ACA

by Emmy Yuanita

Submission date: 12-May-2023 12:48AM (UTC-0500)
Submission ID: 2091091448
File name: aca.pdf (738.51K)
Word count: 3893
Character count: 21017

Acta. Chim. Asiana., 2023, 6(1), 279 – 286
e-ISSN/p-ISSN 2550-0503/2550-049x
Available online at: www.aca.unram.ac.id

RESEARCH PAPER

C-prenylation of 1,3 dihydroxyxanthone: synthesis, characterization and antibacterial activity

Emmy Yuanita*, Baiq Khaeratul Jannah, Maria Ulfa, Sudirman, Baiq Nila Sari Ningsih, Ni Komang Tri Dharmayani

Department of Chemistry, Faculty of Science, University of Mataram,
*e-mail: emmy_yuanita@unram.ac.id

DOI: 10.29303/aca.v6i1.149

Article info:

Received 21/01/2023

Revised 15/02/2023

Accepted 16/02/2023

Available online 25/02/2023

Abstract: Prenylated 1,3-dihydroxyxanthone has been successfully synthesized using Prenyl bromide and KOH. Characterization of the synthesized compound using Infra Red (IR) and Nuclear Magnetic Resonance (^{1}H-NMR) showed that monosubstituted c-prenylation was occurred at carbon number 2 to form 1,3-dihydroxy-2-prenylxanthone. The synthesis result was a yellow-brown paste with a yield of 43.09%. This prenylated 1,3-dihydroxyxanthone had moderate antibacterial activity against *Escherichia coli* with an inhibition zone > 5 mm at a concentration of 15%.

Keywords: 1,3-dihydroxyxanthone, 1,3-dihydroxy-2-prenylxanthone, prenylation, antibacterial

Yuanita, E., Jannah, B. K., Ulfa, M., Sudirman, Ningsih, B. N. S., Dharmayani, N. K. T. (2023). C-prenylation of 1,3 dihydroxyxanthone: synthesis, characterization and antibacterial activity. *Acta Chimica Asiana 6*(1), pp 279 – 286, DOI: 10.29303/aca.v6i1.149

INTRODUCTION

Xanthones displayed many biological activities, including antioxidant [1], antibacterial [2], and anticancer [3,4]. Prenyl xanthone derivatives have been reported to also possess antitumor [5], anti-inflammatory [6], and antibacterial [7,8] activities. α-Mangostin, a prenyl xanthone derivative obtained from the purification of mangosteen fruit extract [9], exhibited antibacterial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) with a MIC value of 1.95 μg/mL [10]. This compound also exhibited in vitro activity against *Bacillus subtilis* and *Mycobacterium smegmatis* with IC_{50} values of 3.9 and 3.7 μM, respectively [11]. In addition, 3-pentyloxy-1-hydroxyxanthone synthesized from 1,3-dihydroxyxanthone showed antibacterial activity against *Proteus mirabilis* and *Pseudomonas aeruginosa* with MIC values of 8 and 32 μg/mL, respectively [12]. Another prenyl xanthone derivative, 1,3-dihydroxy-2-methylxanthine, had the potential to inhibit the growth of human tumor cells [13].

On the other hand,1-hydroxy-2-prenyl-3,6-diprenyloxyxanthone gained by reacting 1,3,6-trihydroxyxanthone with prenyl bromide in t-butyl alcohol also showed antioxidant activity with an IC_{50} value of > 200 μg/mL [14]. Moreover, 3-dihydroxy-2,4-di(3-methyl-but-2-enyl)-xanten-9-one exhibited antioxidant activity with an IC50 value of 85 μg/mL [15].

MATERIALS AND METHODS

Equipment

The equipment used in this study were 500 mL three-neck flask, 250 mL Erlenmeyer flask, 250 mL round bottom flask, buchner funnel, separatory funnel, rotary evaporator, vacuum pump, heater, stopwatch, thermometer, desiccator, TLC chamber and cover, reflux apparatus, condenser, magnetic stirrer, ^{1}H-NMR and FTIR.

