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by Dr. Eva Marliana

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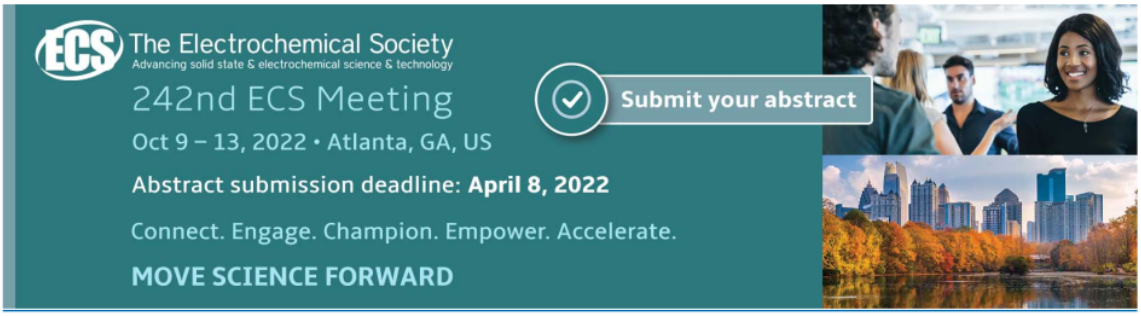

Antiplasmodial activity of flavonoids from *Macaranga tanarius* leaves

E Marliana[1,*], R Hairani[1], T S Tjahjandarie[2] and M Tanjung[2]

[1]Department of Chemistry, Faculty of Mathematics and Natural Science, Mulawarman University, Samarinda, Indonesia
[2]Natural Product Chemistry Group, Organic Chemistry Division, Department of Chemistry, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia

* Corresponding author eva_samarinda@yahoo.com

Abstract. Malaria is one of the leading causes of death in the world which is caused by *Plasmodium sp*. This parasite tends to have mutation and shows resistance towards malaria drug. Due to the emergence and spread of *Plasmodium sp*. resistance towards malaria drugs, an exploration to find new effective and selective malaria drug is essential. In this study, four flavonoids, namely nymphaeol C (**1**), solophenol D (**2**), nymphaeol A (**3**), and nymphaeol B (**4**) were isolated from ethyl acetate fraction of *Macaranga tanarius* leaves. The structures of those compounds were characterized by NMR analysis. Furthermore, antiplasmodial activity of ethyl acetate fraction and four isolated compounds (**1**–**4**) were evaluated by Giemsa method against *Plasmodium falciparum* strain 3D7. According to this assay, it showed the IC_{50} values were 0.30, 0.24, 0.31, 0.05, and 0.05 µg/mL, respectively. The results provide important evidence of the antiplasmodial activity of flavonoids in traditional use. In addition, it can be indicated that *Macaranga tanarius* is potential to be developed as antiplasmodial agents.

1. Introduction

Malaria is known as the world's most important parasitic disease especially when *Plasmodium sp*. is the causative agent. World Health Organization (WHO) has reported that about 300 million people were at risk of malaria with 1.5 million deaths per year caused by *Plasmodium sp*. infections [1]. Kalimantan is one of the malaria endemic areas in Indonesia, therefore, local people in this island try to find the treatment to cure this disease using some medicinal plants [2].

Macaranga is one of plants that is used for malaria treatment. This genus belongs to Euphorbiaceae family containing flavonoids and stilbenoids integrated with terpenoids. Based on the chemical structure, the combination of flavonoids with terpenoids yields many varieties of flavonoid derivatives structures which further provides an opportunity for scientist to discover new compounds and new frameworks that have potency as antiplasmodial agent.

One species of Macaranga genus that had been reported for its antiplasmodial activity is only *Macaranga triloba* which is known containing 6-prenyl-3'-methoxyeriodictyol, nymphaeol B, nymphaeol C, and 6-farnesyl eriodictyol [3]. Another species from this genus named *Macaranga*

tanarius is suspected also to have antiplasmodial activity due to some compounds in *M. tanarius* also belong to flavonoids. Several isolated compounds from *M. tanarius* had been reported, such as tanariflavanone A, tanariflavanone D, nymphaeol A and nymphaeol B [3, 4].

