The Effect of *Asparagus racemosus* Root Extract Against *Plasmodium yoelii* 17XL Infected Mice

ผลของสารสกัดจากรากสามสิบในการต่อต้านการติดเชื้อปรสิตชนิดพลาสโมเดียม โยอิไลด์ เซเว่นทีน เอ็กซ์แอล ในหนูทดลอง

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ABSTRACT

Nowadays, *Plasmodium falciparum* become increasingly resistant to standard antimalarial drugs. The herbal plants play an important role to scavenging free radical product which effect from malaria infection. The aim of the study was investigated the effect of *A. racemosus* root extracts on malaria infection. *A. racemosus* extract 750 and 250 mg/kg doses were treated in *P. yoelii* 17XL infected mice then percent parasitemia all stage was checked daily to calculated percent suppression parasitemia and observed histology changes of liver. The extract at 750 mg/kg was able to suppress ring stage in erythrocytic stages and decrease percentage of hemozoin in liver. Moreover, the antioxidant capacity was assessed by Oxygen radical absorbance capacity assay and showed value at 18,385 µM TE/100 g. The investigating indicated that *A. racemosus* root extract deserve to be investigated further as potential antimalarial agents.

บทคัดย่อ

ในปัจจุบันเชื้อ *Plasmodium falciparum* เริ่มมีการดื้อต่อยาที่ใช้ในการรักษาเพิ่มขึ้น และพืชสมุนไพรมีบทบาทสำคัญในการขจัดอนุมูลอิสระที่เป็นผลมาจากการติดเชื้อมาลาเรีย ซึ่งจุดมุ่งหมายของการศึกษานี้คือศึกษาผลของสารสกัดจากรากสามสิบต่อการติดเชื้อมาลาเรีย สารสกัดที่ความเข้มข้น 750 และ 250 มก. / กก. ถูกใช้ในการทดสอบกับหนูที่ติดเชื้อพลาสโมเดียม โยอิไลด์ เซเว่นทีน เอ็กซ์ แอล จากนั้นตรวจสอบเปอร์เซ็นต์พาราไซต์เพื่อนำไปคำนวณหาเปอร์เซ็นการยับยั้งการเจริญของเชื้อ และศึกษาลักษณะการเปลี่ยนแปลงทางจุลกายวิภาคศาสตร์ของตับ ผลการทดสอบพบว่าสารสกัดที่ความเข้มข้น 750 มก. / กก. สามารถยับยั้งเชื้อมาลาเรีย ในระยะ ring ที่ติดเชื้อภายในเม็ดเลือดแดงและลดเปอร์เซ็นต์ hemozoin ในตับได้ นอกจากนี้ความสามารถในการต้านอนุมูลอิสระได้รับการประเมินโดยการวิเคราะห์ความสามารถในการดูดกลืนรังสีอนุมูลอิสระเท่ากับ 18,385 µM TE / 100 กรัม จากการศึกษาชี้ให้เห็นว่าสารสกัดรากสามสิบมีศักยภาพในการต้านเชื้อมาลาเรีย และอาจจะนำไปเป็นสารสำคัญในการต้านเชื้อมาลาเรียได้

Keywords: *Asparagus racemosus*, Antioxidant, Malaria

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Introduction

Malaria is a common and life-threatening disease in many tropical and subtropical areas. It is caused by the protozoan parasite Plasmodium. Human malaria is caused by four different species of Plasmodium: *P. falciparum, P. malariae, P. ovale and P. vivax*. Humans occasionally become infected with Plasmodium species that normally infect animals, such as *P. knowlesi*. *P. falciparum* is the most prevalent malaria parasite on the African continent. It is responsible for most malaria-related deaths globally. The malaria parasite is transmitted by female Anopheles mosquitoes, which bite mainly between dusk and dawn. (WHO, 2017) The severity of *P. falciparum* infection depends on ability of parasitized red blood cells to adhere on endothelial cells induced inflammatory processes, coagulation cascades, oxidative stress, apoptosis and sequester in the capillary network of vital organs such as brain, lungs, kidneys, liver. (Taoufi et al., 2011) Other researchers have described the occurrence of ischemia-reperfusion syndrome and the subsequent development of oxidative stress to be the result of the cyto-adherence characteristic of P. *falciparum* infection.They have discussed the implications of free radicals through oxidative stress in the physiopathogenesis of malaria. Malaria infection induces the generation of hydroxyl radicals ($OH^{\bullet}$) in the liver, which most probably is the main reason for the induction of oxidative stress and apoptosis. Additionally, some others observed that erythrocytes infected with *P. falciparum* produced $OH^{\bullet}$ radicals and H_2O_2 about twice as much compared to normal erythrocytes. (Percário et al., 2012) Nowadays, the best anti-malarial drugs are artemisinin-based combination therapy. Some anti-malarial drugs have limiting factors such as drug resistance, high cost and limited knowledge on safety in pregnancy. (Mutabingwa, 2015) Moreover, parasite has resisted to insecticides.