Materials

The materials used in the study were salicylic acid ($C_7H_6O_3$), phloroglucinol ($C_6H_6O_3$), Eaton reagent (P_2O_5/CH_3SO_3H), distilled water (H_2O), ethanol (C_2H_5OH), n-hexane (C_6H_{14}), ethyl acetate ($C_5H_{10}O_2$), acetone (C_3H_6O), dichloromethane (CH_2Cl_2), potassium hydroxide (KOH), 3,3-dimethylallyl bromide (C_5H_9Br), dichloromethane (DCM), silica gel, TLC plate, ice cubes, filter paper, aluminum foil, and *Staphylococcus aureus* and *Escherichia coli* bacteria.

Synthesis of 1,3-dihydroxyxanthone

Synthesis of 1,3-dihydroxyxanthone was carried out by mixing 0.69 g of salicylic acid

and 0.63 g of phloroglucinol with 5 mL of Eaton's reagent as a catalyst. These materials were placed in a three-neck flask at 80 ± 3 °C for 3 hours. Then, the mixture was allowed to stand at room temperature and poured into ice water to form precipitation. The precipitate was stirred for 1 hour and filtered. Then, the solid residue was dried in a desiccator for 24 hours. The products were monitored by TLC and characterized by FTIR and ^{1}H-NMR spectrophotometers [1].

Synthesis of prenylated 1,3-dihydroxyxanthone

The prenylated 1,3-dihydroxyxanthone was synthesized by reacting 0.228 g of 1,3-dihydroxyxanthone and 0.42 g of potassium hydroxide (KOH) in 30 mL of distilled water. This mixture was stirred in a 250 mL round bottom flask for 10 minutes at room temperature. Then, 0.97 g of prenyl bromide in 3 mL of acetone was injected into the mixture via a syringe. The mixture was then stirred for 24 hours at room temperature and acidified with 100 mL of 10% HCl solution. The mixture was extracted with 35 mL DCM. The top organic layer was removed, and the solvent was evaporated using a rotary evaporator [15].

Antibacterial Test

The antibacterial test was conducted using the disc diffusion method [16]. The bacteria used were gram-positive *S. aureus* and gram-negative *E. coli.*

RESULTS AND DISCUSSION

Synthesis of 1,3-dihydroxyxanthone

The 1,3-dihydroxyxanthone (**1**) was synthesized through two reaction steps, the cyclodehydration reaction of the acid derivatives and the substitution of phloroglucinol aided by Eaton reagent. The synthesis of **1** began by activating the acyl group in salicylic acid using methane sulfonic acid to form a carbocation compound. The following process was a phloroglucinol substitution reaction for the salicylic acid carbocation formed, followed by xanthone cyclization through a dehydration reaction. The phosphorus pentaoxide compound (P_2O_5) in the Eaton reagent caused the absorption of water molecules obtained in the reaction to form phosphoric acid. Compound **1** with the molecular formula $C_{13}H_8O_4$ was formed as a red solid with a yield of 70.17%. The formation of **1** was confirmed based on the same R_f values, 0.71 in n-hexane:ethyl acetate (6:4) eluent, obtained between **1** and the standard compound.

The FTIR spectra showed typical absorptions of 1,3-dihydroxyxanthone. Absorption of cyclic xanthone appeared at wave number of 1296 and 1614 cm^{-1} indicating the presence of ether (C-O-C) and xanthone carbonyl (C=O) groups, respectively [1].

