Antioxidant Activities and Electrochemical Behaviors of Xanthones from *Cratoxylum cochinchinense* and *Cratoxylum formasum*

Benjamat Chailap and Thanesuan Nuanyai*

General Education Department, Faculty of Liberal Arts, Rajamangala University of Technology Rattanakosin, Wang Klai Kangwon Campus, Nongkae, Huahin, Prachuap Khiri Khan, 77110, Thailand

* Corresponding author. E-mail address: thanesuan.nua@rmutr.ac.th

Received: 27 August 2018; Accepted: 9 January 2019

Abstract

Seven xanthones; dulcisxanthone B (**1**), ß-mangostin (**2**), 1,3,7-trihydroxy-2,4-di-(3-methylbut-2-eyl)-xanthone (**3**), cudratricusxanthone E (**4**), cochinchinone A (**5**), 2-geranyl-1,3,7-trihydroxy-4-(3,3-dimethylallyl)-xanthone (**6**), and cochinchinone B (**7**) were isolated from twigs of *Cratoxylum cochinchinense* and *Cratoxylum formasum*. The structures of all isolated compounds were elucidated by spectroscopic methods (^{1}H, ^{13}C, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, and ^{1}H-^{13}C HMBC) and compared with previous literatures. All compounds were evaluated *in vitro* for antioxidant activity using DPPH assay. Their electrochemical behaviors were investigated by cyclic voltammetry. The results derived from two techniques were consistent. Compounds **1**, **2**, **4** and **7** exhibited strong antioxidant activities. Considering the structure-activity relationship, the hydroxy group at C-6 played an important role to antioxidant power.

Keywords: Xanthone, *Cratoxylum cochinchinense*, *Cratoxylum formasum*, Antioxidant, Electrochemical

Introduction

Xanthones are oxygen based heterocyclic compounds which have dibenzo-γ-pyrone as the skeleton of the core structure. In the previous phytochemical studies, xanthones were the major of secondary plant metabolites of twenty plant families (Tchamo, Silvere, & Etienne, 2006) including *Cratoxylum cochinchinense* (Tui-Kliang in Thai) (Anantachoke, Tuchinda, Kuhakarn, Pohmakotr, & Reutrakul, 2012) and *Cratoxylum formosum* (Tui-Khon in Thai) (Duan, et al., 2010), the plants of the genus *Cratoxylum* in the Hypericaceae family. These xanthones have various chemical structures including simple oxygenated and prenylated substituents. The isolated xanthones showed redox-active abilities and also displayed interesting biological activities such as cytotoxicity with cancer cell lines (Rattanaburi, Daus, Watanapokasin, & Mahabusarakam, 2014), antimalarial (Laphookhieo, Maneerat, & Koysomboon, 2009), antibacterial (Raksat, Laphookhieo, Cheenpracha, Ritthiwigrom, & Maneerat, 2014), anti-inflammatory (Boonnak, Chantrapromma, Tewtrakul, & Sudsai, 2014), and antioxidant activities (Udomchotphruet, Phuwapraisirisan, Sichaem, & Tip-Pyang, 2012).

Generally, the free radicals are the products of reaction between reactive oxygen species and biomolecules. These phenomena lead to a loss of functionality of biological molecules (Moon, & Shibamoto, 2009). Antioxidants are molecules that can be involed with the protection of macromolecules from oxidation. The definition of antioxidant in biological term is "any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate" (Antolovich, et al., 2002). Antioxidants have a wide application in diverse fields as they have an importance either as industrial additives or health agents (Moon, & Shibamoto, 2009). Various methods for the measurement of antioxidant capacity have been reported (Antolovich, Prenzle, Patsalide, McDonald, & McDonald, 2002). DPPH assay

(2,2-diphenyl-1-picryhyrazyl radical scavenging capacity assay) is one of technically simple strategies for measuring the antioxidant activity (Huang, Ou, & Prior, 2005). DPPH is a stable and commercially available organic nitrogen radical and has a UV-vis absorption maximum at 515 nm. Antioxidant compounds cause changing to the color of DPPH solution (violet to yellow) which was monitored by a spectrophotometer and reported as the percentage of the DPPH remaining (Huang, Ou, & Prior, 2005). The DPPH assay is widely used for assessing the ability of polyphenols to transfer labile H-atoms to radicals (Lee, et al., 2005). The xanthone is the phenolic compound, which shows the ability of receiving the radical moiety into the antioxidation of living cells. The antioxidant activities of xanthone have been wildly investigated by various assays *in vitro* systems; anti-lipid peroxidation (ALP), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and xanthine-xanthine oxidase system (O_2^-) (Fotie & Bohle, 2006).

