Isolation and Structure Elucidation of Soulatro Coumarin From Stem Bark of Calophyllum soulattri Burm F and In Vivo Antiplasmodial Activity by Using Mice Infected by Plasmodium berghei

Jamilah Abbas¹*, Achmad Darmawan¹ and Syafruddin²

¹Research Centre for Chemistry, Indonesian Institute of Sciences
PUSPIPTEK. Serpong. 15314. Indonesia
²Eijkman Institute for Molecular Biology,
Diponegoro street no 69, Jakarta 104310. Indonesia

Abstract

The soulatro coumarin compound was isolated and elucidated from the stem bark of Calophyllum soulattri Burm F, the samples were collected from Jayapura Papua Irian Island in Indonesia. Isolation process was done by maceration at room temperature in methanol, than partitioned in a mixture of n hexane-water (1:1), followed by dichloromethane-water (1:1) and ethyl acetate-water (1:1). A portion of ethyl acetate extract was subjected to column chromatography over silica gel packed and eluted with n-hexane a gradient of ethyl acetate to 100% followed by CHCl₃ in MeOH (20:1, 10:1, 5:1, 1:1). Fraction B (CHCl₃ in MeOH 20:1) was subjected to column chromatography over silica gel 300 mesh and eluted with EtOAc-MeOH mixtures of increasing polarity. Fraction with the same Rf value were combined and eluted with EtOAc-MeOH (19:1) showed one spot on TLC. They were combined and evaporated to yield a solid than was recrystallized in mixture of CH₂Cl₂-methanol to give soulatro coumarin compound. The structure was determinated by spectroscopic analysis, in particular by 1D and 2D NMR techniques, from these spectra data conclution that compound is soulatro coumarin. Antimalarial assay was tested against Plasmodium berghei parasite as in vivo using 18 mices rodent wich was infected by Plasmodium berghei parasite. The soulatro coumarin showed activity against P. berghei with dosage 0.005867 mM/1 kg body weight; 0.05867 mM/1 kg bw; 0.5867 mM/1 kg bw and 58.67 mM/1 kg bw could inhibit growth rate of parasite = 57.32%; 63.37%; 43.02%; 47.67% respectively.

Keywords: Antiplasmodial activity, coumarin, Calophyllum soulattri Burm. F, in vivo, Chloroquine, Plasmodium berghei.

INTRODUCTION

Calophyllum soulattri Burm F [(local name: Sulatri (Sunda), slatri (Java), malang-malang (Bangka)]. Vernacular Name : bintagor labu (Malaysia). Bintagor lanchar, mintak (Peninsular). C. soulattri is a wide spread but in many places rather uncommon tree of fairly small dimentions, growing in lowland or lower montane rain forest or sometimes in swamp forest, up to 1700 m altitude. (Soerianegara et al., 1994). C. soulattri distributed in West Java, Central Java, Irian and Kalimantan Island and also distributed in Vietnam, Cambodia, Thailand, Andaman, Solomon Island, Northern Australia and Malaysia (Suryanegara et al., 1994). In these research the stem bark of C. soulattri Burm F (Guttiferae Family) was used and collected from Jaya Pura (Irian Island) in Indonesia.

The bark, roots and latex of C. soulattri are used in local as folk medicine. The fruits are edible but sour. The latex may be used to poison dogs.

*Corresponding author email: jamilahabbas@yahoo.com
A number of medicinal and therapeutic properties have been described to various parts of *C. soulattri*, e.i root and leaves, including the treatment of rheumatism, varicose veins, hemorrhoids and chronic ulcers (Crane *et al.*, 2005).

Root and leaves of *C. soulattri* also used as rheumatic pain. Seed or other species are used for scabies, ringworm, as an analgesic in leprosy, dermatoses, as diuretic, gonorrhea, tumors, migraine and vertigo (Crane *et al.*, 2005). In the Bulolo area in Papua New Guinea (PNG) the plant used for several diseases, such as dry skin, high blood pressure and malaria (Khan *et al.*, 2002). The balsem from the bark of *Calophyllum sp* used as a cicatrisan, infusion or decoction of the leaves has been traditionally used as an eye remedy in Asian (Inuma *et al.*, 1993). Prenylated xanthones have been reported to show antibacterial and antifungal activities (Dharmaratne *et al.*, 1997). The anticancer activity of new coumarin was also investigated against HeLa cell line and the new coumarin showed the most potent as anticancer (Abbas, 2008).