Thailand is the one of malaria endemic areas that also has many varieties of herbal plants which play an important role to scavenging free radical product, low cost and easily to found. *Asparagus racemosus* belongs to the family Asparagaceae, genus Asparagus. The plant has some different name such as Rak-Sam-sib, Pak-chee-Chang, Pak-Nham and Sam-Roi-Rak in Thai. *A. racemosus* is one of the most frequently used in traditional medicine. *A. racemosus* is known to possess a wide range of photochemical constituents which are steroidal saponins, oligospirostanoside, polycyclic alkaloid, isoflavones-8-methoxy-5, 6, 4-trihydroxy isoflavone-7-0-beta-D-glucopyranoside, cyclic hydrocarbon-racemosol, dihydrophenantherene, furan compound, carbohydrates-polysacharides, flavonoids, sterols, kaepfrol and miscellaneous. (Alok et al., 2013)

Previous study found the effect methanolic extract of *A. racemosus* (MEAR) in lipopolysaccharide (LPS)-induced oxidative stress in rats. The result showed that MEAR supplementation resulted in normalization of brain glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and decreases in the levels of malondialdehyde (MDA) with reduction of nitric oxide and cytokines in the brain. (Ahmad et al., 2015) Moreover, P. Jeruto et.al studied to screen seven selected medicinal plants from Kenya for antimalarial activity. They evaluated in *in vitro* and *in vivo* antiplasmodial activity of seven medicinal plants. One of these is *A. racemosus*. *In vitro* study, they used parasite cultures the Sierra Leonean CQ sensitive (D6) and Indochinese CQ resistant (W2) *Plasmodium falciparum* strains. They found that *A. racemosus* methanol extract showed good activity (IC_{50}=31.35 µg/ml). *In vivo* study, male albino mice (6 to 8 weeks old, weighing 20±2 g) were used in the experiment. The result represented chemosuppression at

54.05% and parasite density at 19.07%. *A. racemosus* leaves extract exhibited the highest *in vivo* antiplasmodial activity. (Jeruto et al., 2015) However, there are little information that was available about antimalarial activities. In this study, we chose *Plasmodium yoelii* because previous study reported the whole-genome shotgun sequence of one species, *Plasmodium yoelii yoelii*, and comparative studies with the genome of the human malaria parasite *Plasmodium falciparum* clone 3D7. A synteny map of 2,212 *P. y. yoelii* contiguous DNA sequences (contigs) aligned to 14 *P. falciparum* chromosomes reveals marked conservation of gene synteny within the body of each chromosome. Of about 5,300 *P. falciparum* genes, more than 3,300 *P. y. yoelii* orthologues of predominantly metabolic function were identified. (Carlton et al., 2002) We used mice for experimental because rodent models of malaria have been used widely and successfully to complement research on *P. falciparum*.

Objectives of the study

Primary objective

To investigate the effect of *A. racemosus* root extracts on malaria infection whether it could become anti-malarial drug candidate.

Secondary objectives

1. To investigate the effect of *A. racemosus* root extract on antioxidant status.

2. To investigate morphology and histology changes in liver and liver profiles in *P. yoelii* 17XL infected mice after treatment with *A. racemosus* root extract.

Methodology

1. Preparation of *A. racemosus* root extract

The tuberous roots were cleaned and shade dried and milled into coarse powder by a mechanical grinder. The shade dried roots were crushed into small pieces and powdered. The powder was loaded into Soxhlet extractor in 8 batches of 150 g each and was subjected to extraction for about 30-40 h with 95% ethanol. After extraction, the solvent was distilled off and the extract was concentrated under reduced pressure on a water bath at a temperature below 50°C to a syrupy consistency. Then it was dried in the desiccators. The yield is about 30.39 %. The administered dose is 250 (low) and 750 (high) mg/kg bodyweight of mouse intraperitoneally injection, per day in each experimental mouse.