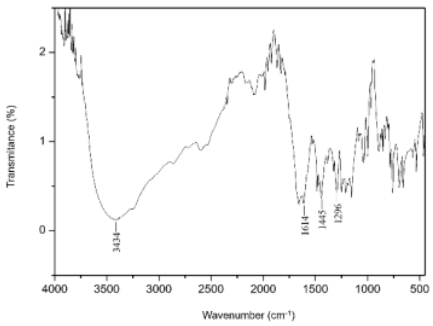

Figure 1. FTIR Spectra of the synthesized 1,3-Dihydroxyxanthone

In addition, there were absorption bands at wave numbers of 1445 and 3434 cm^{-1} suggesting the existence of aromatic C=C and hydroxy group (OH) stretching vibration.

Table 1. Comparison between FTIR data of **1** and 1,3-Dihydroxyxanthone in the References

Functional Groups	Wavenumber (cm^{-1})		
	Synthesized compound	1,3-dihydroxyxanthone	
		[14]	[17]
O-H	3434	3408	3478
C=O	1614	1654	1651
C=C	1445	1330	1454
C-O-C	1296	1175	1312

Bands at lower wave numbers confirmed carbonyl interactions in xanthone compounds due to the interaction between the two aromatic carbons flanking the carbonyl group in the xanthone compound, causing the vibrations to be not free. The identified functional groups corresponded to those of 1,3-dihydroxyxanthone in the reference.

Further characterization using ^{1}H-NMR (Figure 2) showed the presence of distinctive signals for the 1,3-dihydroxyxanthone. A signal

at δ_H 12.88 ppm (OH, *s*) indicating the presence of a hydroxy chelated proton at C-1. Six aromatic protons, two in ring A and four in ring B, were indicated by signals in 6.00-9.00 ppm. The aromatic proton signals of ring A appeared at δ_H 6.21 (1H, *d*, J=1.2 Hz) and 6.40 ppm (1H, d, J=1.2 Hz) were assigned as H-2 and H-4, respectively. The same coupling constant (*J*) values indicated that the two protons were in a meta position with each other. Meanwhile, the four aromatic proton signals in ring A appeared at δ_H 7.78 ppm (1H, *dd*, J = 1.2, 7.9 Hz), 7.85 ppm (1H, *td*, J = 1.3, 7.2, and 7.7 Hz), 7.59 ppm (1H, *dd*, J = 1.0; 8.5 Hz), and 8.13 ppm (1H, *dd*, J=1.2; 7.9 Hz) were attributed to H-5, H-6, H-7, and H8, respectively. The same *J* values of H-5 and H-8 proton signals indicated that the two protons had a para correlation. The chelated hydroxy proton signal (H-1) was in the deshielding region due to the attraction of electrons by the carbonyl group (C=O). The differences in chemical shift values for the fifth and sixth peaks were due to the direct influence of the carbonyl at the xanthone ring.

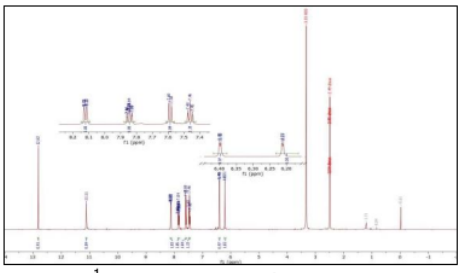

Figure 2. ^{1}H-NMR spectra of the synthesized 1,3-Dihydroxyxanthone

The ^{1}H-NMR data of **1** are similar to those of 1,3-dihydroxyxanthone consisting of six protons at δ_H 6-9 ppm and two OH protons with a singlet multiplicity [14]. Based on the comparison of FTIR and ^{1}H-NMR data to those of the references, the structure of the synthesized compound was confirmed to be 1,3-dihydroxyxanthone.