We now report the isolation, structural elucidation and evaluation of the antiplasmodial activity of natural soulatro coumarin isolated from *C. soulattri*, in vivo evaluation of the antiplasmodial activity has been done by using mice which it’s infected by *Plasmodium berghei*.

The Genus Calophyllum is well known as a source of biologically active compound such us xanthones (Reye-Chipah *et al.*, 1997; Kijjoa *et al.*, 2000; Limuna, M *et al.*, 1993), terpenoids (Cao *et al.*, 1998), neoflavonoid (Dharmaratne *et al.*, 1984). Coumarin (Guilet *et al.*, 2001 and Ito *et al.*, 2003). Xanthones, terpenoids, neoflavonoid were found in related species.

Kalazeyloxanthone has been isolated from the root bark of *C. soulattri* by Dharmaratne (Dharmaratne *et al.* at 1984) (Fig.1)

![Figure 1. Structure of kalazeyloxanthone](image1)

Dombakinaxanthone (diprenylated chromexanthone) has been isolated from the cold hexane extract of the root bark of *C. soulattri* (Dharmaratne *et al.*, 1993) (Fig. 2). Jayapuracoumarin has also been isolated from stem bark of *C. soulattri* that Jayapuracoumarin has antioxidant and antiplasmodial activity (Abbas *et al.*, 2009) (Fig. 3). One new coumarin (aziz coumarin) has also been isolated from stem bark of other species of *Calophyllum*, that coumarin has antimalarial and cytotoxic activity (Abbas *et al.*, 2009) (Fig. 4). One kalocoumarin (brasimarin C) have also success isolated from *C incrassaptum*, that kalocoumarin has antimalarial activity too (Abbas *et al.*, 2009) (Fig. 5).
Three coumarin (teysmanone A, teysmanone B, calanone) and two coumarin (iophyllum C and iophyllum D) have been isolated from C. teysmannii and C. inophyllum respectively (Cao 1998). Two soulatro coumarine (pyranocoumarin) has been previously reported from stem bark and leaves of C. soulattri by Samarawera (1983), and one coumarin (soulatrolide) has also been isolated from stem bark of C. soulattri (Fig. 6).
Other authors reported soulatro coumarin as anticancer (Cao 1998a), anti-HIV (Gustafon, 1994) and three new coumarins also has cancer chemopreventive activity (Ito et al., 2003). In this communication, we report the isolation and structure elucidation of a soulatro coumarin from stem bark of C. soulattri as antiplasmodial. C. soulattri was chosen as a source of antiplasmodial drug candidate, because C. soulattri content some coumarin compounds, and according to some references, coumarin have antiplasmodial activity. Resistance of plasmodium to antimalarial drugs and their toxicity are contributing factors to the spread of the disease. This highlights the need to develop quickly more effective and less toxic new antimalarial drugs from C. soulattri. The isolation of soulatro coumarin will be explained in the experimental procedures.

MATERIALS AND METHODS

General Experimental Procedures

Melting point was measured on a Fisher Scientific serial 903N0056 apparatus. UV spectra was measured on a Hitachi UV-VIS spectrophotometer using methanol solution, \( \lambda_{\text{max}} \) in nm : IR spectra were measured on a FTIR Shimadzu Prestige 21 as KBr disc : \( \nu \) in \( \text{cm}^{-1} \). \(^{13}\)C-NMR (150 MHz), \(^{1}\)H-NMR (500 MHz), and 2D NMR experiment (COSY, HMBC, HMQC) spectra were recorded on a Jeol – JNM ECA 500 MHz spectrophotometer, CDCl\(_3\) was used as solvents, TMS as internal standard : \( \delta \) in ppm, \( J \) in Hz. Mass spectrometry analysis was performed on a Mariner Bio Spectrometry, equipped with a pneumatically assisted electrospray ionization (ESI) source. Silica gel (Merck 200 mesh and 300 mesh) were used for column chromatography. Precoated silica gel at aluminium plates (silica gel 60 F\(_{254}\)) were used for analytical TLC. Spot were visualized by UV (254 and 365 nm), FeCl\(_3\) and 10% \( \text{H}_2\text{SO}_4 \) were used as spraying reagent followed by heating at 110°C.