To date, there is no study about antiplasmodial activity from *M. tanarius*, hence in this study, the isolation of bioactive compounds and the investigation of their antiplasmodial activity from *M. tanarius* leaves were performed to understand the potency of this species.

2. Materials and Methods

2.1. General information

All reagents used in this research were purchased from Merck Chemical, Co. Vacuum liquid chromatography (VLC) and radial chromatography were conducted using Silica gel 60 GF_{254} and Silica gel 60 PF_{254}. The isolation was monitored by Thin Layer Chromatography (TLC) and visualized under UV light at 254 and 356 nm with $CeSO_4$ as staining agent. Pre-coated silica gel plates (Merck Kieselgel 60 GF_{254}, 0.25 mm thickness) were employed for TLC analysis. ^{1}H and ^{13}C NMR spectra of isolated compounds were recorded with a JEOL ECS 400 spectrometer operating at 400 and 100 MHz, respectively, in $CDCl_3$ and acetone-d6 using TMS as the internal standard.

2.2. Plant material

The leaves of *Macaranga tanarius* were collected from Samboja forest, Kutai Kartanegara, East Kalimantan. The identification of this plant was taken by Herbarium of Wanariset, East Kalimantan.

2.3. Extraction and isolation

The air-dried powder of *M. tanarius* leaves (2 kg) were extracted by maceration method using methanol at room temperature for two times. The filtrate was evaporated to obtain crude extract (276 g). A part of this crude extract (150 g) was partitioned with *n*-hexane and ethyl acetate, respectively. Ethyl acetate fraction (50 g) was separated using Vacuum Liquid Chromatography (VLC) and eluted with the mixtures of *n*-hexane and ethyl acetate by increasing the polarity (9:1, 4:1, 7:3, and 1:1) to yield two major fractions, i.e. A and B. Separation of A fraction using column chromatography and eluted with the mixtures of *n*-hexane and ethyl acetate by increasing the polarity (9:1, 4:1, 7:3, and 1:1) yielded three major sub-fractions, i.e. A1, A2, and A3. Further separation of A2 and A3 gave compound **1** (65 mg) and compound **4** (25 mg). While for separation of B fraction using column chromatography and eluted with the mixtures of *n*-hexane and ethyl acetate by increasing the polarity (9:1, 4:1, 7:3, and 1:1) yielded two major sub-fractions, i.e. B1 and B2. Further separation of B1 and B2 gave compound **2** (4.8 mg) and compound **3** (12.7 mg).

2.4. Determination of antiplasmodial activity

Determination of antiplasmodial activity of ethyl acetate fraction and isolate compounds was conducted by Trager and Jensen method [5] and followed by Giemsa staining as described by Widyawaruyanti, et al [6]. In this method, sample was dissolved in 20 µL of DMSO and diluted with 180 µL of RPMI 1640 medium until obtained various kinds of concentration. A total of 50 µL of test solution was inserted into micro well plate, then added 950 µL of parasitic suspension of *Plasmodium falciparum* strain 3D7, incubated for 48 h, then centrifuged. The top of the suspension (supernatant) was removed until the concentrated suspension was obtained, then the preparation of a thin layer of blood (monolayer) stained with Giemsa 20% was then calculated for the percentage of parasitemia and the growth percentage of *P. falciparum* and its resistance, by counting the number of infected erythrocytes every 1000 erythrocytes under microscope [7]. The test was done in triplicate by varying the concentration of ethyl acetate fraction and isolated compounds. Chloroquine diphosphate was used

as positive control, while solvent was used as negative control. The IC_{50} value evaluation was determined based on Probit Analysis with SPSS program.

