p. ISSN: 1411-1047
e. ISSN: 2503-2364

Synthesis andCharacterization of 2,3,4-Trihydroxy-5-methyl Xanthone as Antimalarial Compound

Dhina Fitriastuti[1,*], Jumina[2] and Priatmoko[2]

[a] *Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Islam Indonesia*
[b] *Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada*

* Corresponding author, tel : +62813-3641-4338,
email: dhinaf@uii.ac.id; dhinafitriastuti@yahoo.co.id

ABSTRACT

Synthesis of xanthone derivatives had been conducted to obtain new antimalaria active compounds. The characterization of the synthesized xanthones was also conducted.Synthesis of xanthone was conducted from the raw material of hydroxybenzoic acid and phenol derivatives using Eaton's reagent via acylation-dehydration reaction by modified Grover, Shah and Shah (GSS) method. The synthesis of 2,3,4-trihydroxy-5-methyl xanthone was carried out using gallic acid, *o*-cresol and Eaton's reagent.The mixture was heated for 3 h at 80 °C to yield2,3,4-trihydroxy-5-methyl xanthone in 43% yield as dark red viscous liquid. The results of IR, ^{1}H-NMR and ^{13}C-NMR analysis of sampleshowed that the compound2,3,4-trihydroxy-5-methyl xanthonehas successfully synthesized.

Keywords: xanthone, synthesis, antimalarial

Introduction

Malaria continues to be main health problem and deadly parasitic disease in the world. During 100 years, the world has not given clear contribution to the curing of the disease. In addition, World Health Organization (WHO) (1997) reported that 41% of world population (2.3 billion people) was threatened, 300-500 million were infected, 1.5-2.7 million were died by malaria. In recent years, progress has been made in malaria control as a result of insecticide-treated bed nets and effective treatment. But the development of resistance to insecticides and medicines as well as the poor quality of the health systems in many affected countries pose threats to these achievements (WHO, 1997). Malaria could be treated by oral medication. However, it constantly changes, especially through the development of parasite (such as *P.falciparum*), which is resistance to standard anti malaria drugs, such as chloroquine. Therefore, the discovery and development of new effective anti malaria drugs are urgently required to solve the problems.

p. ISSN: 1411-1047
e. ISSN: 2503-2364

Drug discovery is an effort to address the health problems, including malaria. This could be done by isolating the active compounds from medicinal herbs, which are traditionally and empirically employed to cure malaria and by synthesizing the natural product-analog-compounds. Two medicinal plants which have been clinically proven to display anti malaria activity are *Garcinia* and *Calophyllum*. The herbs are rich in phenolic secondary metabolite of xanthone (Likhitwitayawuid *et al*., 1998; Hay *et al*., 2004; Syamsudin *et al*., 2008; Naidoo, 2009). This class displays several activities of antimalarial, anticancer, anti-HIV, antioxidant, antiinflammatory, as well as antibacterial (Ito *et al*., 1998; Saha *et al*., 2004; Riscoe *et al*., 2005). Despite the fact that they possess good anti malaria activity, the isolation only gave very low yield of 0.55% (Hay *et al*., 2004). Therefore, the synthesis of xanthone is more convenient and advantageous from health, scientific and economic points of view, comparing with the isolation process.

Fotie *et al.* (2003) and Hay *et al.* (2004) postulated that the high antimalarial activity was probably caused by the presence of hydroxyl group on the xanthone skeleton. The number of hydroxyl group also gave positive effect to the antimalarial activity. Ignatuschenko *et al.* (1997) also stated that the potency of hydroxyl-substituted-xanthone on the position 2, 3, 4, 5 and 6 correlated with the ability to inhibit heme polymerization as well as antimalarial by preventing the formation of hemozoin. In addition, Portela *et al.* (2007) concluded that the electronic properties of hydroxyl groups played important role in determining the antimalarial activity. This research consisted of synthesis of xanthone derivative as well as characterization of the synthesized xanthone.