Plant Material

The stem bark of C. soulattri was collected from Irian Island, Indonesia and the voucher specimen was identified and deposited at the Herbarium Bogorience – LIPI. Bogor, Indonesia.

Extraction and Isolation of Plant Materials

The dried and powdered of the stem bark of C. soulattri (1 kg) was masedated by methanol (3 x 2 litre x 7 days), solvent was removed under reduced pressured by rotary evaporator to gave 109,47 g methanol extract, which was then suspended in water and partitioned with \( n \)-hexane, dichloromethane and ethyl acetate. Each solvent were removed under pressure by rotary evaporator to give \( n \)-hexane extract (38.39 g, 3.84%), dichloromethane extract (25.05 g, 2.51%) and ethyl acetate extract (25 g, 2.50%).

The ethyl acetate extract (25 g) was separated by column chromatography on silica gel 70-230 mesh (Merck 800 g) by using \( n \)-hexane a gradient of ethyl acetate to 100% followed by CHCl\(_3\) in MeOH (20:1, 10:1, 5:1, 1:1). Fraction B from (CHCl\(_3\) in MeOH 20:1) was subjected to column chromatography (60 cm x 6 cm i.d) on silica gel 300 mesh (Merck code 1.07734) eluting by EtOAc : MeOH with a step gradient elution). Fraction with the same \( R_f \) values were combined and eluted with EtOAc-MeOH (19:1) and showed one spot on

---

**Figure 7. Structure of soulatrolide from C. Soulattri**
TLC than was recrystalized by using CH$_2$Cl$_2$ and methanol, to produce coumarin compound (500 mg) as pale yellow powder with mp 83-84°C (from fraction 7a). Moleculer weight 426.11. Identically by comparison of $^1$H-NMR, $^{13}$C-NMR and MS data with literature values (Cao et al. 1998b).

**Determination of the Antimalarial Activity.** (Trager and Jensen, 1976 and Fotie et al., 2003)

P. berghei was maintained continuously in culture on human erythrocytes as described by Trager and Jensen (Trager and Jensen, 1976). Antimalarial activity of the soulatro coumarin compound was determined in vivo.

In brief soulatro coumarin 2.5 mg (BW 426,11) was dissolved in 100 μl DMSO to give 58,670 mM as stock solution and kept at -20°C until used. The DMSO concentration never exceeded 0.1% and did not inhibit the parasite growth (Fotie, 2004). Antimalarial activity was done in-vivo against P. berghei using mice strain Swiss. Groups of 18 mice strain Swiss, weighing 20-24 g (2-3 month old) were used. The animals had free access to standard commercial diet and water ad libitum in a 12 h/12 h light-dark cycle at 22°C and must be aclimation by giving antibiotic for 3 days before be used. Mice were injected by 100 μl P.berghei parasite (pi), inoculaton for 10-14 days, take blood from the vena of mice’s tail after 2 days, calculated amount of parasite, if total amount of parasites in the mice blood under 20 %, take blood from the vena of mice’s tail after 3 or 4 or 5 or 6 or 10 days inoculation, calculated amount of parasite, if total of parasite in the mice blood up to 20 %, its mean those mices was positive infected by P.bergehi parasite, than all mice already be used for antipasmodial evaluation. Inject six dosages of soulatro coumarins to the mice each 100 μl : 0.0005867 mM/kg body weight: 0.005867 mM/kg body weigh; 0.05867 mM/kg body weight, 0.5867 mM/kg body weight, 5.867 mM/kg body weight and 58.670 mM/kg kg body weight. Take blood from the vena of mice’s tail after zero day (D0), one day (D1), two days (D2), three days (D3) and four days (D4) inoculation, were then calculated % parasites by making a blood smear, fixed with methanol and stained with Giemsa stain. Number of the infected red blood cells were counted under the microscope. Injected the chloroquine to the mice 2.5 mg/kg body weight, chloroquine was used as a positive control.

The concentration response parasite growth data were analyzed by a linear regression function using the Sigma-plot 2000 computer program to determine the 50% inhibitory concentration (IC$_{50}$). The IC$_{50}$ value is defined as the concentration of compound producing 50% parasite growth inhibition relative to untreated control (without give the soulatro coumarins sample).