Synthesis of 1,3-dihydroxy-2-prenylxanthone

Synthesis of 1,3-dihydroxy-2-prenylxanthone (**2**) occurred through a nucleophilic substitution reaction on the hydroxyxanthone base using prenyl bromide in alkaline conditions (KOH). This synthesis had a 43.09% yield in the form of yellow-brown paste obtained after 24 hours of reaction. The Rf value was 0.87 in n-hexane:ethyl acetate (7:3) eluent. In addition to maintaining the reaction in an alkaline environment, potassium hydroxide (KOH) in the hydroxyxanthone prenylation reaction acted as both base and catalyst. The mechanism began with the substitution of the hydroxyl group from the KOH base. Then, a carbanion was formed, followed by the substitution of the prenyl group from prenyl bromide in the C-2 or C-4 ring of the aromatic 1,3-dihydroxyxanthone ring. This substitution reaction resulted in the formation of a carbanion in the aromatic ring, which then underwent delocalization and was followed by the release of a proton (H^+ ion). The prenylation reaction of 1,3-dihydroxyxanthone occurred at either C-2 or C-4 positions. The proposed reaction mechanism of the prenyl group at C-2 was shown in Figure 3 while that of C-4 was illustrated in Figure 4.

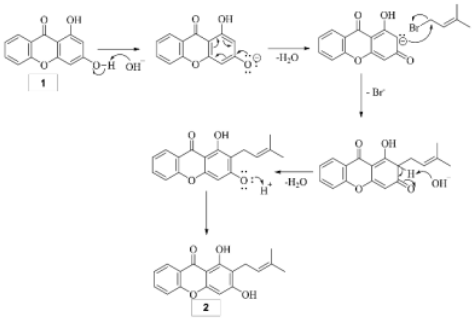

Figure 3. Proposed mechanism of 1,3-dihydroxyxanthone prenylation at C-2 position

Substitution of the prenyl group was estimated to be conducted at the C-2 position due to the presence of hydroxy group at the C-1 position forming a chelate with the carbonyl group. This caused a smaller steric hindrance allowing the prenyl group to be substituted at the C-2 position. In addition, the induction effect of the chelated hydroxy group at the C-1 position caused more steric hindrance at the C-4 carbon atom. Hence, the possibility for the prenyl group to be substituted at the C-4 position became smaller compared to that in the C-2 position. A previous study also

revealed that the synthesis of 1,3-dihydroxyxanthone derivatives showed a more stable substituent in the C-2 position [18]. Therefore, the more plausible reaction mechanism for the prenylation of 1,3-dihydroxyxanthone framework led to the formation of **2**.

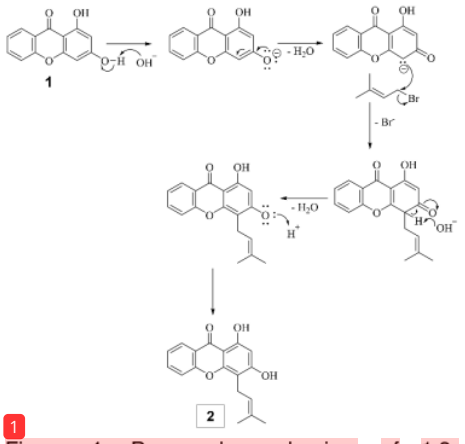

Figure 4. Proposed mechanism of 1,3-dihydroxyxanthone prenylation at C-4 position

Compound **2** had a different FTIR spectra to that of **1**, including the sharper absorption peak of the -OH group. Meanwhile, the carbonyl group became freer (possessed a larger wave number) due to the presence of a prenyl group (as an electron attractant) at position C-2, causing the absorption band of the hydroxy group at position C-1 to become weaker. Therefore, the absorption band of the hydroxyl group in the prenylated compound tended to be sharp. The absorption of this hydroxyl group indicated that the prenyl was not substituted at the OH position.

In addition, the typical wave number of aromatic alkenes from 1445 cm^{-1} to 1469 cm^{-1} reflected the presence of other substituents on the alkene carbon. The absorption band at wave number 2920 cm^{-1} indicated the presence of prenyl C-H. It was supported by an absorption band in the fingerprint region, 900-1000 cm^{-1}. In addition, the absorption at wave number 1296 cm^{-1} revealed that the alkene group from the prenyl had been attached to one of the C rings of the hydroxyxanthone framework.