EXPERIMENTAL SECTION

Materials

Chemicals used in this research were gallic acid, *o*-cresol, Eaton's reagent (P_2O_5/CH_3SO_3H), n-hexane, ethyl acetate and distilled water. All chemicals, except Eaton's reagent(was purchased from Sigma Aldrich) and distilled water (was obtained from Laboratory of Fundamental Chemistry, Universitas Gadjah Mada) were purchased from E. Merck with high grade and used without any further purification.

Instrumentation

Analytical mass balance (Mettler AT200), infra red spectrophotometer (IR, Shimadzu-Prestige 21), proton nuclear magnetic resonance spectrometer (^{1}H-NMR, Bruker AC-400 MHz), carbon

p. ISSN: 1411-1047
e. ISSN: 2503-2364

nuclear magnetic resonance spectrometer (^{13}C-NMR, Bruker AC-400 MHz).

Procedure

Synthesis of 2,3,4-trihydroxy-5-methyl xanthone

As much as 0.462 g of gallic acid (2.71 mmol), o-cresol (0.29 g, 2.71 mmol), and Eaton's reagent (5 mL) was heated at 70-80 °C for 3 hours. The mixture was allowed to cool to room temperature, poured into cooled water and stirred for 1 hour. The precipitate was filtered out by using Buchner filtration, and dried in an oven at 50^{o}C for overnight. The crude product obtained was subsequently purified by using column chromatography with gradient elution from 100% n-hexane,n-hexane : ethyl acetate(1:1), until 100% ethanol to give 2,3,4-trihydroxy-5-methyl xanthonein 43% yield (assumed 100% purity of product) as dark red viscous liquid.The product was elucidated with Infra Red spectrophotometer, ^{1}H-NMR, and ^{13}C-NMR spectrometers.

RESULTS AND DISCUSSION

Synthesis of 2,3,4-trihydroxy-5-methyl xanthone was carried out by reacting gallic acid and *o*-cresol with Eaton's reagent. The reaction was monitored by thin layer chromatography. The mixture was heated for 3 h at 70-80 °C. The data of TLC showed that the product is a mixture which had 4 spots while elucidated with n-hexane : ethyl acetate (3:1). The mixture of product was then purified with column chromatography using gradient polarity of solvent. The fraction 1 was identified as 2,3,4-trihydroxy-5-methyl xanthone as a dark red viscous liquid in 43% yield. The product was then characterized by FT-IR, ^{1}H-NMR, and ^{13}C-NMR.

The IR spectrum of condensation product of gallic acid and *o*-cresol was presented in Figure 1. As shown in Figure 1, the hydroxyl group on 2,3,4-trihydroxy-5-methyl xanthone gave broad absorption band at 3425 cm^{-1}. Then, absorption at 1589 and 1512 cm^{-1} together with absorption at 3039 cm^{-1} indicated the existence of the aromatic group. The presence of carbonyl (C=O) group of ketone was overlapped with absorption of aromatic carbon at 1589 cm^{-1}. In addition, absorption at 1273 and 1172 cm^{-1} represented asymmetric and symmetric C-O-C stretching vibrations, respectively. The absorption band at 1357 cm^{-1} and 2931 cm^{-1} represented symmetric bending and asymmetric stretching vibration of C-H methyl, respectively. However, there was a weak peak at 1705 cm^{-1}, which was the characteristic of the carbonyl group

p. ISSN: 1411-1047
e. ISSN: 2503-2364

from carboxylic acid. It presumably came from the remaining gallic acid reactant.