**RESULTS AND DISCUSSION**

Coumarin compound gave a positive reaction with FeCl$_3$ reagent in methanol (-blue spot) (that mean coumarin contain OH groups, the OH with FeCl$_3$ become Fe(OH)$_3$ blue spot. This structure was established by spectroscopic means. In the LC-MS spectrum (Fig. 11), showed the moleculer ion (M+1)$^+$ at m/z = 427.11 and moleculer ion (2M)$^+$ at m/z = 852.48 and also (2M + Na)$^+$ at m/z = 874.48, corresponding to the molecular formula of C$_{25}$H$_{30}$O$_6$ and moleculer weigh 426.11 (Fig. 8c).
Figure 8a. $^1$H- NMR
Chemical shift of soulatro coumarin

Figure 8b. $^{13}$C- NMR
Chemical Shift of soulatro coumarin

Figure 8c. Structure of soulatro coumarin

Figure 9. HMBC correlation of new soulatro coumarin
Table I. $^1$H and $^{13}$C NMR spectral data for soulatro coumarin

| Carbon | $^{13}$C-NMR | $^1$H-NMR | HMBC correlation |
|--------|-------------|-----------|------------------|
| 1      | 166.1       |           |                  |
| 2      | 111.4       | 6.13 (1H, s) | C-2, C-4, C-1'  |
| 3      | 156.1       |           |                  |
| 4      | 107.2       |           |                  |
| 4a     | 148.4       |           |                  |
| 5      | 110.4       |           |                  |
| 6      | 211.0       |           |                  |
| 7      | 49.3        | 4.06 (2H, $J = 6.7$) | C-5a, C-6, C-9a |
| 8      | 77.2        | 2.34 (1H, $J = 7.95$) | C-7, C-6, C-9a |
| 9      | -           |           |                  |
| 9a     | 157.6       |           |                  |
| 10     | 109.9       |           |                  |
| 10a    | 165.6       |           |                  |
| 11     | 211.3       |           |                  |
| 12     | 14.3        | 1.23 (3H, $d J = 9.25$) | C-7, C-8 |
| 1'     | 50.6        | 0.92 (2H, $d J = 4.25$) | C-3', C-4', C-4a |
| 2'     | 113.10      | 0.87 (1H, $t J = 7.35$) | C-3, C-4, C-5' |
| 3'     | 135.1       | -         |                  |
| 4'     | 21.9        | 1.25 (3H, s) | C-1', C-2', C-12' |
| 5'     | 23.0        | 1.25 (3H, s) | C-1', C-2', C-12' |
| 12'    | 55.4        | 5.47 (2H, $d J = 15.8$) | C-10, C-11, C-27', C-37' |
| 22'    | 40.4        | 1.74-1.77 (2H, m) | C-10, C-11, C-27', C-37', C-47' |
| 32'    | 38.4        | 1.74-1.77 (2H, m) | C-11, C-12', C-37', C-47' |
| 42'    | 29.1        | 1.67 (1H, m) | C-37', C-52', C-62' |
| 52'    | 19.5        | 1.26 (3H, $d J = 4.3$) | C-42', C-52', C-62' |
| 62'    | 19.5        | 1.26 (3H, $d J = 4.3$) | C-42', C-52', C-62' |

Figure 10. Structure of Soulatro coumarin and teysmanone. (A) Soulatro coumarin from stem bark C. soulatri, (B) Coumarin (teysmanone) from reference (Cao 1998)
Table II. Differenes of $^{13}$C NMR spectral data of new soulatro coumarin and other coumarin (teysmanone Cao 1998)