Electrochemical techniques have been still developing for antioxidant capacities with many advantages such as chemical inertness, low cost, simple fabrication, low background currents, rapid renewal of the surface, and easy modification (Judzentiene, Garjonyte, & Budiene, 2016). Cyclic voltammetry (CV), one of electrochemical techniques, has been widely used for the determination of antioxidant activities of organic compounds (Chevion, Roberts, & Chevion, 2000). The data from voltammogram such as the reversibility of peak, oxidation potential and the number of peaks can be useful information in evaluation of antioxidant capacity. Moreover, the information the CV technique can lead to the understanding of antioxidant mechanism and also be an important tool for the rational design of new and potent antioxidants (Sochor, et al., 2013). The oxidation potentials indicated antioxidant strength of compounds. The compounds that have lower oxidation potentials tend to act as antioxidant because they can donate an electron (to the free radical) easier (Sochor, et al., 2013). Moreover, it has been found that electrochemical behaviors and antioxidant by DPPH assay have also been correlated (Arteaga, et al., 2012). The electrochemical behaviors derivatives and antioxidant activities of xanthone have been reported (Lee, et al., 2005). In this paper, radical scavenging property of seven isolated natural xanthones were evaluated using the DPPH assay. The redox chemistry of these compounds has been investigated using the cyclic voltammetry. Structure-activity relationship of these compounds will be discussed.

Methods and Materials

General experiment procedures

UV spectra were measured with a CARY 100 Bio spectrophotometer. NMR spectra were recorded in DMSO-d_6 on Bruker AV400 spectrometers at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR using TMS (tetramethylsilane) as internal standard. Merck's silica gel 60 No. 7734 was used as adsorbents for normal phase column chromatography. Merck's thin layer chromatography (TLC) aluminum, silica gel 60 F_{254} precoated, 20x20 cm, layer thickness 0.2 mm were used for TLC analysis. The detection was visualized under ultraviolet light at the wavelength of 254 nm and dipped with $(NH_4)_6Mo_7O_{24}$ in 5% H_2SO_4 solution. All isolated compounds were identified by comparison of their spectroscopic data with those in the literature.

Plant materials

The twigs of *C. cochinchinense* and *C. formasum* were collected in December 2014 from Nongkae, Huahin, Prachuap Khiri Khan and Nam-phong, Khon Kaen, Thailand, respectively. The voucher specimens of *C. cochinchinense* (Plant-004) and *C. formasum* (Plant-005) were deposited at department of Science, Faculty

of Liberal Arts, Rajamangala University of Technology Rattanakosin, Wang Klai Kangwon Campus, Nongkae, Huahin, Prachuap Khiri Khan, Thailand.

Extraction and isolation

The air-dried twigs of *C. formasum* (1.5 kg) were macerated with 15.0 L of methanol for three days. The methanol extracted was evaporated by rotary evaporator and extracted with ethyl acetate (EtOAc) and water. The EtOAc layer was dried in vacuo (25.82 g) and purified by column chromatography technique with a gradient of hexane-EtOAc (9:1 to 0:10, v/v) to afford four fractions (F1-F4). Fraction F4 (1.21 g) was separated by silica gel column chromatography (CC) with hexane-acetone (7:3, v/v) as an eluent to effort three sub-fractions (F4.1-F4.3). The silica gel chromatographic separation of sub-fraction F4.1 (175 mg) with a CH_2Cl_2-hexane (1:1, v/v) as an eluent yielded β-mangostin (15.0 mg) and cochinchinone A (50.5 mg). In addition, cochinchinone B (22.0 mg) was isolated by silica gel column chromatography of sub-fraction F4.2 (150.0 mg) using hexane-acetone (7.5:2.5, v/v) as a mobile phase. Sub-fraction F4.3 (250 mg) was purified by silica gel CC, hexane-acetone (7:3, v/v) as an eluent, to afford cudratricusxanthone E (12.0 mg). The structures of all isolated compounds were elucidated by basic NMR techniques (^1H, ^{13}C, COSY, HSQC and HMBC) and compared with previously reported. In addition, our other reported xanthone derivatives from *Cratoxylum cochinchinense* (dulcisxanthone B, 1,3,7- trihydroxy-2,4-di-(3-methylbut-2-eyl)-xanthone, and 2-geranyl-1,3,7-trihydroxy-4-(3,3-dimethylallyl)-xanthone) were also studied for comparing with the xanthones from *C. formasum* (Nuanyai, Benjamat, Songchan, & Anumart, 2015).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