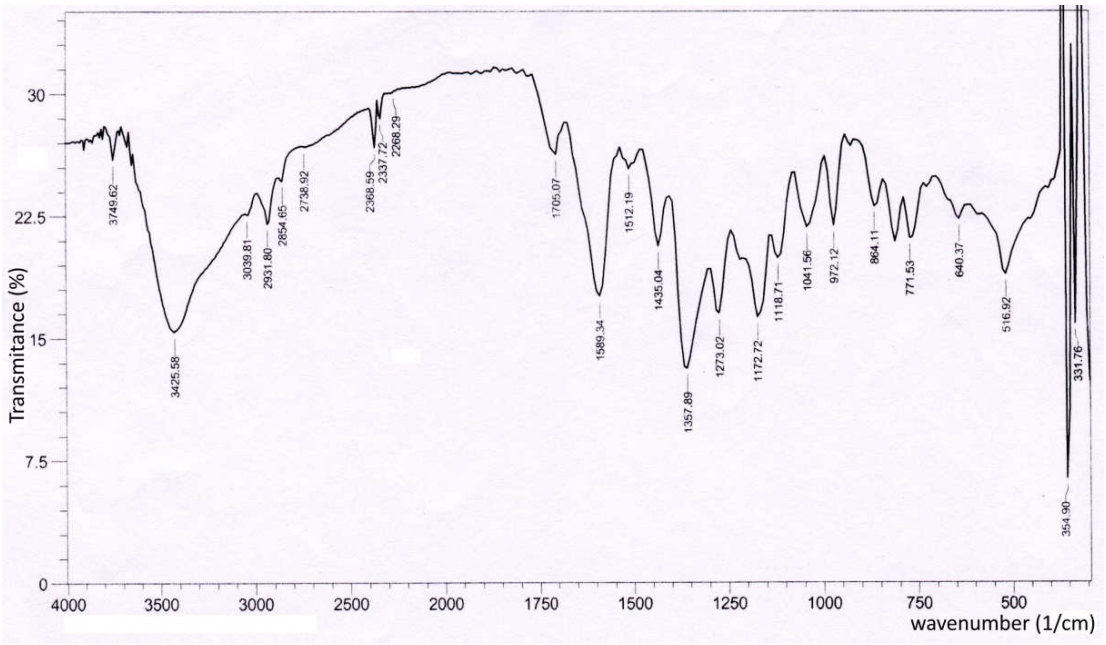

Figure 1. IR spectrum of condensation product of gallic acid and *o*-cresol

^{1}H-NMR spectrum was displayed in Figure 2. ^{1}H-NMR spectrum of the synthesized product showed that there were 4 protons inchemical environment of aromatic group. Signal a (δ 7.48-7.49 ppm, doublet, 1H) belonged to proton of aromatic group which had coupling with proton from *ortho*- position. Signal b(δ 7.29-7.32 ppm, doublet of doublet, 1H) represented proton of aromatic group which had coupling with protons from *ortho*-position in different chemical environment. Signal c (δ 7.65-7.66 ppm, doublet, 1H) came from proton of aromatic group which had coupling with protons from *ortho*- position while signal d which appeared at δ 7.14 ppm with multiplicity singletwas come from proton of aromatic group in different benzene ring. Signal e (δ 2.20 ppm, singlet, 3 H) represented protons of methyl (CH_3-). The presence of hydroxyl group did not appear in this spectrum. However, there were some peaks of impurities, which came from used solvents in purification process (ethanol δ 1.91; 3.52 ppm and methanol δ 3.32 ppm). The real purity of product can not be determined because internal standard does not used in the NMR analysis.