| Carbon | $^{13}$C-NMR new coumarin (Soulatro coumarin) | $^{13}$C-NMR Coumarin (teysmanone) (Reffence, Cao 1998) |
|--------|---------------------------------------------|--------------------------------------------------|
| 1      | -                                           | 158.9                                            |
| 2      | 166.1                                       | 152.5                                            |
| 3      | 111.4                                       | 101.1                                            |
| 4      | 156.1                                       | 149.9                                            |
| 4a     | 107.2                                       | 106.3                                            |
| 5      | 148.4                                       | 115.4                                            |
| 5a     | 110.4                                       | 47.2                                             |
| 6      | 211.0                                       | 79.5                                             |
| 7      | 49.3                                        | 113.4                                            |
| 8      | 77.2                                        | 101.4                                            |
| 9      | -                                           | 9a 157.6                                         |
| 10     | 109.9                                       | 157.6                                            |
| 10a    | 165.6                                       | 109.9                                            |
| 11     | 211.3                                       | 106.4                                            |
| 12     | 14.3                                        | 191.9                                            |
| 1'     | 50.6                                        | 135.9                                            |
| 2'     | 113.10                                      | 127.5                                            |
| 3'     | 135.1                                       | 130.1                                            |
| 4'     | 21.9                                        | 130.6                                            |
| 5'     | 23.0                                        | 130.1                                            |
| 12'    | 55.4                                        | 137.6                                            |
| 22'    | 40.4                                        | 129.6                                            |
| 32'    | 38.4                                        | 128.5                                            |
| 42'    | 29.1                                        | 135.3                                            |
| 52'    | 19.5                                        | 128.5                                            |
| 62'    | 19.5                                        | 129.6                                            |

Figure 11. LC-MS spectra of soulatro coumarin compound (Column C18 with 80% methanol-water as eluent)

In the IR spectrum, absorption bands attributable to an OH (3352 cm$^{-1}$ sharp) and C=O groups from nucleus coumarin (1697 and 1390 cm$^{-1}$ for C-O), aromatic ring (C=C) at 1604 and 1558 cm$^{-1}$ and C-H streching methyl group at 2964 and 2872 cm$^{-1}$ and C-H bending methyl at 850, 889 and 933 cm$^{-1}$ (Fig. 12).
UV absorption maximum at λ 221.5 nm with absorbance 0.394 typical of an oxygenated coumarin and at λ 257.0 with absorbance 0.205 and at λ 331.0 nm with absorbance 0.590 (C 10 ppm in methanol). These UV spectrum showed the typical absorptions of a prenylcoumarin nucleus at \( \lambda_{\text{max}} = 221.5 \), \( 257.0 \) and \( 331.0 \) nm (Fig. 13). UV spectra of soulatro coumarin compounds is similar with Ref (\( \lambda_{\text{max}} 228 & 286 \) and 324 nm, Cao 1998b, Ito et al., 2003).

The \(^1\text{H}-\text{NMR}\) spectrum (500 MHz in CDCl\(_3\)) revealed signal assignable to a prenyl group at C-4, and additional one proton singlet at \( \delta 6.13\) (1H, s) specific at position C-3. Proton singlet at \( \delta 6.13\) correlated to the C-atom at \( \delta 111.4\) in the HMOC spectrum. The HMOC spectrum proton singlet at \( \delta 6.13\) showed long-range correlation to C-signals at \( \delta 165.6\) (C-10a), 156.1 (C-4), 148.4 (C-5) and 50.6 (C-1\'). The C-atom at 50.6 (C-1\') was also correlated to the aliphatic proton at C-3, C-4, C-1' and C-2'. The correlation between proton at C-3 and carbon at C-2, C-4, C-1' and C-2' allowed the position of prenyl group at C(4) (Table I and Fig. 10) which confirmed that the isoprenyl group was attached at C-4 of the coumarin moiety.

HMOC-2D data illustrate the existence of a single isoprenyl group substitution at position C-4 and one hydroxyl at position C-5 and one carbonyl at C-6, that new compound were given name soulatro coumarin. The \(^1\text{H}-\text{NMR}\) and \(^{13}\text{C}-\text{NMR}\) spectral data of new soulatro coumarin as in Table I, Fig. 8a and 8b. The structure of the new soulatro coumarin was showed in Fig. 8c and HMOC correlation of a new soulatro coumarin was showed in Fig. 9.

The \(^{13}\text{C}-\text{NMR}\) spectrum revealed an aliphatic ketone (\( \delta 211.3\)), a conjugated lactone (\( \delta 116.1\)), a monosubstituted methyl (\( \delta 14,3, 1x\))
Me), a fully substitute benzen ring bearing three oxygen moities δ 157.6, 109.9, 165.6, 148.4, 110.4, 107.2. These data (Table I) suggested that compound from *C. soulatri* as a new coumarin (soulatro coumarin). A comparison of soulatro coumarin with teymanone (Cao et al., 1998a) showed considerable differences of both chemical shifts of 13C-NMR (Table II).