The experiments were modified from the method of Braca et al., 2001. The ethanolic solutions of various concentrations of isolated xanthones (**1**-**7**) or Trolox were added to a DPPH in EtOH (2.5 mg/L) and the reaction mixture was shaken vigorously. The absorbance of the final solution was measured at 517 nm using a UV spectrophotometer after incubated at room temperature in the dark for 30 minutes. The results were calculated as percentage radical scavenging (equation 1).

$$\% \text{ radical scavenging} = [(A_{control} - A_{sample})/A_{control}] \times 100. \quad (1)$$

The DPPH solution without compounds **1**-**7** was used as control. The IC_{50} values were obtained by linear regression analysis of the dose response curves, which were plots of % radical scavenging versus concentration. Each sample was analyzed by means of DPPH assay by triplicate studies.

Cyclic voltammetry

The experimental procedures were analyzed using our previous experiment with some modifications (Chailap, & Tuntulani, 2012). The supporting electrolyte was prepared by dissolving tetrabutylammonium hexafluorophosphate ($TBAPF_6$) in 10% CH_3CN in CH_2Cl_2 to get the concentration at 0.1 M. Solutions of compounds (**1**-**7**) were prepared at the same concentration (1.0×10^{-3} M) using the supporting electrolyte as a solvent. Three electrode cells used in this experiment were a glassy carbon electrode (working electrode), a Pt wire (counter electrode) and the Ag-$AgNO_3$ electrode (reference electrode).

Results and Discussion

Seven compounds were isolated and identified as dulcisxanthone B (**1**) (Deachathai, Mahabusarakam, Phongpaichit, & Taylor, 2005), ß-mangostin (**2**) (Likhitwitayawuid, Phadungcharoen, & Krungkrai, 1998),

1,3,7-trihydroxy-2,4-di-(3-methylbut-2-eyl)-xanthone (**3**) (Iinuma, Tosa, Tanaka, & Riswan, 1996), cudratricusxanthone E (**4**) (Zou, et al., 2004), cochinchinone A (**5**) (Mahabusarakam, Nuangnaowarat, & Taylor, 2005), 2-geranyl-1,3,7-trihydroxy-4-(3,3-dimethylallyl)-xanthone (**6**) (Bennett, Harrison, Sia, & Sim, 1993), and cochinchinone B (**7**) (Mahabusarakam, Nuangnaowarat, & Taylor, 2005), based on the NMR data, as well as comparison of the spectral data with the previous reports. The structures of all isolated compounds were shown in Figure 1.