p. ISSN: 1411-1047
e. ISSN: 2503-2364

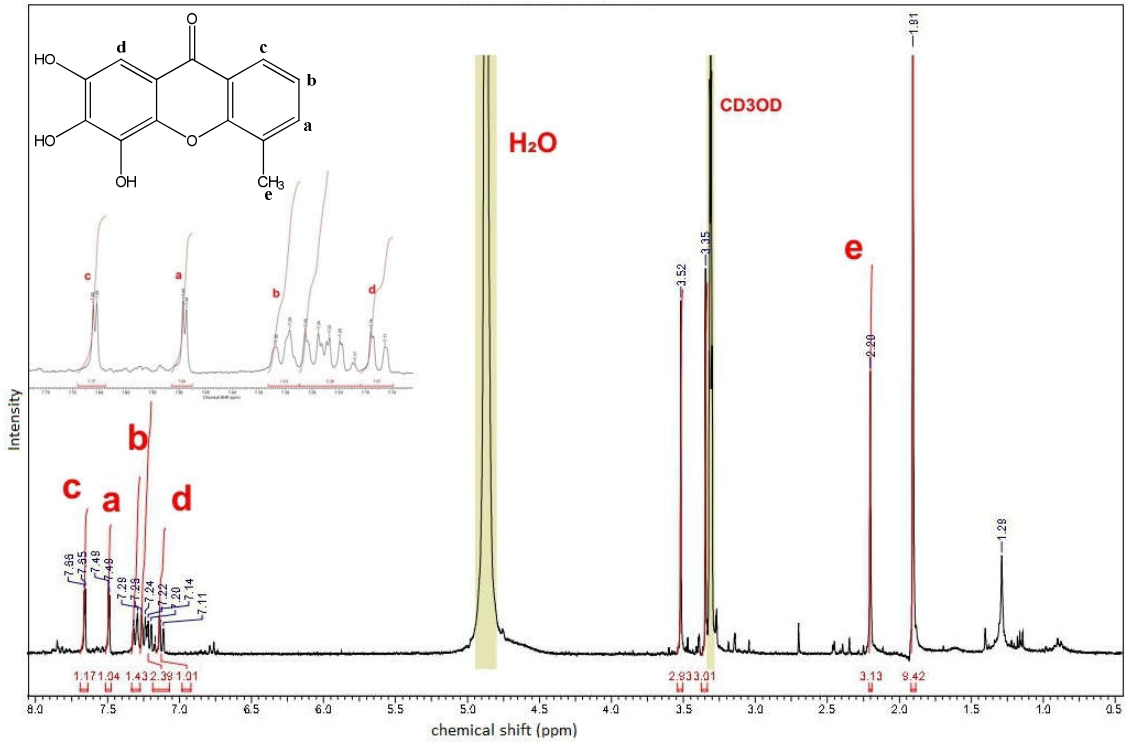

Figure 2.[1]H-NMR spectrum of condensation product of gallic acid and*o*-cresol

Table 1. The data analysis of [1]H-NMR spectrum of condensation product gallic acid and *o*-cresol

Peak	Chemical shift (ppm)	Multiplicity	Integration	Type of proton
a	7.4854-7.4918	Doublet	1H, J=2.56 Hz	Aryl
b	7.2955-7.3177	Doublet of doublet	1H, J=8.88 Hz	Aryl
c	7.6538-7.6605	Doublet	1H, J=2.68 Hz	Aryl
d	7.14	Singlet	1H	Aryl
e	2.20	Singlet	3H	Methyl

Further analysis using [13]C-NMR gave spectrum which was displayed in Figure 3. The twelve peaks in the region of δ 113.53-165.32 ppm belonged to the aromatic carbons from xanthone ring. Then peak at δ 189.38 ppm was produced from carbonyl (C=O) group. The presence of peak at δ 24.02 ppm was coming from methyl (CH_3-) group. Furthermore, carbons of methanol and ethanol also displayed peaks at δ 16.39 and 39.16 as well as 40.99 ppm. The[13]C-NMR spectrum of 2,3,4-trihydroxy-5-methyl xanthone from ChemBioDraw Ultra 11.0 was also displayed in Figure 4 and gave similarities with the experimental spectrum.