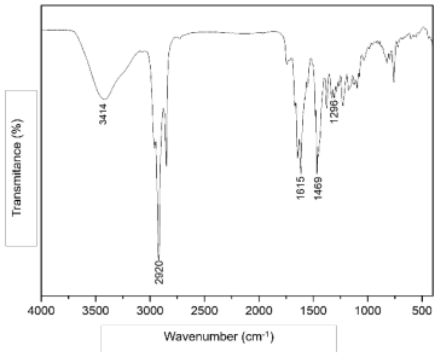

Figure 5. FTIR spectra of the shynthesized prenylated 1,3-Dihydroxyxanthone

The FTIR spectra of the **2** showed a similar absorption for prenyl CH, 2920 cm^{-1}, with that of the reference, at 2924 cm^{-1}. This absorption indicated that the prenyl group had been substituted at one of the 1,3-dihydroxyxanthone carbon rings [14].

Table 2. Comparison between FTIR data of **2** and 1,3-dihydroxy-2-prenylxanthone in the reference

Functional Groups	Wavenumber (cm^{-1})	
	Synthesized compound	1,3-dihydroxy-2-prenylxanthone [14]
O-H	3414	3435
C-H	2920	2924
C=O	1615	1610
C=C Aromatic	1469	1448
C-O-C	1296	-

The ^{1}H-NMR spectra of **2** showed signals at δ_H 1.25 and 1.78 ppm revealing the existence of prenyl substituents. Specifically, the loss of the proton signal at δ_H 6.20 of the 1,3-dihydroxyxanthone mainframe indicated that the prenyl was substituted at the C-2 position. Furthermore, the appearance of a proton signal at δ_H 6.42 indicated that the proton signal from C-4 was still present and not substituted by the prenyl group.

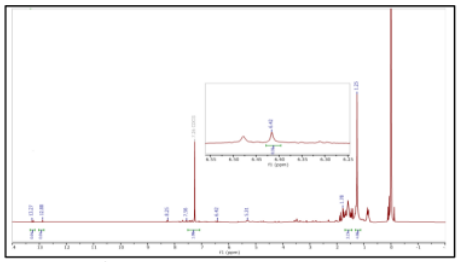

Figure 6. ^{1}H-NMR spectra of 1,3-dihydroxy-2-prenylxanthone

The ^{1}H-NMR spectroscopic data for **2** were then compared with the structurally related α-mangostin [19] (Table 3).

Table 3. Comparison of ^{1}H-NMR data of **2** and α-mangostin

C Position	Compound 2 (δ_H) (ppm)	α-mangostin (δ_H)(ppm) [19]
4	6.42 (1H, *s*)	6.41 (1H, s)
5	7.10 (1H, *d*, *J* = 7.6 Hz)	6.83 (1H, *s*)
6	7.56 (1H, *d*, *J* = 9.3 Hz)	-
7	7.44 (1H, *d*, *J* = 6.1 Hz)	-
8	8.25 (3H, *d*, *J* = 8.2 Hz)	-
11	3.57(3H, *d*, *J* = 7.4 Hz)	3.34 (2H, *d*, J = 7.3)
12	5.31(3H, *d*, *J* = 7.0 Hz)	5.28 (*m*)
14	1.25 (3H, *s*)	1.66 (3H, *s*)
15	1.78 (3H, *s*)	1.83 (3H, *s*)
1-OH	13.27 (OH, *s*)	13.80 (OH, *s*)
3-OH	12.88 (OH, *s*)	-

The characteristics of substituted prenyl groups on the xanthone framework were shown by the presence of δ_H at 1-3 ppm for prenyl group and 11-13 ppm for the hydroxyl groups [14].

