H 5.32 (1H, *dd*, *J* = 0.8; 10.6 Hz, H-1b")] and the gem-dimethyl protons at δ_H 1.79 (6H, *s*, H-4"/ H-5")]. The ^{13}C-NMR spectrum of geronthoxanthone I showed 22 carbon signals representing 23 total carbons. The signal carbon consist of one gem-dimethyl carbon [δ_C 28.6 (C-4"/C-5")], two methyl carbons [δ_C 25.9 (C-5'), 17.9 (C-4')], one methylene carbon [δ_C 22.2 (C-1')], four olefinic carbons [δ_C 112.4 (C-3"), δ_C 123.2 (C-2'), δ_C 131.9 (C-3'), δ_C 151.2 (C-2")], two aromatic proton bearing carbons [δ_C 117.1 (C-8), 113.4 (C-7)], six quaternary carbons [114.6 (C-8a), 111.9 (C-2), 111.3 (C-4), 103.6 (C-9a), 42.1 (C-1")], six phenolic carbons [δ_C 161.4 (C-3), 159.9 (C-1), 154.9 (C-4a), 151.6 (C-6), 146.5 (C-10a), 133.7 (C-5)] and one carbonyl [δ_C 181.8 (C-9)]. The placement of the hydroxy groups, 3-methyl-2-butenyl and 3-methyl-1-butenyl chains was determined based on the HMQC and HMBC spectra. The hydroxy proton signal at δ_H 13.85 (1-OH) correlates with the oxy-carbon at δ_C 159.9 (C-1) and two quaternary carbons at δ_C 111.9 (C-2) and δ_C 103.6 (C-9a).[15] The methylene proton at δ_H 3.35 (H-1') correlates with C-1, C-2, methine carbon at δ_C 123.2 (C-2'), and quaternary carbon at δ_C 131.9 (C-3'). The results of this correlation emphasize the 3-methyl-2-butenyl chain bound at C-2. The hydroxy proton signal at δ_H 7.75 (3-OH) correlates with C-2, the oxy-carbon carbon at δ_C 161.4 (C-3), and the quaternary carbon at δ_C 111.3 (C-4) indicates a H-bonded hydroxy group at C-3 and reinforces the 3-methyl-1-butenyl chain attached at C-4. The gem-dimethyl proton signal at δ_H 1.79 (H-4˝/H-5'") correlates with C-4, quaternary carbon δ_C 42.1 (C-1") and olefinic carbon δ_C 151.2 (C-2"). The proton signal at δ_H 6.58 (H-2") shows a correlation with the gem-dimethyl carbon δ_C 28.6 (C-4"/ C-5"). The methylene proton signal (H-3") at δ_H 5.45 and δ_H 5.32 correlates with C-1" and C-2". The aromatic proton signal at δ_H 7.61 (H-8) shows a correlation with carbonyl carbon δ_C 181.8 (C-9), two oxy-carbons at δ_C 151.6 (C-6), δ_C 146.5 (C-10a) and other aromatic proton signals at δ_H 7.00 (H-7) correlated with C-6, oxy-carbons at δ_C 133.7 (C-5) and quaternary carbons at δ_C 114.6 (C-8a). Based on the spectral data above, the compound was identified as gerontoxanthone I.[16]