2. Antioxidant determination of *A. racemosus* roots extract by ORAC assay

Oxygen radical absorbance capacity (ORAC) is used to evaluate oxidant inhibition and antioxidant properties in plants. This assay was used to evaluate antioxidant activity from *A. racemosus* extract. The ORAC assay uses free radical damage to fluorescent probe and result of fluorescent intensity in downward change. The degree of change is amount of radical damage. The antioxidant results presence inhibition in free radical damage to fluorescent compound. The antioxidants and blanks are run in parallel so protection reaction can calculate by area under curve AUC Comparison to set of known standards to calculate equivalents and compare result from sample Trolox6hydroxy 2,5,7,8

tetramethylchroman2carboxylic acid is a vitamin E derivative used as calibration standard ORAC results are expressed as Trolox equivalent μM

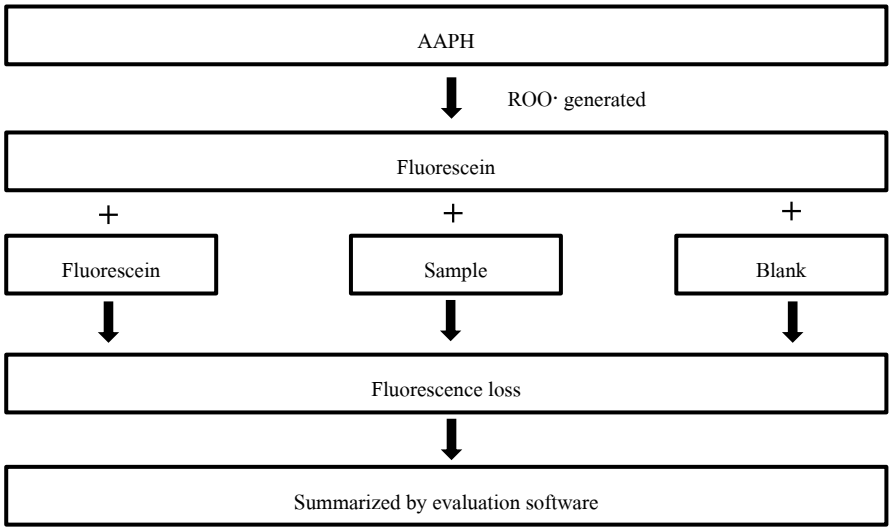

Figure 1 Principal of ORAC assay

3. ORAC assay antioxidant capacity determination of *A. racemosus* extract in mice plasma

Antioxidant capacity in mice plasma was determined using oxygen radical absorbance capacity (ORAC) assay to measured antioxidant inhibition against peroxyl radical generated by thermal decomposition of AAPH. For determination, the blank solution, fluorescein solution, standard Trolox solution and serum solution were prepared. Phosphate buffer (pH 7.2) was used as blank solution. 0.5 ml of blank solution.

Sample extraction

1. Weigh 1-5 g of sample into 125 ml Erlenmeyer flask.
2. Add 20 ml of 50% acetone.
3. Shake with 400 rpm speed by Shaking machine at room temperature, 1 hour.
4. Transfer to 15 ml centrifuge tube.
5. Centrifuge at 2,500 rpm for 15 min.
6. Transfer the supernatant of extracted sample into a test tube.

Spectrofluorometer measurement

1. Set temperature of water bath at 37°C.
2. Set the spectrofluorometer with an excitation wavelength at 493 nm, emission wavelength at 515 nm. Warm the instrument for 30 min.
3. Add 3 ml of fluorescein working solution into the cuvette.
4. Add 0.5 ml of Trolox standard or blank or sample supernatant.
5. Add 0.5 ml of AAPH
6. Measured the intensity of fluorescence, every 1 min until the value nearly constant or equal zero.