p. ISSN: 1411-1047
e. ISSN: 2503-2364

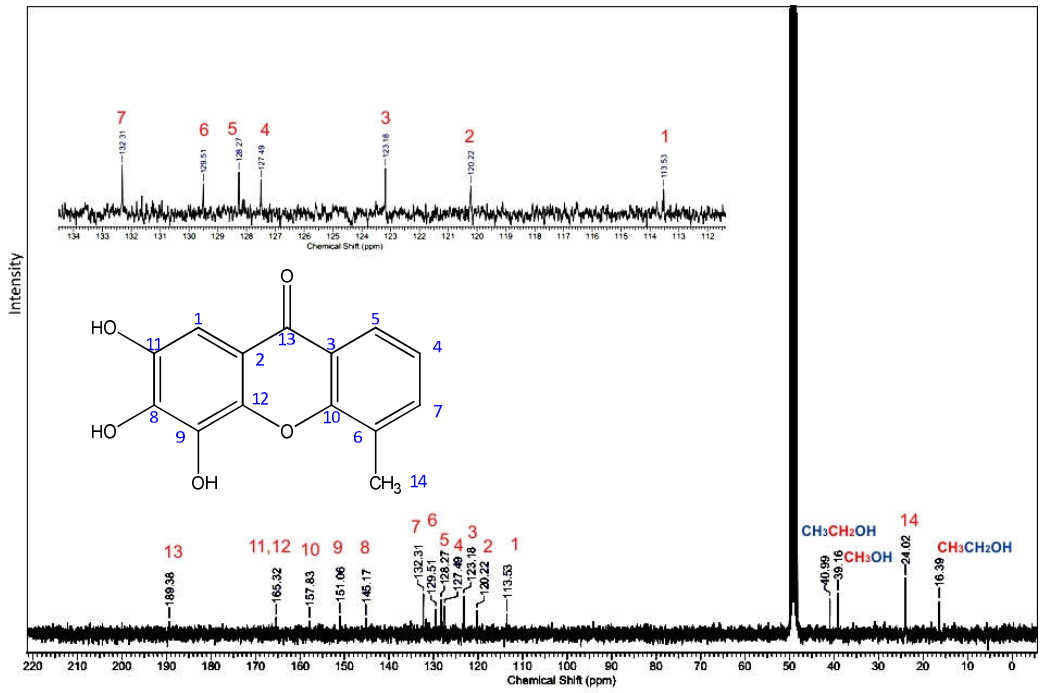

Figure 3.[13]C-NMR spectrum of condensation product of gallic acid and*o*-cresol

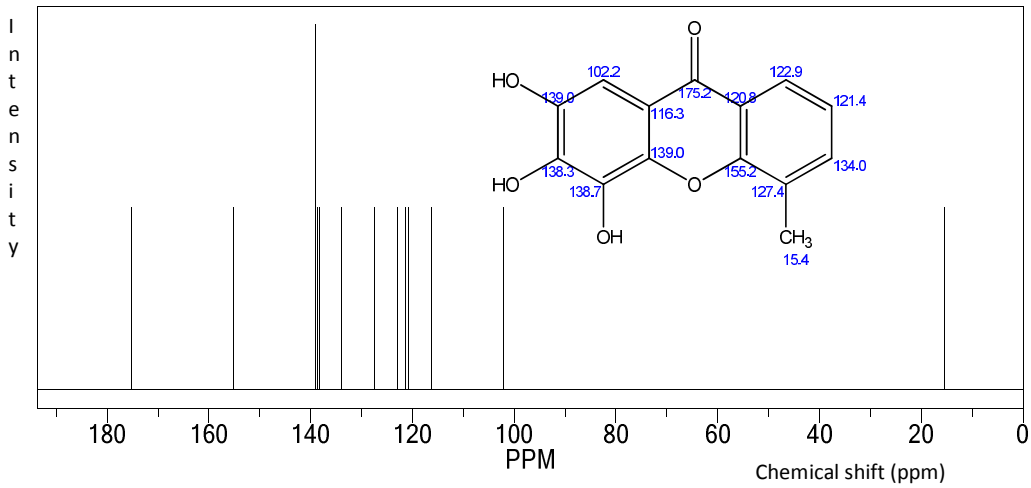

Figure 4. Prediction of [13]C-NMR of 2,3,4-trihydroxy-5-methyl xanthone

Based on FT-IR, [1]H-NMR, and [13]C-NMR analyses, it could be concluded that the 2,3,4-trihydroxy-5-methyl xanthone was successfully synthesized in 43% yield. The reaction mechanism of synthesis of 2,3,4-trihydroxy-5-methyl xanthone was displayed in Figure 6.

