Trop J Nat Prod Res, January 2024; 8(1):5932-5935 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Xanthine Oxidase Inhibitory Activity of Xanthones from *Calophyllum pseudomole* P. F. Stevens

Ratih D. Saputri[1]*, Tukiran[1], Suyatno[1], First A.Wati[1], Shod A. Dzulkarnain[1], Mufidatuz Zakiyah[2], Tjitjik S. Tjahjandarie[2], Mulyadi Tanjung[2]

[1]*Organic Chemistry, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Surabaya 60231, Indonesia*
[2]*Natural Products Chemistry Research Group, Organic Chemistry Division, Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia*

ARTICLE INFO

Article history:
Received 15 December 2023
Revised 17 January 2024
Accepted 24 January 2024
Published online 01 February 2024

ABSTRACT

The genus *Calophyllum* (Calophyllaceae) is a plant proven to produce various xanthones, chromanoic acids, and 4-phenyl coumarins. This study aims to determine the xanthine oxidase inhibitory activity of xanthones from *Calophylum pseudomole* P.F. Stevens. Two xanthones, ananixanthone (**1**) and caloxanthone L (**2**), were isolated from *Calophyllum pseudomole* stem barks. The structures of both xanthones were determined based on UV, HRESI-MS, 1D, and 2D NMR spectral data. Compounds 1-2 showed xanthine oxidase inhibitory activity with an IC_{50} of 15.05 and 17.7 µM, respectively, and were categorized as potent.

Keywords: *Calophyllum pseudomole*, xanthone, ananixanthone, caloxanthone L, *xanthine oxidase*.

Introduction

Gout is one of the most degenerative diseases in the world. Symptoms of this disease in sufferers are characterized by arthritis due to a buildup of uric acid, which is caused by consuming foods that contain purines derived from protein.[1-3] The formation of uric acid is the final product of DNA and RNA metabolism, which contains adenosine and guanosine, which play a vital role in producing hypoxanthine. Xanthine oxidase (XO) is an enzyme that oxidizes hypoxanthine into xanthine and is then oxidized to produce uric acid. Inhibition of XO in uric acid formation is an alternative for sufferers.[4] Allopurinol is a drug commonly consumed by sufferers in Indonesia. The ethanol extract of *C. inophyllum* shows as an XO inhibitory *in vivo* against albino mice, and 1,3,6,7-tetrahydroxyxanthone showed more activity than allopurinol.[5-6] The *Calophyllum* genus belongs to the Calophyllaceae family, and this plant is commonly found in forest areas in Southern and Southeastern Asia. This genus has been shown to produce benzofurans, chromanone acids, 4-phenyl coumarins, and xanthones containing terpenyl chains in the aromatic rings. The secondary metabolites of *Calophyllum* were reported to possess anti-corona virus, anticancer, antimalarial, and antioxidant.[7-16] *Calophyllum pseudomole* is one of the endemic plants of the Borneo Islands and is locally known as Bintangor. This research aims to explore the potential of xanthones from *C. pseudomole* as xanthine oxidase inhibitory activity has not been reported. Two known xanthones, ananisanthone (1) and caloxanthone L (2), were obtained from *Calophyllum pseudomole* stem barks. The XO inhibitory of xanthones 1-2 was reported.

*Corresponding author. E mail: ratihsaputri@unesa.ac.id
Tel: +6231-8298761

Citation: Saputri RD, Tukiran, Suyatno, Wati FA, Dzulkarnain SA, Zakiyah M, Tjahjandarie TS, Tanjung M. Xanthine Oxidase Inhibitory Activity of Xanthones from *Calophyllum pseudomole* P. F. Stevens.Trop J Nat Prod Res. 2024; 8(1):5932-5935. http://www.doi.org/10.26538/tjnpr/v8i1.31

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Materials and Methods

General experimental procedures

Column chromatography gravity used silica gel G_{60} and Sephadex LH-20 as the stationary phases, and silica gel 60 PF254 (Merck) was used for radial chromatography. Determining the structure of xanthones using a Shimadzu 1800 UV-Vis spectrophotometer, high-resolution ESIMS using Merck Waters LCT, 1D, and 2D NMR using NMR machine (JEOL ECA_JNM operating 400 MHz). Visualization of xanthones using Kiesegel 60 GF254 0.25 mm TLC plates (Merck) and cerium sulfate reagent. The XO inhibitory of compounds 1-2 using the Owen and Duong method with a UV spectrophotometer.