Antibacterial Activity Test

The antibacterial activity test of **2** was carried out using the disc diffusion method. This method was based on the diffusion of the antibacterial compound into the solid medium containing inoculated microbe. Observations were obtained to identify the presence or absence of a clear area formed around the disc paper, which indicated an inhibition zone for bacterial growth [20]. The unprenylated 1,3-dihydroxyxanthone, compound **1**, was also tested as a comparison.

Table 4. Antibacterial activity of the synthesized compounds at various concentrations against *E. coli*

Compounds	Concentration (%)	Diameter of the inhibition zone (mm)			Average (mm)
		U_1	U_2	U_3	
1,3-dihydroxy-2-prenylxanthone (**2**)	5	2	3	2	2,3
	10	5	5	3	4,3
	15	6	5	5	5,3
1,3-dihydroxyxanthone (**1**)	5	3	2	2	2,3
	10	4	3	3	3,3
	15	4	3	3	3,3
Positive control	5	15			15

Based on antibacterial activity evaluation, **2** showed moderate activity against gram-negative *E. coli*, >5 mm at a concentration of 15%. This value was higher than that of **1**, >3 mm, at the same concentration. Hence, the presence of a prenyl group on the C-2 side chain can increase antibacterial activity. There are four levels of inhibition classification, very strong (>20 mm), strong (10-20 mm), moderate (5-10 mm), and weak (<5 mm) [21].

Based on the Clinical and Laboratory Standards Institute (CLSI), the inhibition zone of this compound was classified as resistant because the inhibition diameter was <10 mm. An antibacterial is identified as intermediate if the inhibitory diameter is between 11-15 mm and susceptible if it is more than 16 mm [22]. Therefore, 1,3-dihydroxy-2-prenylxanthone can only moderately inhibit the growth of *E.*

coli bacteria. Meanwhile, both prenylated and unprenylated 1,3-dihydroxyxanthone showed a weak inhibition response in suppressing the growth of gram-positive bacteria, *S. Aureus*. Specifically, **2** showed a less sensitive inhibitory response, >1 mm, than **1**, >3 mm at the concentration of `15%.

Table 5. Antibacterial activity of the synthesized compounds at various concentrations against *S. Aureus*

Compounds	Concentration (%)	Diameter of the inhibition zone (mm)			Average (mm)
		U_1	U_2	U_3	
1,3-dihydroxy-2-prenylxanthone (**2**)	5	0	0	2	0.6
	10	0	0	5	1.6
	15	0	0	4	1.3
1,3-dihydroxyxanthone (**1**)	5	1	2	2	2.3
	10	3	3	3	3.0
	15	4	3	3	3.3
Positive control	5	10			10

This was possibly due to the absence of specific receptors (protein molecules that receive chemical signals) of the synthesized compounds for gram-positive bacterial cells [23]. In addition, Gram-positive bacteria had a thicker peptidoglycan layer than Gram-negative bacteria [24].

The antibacterial test showed that **2** had a better inhibitory response in suppressing Gram-negative bacteria growth than Gram-positive bacteria. The unformed inhibition zone could be due to various factors, including the pH of the media, the amount of bacterial inoculum, the ability of bacteria to grow, and the composition of the bacterial cells. Besides, the Gram-negative bacteria have a cell wall that tends to be more sensitive to the tested compounds. In addition, both bacteria had different resistance mechanisms due to natural differences between them.

The presence of substituents on the hydroxyxanthone framework can increase the antibacterial activity, especially against Gram-negative bacteria with a moderate inhibitory response. The synthesized prenylated 1,3-dihydroxyxanthone was classified as having resistant activity against Gram-negative and Gram-positive bacteria, meaning that this compound can only suppress the growth of these bacteria but cannot kill bacteria.

CONCLUSION

Synthesis of **2** using prenyl bromide in alkaline conditions (KOH) has been successfully carried out with a yield of 43.09%. The antibacterial activity test revealed that this compound showed moderate antibacterial activity in inhibiting *E. coli* bacteria with an inhibition zone value of >5 mm at a concentration of 15%.

