A New Thiazinedione Glycoside From the Fruits of Xanthium strumarium L.

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Abstract
A total of 4 thiazinedione derivatives including 1 new thiazinedione glycoside (1) and 3 known compounds (2-4) were isolated from the fruits of Xanthium strumarium L. Their chemical structures were determined as 7-hydroxymethyl-8,8-dimethyl-4,8-dihydrobenzo[1,4]-thiazine-3,5-dione-11-O-[β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranoside] (1), 7-hydroxymethyl-8,8-dimethyl-4,8-dihydrobenzo[1,4]-thiazine-3,5-dione-11-O-[β-D-apiofuranosyl-(1→6)-O-β-D-glucopyranoside] (2), xanthiaide (3), and xanthiazone (4) by extensive nuclear magnetic resonance spectroscopic and high-resolution electron spray ionization mass spectrometry analysis and by comparison of the spectral data with those reported in the literature. Compounds 3 and 4 exhibited cytotoxic activity against lung carcinoma (SK-LU-1), human breast carcinoma (MCF-7), hepatocellular carcinoma (HepG2), and skin melanoma (SK-Mel-2) cell lines with half-maximal inhibitory concentration (IC₅₀) values ranging from 27.0 ± 1.1 to 43.2 ± 1.8 µM.

Keywords
Asteraceae, Xanthium strumarium, thiazinedione, xanthiazone A, cytotoxic activity

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Introduction
Xanthium strumarium L. (Asteraceae) is a common and well-known traditional herbal medicine in China and Vietnam.¹,² The fruits of X strumarium, named as “Cang-Er-Zi” or “Chang-Er-Zi” in China and “Ke Da dau Qua” in Vietnam, were traditionally used to treat many diseases such as arthritis,² urticaria,³ headache,⁴ rheumatism, nasal sinusitis, gastric ulcer, and bacterial and fungal infections.¹,² The main chemical constituents of X strumarium are sesquiterpenoids, coumarins, thiazides, phenylpropenoids, lignanoids, steroids, glycosides, flavonoids, anthraquinones, and naphthoquinones.¹,²,⁶-¹² Some of these compounds exhibited medicinal properties such as diuretic, antihelminthic, antifungal, anti-inflammatory, anti-diabetic, anticancer, and other activities.²,⁶-⁹,¹¹ Continuing research on the bioactive compounds from the fruits of X strumarium¹² has led to the isolation and structural elucidation of 1 new and 3 known thiazinedione compounds, and their cytotoxic activity on some cancer cell lines; these results are reported in this paper.

Results and Discussion
Compound 1 was obtained as a white amorphous powder. The molecular formula of 1 was determined to be C₂₃H₃₃NO₁₃S based on a quasimolecular ion peak at m/z 562.15880 [M–H]⁻ (Calcd. for C₂₃H₃₂NO₁₃S, 562.15998, Δ = –2.1 ppm) in the high-resolution electron spray ionization mass spectrometry (HR-ESI-MS) (Supplemental Figure S1). The nuclear magnetic resonance (¹H NMR), ¹³C NMR, and heteronuclear single quantum coherence/correlation (HSQC) spectra of 1 (Supplemental Figures S2 to S6) showed the presence of 2 methyl singlets [δH 1.50 (6H, s)/δC 27.6 and 27.4], 1 olefinic proton [δH 6.69 (s)/δC 123.2], an oxygenated methylene group [δH 4.78 and 4.53 (each, 1H, dd, J = 16.0, 1.5 Hz), 1 methylene singlet at δH 3.51 (2H)/δC 29.8], 2 carbonyl groups at δC 177.1 and 164.6, and 4 quaternary carbons at 43.5, 130.9, 143.5, and 167.4. In addition, 2 glucopyranosyl sugars were identified by anomeric signals at δH 4.40 (1H, d, J = 7.5 Hz)/δC 104.0 and δH 4.40 (1H, d, J = 7.5 Hz)/δC 105.1, and 2 oxygenated methylene groups at δH 4.18 (1H, dd,

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was established as 7-hydroxymethyl-8,8-dimethyl-4,8-dihydrobenzo[1,4]-thiazine-3,5-dione-11-0-[β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranoside], a new compound named xanthistrumoside A (Figure 2).

The known compounds were identified as 7-hydroxymethyl-8,8-dimethyl-4,8-dihydrobenzo[1,4]-thiazine-3,5-dione-11-O-[β-D-apiofuranosyl-(1→6)-O-β-D-glucopyranoside] (2), xanthistrumide (3), and xanthiazione (4). The NMR spectral data of compounds 2 to 4 were consistent with those previously reported in the literature (Supplemental Figures S11 to S19).