3. Results and Discussion

3.1. Flavonoids from Macaranga tanarius leaves

There were four isolated compounds obtained from ethyl fraction of *M. tanarius* leaves, named nymphaeol C (**1**), solophenol D (**2**), nymphaeol A (**3**), and nymphaeol B (**4**). The position of protons and carbons NMR (1D NMR) of compounds **1**–**4** are presented in table 1. In addition, the Heteronuclear Multiple Bond Correlation (HMBC, 2D NMR) of those compounds are shown in figure 1.

Table 1. NMR data of compounds **1**–**4** in $CDCl_3$ or acetone-d6

Compounds	^{1}H NMR, 400 MHz δ_H (ppm)	^{13}C NMR, 100 MHz δ_C (ppm)
1	5.49 (1H, *dd*, *J*=13.2, 2.8 Hz, H-2), 3.12 (1H, *dd*, *J*=17.2, 13.2 Hz, H-3_{ax}), 2.73 (1H, *dd*, *J*=17.2, 2.8 Hz, H-3_{eq}), 5.97 (1H, *s*, H-8), 6.81 (1H, *d*, *J*=8.4 Hz, H-5'), 6.95 (1H, *d*, *J*=8.4 Hz, H-6'), 3.33 (2H, *d*, *J*=7.2 Hz, H-1"), 5.22 (1H, *t*, *J*=8.4 Hz, H-2"), 1.80 (3H, *s*, H-4"), 1.74 (3H, *s*, H-5"), 3.44 (2H, *d*, *J*=6.8 Hz, H-1"'), 5.15 (1H, *t*, *J*=6.8 Hz, H-2"'), 2.05 (2H, *t*, H-4"'), 2.07 (2H, *m*, H-5"'), 5.02 (1H, *t*, H-6"'), 1.58 (3H, H-8"'), 1.65 (3H, *s*, H-9"'), 1.75 (3H, *s*, H-10"'), 12.34 (1H, *s*, 5-OH), 5.64 (1H, *s*, 4'-OH).	76.5 (C-2), 42.8 (C-3), 197.1 (C-4), 103.1 (C-4a), 161.5 (C-5), 107.6 (C-6), 161.6 (C-7), 95.7 (C-8), 164.1 (C-8a), 128.7 (C-1'), 126.7 (C-2'), 142.8 (C-3'), 144.8 (C-4'), 113.1 (C-5'), 118.9 (C-6'), 21.4 (C-1"), 121.6 (C-2"), 135.2 (C-3"), 18.1 (C-4"), 26.1 (C-5"), 25.5 (C-1"'), 121.8 (C-2"'), 138.7 (C-3"'), 39.8 (C-4"'), 26.6 (C-5"'), 123.9 (C-6"'), 132.4 (C-7"'), 18.0 (C-8"'), 26.0 (C-9"'), 16.5 (C-10"').
2	6.25 (1H, *d*, *J*= 2.0 Hz, H-6), 6.38 (1H, *d*, *J*=2.0 Hz, H-8), 6.84 (1H, *d*, *J*=8.4 Hz, H-5'), 6.93 (1H, *d*, *J*=8,4 Hz, H-6'), 3.48 (2H, *d*, *J*=6.8 Hz, H-1"), 4.98 (1H, *t*, 6.8 Hz, H-2"), 1.79 (2H, *m*, H-4"), 1.86 (2H, *m*, H-5"), 5.14 (1H, *t*, H-6"), 1.57 (3H, *s*, H-8"), 1.45 (3H, *s*, H-9"), 1.49 (3H, *s*, H-10"), 12.29 (1H, *s*, 5-OH).	