Plant material

The plant material (stem bark) of C. pseudomole bark originates from Panaen Village, Muara Teweh, Central Kalimantan (GPS coordinates: 0.9349^0 S, 114.8985^0 E, altitude: 400 m), in Dec 2022. The voucher specimens (No. 20221312) were deposited in the Herbarium Bogoriense, National Research and Innovation Agency, Bogor, Indonesia. The specimen was identified by Dr Ismail Rachman, botanist National Research and Innovation Agency.

Extraction and Isolation

The dried powder of *C. pseudomole* stem barks (1.9 kg) was extracted twice with 5 L of 90% methanol by the maceration method for 24 h. Evaporation of the solvent using a low-pressure evaporator produces a thick methanol extract.[17-19] The methanol extract was successively partitioned with *n*-hexane and ethyl acetate, and after evaporation, it produced *n*-hexane extract (30 g) and ethyl acetate extract (20 g). The ethyl acetate extract (19 g) was separated on silica gel G_{60} CC using solvents of *n*-hexane-ethyl acetate (with a gradient of 9:1 to 7:3 v/v) and collected in 100 mL to yield two fractions A-B. The separation of fraction A (900 mg) with radial chromatography (chromatotron, plate thickness 2 mm) using a mixture of n-hexane: chloroform (7:3, 1:1, 3:7 v/v) as mobile phase and collected in 20 mL to obtain two xanthones; 1 (87 mg) and 2 (13 mg).

Xanthine oxidase inhibitory assay

The XO inhibitory assay of xanthones 1-2 was evaluated by UV-vis spectrophotometry using the Owen and Duong method.[1-2] The parameters for determining XO inhibitory of compounds 1-2 consist of test solution, blank (negative control), xanthine oxidation by XO to uric acid without the test solution, and allopurinol (positive control). The XO inhibitory assay of compounds 1-2 was carried out at 1, 5, 10, 30, and 100 µg/mL. 1 mL of xanthone solution was added to 2.9 mL of 50 mM phosphate buffer solution (pH 7.5), 2 mL of substrate solution (0.20 mM xanthine in phosphate buffer pH 7.5) was incubated at 25 ºC for 15 minutes, and then the addition of 0.1 mL XO (0.3 U/mL in phosphate buffer, pH 7.5). The test solution was incubated at 25 ºC for 30 minutes, and 1 mL HCl 1N was added. The absorbance was measured by UV-Vis spectrophotometry. The maximum absorption of uric acid from xanthine oxidation by XO was measured at λ_{max} 286 nm.

