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In vivo Antiplasmodial of the Most Active Fraction and Its Compound of *Kapur Leaves* (*Harmsiopanax aculeatus* Harms) Extract Against *Plasmodium berghei*

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ABSTRACT

Introduction : The rising of *Plasmodium* resistance towards chloroquine and other antimalarial drugs have encouraged to discover and develop new drugs mainly derived from natural products. *Harmsiopanax aculeatus* (kapur plant) has traditionally used by people of in Maluku Province to treat malaria.

Objectives: The aims of this study were to identify antiplasmodial activity and its chemical constituents of the most active fraction of kapur leaves.

Methods: The dried powder of Kapur leaves (1.3 kg) were extracted successively by maceration with n-hexane, ethyl acetate and methanol. After removal the solvents the hexane 15.6 g (1.2%), ethyl acetate 53.3 g (4.1%) and methanol 61.1 g (4.7%) extracts were obtained. Those extracts were assayed for their *in vivo* antiplasmodial activities by using 4-days suppressive test in Swiss mice infected with *Plasmodium berghei*, HPIA and identified the compound by GC-MS.

Results: The ED_{50} of hexane, ethyl acetate and methanol extracts were 467.58, 2074.02 and 16.16 mg/kgBW, respectively. Fractionation of the methanol extract gave 18 combined fractions (FG_1 – FG_{18}). FG_8 was the most active fraction with the IC_{50} HPIA of 18.22 μg/ml. Phytochemical test of this fraction using spray reagent showed the existence of essential oils, triterpenoids, and phenolic compounds. Separation of FG_8 using pressed chromatography gave 19 combined fractions ($FG_{8.1}$-$FG_{8.19}$). The fraction containing intense blue fluorescent spot ($FG_{8.5}$) was further separated by PLC fourthly eluted with chloroform. Seven major components with the percentage of compotition more than 3.11% were identified as eugenol (t_r = 12.692; 18.22%), isoprophyl myristate (t_r = 16.333; 3.99%); bis(2-methylpropyl) phtalat (t_r = 16.939; 7.15%); methyl palmitic (t_r = 17.442; 3.11%); palmitic acid (t_r = 17.883; 25.72%); butyl 2-methylpropyl phtalat (t_r = 17.957; 9.37%) and bis(2-ethylhexyl) phtalat (t_r = 23.258; 23%).

Conclusion: Methanol extract of *H. aculeatus* was the most potential *in vivo* antiplasmodial activity. Combined fraction 8 which contain 7 compounds was the most active fraction.

Key words: *Harmsiopanax aculeatus* Harms, *in vivo* antiplasmodial, HPIA, PLC, GC-MS

INTISARI

Pendahuluan: Adanya resistensi *Plasmodium* terhadap klorokuin dan obat antimalaria lainnya telah mendorong dilakukannya penelitian untuk mencari dan mengembangkan obat baru terutama yang berasal dari bahan alam. Daun tanaman kapur (*Harmsiopanax aculeatus* Harms) telah digunakan secara tradisional oleh masyarakat Maluku untuk mengobati malaria.

Tujuan: Serbuk kering daun kapur sebanyak 1,3 kg diekstraksi secara maserasi bertingkat menggunakan *n*-heksan, etil asetat dan metanol. Setelah pelarut diuapkan diperoleh ekstrak heksan sebesar 15,6 g (1,2%), ekstrak etil asetat 53,3 (4,1%) dan ekstrak metanol 61,1 (4,7%). Ekstrak diuji aktivitas antiplasmodiumnya secara in vivo menggunakan metode 4 days suppressive test pada mencit Swiss yang diinfeksi Plasmodium berghei, HPIA dan identifikasi senyawa dengan GC-MS