7. Put curve between time and relative fluorescein.

4. *In vivo* anti-malarial activity assay

4.1 Parasites preparation

P. yoelii 17XL infected blood stock was thawed at room temperature. After that 100 µL of blood stock was incubate to donor mice and calculate parasitemia. Blood was collected by tail snip bleed for thin blood smear, fixed with methanol and Giemsa stained. Specimen was examined under light microscope at magnification of 100X. Mice only use after ensuring that contain 20% parasitaemia. The donor mice were sacrificed by anesthesia with Nambutal and collected blood through cardiac puncture. There are 4.8×10^8 infected erythrocytes contain in 1 mL of blood from donor mice. After that diluted blood in normal saline to make the solution of 10^7 infected erythrocytes per mL Then, each mouse was inoculated with 0.2 ml of 10^7 infected red blood cells by intraperitoneally (i.p.).

4.2 Experimental design

The ethical certificate was approved by Siriraj Animal Care and Use Committee (No. SI-ACUP 001/2561). Thirty female ICR mice were randomly divided into 6 groups (5 mice of each group). Before starting the experiment, percent pack cell volume of each mouse was measured by using 100 µl of blood from tail. Before the real experiment, stock of *P. yoelii* 17XL was incubated into the other 5 donor mice. After that, percent parasitemia was checked daily when percent parasitemia increased to 20-80% of infected red blood cell. Mice was sacrificed by injecting 0.2 ml of Numbutal via intraperitoneally. Blood was withdrawn by heart puncture and later was used for mice of all group.

Group I: Negative Control (NC): Only standard diets were given to each mouse for 7 days. This group was not incubated with parasite and received only normal saline intraperitoneal injection.

Group II: Infect only (PC): This group was served as Parasite only group. $1x10^7$ of *P. yoelii* 17XL were given to each mouse at day 0 and standard diets were given to each mouse for 7 days. This group was received no treatment.

Group III: High dose AE: The mice in this group were daily administered 750 mg/kg per body weight of mouse (high) dosage of *A. racemosus* root extract via intraperitoneally for 7 days. This group serve as "high dose extract only group"(HD).

Group IV: Low dose AE: The mice in this group were daily administered 250 mg/kg per bodyweight of mouse (low) dosage of *A. racemosus* root extract via intraperitoneally for 7 days. This group serve as "low dose extract only group" (LD).

Group V: Parasite high dose AE: The mice in this group were infected by 10^7 parasited erythrocytes per ml. via intraperitoneally at day 0. At day 1, the mice were administered 750 mg/kg bodyweight of mouse high dosage of the *A. racemosus* root extract via intraperitoneally daily until day 7. This group serves as "high dose treatment group" (IHD).

Group VI: Parasite low dose AE: The mice in this group were infected by 10^7 parasited erythrocytes per ml. via intraperitoneally at day 0. At day 1, the mice were administered 250 mg/kg bodyweight of mouse low dosage

of the *A. racemosus* root extract via intraperitoneally daily until day 7. This group serves as "low dose treatment group" (ILD).

Parasitemia was checked daily from tail snip bleed by classical Giemsa staining method. Then, at the end of experiment, all of mice was sacrificed by using Numbutal. Whole blood of heart was withdrawn by heart puncture technique and the blood was taken to centrifuge at 2,800 rpm in 4 °C for 7 minutes in order to collect serum and store at -20 °C until used. Then, liver was removed for later processing of paraffin block and hematoxylin and eosin technique were investigated in order to observe pathohistological changes of liver after *A. racemosus* treatments.

4.3 Determination percent reduction of parasitemia

A. racemosus roots extract effects against malaria infection on blood stage was evaluate by calculate percent reduction of parasitemia. Percent reduction of parasitemia is percentage related between number of infected red blood cells and total number of red blood cells. Collecting mice blood from tail snip bleed after that thin blood smear was prepare. Slides were fixed with methanol, Giemsa stained and determined under light microscope at 100X magnification. The parasitemia was counted on the photograph which took from microscope. Then calculated daily from day 1 of infected mice to the end of experimental. Day 1 is the day that parasites were supposed to be found in red blood cells. The percent reduction of parasitemia was expressed as mean ± SEM. One-way ANOVA was use as statistical analysis test for comparison of mean between groups.

Calculate percent parasitemia from the formula:

$$\% \text{ Parasitemia} = \frac{\text{Number of infected red blood cells}}{\text{Number of total red blood cells}} \times 100$$

Calculate percentage parasite suppression from the formula:

$$\% \text{ Suppression} = \frac{A - B}{A} \times 100$$

A = mean of parasitaemia on day 4 in infected control group

B = mean of patatsitaemia on day 4 in infected treatment group

5. Morphological study

After sacrificing the mice, livers were collected and photo taken. Then the changes in color, size, and weight of liver were studied. Firstly, the color of liver was observed and compared with other groups. Secondly, size of liver was measured length and width in centimeter. The lastly, liver weight was calculated to study percent relative weight.