Result and Discussion

Ananixanthone (1) was obtained as a yellow solid with melting point 159-160 ºC and showed a positive quasi-molecular ion $[M+H]^+$ at *m/z* 379.1550 (calculated mass 379.1545), corresponding to the molecular formula $C_{23}H_{23}O_5^+$ based on HRESIMS spectrum. The UV absorption of ananixanthone (1) in methanol shows maximum absorption (λ_{max} (log ε) nm: 253 (4.53); 270 (4.47); 332 (4.08); and 378 (3.47), which is characteristic of the chromophore of xanthone.[8-9] The ^{1}H-NMR of 1, showing three protons of the benzene 1,2,3-trisubstituted at δ_H 7.64 (1H, *dd*, *J* = 7.9; 1.3 Hz, H-8), δ_H 7.34 (1H, *dd*, *J* = 7.9 and 1.3 Hz, H-6), and δ_H 7.24 (1H, *t*, *J* = 7.9 Hz, H-7). Compound 1 also showed a chelate proton of the hydroxy group at δ_H 13.38 (1-OH), four protons of a 3-methylbut-2-en-1-yl chain consists of two methyl protons [δ_H 1.82 (3H, *s*, H-5'), δ_H 1.63 (3H, *s*, H-4'), one methylene proton [δ_H 3.30 (2H, *d*, *J* = 7.2 Hz, H-1'), a vinylic proton [δ_H 5.21 (1H, *t*, *J* = 7.4 Hz, H-2'), and three protons of a 2,2-dimethylpyrano ring consists of a pair of ortho coupling (*J* = 10.0 Hz) of *cis* vinylic protons [δ_H 7.01 (1H, *d*, *J* = 10.0 Hz, H-4"), δ_H 5.73 (1H, *d*, *J* = 10.0 Hz, H-3"), and a *gem*-dimethyl proton at δ_H 1.47 (6H, *s*, H-5"/6") were confirmed by 2D NMR. The ^{13}C-NMR spectrum of ananixanthone (1) shows 22 separate carbon signals representing 23 carbon signals. HMBC correlations (Figure 1), the hydroxy proton at δ_H 13.38 (1-OH) shows a correlation with an oxyaryl carbon at δ_C 160.8 (C-1), and two quaternary carbons [δ_C 112.0 (C-2), δ_C 103.6 (C-9a)]. The methylene proton of a part of 3-methylbut-2-en-1-yl chain *at* δ_H 3.30 (H-1') correlated to C-1, C-2, an oxyaryl carbon at δ_C 159.2 (C-3), and a double bond [δ_C 122.9 (C-2'), δ_C 131.6 (C-3'), confirming that the 3-methylbut-2-en-1-yl chain is bound at C-2. A proton of *cis* vinylic at δ_H 7.01 (H-4") correlated to C-3, a quaternary carbon at δ_C 101.8 (C-4), an oxyaryl carbon at δ_C 150.6 (C-4a), and an oxy-carbon at δ_C 79.0 (C-2") showing that the 2,2-dimethylpyrano ring is connected at C-3 and C-4. The positioning of the 2,2-dimethylpyrano ring at C-3 and C-4 is supported by a proton of the other *cis* vinylic at δ_H 5.73 (H-3") correlated to C-4, C-2", and a *gem*-dimethyl proton at δ_H 1.47 (H-5"/6") correlated to C-2", and a methine carbon at at δ_C 127.9 (C-3"). An aromatic proton at δ_H 7.64 (H-8) correlates with the carbonyl carbon at δ_C 181.9 (C-9), an oxyaryl carbon at δ_C 146.1 (C-10a), and a methine carbon at δ_C 121.6 (C-6), confirming that the position of a hydroxy group attached at C-5. The aromatic proton at δ_H 7.24 (H-7) correlates with the oxyaryl carbon at δ_C 147.1 (C-5) and a methine carbon at δ_C 116.1 (C-8). An aromatic proton at δ_H 7.34 (H-6) correlated to C-5 and a quaternary carbon at δ_C 122.1 (C-8a). Compound 1 was elucidated as 6,11-dihydroxy-3,3-dimethyl-5-(3-methylbut-2-en-1-yl)-3H,7H-pyrano[2,3-c]xanthen-7-one and named as ananixanthone.[8]