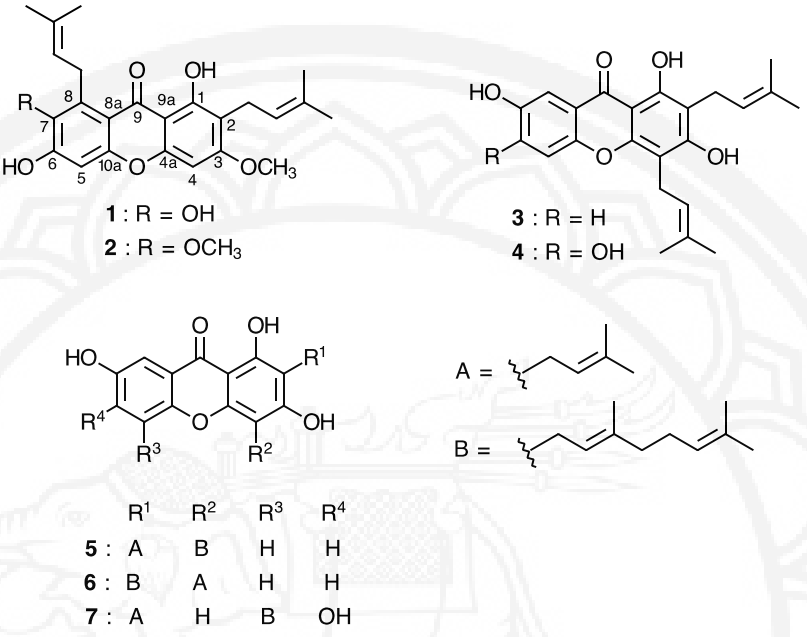

Figure 1 Structure of isolated xanthones from twigs of *C. cochinchinense* and *C. formasum* (**1**-**7**).

All isolated compounds were examined for their antioxidant activities using DPPH radical scavenging assay. The half maximal inhibition concentration of DPPH radical (IC_{50}) of all isolated compounds and Trolox values were shown in Table 1. The compounds (**3**, **5** and **6**) appeared to be inactive at the concentration up to 100 μM. Interestingly, the isolated xanthones (**1**, **2**, **4** and **7**) having hydroxy group at C-6 exhibited strong free radical scavenging activity which were displayed the IC_{50} values at 24.81, 22.97, 23.51, and 15.12 μM, respectively. Their strong free radical scavenging activities were consistent with the report of catecholic xanthones which have dihydroxy group at C-6 and C-7 (Lee, et al., 2005). It can be rationalized that the hydroxy group at C-6 and C-7 could transfer to quinone easily by releasing two electrons (Lee, et al., 2005).

Table 1 The IC_{50} values of antioxidant activity by DPPH assay of compound **1**-**7** and Trolox.

Compounds	IC_{50} [μM]
1	24.81 ± 3.64
2	22.97 ± 3.68
3	> 100
4	23.51 ± 0.83
5	> 100
6	> 100
7	15.12 ± 2.69
Trolox	23.27 ± 4.78