p. ISSN: 1411-1047
e. ISSN: 2503-2364

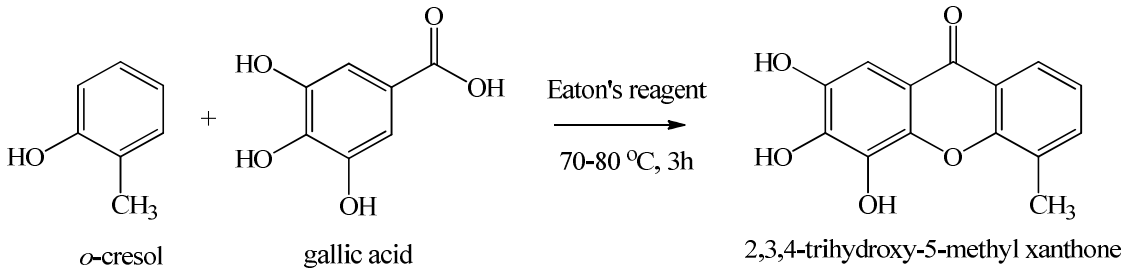

Figure 5. Reaction of synthesis of 2,3,4-trihydroxy-5-methyl xanthone

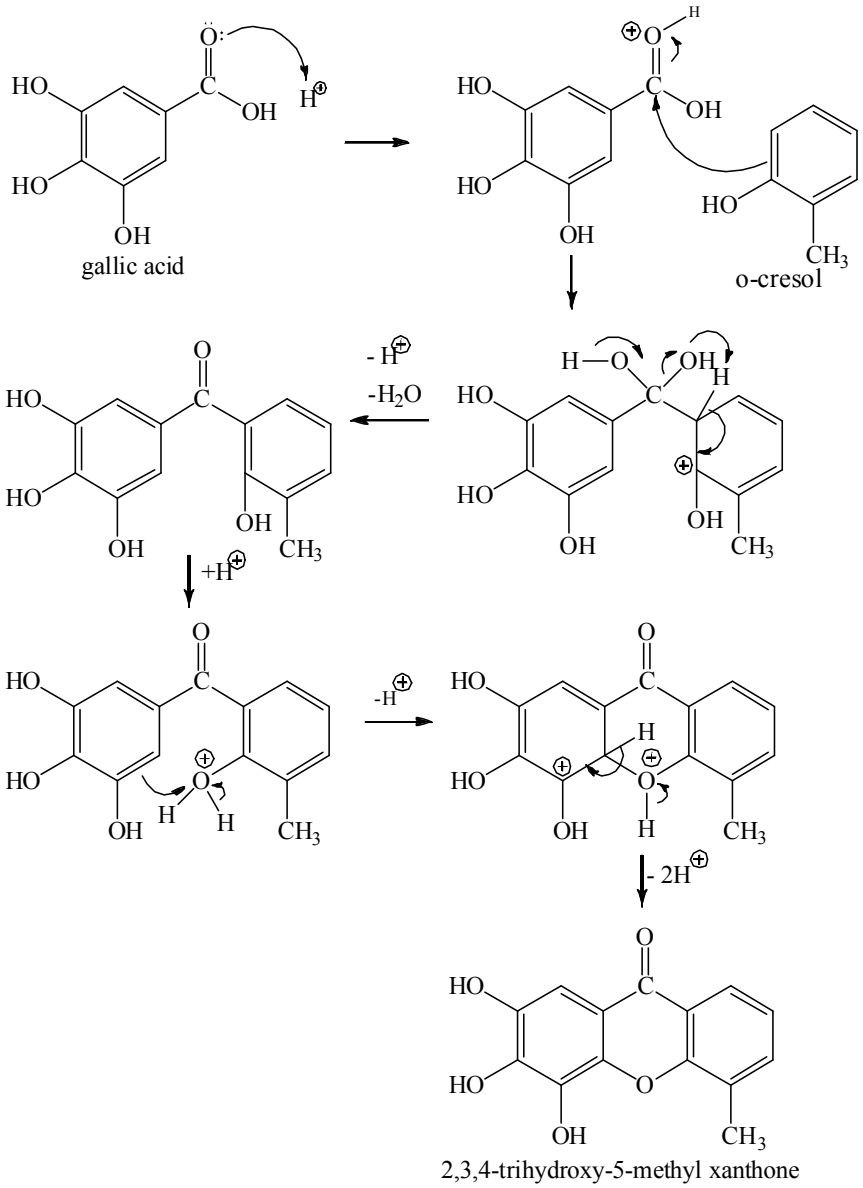

Figure 6. The reaction mechanism of synthesis of 2,3,4-trihydroxy-5-methyl xanthone

CONCLUSION

The reaction between gallic acid, o-cresol and Eaton's reagent yielded 2,3,4-trihydroxy-5-methyl xanthone in 43% yield as dark red viscous liquid.The results of IR, ^{1}H-NMR and ^{13}C-NMR analysis of sample showed that the compound 2,3,4-trihydroxy-5-methyl xanthone has successfully synthesized.

ACKNOWLEDGEMENTS

This research was funded by LPDP-Thesis scholarship from Ministry of Financial, Indonesian Government.

REFERENCES

Fotie, J., Nkkengfack, A.E., Rukunga, G., Tolo, F., Peter, M.G., Heydenreich, M., and Fomum, Z.T., 2003. In vivo antimalarial activity of some oxygenated xanthones. *Annals of Tropical Medicine and Parasitology*, 97 (7), 683–688.

Hay, A., E., He'lesbeux, J., J., Duval, O., LabaRed, M., Grellier, P. and Richomme, P., 2004, Antimalarial Xanthones from *Calophyllum caledonicum* and *Garcinia vieillardii*, *J. Lif. Sci.*, 75, 3077-3085.

Ignatuschenko, M.V., Winter, R.W., Bachinger, H.P., Hinrichs, D.J., and Riscoe, M.K., 1997.