Caloxanthone L (2) was obtained as a yellow solid, and the UV spectrum showed the UV absorption [λ_{max} (log ε) nm: 243 (4.17); 262 (4.15); and 326 (3.63)], typical of the xanthone chromophore. The ^{1}H-NMR spectrum of caloxanthone L (2), showing the same pattern with compound 1, including the proton signal of a benzena 1,2,3-trisubstituted benzene [δ_H 7.23 (1H, *t*, *J* = 8.0 Hz, H-7), δ_H 7.35 (1H, *dd*, *J* = 8.0; 1.6 Hz, H-6), δ_H 7.68 (1H, *dd*, *J* = 8.0; 1.6 Hz, H-8)], a chelate proton of the hydroxy group at δ_H 13.49 (1-OH), and four protons of 3-methylbut-2-en-1-yl chain [δ_H 5.27 (1H, *t*, *J* = 7.4 Hz, H-2'), δ_H 3.28 (2H, *d*, *J* = 7.3 Hz, H-1'), δ_H 1.76 (3H, *s*, H-4'), δ_H 1.65 (3H, *s*, H-5')]. The main difference compound 2 shows the presence of four protons of the 2,3,3-trimethylfurano ring consisting of one oxy-methine proton at δ_H 4.60 (1H, *q*, *J* = 6.6 Hz, H-1"), three methyl proton protons at δ_H 1.63 (3H, *s*, H-3"), δ_H 1.42 (3H, *d*, *J* = 6.6 Hz, H-5"), and δ_H 1.34 (3H, *s*, H-4"). The ^{13}C-NMR spectrum of compound 2 shows 23 completely separated carbon signals (Table 1). HMBC correlation of 1,2,3-trisubstituted benzene protons [δ_H 7.23 (H-7), δ_H 7.35 (H-6), δ_H 7.68 (H-8)]. The proton of the hydroxy group at δ_H 13.49 (1-OH), and the proton signal of the 3-methylbut-2-en-1-yl chain [δ_H 5.27 (H-2'), δ_H 3.28 (H-1'), δ_H 1 .76 (H-4'), δ_H 1.65 (H-5')] shows two-bond and three-bond correlations identic with compound 1. Two methyl protons at δ_H 1.34 (H-4") and δ_H 1.63 (H-3"), respectively correlated with two quaternary carbons [δ_C 113.3 (C-4), δ_C 44.9 (C-2")], an oxy-methine carbon at δ_C 91.6 (C-1′′). Another methyl proton at δ_H 1.42 (H-6") correlated to C-1" and C-2", and an oxy-methine proton δ_H 4.60 (H-1") correlates with two methyl carbons at δ_C 25.9 (C-3") and δ_C 21.4 (C-4"), supporting that a 2,3,3-trimethylfurano ring attached at C-3 and C-4. Compound 2 was determined as 5,10-dihydroxy-1,1,2-trimethyl-4-(3-methylbut-2-en-1-yl)-1,2-dihydro-6H-furo[2,3-c]xanthen-6-one and named as caloxanthone L.[16]

The Owen and Duong method evaluated the xanthine oxidase inhibitory of xanthones **1-2**.[1-2] The positive and negative controls for assay use allopurinol and blank. Ananixanthone (1) and caloxanthone L (2) showed xanthine oxidase inhibitory with an IC_{50} of 15.05 and 17.7 µM, respectively, and were categorized as potent activity.[1-2] Ananixanthone (1) showed slightly more activity than caloxanthone L (2). The presence of the 2,2-dimethylpyrano ring in compound 1 can increase the xanthine oxidase inhibition inhibitory compared to the 2,3,3-trimethylfurano ring in compound 2.