The COSY spectrum (Fig. 14) showed the connection of a doublet signal at δ 4.06 and a doublet signal at δ 1.23 (J = 9.2 Hz), other connectivity could be shown between a multiplet signal at δ 1.74-1.77 and doublet signal at 1.26 (J= 4.3), also other connectivity could be shown between a multiplet signal at δ 1.74-1.77 and multiplet signal at 1.67.

Soulatro coumarin have antiplasmodial activity with inhibiton growth rate of paracites but were not depend of samples concentrations (Table III). Chloroquine was used as positive control (Table IV and Fig. 15).

The result of antiplasmodial activity of the sample of soulatro coumarin are recorded in Table III. The sample soulatro coumarin showed activity against *P. berghei* with dosage 0.0005867 mM/1 kg bw; 0.005867 mM/1 kg bw; 0.05867 mM/1 kg bw; 0.5867 mM/1 kg bw and 58.67 mM/1 kg bw could inhibit growth rate of parasite 57.32%; 63.37%; 53.49%; 43.02%; 47.67% respectively. Soulatro coumarin shown activity against *Plasmodium berghei*, but it is not dose dependent.

### Table III. Result of antiplasmodial activity of soulatro coumarin compound as *in vivo*

| Soulatro coumarin Concentration (mM) | Mean Parasetemia (%) | Growth rate (%) |
|------------------------------------|----------------------|-----------------|
|                                    | Mean DO | Mean D1 | Mean D2 | Mean D3 | Mean D4 |                |
| 0.0005867                          | 3.575   | 5.000   | 4.300   | 4.225   | 4.175   | 100.00         |
| 0.005867                           | 3.575   | 5.000   | 2.465   | 4.450   | 4.150   | 57.32          |
| 0.05867                            | 3.575   | 4.100   | 2.725   | 2.900   | 3.150   | 63.37          |
| 0.5867                             | 3.575   | 3.275   | 1.850   | 3.400   | 4.325   | 43.02          |
| 5.8670                             | 3.575   | 2.950   | 2.300   | 3.650   | 4.450   | 53.49          |
| 58.670                             | 3.575   | 2.950   | 2.050   | 2.175   | 3.600   | 47.67          |
Table IV. Growth rate inhibition of *Plasmodium* parasite by six dosage of the chloroquine

| Cloroquine Concentration (mM) | Parasetemia (%) | Growth rate (%) | IC<sub>50</sub> (mMol) |
|------------------------------|-----------------|----------------|---------------------|
|                              | Mean D0 D2 D2  | Mean D2 D2  |                     |
| 3 x 10<sup>-7</sup>          | 0.45 0.5 0.475 | 3.7 4.0 3.85 | 100.00              |
| 3 x 10<sup>-6</sup>          | 0.45 0.5 0.475 | 2.3 2.8 2.55 | 66.23               |
| 3 x 10<sup>-5</sup>          | 0.45 0.5 0.475 | 0.3 0.8 0.55 | 14.29 3 x 10<sup>-4</sup> |
| 3 x 10<sup>-4</sup>          | 0.45 0.5 0.475 | 0.08 0.09 0.085 | 2.21 |
| 3 x 10<sup>-3</sup>          | 0.45 0.5 0.475 | 0.03 0.04 0.035 | 0.91 |
| 3 x 10<sup>-2</sup>          | 0.45 0.5 0.475 | 0.01 0 0.005 0.13 |                   |

Figure 15. Effect of six dosage of chloroquines in growth rate inhibition of *Plasmodium* parasite (%)