Hasil: ED_{50} ekstrak heksan, etil asetat dan metanol berturut turut adalah 467,58; 2074,02 dan 16,16 mg/kgBB. Fraksinasi ekstrak metanol dilakukan dengan VLC menggunakan silika gel yang dielusi dengan meningkatkan polaritas pelarut, menghasilkan 18 fraksi gabungan (FG). 18 FG ini diuji HPIA secara *in vitro*. FG_8 adalah fraksi paling aktif dengan IC_{50} HPIA 18,22 µg/ml. Uji fitokimia terhadap fraksi ini menggunakan pereaksi semprot memperlihatkan keberadaan minyak atsiri, triterpenoid dan senyawa fenolik. Pemisahan FG_8 menggunakan kromatografi tekan menghasilkan 19 fraksi gabungan ($FG_{8.1}$ – $FG_{8.19}$). Fraksi yang mengandung spot berfluoresensi biru ($FG_{8.5}$) dipisahkan menggunakan PLC 4 kali elusi dengan kloroform. Pita berfluoresensi biru dikerok dan diekstraksi dengan kloroform. Isolat kemudian dianalisis menggunakan GC-MS. Tujuh komponen utama dengan komposisi persentase lebih dari 3,11% diidentifikasi sebagai eugenol (t_r = 12,692; 18,22%), isopropil miristat (t_r = 16,333; 3,99%); bis(2-metilpropil) ftalat (t_r = 16,942; 7,15%); metil palmitat (t_r = 17,442; 3,11%); asam palmitat (t_r = 17,883; 25,72%); butil 2-metilpropil ftalat (t_r = 17.957; 9.37%) dan bis(2-etilheksil) ftalat (t_r = 23,258; 20,29%).
Simpulan: Ekstrak metanol *H. aculeatus* mempunyai aktivitas antiplasmodium in vivo yang paling potensial. Fraksi gabungan 8 merupakan fraksi yang paling aktif mengandung 7 komponen utama.

Kata kunci: *Harmsiopanax aculeatus* Harms, antiplasmodium *in vivo*, HPIA, PLC, GC-MS

INTRODUCTION

Malaria is an infectious disease caused by parasites of the genus *Plasmodium*[1]. Based on data from 2009 and WHO's Global Malaria Action Plan, 3.3 billion people (half the human population) live in areas with malaria transmission and thirty-five countries (30 in sub-Saharan Africa and 5 in Asia) has a high mortality caused by malaria by 98%. In 2008, malaria is estimated at 190-311000000 clinical symptoms with mortality rates as much as 708,000-1,003,000 people[2]. The spread of malaria is widespread in many developing countries including Indonesia. Indonesia is one country that is still considered at risk of malaria and frequent outbreaks (epidemics). Various measures of prevention and treatment to combat malaria have been conducted such as vector control, use of antimalarial drugs, the use of anti-mosquito bed nets and other means have given positive results in limiting the spread of this disease, but the eradication of malaria is still far from perfect, even some areas the situation becomes more worse with the emergence of parasite strains resistant to more than one type of anti-malarials[3]. One cause of failure of eradication of malaria is the emergence of *Plasmodium* resistance to antimalarial drugs, particularly chloroquine, known previously as first-line drugs. Perez *et al*[4]. states that the global spread of malaria parasites resistant to several drugs that are available is a major health problem that requires effort to find new antimalarial drugs. One attempt was made through the study of medicinal plants used by traditional communities to treat malaria. Plant of *Harmsiopanax aculeatus* (Bl. Ex DC.) Harms is a traditional medicinal plants, which is widely used by people in Maluku to treat malaria. Maluku people call it "Kapur ". The aplication is that clean young lime leaves pressed, filtered with a clean cloth and then dropped into the eyes of patients with malaria one drop / day for three consecutive days. Kapur plant are classified into Araliaceae family and based on a literature search has not been carried out phytochemical and pharmacological studies on this plant. This research was conducted to obtain the most active antiplasmodial fraction of kapur leaf and identity of the compounds using GC-MS. This study uses Bioassay Guided fractionation. Kapur leaves was extracted using *n*-hexane, ethyl acetate and methanol. Hexane, ethyl acetate and methanol extracts of kapur

leaves were then tested for antiplasmodial activity *in vivo* against *Plasmodium berghei*. The most active extract was then separated by chromatography and fractions obtained tested for haem polymerization inhibition activity. The most active fraction is further separated and the compounds were analyzed by GC-MS.

MATERIALS AND METHODS

Plant Extract. *Harmsiopanax aculeatus* was obtained from the Amahai village and district of Central Maluku, determined at the Laboratory of Plant Taxonomy Faculty of Biology, Universitas Gadjah Mada. *H. aculeatus* leaves were dried and 1.3 kg of powder was made. The dried powder of Kapur leaves (1.3 kg) were extracted successively by maceration with *n*-hexane, ethyl acetate and methanol.