147.0 (C-2), 135.3 (C-3), 176.9 (C-4), 104.7 (C-4a), 164.7 (C-5), 99.0 (C-6), 162.5 (C-7), 94.4 (C-8), 158.3 (C-8a), 129.3 (C-1'), 125.0 (C-2'), 142.7 (C-3'), 144.3 (C-4'), 113.1 (C-5'), 122.7 (C-6'), 26.6 (C-1"), 123.4 (C-2"), 131.6 (C-3"), 40.3 (C-4"), 27.3 (C-5"), 123.6 (C-6"), 130.7 (C-7"), 25.7 (C-8"), 17.6 (C-9"), 16.2 (C-10").
3	5.35 (1H, *dd*, *J*=12.8, 2.8 Hz, H-2), 3.11 (1H, *dd*, *J*=17.2, 12.8 Hz, H-3_{ax}), 2.70 (1H, *dd*, *J*=17.2, 2.8 Hz, H-3_{eq}), 6.01 (1H, *s*, H-8), 7.02 (1H, *s*, H-2'), 6.85 (2H, *d*, *J*=8.4 Hz, H-5'/H-6'), 3.24 (2H, *d*, *J*=6.8 Hz, H-1"), 5.24 (1H, *brt*, H-2"), 1.94 (2H, *t*, *J*=8.4 Hz, H-4"), 2.03 (2H, *m*, H-5"), 5.07 (1H, *t*, H-6"), 1.55 (3H, *s*, H-8"), 1.61 (3H, *s*, H-9"), 1.75 (3H, *s*, H-10"), 12.47 (1H, *s*, 5-OH).	79.9 (C-2), 43.6 (C-3), 197.2 (C-4), 102.8 (C-4a), 164.8 (C-5), 108.9 (C-6), 162.1 (C-7), 95.2 (C-8), 161.8 (C-8a), 131.5 (C-1'), 114.6 (C-2'), 146.1 (C-3'), 146.4 (C-4'), 119.1 (C-5'), 115.8 (C-6'), 21.5 (C-1"), 123.3 (C-2"), 134.9 (C-3"), 39.4 (C-4"), 27.3 (C-5"), 125.0 (C-6"), 131.5 (C-7"), 17.7 (C-8"), 25.8 (C-9"), 16.0 (C-10").
4	5.55 (1H, *dd*, *J*=13.2, 2.8 Hz, H-2), 3.12 (1H, *dd*, *J*=17.2, 13.2 Hz, H-3_{ax}), 2.73 (1H, *dd*, *J*=17.2, 2.8 Hz, H-3_{eq}), 5.99 (1H, *d*, *J*=2.4 Hz, H-6), 5.95 (1H, *d*, *J*=2.4 Hz H-8), 6.82 (1H, *d*, *J*=8.4 Hz, H-5'), 6.94 (1H, *d*, *J*=8,4 Hz, H-6'), 3.44 (2H, *d*, *J*=7.8 Hz, H-1"), 5.15 (1H, *brt*, *J*=6.8 Hz, H-2"), 2.05 (2H, *brt*, *J*=6.8 Hz, H-4"), 2.09 (2H, *brq*, H-5"), 5.01 (1H, *brt*, *J*=6.8 Hz, H-6"), 1.57 (3H, *s*, H-8"), 1.65 (3H, *s*, H-9"), 1.75 (3H, *s*, H-10"), 12.04 (1H, *s*, 5-OH), 5.63 (1H, *s*, 4'-OH).	76.4 (C-2), 42.4 (C-3), 196.5 (C-4), 103.0 (C-4a), 164.7 (C-5), 96.7 (C-6), 164.2 (C-7), 95.5 (C-8), 163.4 (C-8a), 128.2 (C-1'), 126.4 (C-2'), 142.5 (C-3'), 144.8 (C-4'), 113.0 (C-5'), 119.0 (C-6'), 25.4 (C-1"), 121.2 (C-2"), 139.0 (C-3"), 39.5 (C-4"), 26.2 (C-5"), 123.6 (C-6"), 132.3 (C-7"), 17.2 (C-8"), 25.7 (C-9"), 16.3 (C-10").