The chloroquine showed activity against *Plasmodium* with dosage 3 x 10<sup>-7</sup>, 3 x 10<sup>-6</sup>, 3 x 10<sup>-5</sup>, 3 x 10<sup>-4</sup>, 3 x 10<sup>-3</sup> and 3 x 10<sup>-2</sup> could inhibit growth rate of parasite 0%; 33.77%, 85.71%; 97.79%; 99.0.9% and 99.87% respectively. Growth rate inhibition of *Plasmodium* parasite by chloroquines are depend of dosage of chloroquines. The chloroquine displayed significant inhibition of growth of *Plasmodium* parasite with IC<sub>50</sub> = 3 x 10<sup>-9</sup> M or = 3 x 10<sup>-6</sup> mM or = 0.03 μM, but growth rate inhibition of *Plasmodium* parasite by soulatro coumarin are note depend of dosage of soulatro coumarin. Chloroquine have shown the best growth rate inhibition of *Plasmodium* parasite, with IC<sub>50</sub> = 3 x 10<sup>-9</sup> M or = 0.03 μM indicating that chloroquine are the most potent compound compared with benzophenon coumarin structures, in association with the biological evaluation, is providing interesting improvement of the antiplasmodial activity. Firstly, the position of the hydroxyl groups at C-5 appears to be important as indicated of activity observed in the present report. Secondly, the substitution by a 1,1-dimethylallyl chain, appear to be factors for good activity. So far, the antiplasmodial potency of solatro coumarin correlated well with its ability to inhibit in vivo heme polymerization, suggesting that this compound exerts its antimalarial action by preventing hemoglobin formation (Ignatuschenko et al., 1997). IC<sub>50</sub> values were obtained from doublet experiments.

**CONCLUSION**

As a conclusion, new soulatro coumarin showed moderate activity on a chloroquino-resistant strain of *Plasmodium berghei*. The natural approaches developed here to isolate coumarin structures, in association with the biological evaluation, is providing interesting improvement of the antiplasmodial activity. Firstly, the position of the hydroxyl groups at C-5 appears to be important as indicated of activity observed in the present report. Secondly, the substitution by a 1,1-dimethylallyl chain, appear to be factors for good activity. So far, the antiplasmodial potency of solatro coumarin correlated well with its ability to inhibit in vivo heme polymerization, suggesting that this compound exerts its antimalarial action by preventing hemoglobin formation (Ignatuschenko et al., 1997). IC<sub>50</sub> values were obtained from doublet experiments.

**ACKNOWLEDGEMENT**

Thanks are due to Mr. Ismail Rahman at the Herbarium Bogoriences - LIPI Bogor for identifying the plant material. The authors are grateful to Puji at Eijkman Institute for bioassay and Sofa Fajriah for the NMR measurement and Puspa Dewi for MS measurement.
REFERENCES

Abbas, J., 2008, Doctoral Disertation, Isolation and structure elucidation biological active compound from C. soulatttii Burm. F., C. incrasaptum M. R. Henderson and Wyatt Smith and C. tomentosum Wright., University of Indonesia.

Abbas, J., Hanafi.M., Kardon L.B.S., Que-Wei Qin., Kosela S, 2009, Cytotoxic coumarin from Calophyllum Incrasaptum M.R Henderson & Wyatt-Smith (Guttiferae). International Journal of Applied Chemistry, 4(3), 205-208.

Abbas, J., M. Hanafi., Kardon L.B.S., 2009, Antioksidant activity of coumarin compound from stem bark of Calophyllum incrasaptum. Proceeding of Natural Products Simposium XIV. Natural Products resources as well known for treatment of Cancer. BPPT Jakarta 2009 August, 11-12.ISBN : 978-602-95911-0-1

Abbas, J., Nina Artanti, Syafuddin, M. Hanafi, 2009, Antimalarial Activity of Some Plants Traditionally Used in Treatment of Malaria in Indonesia.Prociding Chemistry Seminar XVIII. Santika Hohel, Yogyakarta, December 32009, ISSN: 0854-4778

Akhouri, R.R., Sharma, A.S., Malhota, P. and Sharma, A., 2008, Role of Plasmodium falciparum thrombospondin-related anonymous protein in Host-cell interactions, Malarial Journal, 7(63), 1-11.

Ampofo, S.A. and Waterman, P.G., 1986, Xanthones and neoflavonoids from two Asian species of Calophyllum, Phytochemistry, 25(11), 2617–2620.

Bennett, G.J. and Lee, H.H., 1988, Xanthones from Guttiferae. Phytochemistry, 28(4), 967–998.

Cao, S.G., Sim, K.W., Pereira, J. and Goh, S-H., 1998a, Coumarins from Calophyllum teysmannii, Phytochemistry, 47(6), 1051-1055.