ACKNOWLEDGEMENTS

The authors would like to express gratitude for Department of Chemistry, Faculty of Science, and Department of Pharmacy, Faculty of Medicine, University of Mataram for the support.

REFERENCES

1. Yuanita, E., Pranowo, H. D., Siswanta, D., Swasono, R. T., Mustofa, Zulkarnain, A. K., Syahri, J., & Jumina. (2018). One-Pot Synthesis, Antioxidant Activity and Toxicity Evaluation of Some Hydroxyxanthones. Chemistry & Chemical Technology, *12*(3), 290– 295.
2. Yuanita, E., Sudarma, I.M., Sudewiningsih, N.M., Syahri, J., Dharmayani, N.K.T., Sudirman, Ulfa, & Sumarlan, I. (2021). Antibacterial Activity and Molecular Docking Studies of Hydroxyxanthone. AIP Conference Proceedings, *2243*(1), 020032, AIP Publishing LLC.
3. Yuanita, E., Pranowo, H. D., Mustofa, Swasono, R. T., Syahri, J., & Jumina. (2019). Synthesis, Characterization and Molecular Docking Chloro Substituted Hydroxyxanthone Derivatives. Chemistry Journal of Moldova, *14*(1), 68–76.
4. Yuanita, E., Ulfa, M., Sudirman, S., Sumarlan, I., Dharmayani, N. K. T, Sudarma, I. M, Syahri, J., & Jumina. (2021). Synthesis, Cytotoxic Evaluation and Molecular Docking of Bromo-

Substituted 1,3,6-Trihydroxyxanthone as Protein Tyrosine Kinase Inhibitor. Malaysian Journal of Chemistry, *23*(1), 24-32.