Table 1. 1H NMR and 13C NMR Spectroscopic Data for Compounds 1 and 3 in Deuterated Methanol.

| Pos. | 1      | 2      | 3      |
|------|--------|--------|--------|
|      | δC     | δH (mult., J in Hz) | δC     | δH (mult., J in Hz) |
| 2    | 29.8   | 3.51 (s) | 29.8   | 3.51 (s) |
| 3    | 164.6  | –       | 164.6  | –       |
| 4a   | 130.9  | –       | 130.1  | –       |
| 5    | 177.1  | –       | 177.1  | –       |
| 6    | 123.2  | 6.69 (dd, 1.5, 1.5) | 123.2  | 6.69 (dd, 1.5, 1.5) |
| 7    | 167.4  | –       | 167.3  | –       |
| 8    | 43.5   | –       | 43.4   | –       |
| 8a   | 143.5  | –       | 143.4  | –       |
| 9    | 27.6   | 1.50 (s) | 27.5   | 1.50 (s) |
| 10   | 27.4   | 1.50 (s) | 27.2   | 1.48 (s) |
| 11   | 68.1   | 4.78 (dd, 16.0, 1.5) | 67.7   | 4.80 (dd, 16.0, 1.5) |
|      | 4.53 (dd, 16.0, 1.5) | 4.55 (dd, 16.0, 1.5) |
| GlcI |        |        |        |        |
| 1'   | 104.0  | 4.40 (d, 7.5) | 103.8  | 4.39 (d, 7.5) |
| 2'   | 75.2   | 3.23 (dd, 9.0, 7.5) | 75.1   | 3.24 (dd, 9.0, 7.5) |
| 3'   | 78.0   | 3.33*   | 78.2   | 3.33*   |
| 4'   | 71.7   | 3.31*   | 71.7   | 3.30*   |
| 5'   | 77.3   | 3.53 (m) | 78.1   | 3.38 (dd, 9.0, 6.0, 1.5) |
| 6'   | 70.1   | 4.18 (dd, 12.0, 2.0) | 62.9   | 3.92 (dd, 12.0, 1.5) |
|      | 3.80 (dd, 12.0, 6.5) | 3.69 (dd, 12.0, 6.0) |
| GlcII|        |        |        |        |
| 1''  | 105.1  | 4.40 (d, 7.5) |        |        |
| 2''  | 75.1   | 3.21 (dd, 9.0, 7.5) |        |        |
| 3''  | 78.1   | 3.30*   |        |        |
| 4''  | 71.6   | 3.31*   |        |        |
| 5''  | 78.0   | 3.40 (m) |        |        |
| 6''  | 62.8   | 3.88 (dd, 11.5, 2.0) | 3.67 (dd, 11.5, 2.0) |

Abbreviation: NMR, nuclear magnetic resonance.

* indicates overlapped signals.

Figure 1. Important heteronuclear multiple bond correlation (HMBC) of compound 1.

Figure 2. Chemical structures of compounds 1 to 4.
against SK-LU-1, MCF-7, HepG2, and SK-Mel-2 cell lines with half-maximal inhibitory concentration (IC50) values of 35.2 ± 2.1, 41.2 ± 1.3, 37.6 ± 1.3, and 41.5 ± 2.3 μM (for compound 3), and 28.1 ± 2.3, 35.9 ± 1.6, 43.2 ± 1.8, and 27.0 ± 1.1 μM (for compound 4), respectively, compared to the IC50 values of the positive control compound, ellipticine: 1.41 ± 0.19 µM, 1.54 ± 0.12, 1.65 ± 0.12, and 1.71 ± 0.19 µM, respectively (Supplemental Table S2). These results suggested that compounds 3 and 4 exhibited weak cytotoxic activity, while compounds 1 and 2 were inactive on the tested cell lines.

**Material and Methods**

**General Experimental Procedures**

The NMR spectra were obtained on a Bruker Avance III 500 MHz spectrometer, and HR-ESI-MS on an Agilent 6530 Accurate Mass Q-TOF system. Optical rotation was recorded on a Jasco P-2000 polarimeter. Column chromatography was performed using either silica gel or reversed-phase C-18 resins as adsorbents. Thin-layer chromatography was carried out on precoated silica gel 60 F254 and/or RP-18 F254S plates. The optical rotation of the glucose of compound 1 was [α]D25: -44.3 (c 0.1, H2O). By comparing this value with that of D-glucose: [α]D25: -45.5 (c 0.1, H2O), the glucose in compound 1 was determined to have a D-configuration.

**Extraction and Isolation**

The dried fruits of *X strumarium* L. were collected in Yen My District, Hung Yen Province, Vietnam in December 2019 and identified by Dr Nguyen The Cuong, Institute of Ecology and Biological Resources. A voucher specimen (NCCT-P91) was deposited at the Institute of Marine Biochemistry, VAST.