Materials to extraction, fractionation, isolation and identification compound. Material of extraction are *n*-hexane, ethyl acetate, methanol technical level. Materials for insulation is a paper filter and lime leaves young tubers, chloroform, ethyl acetate, methanol analysis of degree; technical ethanol, silica gel GF40 (35-37 mesh), and silica gel 60 GF pore size of 230-400 mesh (E. merck production), silica gel 60H (1105932), silica gel GF254, filter paper, aquades, silica gel F254 plates (1.05735) and plate PLC.

Materials to haem polymerization inhibition test. Hematin(Sigma), 0.1 M NaOH, 0.2 M NaOH, 100% glacial acetic acid, DMSO, aquades, Chloroquine diphosphat.

***In vivo* antiplasmodial activity**. Antiplasmodial activity was evaluated by 4-day suppressive test method[5]. Male and female Swiss mice (20-25g) were inoculated intraperitoneally with 10^7 *P. berghei*-infected erythrocytes, resuspended in RPMI 1640 medium on day 0 (H0) to a volume of 0.2 ml. One hundred eighty mice were divided over 18 groups. The first 5 groups received 25, 50, 100, 200 and 400 mg/kgBW/d hexane extract, respectively. The second 5 groups received 12.5, 25, 50, 100 and 200 mg/kgBW/d ethyl acetate extract. The third 7 groups received 1.25, 2.5, 5, 10, 20; 40 and 80 mg/kg /d methanol extract, respectively. The other group without compound was negative control. Each group consisted of 5 male and 5 female mice. Each dose of the extract was prepared by dissolved in DMSO at 8%. Each dose of extract was administered once daily for 4 consecutive days, beginning on the day of infection, starting two hours after inoculation until third day (H_3). Parasitaemia was calculated using thin blood smears which were stained by Giemsa and examined under a light microscope. The level of parasitaemia was determined the day following the last treatment (fourth day). The ED_{50}, which is the dose leading to 50% parasite growth inhibition compared to growth in the control, was evaluated from a plot of activity (expressed as a percentage of the activity in the control) versus the log dose.

Fractionation of Methanol Extract *H. aculeatus*. The most potential antiplasmodial activity was methanol extract. The extract then fractionated using vacuum liquid cromatography (VLC). The amount of 10 grams of methanol extract was dissolved in methanol and then impregnated with silica gel 40 Merck (37-50 mesh). Methanol solvent was evaporated until the mixture becomes dry. Column chromatography provided the solvent n-hexane ± ¾ stationary phase column and then powdered silica gel 60H which had been mixed with the solvent *n*-hexane in the form of silica slurry, was added to the column a little while vaccumed up to ± 15 cm high column. Columns that have been packed, left overnight. The powder sample was sown on the stationary phase until blended and the powder surface was closed again with

silica gel 40 Merck (37-50 mesh). Performed by gradient elution starting with hexane-chloroform, followed by chloroform-methanol and finally methanol with various comparisons. Fractions obtained, evaporated the solvent and TLC profile of his views. Fractions with similar TLC profiles were combined. A number of 18 combined fractions were obtained and tested for haem polymerization inhibition activity.

Haem Polymerization Inhibition Activity. Haem polymerization inhibition test was carried out on each combined fraction by using Bassilico *et al*. method[6] with modification. Put a 100 µL solution of 1 mM hematin in 0.2 M NaOH and 50 µL material tested into eppendorf tube, then added 50 µL of glacial acetic acid (pH 2.6). Various concentration of materials tested were used : 25; 50; 100; 200; 400 and 800 µg/ml for the combined fractions 1-7 and 9-18, while a series of concentration 6.25, 12.5, 25; 50; 100; 200; 400 and 800 µg/ml were used for combined fraction of 8. Each concentration was made triplicate. Aquadest and chloroquine were used for negative and positive control. Chloroquine concentrations were 25; 50; 100; 200; 400 and 800 µg/ml. Eppendorff which already contains the solution was incubated at 370C for 24 hours. After incubation, eppendorff was centrifuged at 8000 rpm for 10 minutes and then washed three times with DMSO. Precipitate of haematin crystal was dissolved with 200 mL of NaOH 0.1 M. A total of 100 mL solution was transferred to microplate 96 wells and measure the absorbance values by ELISA Reader at λ 405 nm. Absorbance values obtained are plotted to a linear regression equation of standard curve so that it can be determined the concentration of β-hematin of the samples. Values of haem polymerization inhibition IC50 were expressed in levels that could inhibit the polymerization hem up to 50%.