Cao, S.G., Wu, X.H., Sim, Tan, Benny, K.Y., Vittal Jagadese, J.J., Perera, J.T. and Go. S-H., 1998b, Minor coumarins from Calophyllum teysmannii var inophyllode and synthesis of cytotoxic calanone.

derivates, Helvetica Chimica Acta Phytochemistry, 47(6), 1051-1055.

Crane, S., Aurore, G., Joseph, H., Mouloungui, Z. and Bourgeois, P., 2005, Composition of Fatty Acids Tricylglycerols and Unasaponifiable Matter in Calophyllum calaba L. Oil from Guadeloupe, Phytochemistry, 66, 1825-1831.

Dharmaratne, H.R.W., Nishanthi, W.M. and Wijesinghe, 1997, Trioxxygenated Diprenylated Chromenxanthone from Calophyllum Moonii, Phytochemistry, 46(7), 1293-1295.

Dharmaratne, H.R.W., Sotheeswaran, S.Balasubramaniam, S., 1984, Triterpenes and Neoflavonoids of Calophyllum lankaesis and Calophyllum thwaitesi, 23(11), 2601-2603.

Dharmaratne, H.R.W., Perera, D.S.M., Marasinghe, G.P.K. and Jamie, 1999, A chromene acid from Calophyllum cordato-oblongum, Phytochemistry, 15, 111-113.

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

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

Guilet, D., Seraphin, D., Rondeau, D., Richommme, P. and Bruneton, J., 2001, Cytotoxic coumarin from Calophyllum dispar, Phytochemistry, 58, 571-575.

Gustafon, K.R., Bokesch, H.R., Fuller, R.W., Cardellina, J.H., Kadushin, M.R., Soejarto, D.D. and Boyd, M.R., 1994, Calanone A Novel Coumarin From Calophyllum teysmannii, Tetrahedron Lettres, 35(32), 5821-5824.

Hay, A.E., Heilesbeux, J.J., Duval, O., LabaRed, M., Grellier, P. and Richommme, P., 2004, Antimalarial xanthones from Calophyllum caledonicum and Garcinia vieillardii, Life Sciences, 75, 3077–3085.

Ignatuschenko, M.V., Winter, R.W., Bachinger, H.P., Hinrichs, D.J., Riscoe, M.K., 1997,
Xanthones as antimalarial agents: studies of a possible mode of action. *FEBS Letters*, **409**, 67–73.

Ito C., Itoigawa, M., Mishina, Y., Filho, C.V., Enjo, F., Tokuda, H., Nishino, H. and Furukawa, H., 2003, Chemical constituent of *Calophyllum brasiliense*: Structure of the three new coumarin and cancer chemopreventive activity of 4-substituted coumarin, *Phytotherapy*, **66**(3), 369-371.

Khan, M.R., Kihara, M., Omoloso. A.D., 2002, Antimicrobial activity of Calophyllum soulattri, *Fitoterapia*, **73**, 741-743.

Kijjoa, A., Gonzalez, M., Afonso, C.M., Pinto, M., Anantachoke, C. and Herz, 2000, Xanthone from *Calophyllum teysmannii* var. *Inophylloide*, *Phytochemistry*, **53**, 833-836.

Krungkrai, S.R., 2001, Activity of some oxygenated xanthones, *Annals of Tropical Medicine and Parasitology*, **97**(7), 683–688.

Limuna, M., Tosa, H., Tanaka, T. and Yonemori, S., 1993, Two xanthones from root bark of *Calophyllum inophyllum*, *Phytochemistry*, **35**(7), 527-532.

Reyes-Chilpa., Estrada-Muniz, Apan, Amekraz., Aumelas., Christopher and Jankowski., Vazquez-Torres, 2004, Cytotoxic effects of mamea type coumarins from *Calophyllum brasiliense*, *Life Science*, **75**, 1635-1647.

Samarawera., Ravelanjato, B. and Kuneneshch, N., 1983, Pyrano coumarin from the stem bark and leaves of *C. Soulatii*, *Phytochemistry*, **26**(11), 2973–2987.

Soerianegara and Lemmens, R.H.M.J., 1994, Plant Resources of South-East Asia, Timber trees major commercial timbers, Bogor, Indonesia, *Prosea*, **5**(1), 114-132.

Trager, W. and Jensen, J.B., 1976, Human malarial parasites in continuous culture, *Science*, **193**, 673–677.