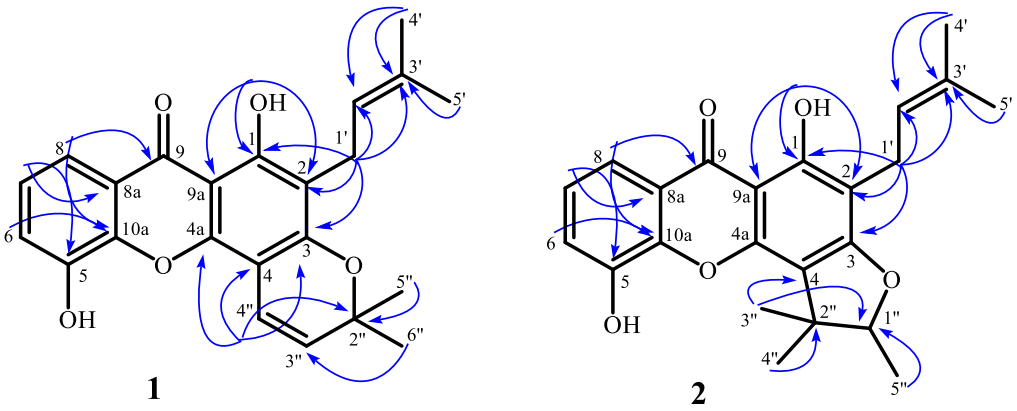

Figure 1: HMBC correlations of 1-2

Table 1: ^{1}H and ^{13}C NMR spectra of xanthones 1-2

	Ananixanthone (1)		**Caloxanthone L (2)**	
No.C	δ_H **(mult, *J* in Hz)**	δ_C	δ_H **(mult, in *J* Hz)**	δ_C
1	-	160.8	-	161.8
2	-	112.0	-	107.2
3	-	159.2	-	166.0
4	-	101.8	-	113.3
4a	-	150.6	-	151.7
5	-	147.1	-	147.1
6	7.34 (*dd*, 7.9; 1.2)	121.6	7.35 (*dd*, 1.6; 8.0)	121.1
7	7.24 (*t*, 8.0)	124.8	7.23 (*t*, 8.0)	124.5
8	7.64 (*dd*, 8.0; 1.3)	116.1	7.68 (*dd*, 1.6; 8.0)	116.4
8a	-	122.1	-	122.4
9	-	181.9	-	181.2
9a	-	103.6	-	104.1
10a	-	146.1	-	146.1
1'	3.30 (*d*, 7.2)	21.6	3.28 (*d*, 7.3)	22.3
2'	5.21 (*t*, 7.4)	122.9	5.27 (*t*, 7.4)	122.6
3'	-	131.6	-	132.1
4'	1.63 (*s*)	25.8	1.76 (*s*)	17.8
5'	1.78 (*s*)	18.0	1.65 (*s*)	25.8
1''	-	-	4.60 (*q*, 6.6)	91.6
2''	-	79.0	-	44.9
3''	5.73 (*d*, 10.0)	127.9	1.63 (*s*)	25.9
4''	7.01 (*d*, 10.0)	116.0	1.34 (*s*)	21.4
5''	1.47 (*s*)	28.3	1.42 (*d*, 6.6)	14.7
6''	1.47 (*s*)	28.3	-	-
1-OH	13.38 (*s*)	-	13.49 (*s*)	-

Conclusion

Two known xanthones, ananixanthone (1) and caloxanthone L (2), were isolated from *Calophyllum pseudomole* stem barks. Compounds 1-2 showed high activity to xanthine oxidase inhibitory and had the prospect of being assayed in vivo.

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.

Acknowledgments

The research was funded through Program Penelitian Kebijakan Fakultas Matematika Dan Ilmu Pengetahuan Alam Dana Non-APBN, Universitas Negeri Surabaya, 2023, No. 1117/UN38/HK/PP/2023.

References

1. Falodun A, Qadir MI, Choudary, MI, Isolation and characterization of xanthine oxidase inhibitory constituents of *Pyrenacantha staudtii*. 2009; Acta Pharm Sin, 44(4), 390-394.
2. Juanda D, Fidrianny I, Wirasutisna KR, Insane M. Evaluation of xanthine oxidase inhibitory and antioxidant activities of three organs of idat (*Cratoxylum glaucoma* korth.) and correlation with phytochemical content. 2021; Pharmacogn.J, 13(4), 971–976.
3. Kostić DA, Dimitrijević DS, Stojanović GS, Palić IR, Dordević AS, Ickovski JD. 2015. *Xanthine oxidase*: isolation, assays of activity, and inhibition. 2015; J. Chem. 1-8
4. Pacher P, Nivorozhkin A, Szabo A. Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. Pharmacol. Rev., 2008; 58(1), 87-114.
5. Hu L, Hu H, Wu W, Chai X, Luo J, Wu Q. Discovery of novel xanthone derivatives as xanthine oxidase inhibitors. Bioorg. Med. Chem. Lett. 2011; 21(13), 4013–4015.
6. Shaffique S, Ahmad S, Rehman T, Anwar H, Hussain G. Antihyperuricemic activity of gum of *Calophyllum inophyllum* ultra high dilutions in potassium oxonate induced wister albino rats. Indian J. Trad. Knowl. 2019; 18(2), 356-359
7. Tanjung, M., Saputri, R. D., Tjahjandarie, T. S. 5,9,11-Trihydroxy-2,2- dimethyl-10-(3′-methyl-2′-butenyl)-3-(2″-methyl-3″-butenyl)pyrano[2,3- a]xanthen-12(2H)-one from