Analysis of Group Compounds in The Most Active Fraction (FG_8) of Methanol Extract. FG_8 solution in chloroform spoted on 7 plates thin layer of silica gel 60 F254 (1.05735) measuring 1x9 cm and eluted using eluent system chloroform ethyl acetate (9:1). TLC plates were then sprayed with Dragendorff reagent, FeCl3, Liebermann-Burchard, KOH and Anisaldehid sulfate. Change the color of the spots seen in visible light and UV 365 nm and compared with the standard TLC plates.

Fractionation of combined fractions 8 methanol extract of *H. aculeatus*. Fractionation of FG_8 methanol extract of kapur leaves performed using column chromatography press with 1cm column diameter. Active fraction that was used as much as 0.1323 grams dissolved in chloroform and then impregnated with 0.5292 gram of silica gel Merck 40 PF254 (37-50 mesh) . Chloroform was evaporated so that the obtained samples powder is ready for the fractionated. The column was prepared by the solvent *n*-hexane and then silica slurry made of silica gel 60 PF254 Merck (230-400 mesh) filled slowly inserted into the column and left overnight. Furthermore, the samples powder are inserted at the top and coated with silica gel Merck 40 PF254 (35-70 mesh). Fractionation performed gradient using a ratio of chloroform-ethyl acetate, ethyl acetate-methanol and methanol. Fractions obtained accommodated, the solvent

The percentage inhibition of haem polymerization calculated by the formula:

$$\% \text{ Inhibition} = \frac{[\text{B-hematin}]_{\text{negative control}} - [\beta\text{-hematin}]_{\text{materials testing}}}{[\text{B-hematin}]_{\text{negative control}}} \times 100\%$$

evaporated and its profile monitored using thin layer chromatography. TLC profile that has a fluorescence blue spot found in fractions 10-13 and then combined into $FG_{8.5}$ which was isolated using preparative layer chromatography (PLC).

Isolation of blue fluorescent compounds in $FG_{8.5}$. Isolation of blue fluorescence compounds in $FG_{8.5}$ done with PLC by multiple elution. Sample spoted forming elongated ribbon above the plate PLC using the stationary phase silica gel 60 GF254 Merck with a thickness of 0.5 mm. Plate eluted using a mobile phase of 100% chloroform. Making preparative plates by mixing 40 grams of silica gel 60 GF_{254} Merck (1.07730.1000) and 80 ml of distilled water in a closed erlenmeyer, beaten for ± 2 minutes, then spread out on 6 pieces of glass plates measuring 20 x 20 cm. Silica plate was allowed to dry (± 18-20 hours) and activated in an oven at a temperature of 100-110^{0}C for ± 60 minutes if want to use. Plates that have been activated, refrigerated until ready to use. Sample solution spoted on the elongated ribbon-shaped plate and then aerated until all the solvent evaporates. Plate were inserted into the chamber containing chloroform. After elution is completed, the plates is taken from chamber and the chloroform evaporated by means aerated to dry. Plate inserted into the chamber again. Elution performed 4 times. Blue fluorescence bands scraped and dissolved in chloroform, stired and filtered. The filtrate was dried with aerated. Isolates were obtained, checked for purity by TLC using stationary phase silica gel 60 GF_{254} Merck is eluted with chloroform 100%. Chromatograms were observed under the UV.

Identification of Blue Fluorescent Compounds. Based on TLC profiles, blue fluorescence isolate can be estimated that the isolate is not pure. This can be seen that even though these isolates seem to give one spot, but revealed distinct blue fluorescence intensity when observed under UV 254 nm and 366 nm. Therefore, very few in number, the isolates were analyzed by GC-MS. Mass spectrum of each component compared with mass spectra available in the Library WILLEY229 GC-MS instrument.

RESULTS AND DISCUSSIONS

Extraction of Kapur Leaves (*H. aculeatus*). Extraction of 1.3 kg *H. aculeatus* leaves powder was done by maceration with elution graded by using *n*-hexane, ethyl acetate and methanol solvents. After the solvent evaporated, 15.6 g (1.2%) *n*-hexane, 53.3 g (4.1%) ethyl acetate and 61.1 g (4.7%) methanol extracts were obtained.