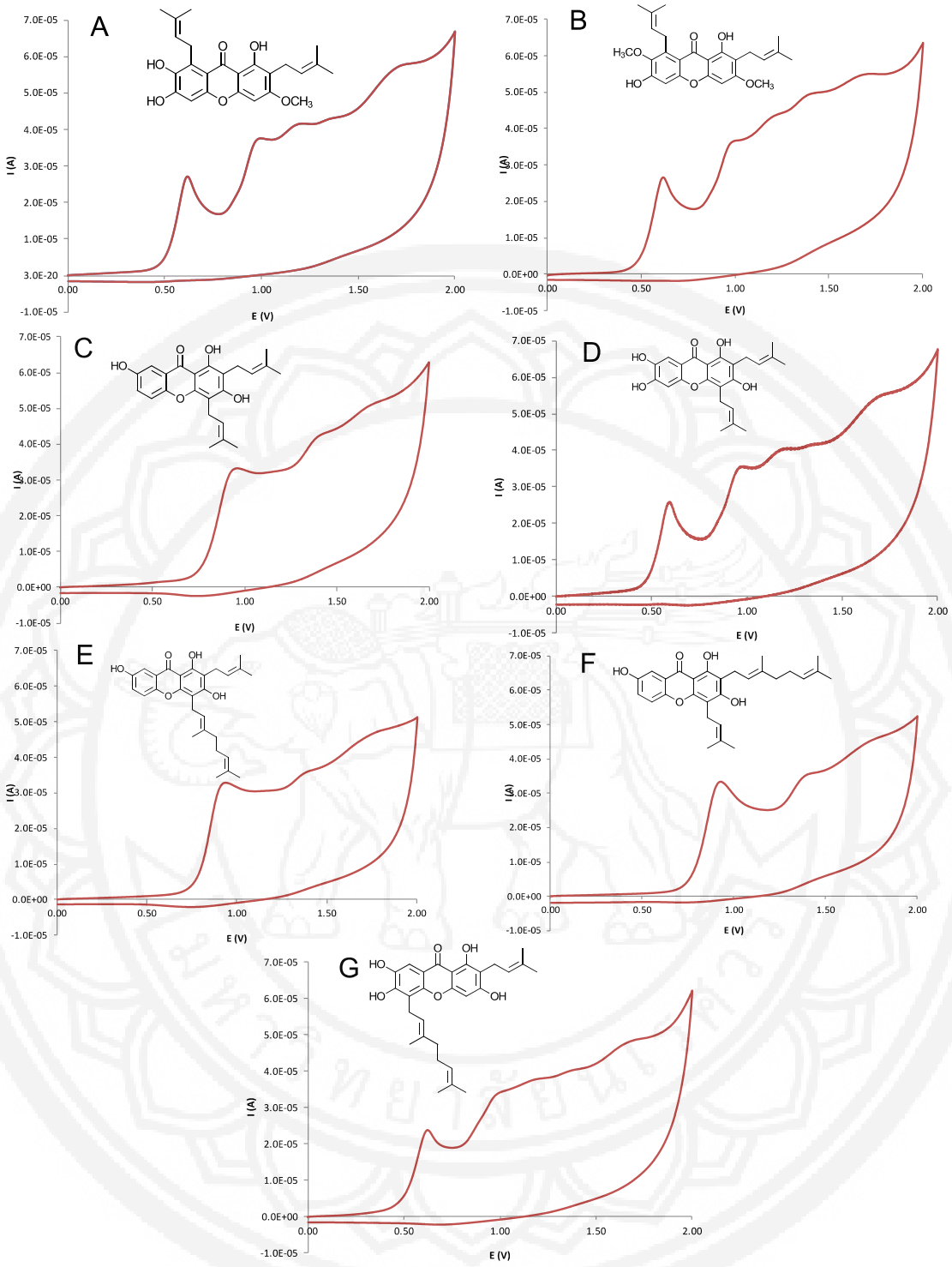

Figure 2 Voltammograms of 1.0 mM compounds **1**-**7**; dulcisxanthone B (A), ß-mangostin (B), 1,3,7-trihydroxy-2,4-di-(3-methylbut-2-eyl)-xanthone (C), cudratricusxanthone E (D), cochinchinone A (E), 2-geranyl-1,3,7-trihydroxy-4-(3,3-dimethylallyl)-xanthone (F), and cochinchinone B (G), glass carbon electrode in 0.1 mM $TBAPF_6$, scan rate 50 mV/s.

Cyclic voltammetry was the selected technique for studying of electrochemical behavior of all isolated compounds (**1**-**7**). Antioxidant compounds by virtue of their ability tend to be easily oxidized at inert electrode and show low oxidation potentials (Sochor, et al., 2013). The voltammograms of compound **1**-**7** were displayed

in Figure 2. All compounds were oxidized in the potential range of 0- 2V. It was found that cyclic voltammograms of all compounds presented the irreversibility of cycles and showed the first apparent oxidation peak at different potentials. The oxidation peaks of compounds **3**, **5** and **6** were equal potential (0.95 V) which was higher than the oxidation peaks of other compounds. Compounds **1**, **2**, **4**, and **7** containing the hydroxy group at C-6 position, showed lower potential of oxidation peaks at 0.64, 0.56, 0.61 and 0.65 V, respectively. The lower potential values of compounds **1**, **2**, **4** and **7** correlated with their higher reducing strength of anti-radical properties of DPPH (low IC_{50}) (Sochor, et al., 2013).