5. Fei, X., Jo, M., Lee, B., Han, S. B., Lee, K., Jung, J. K., Seo, S. Y., & Kwak, Y. S. (2014). Synthesis of Xanton Derivatives Based on α-Mangostin and Their Biological Evaluation for Anti-Cancer Agents. Bioorganic & Medicinal Chemistry Letters, 24(9), 2062-2065.
6. Yen, C. T., Nakagawa-Goto, K., Hwang, T. L., Morris-Natschke, S. L., Bastow, K. F., Wu, Y. C., & Lee, K. H. (2012). Design and Synthesis of Gambogic Acid Analogs as Potent Cytotoxic and Anti-Inflammatory Agents. Bioorganic & Medicinal Chemistry Letters, *22*(12), 4018-4022.
7. Nakatani, K., Nakahata, N. Arakawa, T., Yasuda, H., & Ohizumi, Y. (2002). Inhibition of Cyclooxygenase and Prostaglandin E2 Synthesis by Gamma-mangostin, A Xanthone Derivative in Mangosteen, In C6 Rat Glioma Cells. Biochemical Pharmacology*, 63(1)*, 73-79.
8. Narosa, G. B. (2009). Natural Prenylated Xanthones: Chemistry and Biological Activities. *In Natural Products:* Chemistry, Biochemistry and Pharmacology. Publ ishing House PVT. New Dehli. India.
9. Zhang, Y., Song, Z., Hao, J., Qiu, S., & Xu, Z. (2010). Two New Prenylated Xanthones and A New Prenylated Tetrahydroxanthone from the Pericarp of *Garcinia mangostana*. Fitoterapia, *81*(6), 595–599.
10. S., Wongsariya, K., & Bunyapraphatsara, N. (2009). Antibacterial activity of Thai medicinal plants against methicillin resistant *Staphylococcus aureus*. Fitoterapia. *80*(2), 102–104.
11. Al-Massarani, S. M., El Gamal, A. A., Al-Musayeib, N. M., Mothana, R. A., Basudan, O. A., Al-Rehaily, A. J., Farag, M., Assaf, M. H., El Tahir, K. H., & Maes, L. (2013). Phytochemical, Antimicrobial and Antiprotozoal Evaluation of *Garcinia mangostana* Pericarp and *α*-mangostin, Its Major Xanthone Derivative. Molecules (Basel, Switzerland), 18(9), 10599–10608.
12. Das A., Shaikh, M. M., & Jana, S. (2014). Design, Synthesis, and In Vitro Antibacterial Screening Of Some Novel 3-Pentyloxy-1-hydroxyxanthone Derivatives. *Medicinal Chemistry Research, 23,* 436-444.
13. Pedro, M., Cerqueira, F., Sousa, M. E., Nascimento, M. S. J., & Pinto, M. (2002). Xanthones as Inhibitors of Growth of Human Cancer Cell Lines and Their Effects on The Proliferation of Human Lymphocytes In Vitro. Bioorganic & Medicinal Chemistry, *10*(12), 3725-3730.
14. Ling, C. S. (2013). Synthesis And Antioxidant Activity of Prenylated Xanthones Derived From 1,3,6-Trihydroxyxanthone. Doctoral dissertation. Universiti Tunku Abdul Rahman.
15. Kuan, L. C. (2012). Synthesis and Characterisation of 1,3-Dihydroxyxanthone Derivatives and Their Antioxidant Activities. Doctoral dissertation. Universiti Tunku Abdul Rahman.
16. Dharmayani, N. K. T., Garnasih, I., Yuanita, E., Ulfa, M., Sudirman, S., Darmayanti, M. G., & Septiyana, M. (2022). Antibacterial Test and Isolation of Xanthones from Pericarps Mangosteen (*Garcinia mangostana* L.) using Calcium Oxide (CaO) as a Vacuum Liquid Chromatography (VLC) Stationary Phase. Acta Chimica Asiana, *5*(1), 181-185.
17. Vanessa, V. V., Teh, S. S., Lam, K. W., & Mah, S. H. (2022). Synthesis of 1-Hydroxy-3-O-Substituted Xanthone Derivatives and Their Structure-Activity Relationship on Acetylcholinesterase Inhibitory Effect. Natural Product Research, 1-13. DOI: 10.21203/rs.3.rs-763326/v1.
18. Qin, J., Lan, W., Liu, Z., Huang, J., Tang, H., & Wang, H. (2013). Synthesis and Biological Evaluation of 1, 3-Dihydroxyxanthone Mannich Base Derivatives as Anticholinesterase Agents. Chemistry Central Journal, *7*, 1-11.

19. Sim, W. C., Ee, G. C. L., & Aspollah, S. M. (2011). α-Mangostin and β-Mangostin from *Cratoxylum glaucum*. Research Journal of Chemistry and Environment, *15*, 62-66.
20. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for In Vitro Evaluating Antimicrobial activity: A review. Journal of Pharmaceutical Analysis, *6*(2), 71-79.
21. Davis, W. W. & Stout, T. R. (1971). Disc Plate Methods of Microbiological Antibiotic Assay, Microbiology, *22*(4), 659-665.
22. The Clinical and Laboratory Standards Institute (CLSI). (2017). Performance Standards for Antimicrobial Susceptibility Testing. CLSI Supplement M100. 27th ed. Wayne, PA: Clinical and Laboratory Standards Institute.
23. Russell, A. D. (1995). Mechanisms of Bacterial Resistance to Biocides, . International Biodeterioration & Biodegradation *36*(3-4), 247-265.
24. Beveridge, T. J., Makin, S. A., Kadurugamuwa, J. L., & Li, Z. (1997). Interactions Between Biofilms and the Environment. FEMS Microbiology reviews, *20*(3-4), 291-303.

ACA

ORIGINALITY REPORT

4%	4%	2%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	eprints.utar.edu.my Internet Source	2%
2	revista-agroproductividad.org Internet Source	2%

Exclude quotes	On	Exclude matches	< 2%
Exclude bibliography	On		