***In Vivo* Antiplasmodial Activity Test**. Extracts were tested on *in vivo* antiplasmodial activity in *P. berghei* infected mice. The ED_{50} values were 467.58; 2074.02 and 16.16 mg/kgBW, for *n*-hexane, ethyl acetate and methanol extracts respectively. Table 1 showed the effect of *H. aculeatus* extract on the growth of *P. berghei* in Swiss mice.

Table 1 showed that the mean parasitemia obtained in general decreased with increasing dose, except in the group of ethyl acetate extract. The greater the dose of ethyl acetate extract, the less parasitemia but at doses of 100 mg/kgBW/d there was an increase of parasitemia 14.240 ± 7.125. The ED_{50} of methanol extract was lower (16.16 mg/kgBW) than hexane and ethyl acetate extracts, therefore methanol extracts had greater activity than the hexane and ethyl acetate extracts to inhibit growth of *Plasmodium*. Based on the criteria for antiplasmodial activity in vivo according to Munoz *et al.*[7], the ED_{50} ≤ 100 mg/kgBW/d categorized into very good, ED_{50} : 101-250 mg/kgBW/d is good, ED_{50}: 251-500 mg/kgB/d is moderate and inactive when ED_{50} > 500 mg/kgBW/d. Thus, the methanol extract (ED_{50}:16.16 mg/kgBW) had very good antiplasmodial

Table 1. *In vivo* antiplasmodial activity of *H. aculeatus* extract in *Plasmodium berghei* infected Swiss mice

Extract	Dose (mg/kgBW)	Mean Parasitemia ± SD	Mean % Inhibition ± SD	ED50 (mg/kgBW)
Hexane Extract	400	8.45 ± 6.51	46.94 ± 40.92	467.58
	200	12.51 ± 7.99	21.32 ± 50.30	
	100	13.95 ± 6.36	12.22 ±40.00	
	50	15.30 ± 7.57	3.76 ± 47.64	
	25	19.66 ± 7.61	-23.69 ± 52.86	
Ethyl acetate Extract	200	10.75 ± 3.18	17.29 ± 29.89	2072.38
	100	14.24 ± 7.12	32.38 ± 20.03	
	50	13.15 ± 4.75	10.42 ± 44.82	
	25	13.15 ± 6.02	17.29 ± 37.89	
	12,5	14.89 ± 6.58	6.33 ± 41.40	
Methanol Extract	80	5.79 ± 2.80	63.59 ± 17.62	16.16
	40	6.86 ± 1.06	56.83 ± 6.64	
	20	7.05 ± 6.80	55.63± 42.78	
	10	8.31 ± 6.93	47.71 ± 43.59	
	5	9.59 ± 2.21	39.66 ± 13.93	
	2,5	10.70 ± 5.64	32.68 ± 35.49	
	1,25	12.40 ± 6.02	22.01 ± 37.84	
Negative control	0	15.90 ± 5.84	0.00 ± 0.00	

activity, the ethyl acetate extract was inactive (2074.02 mg/kgBW) and hexane extract was moderate (467.58 mg/kgBW). Methanol extract has active compounds that could inhibit the growth of *Plasmodium* compared with hexane and ethyl acetate extract. Some medicinal plants of Indonesia that has been reported to have antiplasmodial activity *in vivo* are ethanol extract of mimba leaves (ED_{50}:1.27 mg/kgBW)[8], methanol extract of the roots of pasak bumi (ED_{50}:11.20 mg/kgBW), brotowali (ED_{50}:97.04 mg/kgBW)[9], meniran (ED_{50}:9.1 mg/kgBW), mahogany (ED_{50}:199.87 mg/kgBW)[10], aqueous extract of sungkai leaf (ED_{50} = 87.79 mg/kgBW)[11] and pauh kijang leaf (ED_{50}:36.95 mg/kgBW)[12]. Based on the ED_{50} value of this some medicinal plants, the ethanol extract of mimba leaves, methanol extract of pasak bumi and methanol extract of meniran have an *in vivo* antiplasmodial activity more potential than kapur leaves.