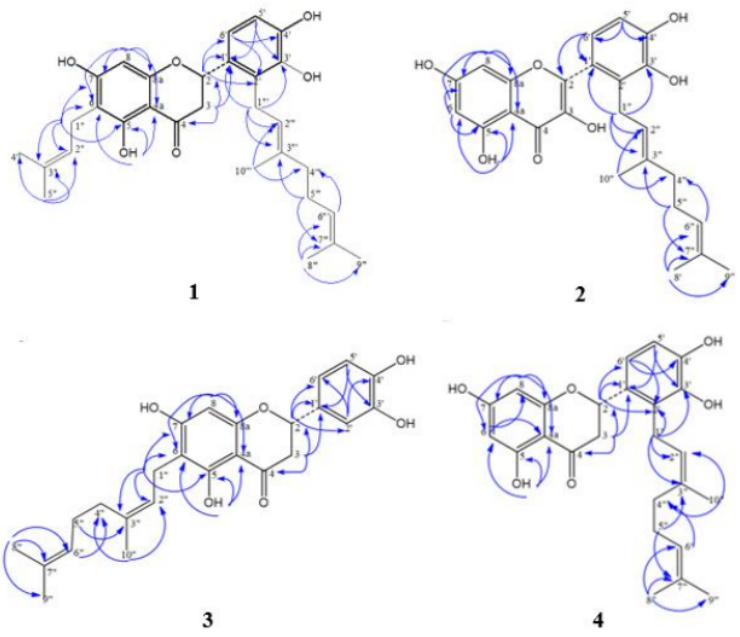

Figure 1. HMBC of nymphaeol C (**1**), solophenol D (**2**), nymphaeol A (**3**) and nymphaeol B (**4**)

Compound **1** was obtained as yellow oil. The UV spectra in methanol gave the maximum absorption at λ_{max} nm (log ε): 243 (4.69), 291.50 (4.67), (MeOH+NaOH): 300 (4.58), 330 (4.66, (MeOH+$AlCl_3$): 297.5 (3.94), (MeOH+$AlCl_3$+HCl): 308 (4.00) 361 (3.57). The UV spectrum profile of the isolated compound indicated that the compound belong to flavanone derivative. In addition to (MeOH+$AlCl_3$), a batochromic shift and absorption change after addition of HCl indicated that the compound can be classified as flavanone having ortho-dihydroxy [8]. ^{1}H NMR spectra analysis of compound **1** (table 1) indicated the presence of flavanone moiety which showed by three proton of doublet-doublet signals at δ_H 5.49 ppm (J=13.2, 2.8 Hz, H-2), 3.12 ppm (J=13.2, 17.2 Hz, H-3ax), and 2.73 ppm (J=17.2, 2.8 Hz, H-3eq). In addition, the spectra exhibited proton signals of five methyl singlets (δ_H 1.80, 1.74, 1.58, 1.65, 1.75 ppm) along with three vinyl triplet signals (δ_H 5.24, 5.15, 5.02 ppm) and four methylene signals (δ_H 3.33, 3.44, 2.05, 0.07 ppm), indicated that the isolated compound has one isoprenyl group and one geranyl group. Spectral analysis of ^{13}C NMR of compound **1** (table 1) showed 30 perfectly separate carbon signals comprising seven methyl carbon atoms, five methylene carbon atoms, five methyl carbon atoms, and thirteen quartz carbon atoms. The ^{13}C NMR spectra exhibited a carbonyl carbon signal (δ_C 197.1 ppm), an oxy carbon methyl carbon signal (δ_C 76.5 ppm), and five oxy acetyl carbon signals (δ_C 164.1, 161.6, 161.5, 144.8, 142.8 ppm) which are the basic skeleton of eriodictyol derivatives. Based on HMBC (figure 1), the correlation of two methyl signals with one methyl carbon signal (δ_C 121.6 ppm) and one quarterner carbon signal (δ_C 135.2 ppm) indicated the isoprenyl substituent bound at C-6. Based on 1D and 2D NMR spectral data, compound **1** was identified as 6-isoprenyl-2'-geranyleriodictyol or known as nymphaeol C according to the previous study that reported nymphaeol C from *M. triloba* having molecular formula $C_{30}H_{36}O_6$ and molecular mass m/z=492 [9].