Ito, C., Itoigawa, M., Furukawa, H., Rao, K.S., Enjo, F., Bu, P., Takayasu, J., Tokuda, H., Nishino, H., 1998. Xanthones as inhibitors of Epstein-Barr virus activation. *Cancer Lett*. 132, 113–7.

Likhitwitayawuid, K., Phadungcharoen, T., Krungkrai, J., 1998. Antimalarial xanthones from Garcinia cowa. *Planta Med*. 64, 70–2.

Naidoo, J. M., 2009. Novel methodology for the synthesis of xanthones. *Thesis*, University of the Witwatersrand, Johannesburg.

Portela, C., Afonso, C.M.M., Pinto, M.M.M., Lopes, D., Nogueira, F., do Rosário, V., 2007. Synthesis and antimalarial properties of new chloro-9H-xanthones with an aminoalkyl side chain. *Chem. Biodivers*. 4, 1508–19.

Riscoe, M., Kelly, J., and Winter, R., 2005. Xanthones as Antimalarial Agents: Discovery, Mode of Action, and Optimization. *Curr. Med. Chem*. 12, 2539–2549.

Saha, P., Mandal, S., Das, A., Das, P.C., and Das, S., 2004. Evaluation of the anticarcinogenic activity of Swertia chirata Buch.Ham, an Indian medicinal plant, on DMBA-induced mouse skin carcinogenesis

p. ISSN: 1411-1047
e. ISSN: 2503-2364

model. *Phytother. Res.* 18, 373–8.

Syamsudin, Soesanto, T., Wahyuono Subagus, and Mustofa, 2008. In vivo Antiplasmodial Activity and Acute Toxicity of the Fraction of the Garcinia parvifolia Miq. Stem Bark. *J. Pharmacol. Toxicol.* 3, 324–329.

WHO, 1997, *Roll Back Malaria*, *A Global Partnership*, Geneva.