**Acid Hydrolysis of Compound 1.** Compound 1 (5.0 mg) was dissolved in 5.0 mL of 6 N HCl and heated at 60°C for 1.5 h. After cooling, the mixture was extracted with EtOAc. The aqueous layer was concentrated in vacuo followed by TLC examination and optical rotation measurement. The optical rotation of the glucose of 1 was [α]D25: +44.3 (c 0.1, H2O). By comparing this value with that of D-glucose: [α]D25: +45.5 (c 0.1, H2O), the glucose in compound 1 was determined to have a D-configuration.

**Cytotoxic Assay.** Refer to Supplemental material.

**Conclusions**

A total of 1 new thiazinedione glycoside (1) and 3 known thiazinedione compounds (2-4) were isolated from the seeds of *X strumarium*. Their chemical structures were determined by extensive analysis of HR-ESI-MS and NMR spectra and by comparison of these data with those reported in the literature. Compounds 3 and 4 exhibited cytotoxic activity against SK-LU-1, MCF-7, HepG2, and SK-Mel-2 cell lines with IC50 values of 35.2 ± 2.1, 41.2 ± 1.3, 37.6 ± 1.3, and 41.5 ± 2.3 μM (for compound 3), and 28.1 ± 2.3, 35.9 ± 1.6, 43.2 ± 1.8, and 27.0 ± 1.1 μM (for compound 4).

**Declaration of Conflicting Interests**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

Trial Registration

Not applicable, because this article does not contain any clinical trials.

References

1. Chi VV. *Dictionary of Vietnamese Medicinal Plants*. Medicine Publishing House; 2012:1185-1186.
2. Fan W, Fan L, Peng C, et al. Traditional uses, botany, phytochemistry, pharmacology, pharmacokinetics and toxicology of *Xanthium strumarium* L.: A review. *Molecules*. 2019;24:359. doi:10.3390/molecules24020359
3. Qin L, Han T, Li H, Zhang Q, Zheng H. A new thiazinedione from *Xanthium strumarium*. *Fitoterapia*. 2006;77(3):245-246. doi:10.1016/j.fitote.2006.02.001
4. Yin RH, Bai X, Feng T, et al. Two new compounds from *Xanthium strumarium*. *J. Asian Nat Prod Res*. 2016;18(4):354-359. doi:10.1080/10286020.2015.1099525
5. Han T, Li H, Zhang Q, et al. New thiazinediones and other components from *Xanthium strumarium*. *Chem Nat Compd*. 2006; 42(5):567-570. doi:10.1007/s10600-006-0215-2
6. Tong C, Chem RH, Liu DC, Zeng DS, Liu H. Chemical constituents from the fruits of *Xanthium strumarium* and their antitumor effects. *Nat Prod Commun*. 2020;15(8):1-5. doi:10.1177/1934578X2046541
7. Wen YL, Li MJ, Ye ZJ, et al. Compounds isolated from the fruits of *Xanthium strumarium*, including a new neo-lignan, and their anticancer effects. *Nat Prod Commun*. 2020;15(12):1-4. doi:10.1177/1934578X20982782
8. Ferrer JP, Zampini IC, Cuello AS, et al. Cytotoxic compounds from aerial organs of *Xanthium strumarium*. *Nat Prod Commun*. 2016;11(3):371-374. doi:10.1177/1934578X1601100313
9. Kim YS, Kim JS, Park SH, et al. Two cytotoxic sesquiterpene lactones from the leaves of *Xanthium strumarium* and their in vitro inhibitory activity on farnesyltransferase. *Planta Med*. 2003;69(4):375-377. doi:10.1055/s-2003-38879
10. Mangel SM, Naresh KS, Kuldip SD. Xanthanolides from *Xanthium strumarium*. *Phytochemistry*. 1992;32(1):206-207. doi:org/10.1016/S0031-9422(00)90678-2
11. Lin B, Zhao Y, Han P, et al. Anti-arthritis activity of *Xanthium strumarium* L. extract on complete Freund’s adjuvant induced arthritis in rats. *J. Ethnopharmacol*. 2014;155(1):248-255. doi:10.1016/j.jep.2014.05.0238
12. Kiem PV, Hoang NH, Thu VK, Tai BH, Nhiem NX. Diterpene glycosides and phenolic compounds from the fruits of *Xanthium strumarium*. *Vietnam J Chem*. 2020;58(5):649-654. doi:10.1002/vjch.202000061
13. Dai YH, Cui Z, Li JL, Wang D. A new thiaziedione from the fruits of *Xanthium sibiricum*. *J. Asian Nat Prod Res*. 2008;10(4):303-305. doi:org/10.1080/10286020701833495
14. Laurence VN, Reneta G, Nicolas B, et al. Triterpenoid saponins from the roots of *Gypsophila trichonoma* Wender. *Phytochemistry*. 2013;90:114-127. doi:10.1016/j.phytochem.2013.03.001
15. Jiang H, Yang L, Liu C, et al. Four new glycosides from the fruits of *Xanthium sibiricum* patr. *Molecules*. 2013;18(10):12464-12473. doi:10.3390/molecules181012464