Conclusion and Suggestions

In conclusion, seven isolated natural xanthones; dulcisxanthone B (**1**), ß-mangostin (**2**), 1,3,7-trihydroxy-2,4-di-(3-methylbut-2-eyl)-xanthone (**3**), cudratricusxanthone E (**4**), cochinchinone A (**5**), 2-geranyl-1,3,7-trihydroxy-4-(3,3-dimethylallyl)-xanthone (**6**), and cochinchinone B (**7**) were isolated form twigs of *C. cochinchinense* and *C. formasum*. Compounds **1**, **2**, **4**, and **7** showed good antioxidant activity of IC_{50} values 15.12 - 24.81 µM, comparable to that of Trolox (IC_{50} = 23.27 µM). These compounds also displayed the ability to act as antioxidants, which were obviously seen from their lower oxidation potentials, 0.56-0.65 V. Structure-activity relationship has been discussed from the results of DPPH assay and cyclic voltammetry. It implied that hydroxy moiety at C-6 played an important role to antioxidant power.

Acknowledgments

This work was financial supported by Rajamangala University of Technology Rattanakosin (Grant No. 2559A16902074: A26/2559). We thank Professor Dr. Thawatchai Tuntulani for his facilities and suggestions.

References

Anantachoke, N., Tuchinda, P., Kuhakarn, C., Pohmakotr, M., & Reutrakul, V. (2012). Prenylated caged xanthones: Chemistry and biology. *Pharmaceutical Biology, 50*, 78-91. doi:10.3109/13880209.2011.636176

Antolovich, M., Prenzle, P. D., Patsalide, E., McDonald, S., & McDonald, K. (2002). Methods for testing antioxidant activity. *Analyst, 127*, 183-198. doi:10.1039/B009171p

Arteaga, J. F., Ruiz-Montoya, M., Palma, A., Alonso-Garrido, G., Pintado, S., & Rodriguez-Mellado, J. M. (2012) Comparison of the simple cyclic voltammetry (CV) and DPPH assays for the determination of antioxidant capacity of active principles. *Molecules, 17*, 5126-5138. doi:10.3390/molecules17055126

Bennett, G. J., Harrison, L. J., Sia, G. L., & Sim, K.Y. (1993). Triterpenoids, tocotrienols and xanthones from the bark of *Cratoxylum Cochinchinense*. *Phytochemistry*, *32*, 1245-1251. doi:10.1016/S0031-9422(98)00467-1

Boonnak, N., Chantrapromma, S., Tewtrakul, S., & Sudsai, T. (2014). Inhibition of nitric oxide production in lipopolysaccharide- activated RAW264. 7 macrophages by isolated xanthones from the roots of *Cratoxylum formosum* ssp. *pruniflorum*. *Archives of Pharmacal Research*, *37*, 1329-1335. doi:10.1007/s12272-014-0338-0

Braca, A., Tommasi, N. D., Bari, L. D., Pizza, C., Politi, M., & Morelli, L. (2001). Antioxidant principles from *Bauhinia tarapotensis*. *Journal of Natural Product*, *64*, 892-895. doi: 10.1021/np0100845

Chailap, B., & Tuntulani, T. (2012). Optical and electrochemical properties of heteroditopic ion receptors derived from crown ether- based calix[4]arene with amido- anthraquinone pendants. *Organic and Biomolecular Chemistry*, *10*, 3617-3625. doi:10.1039/ C2OB00048B

Chevion, S., Roberts, M.A., & Chevion, M. (2000). The use of cyclic voltammetry for the evaluation of antioxidant capacity. *Free Radical Biology & Medicine*, *28*, 860- 870. https://doi.org/10.1016/S0891-5849(00)00178-7

Deachathai, S., Mahabusarakam, W., Phongpaichit, S., & Taylor, W. C. (2005). Phenolic compounds from the fruit of *Garcinia dulcis*. *Phytochemistry*, *66*, 2368- 2375. doi:10.1016/j.phytochem.2005.06.025

Duan, Y. H., Dai, Y., Wang, G. H., Zhang, X., Chen, H. F., Chen, J. B., ... Zhang, X. K. (2010). Bioactive xanthones from the stems of *Cratoxylum formosum* ssp. *pruniflorum*. *Journal of Natural Products*, *73*, 1283-1287. doi:10.1021/np1001797

Fotie, J., & Bohle, D. S. (2006). Pharmacological and biological activities of xanthones. *Anti-Infective Agents in Medicinal Chemistry*, *5*, 15-31. doi:10.2174/187152106774755563