the stem bark of *Calophyllum pseudomole*. MolBank, 2016; 2016(3), 1-5.
8. Tanjung M, Rachmadiarti F, Prameswari A, Agyani VUW, Saputri RD, Tjahjandarie TS, Syah YM. Airlanggins A-B, two new isoprenylated benzofuran-3-ones from the stem bark of *Calophyllum soulattri*. Nat Prod Res. 2018; 32(13),1493-1498.
9. Tanjung M, Tjahjandarie TS, Saputri RD, Kurnia BD, Rachman MF, Syah YM. Calotetrapterins A-C, three new pyranoxanthones and their cytotoxicity from the stem bark of *Calophyllum tetrapterum* Miq. Nat Prod Res. 2021; 35(3),1256-1261.
10. Tanjung M, Tjahjandarie TS, Saputri RD, Aldin MF, Purnobasuki H. Two new pyranoxanthones from the stem bark of *Calophyllum pseudomole* P.F. Stevens. Nat Prod Res. 2022; 36(3), 822-827.
11. Tjahjandarie TS, Tanjung M, Rachmania DF, Rhidoma CI, Saputri RD. Calodioscurins A and B, two new isoprenylated xanthones from the stem bark of *Calophyllum dioscurii* P.F. Steven. Nat. Prod. Res, 2021; 35(7), 1153-1158.
12. Tjahjandarie TS, Nugroho WAS, Palgunadi H, Saputri RD, Tanjung M. Two new chromanone acids from the stem bark of *Calophyllum peekelii* (Lauterb). Nat. Prod. Res. 2023; 37(19), 3214-3219.
13. Yimdjo MC, Azebaze AG, Nkengfack AE, Meyer AM, Bodo B, Fomum ZT. Antimicrobial and cytotoxic agents from *Calophyllum inophyllum*. Phytochem. 2004; 65(20), 2789–2795.
14. Lim CK, Subramaniam H, Say YH, Jong VYM, Khaledi H, Chee CF. A new chromanone acid from the stem bark of *Calophyllum teysmannii*. Nat Prod Res. 2015; 29,1970-1977.
15. Shen Y-C, Wang L-T, Khalil AT, Chiang LT, Cheng PW. Bioactive pyranoxanthones from the roots of *Calophyllum blancoi*. Chem Pharm Bull. 2005; 53, 224-247.
16. Hay A-E, Helesbeux J-J, Duval O, Labaied M, Grellier P, Richomme P. Antimalarial xanthones from *Calophyllum caledonicum* and *Garcinia vieillardii*. Life Sci. 2004; 75, 3077-3085.
17. Saputri RD, Retnowati R, Supratman U, Tjahjandarie TS, Tanjung M. Three novel quinolinone alkaloids from the leaves of *Melicope denhamii*. Nat. Prod. Res. 2023; 37 (2), 197-203.
18. Tjahjandarie TS, Tanjung M, Saputri RD, Aldin MF, Susanti RA, Pertiwi NP, Wibawa RS, Halizah IN. Cytotoxicity evaluation of two new chalcones from the leaves of *Flemingia macrophylla* (Willd.) Merr. Phytochem. Lett. 2021; 44, 78-81.
19. Tjahjandarie TS, Tanjung M, Saputri RD, Nadar PB, Aldin MF, Permadi A. Flavestin K, an isoprenylated stilbene from the leaves of *Macaranga recurvata* Gage. Nat Prod Sci. 2019; 25(3), 244-247.
20. Tanjung M, Tjahjandarie TS, Aldin MF, Aldin MF, Mardhiyyah S, Maqfiroh I, Saputri RD, Ahmat N. Xantones and 4-phenylcoumarins from the twigs of *Mesua beccariana* (Baill.) Kosterm. Nat. Prod. Sci. 2023; 29(1), 38-41.
21. Mardhiyyah S, Zakiyah M, Renata ED, Tjahjandarie TS, Supratman U, Retnowati R, Saputri RD, Tanjung M. Cytotoxic activity of xanthones from the stem bark of *Cratoxylum sumatranum* and their cytotoxic activity, Trop. J. Nat. Prod. Res, 2023; 7(9), 3908-3910.