Compound **2** was obtained as yellow solid. The UV spectra of compound **2** in methanol displayed maximum absorption at λ_{max} nm (log ε): 255 (4.30), 297.50 (3.96) and 348 sh (4.02) which is a characteristic of flavone or flavonol [7]. The maximum absorption of compound in (MeOH+NaOH) λ_{maks} nm (log ε): 270 (4.21), 325 (3.86), (MeOH+$AlCl_3$): 265 (4.60), 297 (4.14) (MeOH+$AlCl_3$+HCl): 256 (4.83), 302.50 (4.24), 362 (4.17). In addition to MeOH+$AlCl_3$ a shift in batochromic and absorption change after addition of HCl indicated that the compound belong to flavonol having ortho-dihydroxy. ^{1}H NMR spectra analysis of compound **2** (table 1) showed a pair of ortho doublet proton signals in the aromatic region (J=8.4 Hz) at δ_H 6.84 ppm and 6.93 ppm (1H each), corresponding to the compound pattern of nymphaeol C i.e. 2', 3', 4'-trisubstitutes in ring B. The presence of three signals of methyl singlet (δ_H 1.57, 1.45, 1.49 ppm) together with two vinyl triplet signals (δ_H 6.8, 6.8 ppm) and three methyl doublet doubles (δ_H 3.48, 1.79, 1.86 ppm), indicated that this compound has one geranyl group. ^{13}C NMR analysis (table 1) showed 25 perfectly segregated carbon signals, comprising six methyl carbon atoms, three methylene carbon atoms, three methyl carbon atoms, and 13 quartz carbon atoms. The position of geranyl substituent of compound **2** was determined by HMBC (figure 1). The correlation of proton aromatic signals at δ_H 6.93 ppm (H-6') with two quarterner carbon signals (δC 147.0, 129.3 ppm) showed δ_C 147.0 ppm bound in C-2 and δ_C 129.3 ppm in C-1'. The correlation of methylene signals in δ_H 3.48 ppm with four quaternary carbon signals (δ_C 142.7, 131.6, 129.3, 125.0 ppm) and one methyl signal (δ_C 123.4 ppm) indicated geranyl substituent bound at C-2'. Based on UV, 1D and 2D NMR data, compound **2** was identified as 2'-geranylquercetin or known as solophenol D, corresponds to an isolated compound from propolis with a positive quasi-molecular ion mass at m/z=439.1679 and molecular formula=$C_{25}H_{27}O_7$ [10].

Compound **3** was gained as yellow oil and the UV spectra in methanol showed the maximum absorption at λ_{max} nm (log ε): 291.50 (4.60), 331 sh (3.91) (MeOH+NaOH): 330 (4.64), (MeOH+$AlCl_3$): 294.50 (4.69), 348 sh (3.91), (MeOH+$AlCl_3$+HCl): 301.50 (4.59), 354.50 sh (3, 92). ^{1}H NMR spectra analysis of compound **3** (table 1) indicated the presence of flavanone moiety which showed by three signals of doublet doublet proton at δ_H 5.35 ppm (J=12.8, 2.8 Hz, H-2), 3.11 ppm (J=12.8, 17.2 Hz, H-3$_{ax}$), and 2.70 ppm (J=17.2, 2.8 Hz, H-3$_{eq}$). In addition, the spectra also showed three signals of methyl singlet proton (δ_H 1.55, 1.61, 1.75 ppm) to gather with two vinyl triplet signals (δ_H 5.24. 5.07 ppm) and three methylene signals (δ_H 3.24. 1.94, 2.03 ppm) indicated the presence of one geranyl group. ^{13}C NMR spectra analysis of compound **3** (table 1) showed 24 carbons signals which representative 25 carbon atoms comprising seven of methine carbon atoms, four methylene carbon atoms, three methyl carbon atoms, and ten quarterne carbon atoms. Likewise with compound **2**, this compound also has eriodictyol moiety. Geranyl position was determined by HMBC correlation (figure 1). It showed singlet signal at δ_H 12.47 ppm (5-OH) with three quarternary carbon atoms aromatic (δ_C 164.8, 108.9, 102.8 ppm) placed carbon signal δ_C 164.8 ppm at C-5, δ_C 108.9 ppm at C-6, and δ_C 102.8 ppm at C-4a. The spectra indicated one geranyl group bounded at C-6. Based on 1D and 2D NMR, compound **3** was identified as 6-geranyleriodictyol or nymphaeol A which had been isolated from *M. alnifolia* with structure formula $C_{25}H_{29}O_6$ and m/z $[M+H]^+$ =425.1948 [11].