Huang, D., Ou, B., & Prior, R. L. (2005). The Chemistry behind antioxidant capacity assays. *Journal of Agricultural and food chemistry, 53*, 1841-1856. doi:10.1021/jf030723c

Iinuma, M., Tosa, H., Tanaka, T., & Riswan, S. (1996). Three new xanthones from the bark of *Garcinia dioica*. *Chemical and Pharmaceutical Bulletin*, *44*, 232-234. doi:10.1248/cpb.44.232

Judzentiene, A., Garjonyte, R., & Budiene, J. (2016). Variability, toxicity, and antioxidant activity of *Eupatorium cannabinum* (hemp agrimony) essential oils. *Pharmaceutical Biology*, *54*, 945-953. doi: 10.3109/13880209.2015.1078384

Laphookhieo, S., Maneerat, W., & Koysomboon, S. (2009). Antimalarial and cytotoxic phenolic compounds from *Cratoxylum maingayi* and *Cratoxylum cochinchinense*. *Molecules*, *14*, 1389- 1395. doi:10.3390/molecules14041389

Lee, B. W., Lee, J. H., Lee, S. T., Lee, H. S., Lee, W. S., Jeong, T. S., & Park, K. H. (2005). Antioxidant and cytotoxic activities of xanthones from *Cudrania tricuspidata*. *Bioorganic and Medicinal Chemistry Letters*, *15*, 5548-5552. doi:10.1016/j.bmcl.2005.08.099

Likhitwitayawuid, K., Phadungcharoen, T., & Krungkrai J. (1998). Antimalarial Xanthones from *Garcinia cowa Planta Medica*, *64*, 70-72. doi:10.1055/s-2006-957370

Mahabusarakam, W., Nuangnaowarat, W., & Taylor, W.C. (2005). Xanthone derivatives from *Cratoxylum cochinchinense* roots. *Phytochemistry*, *67*, 470-474. doi:10.1016/j.phytochem.2005.10.008

Moon, J. K., & Shibamoto, T. (2009). Antioxidant assays for plant and food components. *Journal of Agricultural and Food Chemistry*, *57*, 1655-1666. doi:10.1021/jf803537k

Nuanyai, T., Benjamat, C., Songchan, P., & Anumart, B. (2015). Cytotoxicity of xanthone from twigs of *Cratoxylum cochinchinense* (Lour.) *Blume*. *Journal of Science Ladhrabang*, *24*, 1-12.

Raksat, A., Laphookhieo, S., Cheenpracha, S., Ritthiwigrom, T., & Maneerat, W. (2014). Antibacterial compounds from the roots of *Cratoxylum formosum* spp. *pruniflorum*. *Natural Product Communications*, *9*, 1487-1489.

Rattanaburi, S., Daus, M., Watanapokasin, R., & Mahabusarakam, W. (2014). A new bisanthraquinone and cytotoxic xanthones from *Cratoxylum cochinchinense*. *Natural Product Research*, *28*, 606- 610. doi: 10.1080/14786419.2014. 886212

Sochor, J., Dobes, J., Krystofova, O., Ruttkay-Nedecky, B., Babula, P., Pohanka, M, ... Kizek, R. (2013). Electrochemistry as a tool for studying antioxidant properties. *International Journal of Electrochemical Science*, *8*, 8464-8489.

Tchamo, D. N., Silvere, N., & Etienne, T. (2006). Xanthones as therapeutic agents: chemistry and pharmacology. *Advances in Phytomedicine*, *2*, 273-298. doi:10.1016/S1572-557X(05)02016-7

Udomchotphruet, S., Phuwapraisirisan, P., Sichaem, J., & Tip-Pyang, S. (2012). Xanthones from the stems of *Cratoxylum cochinchinense*. *Phytochemistry*, *73*, 148-151. doi:10.1016/j.phytochem.2010.04. 028

Zou, Y. S., Hou, A. J., Zhu, G. F., Chen, Y. F., Sun, H. D., & Zhao, Q. S. (2004). Cytotoxic isoprenylated xanthones from *Cudrania tricuspidata*. *Bioorganic and Medicinal Chemistry*, *12*, 1947-1953. doi:10. 1016/j.bmc.2004.01.030