Those data supports the truth of the use of kapur leaves empirically by the people of Maluku as an antimalaria. Although certain compounds that could inhibit *Plasmodium* from methanol extracts of kapur leaves have not been identified, expected potential of the methanol extract of kapur leaves can be used as a reference to search and develope a new antimalarial drugs from natural medicinal plants

Fractionation of Methanol Extract of *H. aculeatus* leaves. Vacuum liquid chromatography column was used for fractionation. The separation is based on differential partitioning between the mobile and stationary phases.

The stationary phase used was silica gel 60H (1105932) and the mobile phase using a variety of ratio *n*-hexane-chloroform and chloroform-methanol and methanol in gradient. A number of 62 fraction was obtained from fractionation of methanol extract and based on thin layer chromatography, 18 (FG_1-FG_{18}) combined fractions, similar chromatograms profiles was obtained. Those combined fractions were tested on heme polymerization inhibitory activity to identify the most active one

Haem Polymerization Inhibition Activity Test of Combined Fraction of Methanol Extracts. Haem polymerization is a mechanism in changing Ferrriprotoporfirin IX (FPIX) which is toxic to *Plasmodium*. Haem polymerization occurs within the food vacuole of *P. falciparum*. FPIX formed when *Plasmodium* degrade hemoglobin as a source of nutrients. FPIX be polymerized into hemozoin, a non toxic malarial pigment. Hemozoin is identic with β-hematin. Haem polymerization inhibitory activity of a compound is directly related to its potential as an antimalaria. Concentration of β-hematin from compound tested and control samples were calculated based on standard curve equation: y = 0.378 x + 0.166, y was absorbance value and x was concentration of β-hematin. The value of R^2 was 0.969. Probit analysis showed that IC_{50} of FG varies between 0.018 - 3x107 mg/ml and the positive control (chloroquine) was 240.98 µg/ml (Table 2).

Table 2. The IC_{50} HPIA of combined fraction of *H. aculeatus* methanol extracts

Combined fraction	Mean of IC50 ± SD (µg/ml)
FG 1	38217.88 ± 66171.37
FG 2	1E+0.10 ± 1.732E+0.10
FG 3	1229.78 ± 543.12
FG 4	536.38 ± 286.58
FG 5	1258.25 ± 126.36
FG 6	170.26 ± 135.96
FG 7	137.12 ± 103.54
FG 8	18.22 ± 7.25
FG 9	859.67 ± 1116.48
FG 10	3E+011 ± 5.774E+011
FG 11	2E+038 ± 4.04E+0.38
FG 12	18165.84 ± 29569.35
FG 13	9601.45 ± 13707.42
FG 14	594313.7 ± 1020187
FG 15	22145.13 ± 31317.80
FG 16	2590962 ± 8961.30
FG 17	9871.13 ± 8961.30
FG 18	1401.69 ± 899.14
Positive control (chloroquine)	240.98 ± 50.14

Among the 18 FG, the most active fraction in inhibiting haem polymerization was FG_8 due to the smallest IC_{50} value that was equal to 18.22 ± 7.25 µg/ml. When compared with IC_{50} value of positive control (chloroquine), FG_8 had lower value showed that activity of FG_8 on haem polymerization inhibitory was greater than chloroquine. Haem polymerization inhibitory activity had been reported from several medicinal plants such as ether soluble fraction (fraction 5, 6 and 7) of kembang bulan leaves (*T. diversifolia*) methanol extract and the IC_{50} were 162.20, 216.30, and 231.54 µg/ml respectively[13]. The FG_8 methanol extract of kapur leaves had IC_{50} lower than those extracts. This indicates that the FG_8 methanol extract of kapur leaves have greater activity.

Phytochemicals Active Fraction (Combined Fraction 8) of Methanol Extracts *H. aculeatus*. Phytochemical analysis of FG_8 methanol extracts of *H. aculeatus* leaves using several spray reagents such as dragendorf, anisaldehid sulfate, sitroborat, Liebermann-Burchard, $FeCl_3$ and KOH. Three groups of triterpenoid compounds, phenolic compounds and essential oils were

obrtained.

Fractionation Combined Fraction 8 (FG_8). The most active fractions (FG_8) from methanol extract further separated using pressed column chromatography using stationary phase of silica gel 60 PF254 Merck (230-400 mesh) and a mobile phase gradient using chloroform-ethyl acetate and ethyl acetate-methanol at various ratio. Fraction of its similar TLC profiles were merged and 19 fractions were obtained. Fractions 10-13 were grouped in $FG_{8.5}$ show a dominant blue fluorescence (Figure 1). Further separation was intended to isolate the fraction of compounds that show blue fluorescence.