Compound **4** was isolated as yellow oil and UV spectra in methanol showed maximum absorption at pada λ_{maks} nm (log ε): 289 (4.86), 327.50 *sh* (4.16) (MeOH+NaOH) 324.50 (4.92), (MeOH+$AlCl_3$): 308.50 (4.94) 375 *sh* (4.15), (MeOH+$AlCl_3$+HCl): 308 (4.85), 374.50 *sh* (4.12) Analysis of ^{1}H NMR spectra of compound **4** also indicated flavanone moiety i.e. the presence of three doublet doublet proton signals at δ_H 5.55 ppm (J=13.2, 2.8 Hz, H-2), 3.12 ppm (J=13.2, 17.2 Hz, H-3ax), and 2.73 ppm (J=17.2, 2.8 Hz, H-3eq). The isolated compound spectra exhibited proton signals of three methyl singlets (δ_H 1.57, 1.65, 1.75 ppm) along with two vinyl triplet signals (δ_H 5.15, 5.01 ppm), and three methylene signals (δ_H 3.44, 2.05, 2.09 ppm), indicated that the isolated compound has one gingival group. ^{13}C NMR spectra analysis of compound **4** showed 25 perfectly segregated carbon signals,

comprising seven methyl carbon atoms, four methylene carbon atoms, three methyl carbon atoms and 11 quartz carbon atoms. This compound also has an eriodictyol skeleton. The geranyl substituent position that bounded at C-2' was defined by HMBC (figure 1). Based on 1D dan 2D NMR spectrum, compound **4** was identified as 2'-geranyleriodictyol or known as nymphaeol B which had been isolated from *M. triloba* with m/z = 424 ($C_{25}H_{28}O_6$) [9].

3.2. Antiplasmodial activity

Antiplasmodial activity test was conducted on ethyl acetate fraction and four isolated compounds of *M. tanarius* leaves using Giemsa method against *P. falciparum* strain 3D7. Levels of antiplasmodial activity were expressed in concentration inhibition (IC_{50}) value, the concentration required to inhibit 50% of parasitemia.

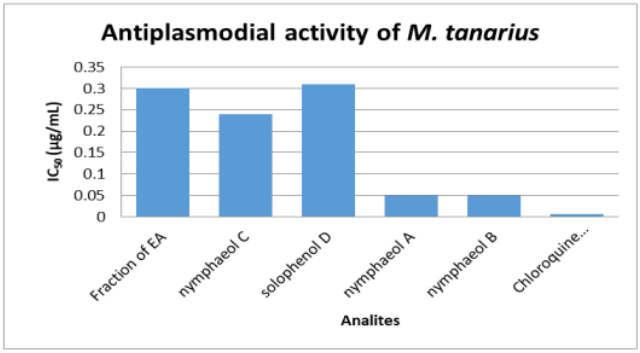

Figure 2. Antiplasmodial activity

Based on the result in figure 2, it showed that ethyl acetate fraction and four isolated compounds (**1**–**4**) are active fraction and active compounds in antiplasmodial activity with IC_{50} values of 0.30, 0.24, 0.31, 0.05, and 0.05 µg/mL, respectively. In this study, chloroquine was used as positive control with IC_{50} value of 0.006 µg/mL. If compared with chloroquine, the ethyl acetate fraction and four isolated compounds (**1**–**4**) have lower activity than chloroquine. Even though ethyl acetate fraction has higher IC_{50} value than chloroquine, the IC_{50} value indicated that this fraction can be categorized as an active fraction. Previous study stated that the extract with IC_{50} value < 25 μg/mL is categorized as an active extract in determination of antiplasmodial activity [12]. The same result also be found for isolated compounds which belong to flavonoids, but particularly, those isolated flavonoids can be categorized as active compounds in inhibiting plasmodial.