Macluraxanthone (2) was obtained as a yellow powder, with a molecular ion $[M+H]^+$ at *m/z* 395.2124 (calculated 395.2120) corresponding to a molecular formula $C_{23}H_{23}O_6$. The UV spectrum of 2 showed the maximum absorption with compound 1 (λ_{max} (log ε): 260 (4.27), 275 (4.28), 288 (4.25), and 332 (3.90) nm). The chemical shifts for compound **2** in the ^{1}H-NMR spectrum showed the same pattern as for compound **1**, especially in the aromatic region [δ_H 7.58 (1H, *d* = 8.7 Hz, H-8), 6.99 (1H, *d* = 8.7 Hz, H-7)], the H-bonded hydroxy group [δ_H 13.90 (1H, *s*, 1-OH)], and the chain of 3-methyl-1-butenyl [δ_H 6.49 (1H, *dd*, *J* = 10.6; 17.5 Hz, Hz, H-4''), 5.02 (1H, *dd*, *J* = 0.8; 17.5 Hz, Hz, H-3a''), 4.86 (1H, *dd*, *J* = 0.8; 10.6 Hz, Hz, H-3b''), 1.73 (6H, *s*, H-4''/5'')]. The main difference in the NMR spectrum of compound **2** were the signals from the 2,2-dimethylpyrano ring, which consists of a pair of olefinic [δ_H 6.68 (1H, *d*, *J* = 10.0 Hz, H-4'), 5.69 (1H, *d*, *J* = 10.0 Hz, H-3'),] and gem-dimethyl proton [δ_H 1.46, 6H, *s*, H-5'/6']. Based on the ^{13}C NMR spectrum, compound **2** showed 21 carbons out of 23. The 13-Carbon signals of macluraxanthone (**2**) consisted of one carbonyl [δ_C 181.8 (C-9)], seven oxy-carbons [δ_C 159.5 (C-3), 157.3 (C-1), 155.8 (C-4a), 151.9 (C-6), 146.7 (C-10a), 133.6 (C-5), 79.0 (C-2´)], four olefinic carbons [δ_C 107.3 (C-3"), 116.3 (C-4´), δ_C 128.2 (C-3´), δ_C 152.7 (C-2")], two aromatic carbon carbons [δ_C 107.3 (C-3"), 116.3 (C-4´), δ_C 128.2 (C-3´), δ_C 152.7 (C-2")], five quaternary carbons [41.8 (C-2"), 114.3 (C-8a), 114.2 (C-4), 105.7 (C-2), 103.6 (C-9a)], and two gem-dimethyl carbons [δ_C 28.0 (C-5'/C-6'), δ_C 29.9 (C-5"/C-6")]. The long-range correlations of the hydroxy group at C-1, the 3-methyl-1-butenyl chain at C-4, and the pair of aromatic protons at H-7 and H-8 show the same pattern as gerontoxanthone I (Fig. 1). The olefinic proton at δ_H 5.69 (H-3') correlates with an oxy-carbons at δ_C 79.0 (C-2´), and a gem-dimethyl carbon at δ_C 29.9 (C-5"/C-6"). Another olefinic proton at δ_H 6.68 (H-4') correlated to C-3; C-2´, an oxy-carbon at δ_C 157.3 (C-1), two oxy-carbons [δ_C 157.3 (C-1), δ_C 159.5 (C-3)], and a quaternary carbon at δ_C 105.7 (C-2). The gem-dimethyl at δ_H 1.46 (H-5'/6') correlated to C-2´, and a methine carbon at δ_C 128.2 (C-3´). Based on the HMBC spectrum data, the 2,2-dimethylpyrano ring was connected to the C-2 and C-3 carbons. The explanation of HRESIMS and NMR spectral shows that the structure of the isolated compound is macluraxanthone.[17]

The cytotoxicity of compounds 1-2 against T47D cells showed high activity with an IC_{50} value of 1.1 and 1.6 µg/mL, respectively. Macluraxanhone (**2**) more active than gerontoxantone I (**1**). The cyclization between the hydroxy group at C-3 and the double bond of the 3-methyl-2-butenyl chain of gerontoxanthone I (**1**) produces macluraxanthone (**2**) and increases cytotoxic activity against T47D cells.[7,18]

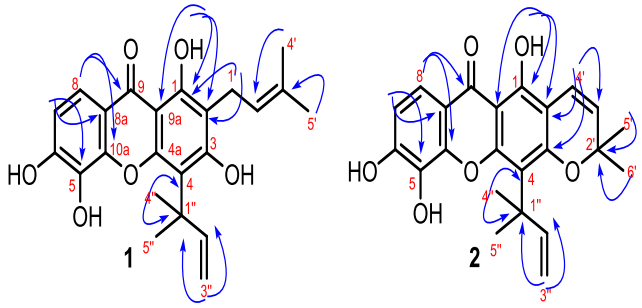

Figure 1: HMBC correlations of **1-2**

Conclusion

Two xanthone derivatives, gerontoxanthone I (1) and macluraxanthone (2), were isolated from *C. sumatranum* stem barks. Compounds **1** and **2** show high activity against T47D cells.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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