Figure 1. TLC profile of FG8.5 methanol extract of H. aculeatus leaves

Isolation Blue fluorescence compounds in the $FG_{8.5}$. Blue fluorescence compounds in $FG_{8.5}$ methanol extracts of *H. Aculeatus l*eaves isolated using preparative thin layer chromatography (PLC) by multiple elution (4 times elution) with 100% chloroform eluent. Elution results showed there were 4 bands at UV 254 nm and 3 bands at 365. PLC chromatogram showed that the third band (UV 254 and 365 nm), had blue fluorescence. Fluorescence bands at UV 365 nm showed thicker than UV 254 nm. The blue fluorescence compounds contain many compounds with similar polarity, so that in the chromatogram did not seem to separate. After the band of blue fluorescence at 365 nm UV marked, scraped and then extracted with chloroform, the filtrate evaporated in a way aerated. Isolates were then analyzed using the blue fluorescence GC-MS.

GC-MS analysis of Isolates Blue Fluorescent. GC-MS analysis of the isolates showed that the blue fluorescence compound was not a single compound but contain many compounds. More than 3.11% of the amount consist of 7 compounds such as essential oils (eugenol), straight-chain saturated fatty acids and esters (isopropyl myristate, methyl ester of palmitate and palmitic acid) and essential oil (eugenol), straight chain saturated fatty acids and esters (isopropyl myristate, methyl ester of palmitic acid and palmitic), phtalat acid ester such as 1,2 Benzendicarboxylic acid bis (2-methylpropyl) ester, 1,2 Benzendicarboxylic acid Butil 2-metilpropil ester, and 1,2 Benzendicarboxylic acid bis (2-ethylhexyl) ester.

According to some research, palmitic acid can induce cardiomyocyte apoptosis through de novo ceramide formation and activation of mitochondria[14]; to regulate apoptosis, such as in the case of pancreatic cells and induces cell death through mtDNA and genomic DNA damaged[15]. It was also reported that palmitic acid is antibacterial, antitumor and antifungal[16,17,18]. Fatty acids that have been reported as an antimalarial is oleic fatty acids and showed a very weak activity *in vitro*[1]. 1,2 benzendicarboxylic acid bis (2-ethylhexyl) ester reported to have activity as an antibacterial, can inhibit tumor growth and potential as an antifungal[19]. Eugenol is widely used in the medical field (dentistry) as well as pharmaceutical. Hemaiswarya and Doble[20], reported that eugenol has a biological activity as antibacterial against gram-positive bacteria. Although many studies have reported on various biological activities of palmitic acid; (2-ethylhexyl) pthalat and eugenol, but its activity

as an antimalarial not yet known and needs to be examined further. It is estimated that seven of these compounds may have antimalarial activity. According Bassilico *et al.*[6], compounds that can inhibit the polymerization of haem are the compounds which have OH groups which can react with iron ions in the haem so that it can inhibit the polymerization of haem. Eugenol and palmitic acids have hydroxyl groups as well as fatty acid ester hydrolyse reaction to form fatty acids and alcohols which contain OH groups. Phthalat esters also hydrolyse reaction to form a carboxylic acid and alcohol containing OH groups. Thus the seven compounds were expected as potential antimalarial candidate.

CONCLUSION

Based on this results, it can be concluded that methanol extract of kapur leaves (*H. aculeatus*) was the most potential in vivo antiplasmodial activity with ED_{50} value of 16.16 mg/kgBW. Combined fraction 8 was the most active fraction with IC_{50} HPIA of 18.217 ± 7.246 µg/ml. The content of compounds in methanol extracts FG_8 were triterpenoids, phenolic compounds and essential oils. Seven blue fluorescence compounds in $FG_{8.5}$ identified using GC-MS were essential oils (eugenol), straight-chain saturated fatty acids and esters (isopropyl myristate, methyl ester of palmitate and palmitic acid) and essential oil (eugenol), straight chain saturated fatty acids and esters (isopropyl myristate, methyl ester of palmitic acid and palmitic), phtalat acid ester such as bis(2-methylpropyl) phtalat, Butil 2-metilpropil pthalat, and bis(2-ethylhexyl) phtalat.

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