There are two main targets of the mechanism of flavonoids that capable for inhibiting the growth of plasmodium: 1) malaria parasite food vacuoles by inhibiting the process of hemoglobin degradation [13]; 2) membranes formed intra eristrositic stage malaria parasite i.e. New Permeation Pathway (NPP) by inhibiting the transport of nutrients needed by parasites [14]. The high antiplasmodial activity of the compounds is suspected from the mechanisms that act on food vacuoles. Flavonoid and its derivatives play a role in blocking the formation of hemozoin by the formation of free heme complexes with active compounds. Heme free (Fe^{3+}) is highly toxic because it can cause highly reactive oxygen species which can trigger oxidative reactions so that the parasites die. Therefore, the parasite converts into a non-toxic substance by forming a polymer from the heme residues by a co-ordination bond between Fe^{3+} heme with another heme hydroxyl group to form the β-hematin molecule, further forming a larger aggregate called hemozoin. This aggregate formation process of hemozoin is a process that can be used as a target for antimalarial therapy.

Pharmacokinetic effects of compounds can be reviewed from three aspects of physical properties of the compound including electronic effects, hydrophobic and molecular size [15]. The presence of terpenyl substituent can increase the lipophilic properties of compound. In this study, nymphaeol C (**1**), solophenol D (**2**), nymphaeol A (**3**), and nymphaeol B (**4**), having terpenyl substituent at C-6 and or C-2', can inhibit the growth of *P. falciparum*. The lipophilic aspect contributes to the activity of the compound in terms of its ability to bypass the semi-permeable lipid membrane of parasite. Solophenol D (**2**) which belongs to flavonol has lower antiplasmodial activity than nymphaeol C (**1**), nymphaeol A (**3**) and nymphaeol B (**4**) which belong to flavanones. The presence of double bonds on aromatic bridges and substituent of 3-OH in solophenol D (**2**) can reduce the antiplasmodial activity. Solophenol D (**2**) is more polar, so its lipophilic property is less than flavanones. Flavonol has a low solubility in fat, hence, it cause a reduced ability of compound to pass through semi permiabel lipid membrane of parasite. Furthermore, it can make the compound complicate to enter the food vacuole for inhibiting the formation of heme polymers (hemozoin) [7]. The nature of lipophilic is also associated with the mechanism of inhibition of the NPP by inhibiting the transport of nutrients needed parasites [14].

For pharmacodynamic effects of compounds can be evaluated based on the types and position of terpenyl substituent bound to flavonoids. Substitution of isoprene chain in the basic framework of flavonoids may increase the antiplasmodial activity [16]. Nymphaeol A (**3**) and nymphaeol B (**4**) exhibited the highest activity as antiplasmodial, this can be noticed that the presence of terpenyl group at C-6 or C-2' play important role in antiplasmodial activity.

4. Conclusions

According to the results, it can be concluded that flavonoids from *M. tanarius* leaves showed good activity in antiplasmodial screening. Compounds **3** and **4** which have ortho-dihydroxy in ring B and have a terpenyl substituent at C-6 or C-2' are active against *P. falciparum*. The presence of two terpenyl substituents at C-6 and C-2' (compound **1**) exhibited lower activity compare with compound **3** and **4**. Moreover, the presence of double bonds on the aromatic ring bridge in compound **2** decreases antiplasmodial activity. Particularly, it can be stated that *M. tanarius* leaves has a potency as antiplasmodial activity in the future.

Acknowledgment

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