6. Histopathological study

Liver tissues, one is for observing histopathological recovering after *A. racemosus* treatment using hematoxylin-eosin (H&E) method. Normal liver was shown normal structure without necrosis, fatty depositions, or any degeneration. The livers were studied under light microscope at magnification 40X. After that, hepatocytes, Kupffer cells, hepatic sinusoids and hemozoin were observed and measured by ImageJ and Image tool programs.

7. Statistical data analysis

Percent parasitemia was calculated and shown in result. The different of parasite infection among groups was reported as Mean ± SEM compared by using one way-ANOVA. The differences at *p*-value less than 0.05 was justified as significantly difference.

Results

1. Anti-oxidant capacity of *A. racemosus* root ethanol extract

The anti-oxidant capacity of *A. racemosus* root ethanol extract was examined by ORAC assay. For ORAC assay use to analyze free radical damage to fluorescent probe and result of fluorescent intensity in downward change. The anti-oxidant results presence inhibition in free radical damage to fluorescent compound. Therefore, *A. racemosus* root ethanol extract sample was calculated equivalents compared to set of standard Trolox solution results expressed as Trolox equivalent (μM). The value of *A. racemosus* root ethanol extract compared with standard Trolox was presented 18,385 uM TE/100g.

2. Anti-oxidant capacity in plasma of *A. racemosus* root ethanol extract

Table 1 Determination of antioxidant capacity of mice plasma in each group (Mean ± SEM).

Groups (n=5)	Antioxidant capacity (μM TE/ml)	*p-value*	
		N	P
Normal control	14.32±0.29		
Parasite control	8.72±0.40	0.000*	
High dose AE	17.81±0.26	0.000*	0.000**
Low dose AE	16.04±0.29	0.006*	0.000**
Parasite high dose AE	11.87±0.34	0.000*	0.000**
Parasite low dose AE	9.12±0.17	0.000*	0.943

* The mean difference is significant at *p-value* < 0.05 compared to NC group (N)

** The mean difference is significant at *p-value* < 0.05 compared to PC group (P)

Anti-oxidant capacity in mice plasma after received *A. racemosus* extract was determined using oxygen radical absorbance capacity (ORAC) assay to measured antioxidant inhibition against peroxyl radical generation. The result expressed as mean ± standard error of the mean (Mean ± SEM). In addition, the differences at the $p < 0.05$ considered as the significantly difference. The antioxidant capacity in Table 1 represented comparison the value among groups. From this study, the result of antioxidant capacity in mice plasma from HD and LD groups present significantly increasing antioxidant property when compare to NC group similar to compared with PC group. After statistical analysis compared means, the data of HD group compared to NC and PC groups showed $p=0.001$. Next, the data of LD group compared NC group showed $p=0.006$ and LD group compared

PC group at $p<0.001$. Furthermore, IHD group antioxidant capacity showed significantly high more than PC group. From this result presented $p\leq0.001$ between IHD and PC groups but showed no significantly difference between ILD and PC groups.

3. Anti-malarial activity on erythrocytic stages of mice infected *P. yoelii* 17XL after treatment with *A. racemosus* root ethanol extract

Percentage parasitemia of infected *P. yoelii* 17XL erythrocytic stage in NC, PC, IHD and ILD groups from Day 1 until day that the last mouse died. From Day 1 to Day 7 the data reported as Mean ± SEM. In NC, HD, and LD groups were not showed percentage parasitemia. For PC group percentage parasitemia had increasing continuously from day 1 (3.49±0.53) to days 6 (66.91±3.41) after that began reducing in day 7 (62.25±2.60) The last mouse of PC group died on day 7 with maximal percent parasitemia (67.43 %). In IHD group showed percent parasitemia from day 1 (3.16±0.92) increasing to day 6 (58.64±5.37) after that begin reducing in day 7 (56.64±6.21). The last mouse in IHD group died on day 7 with maximal percent parasitemia (70.85%). For ILD group, mice showed increasing percentage parasitemia from day 1 (2.19±0.35) to maximum on days 6 (61.18±3.56) then reducing on day 7 (54.87±10.41) until died in day 7 with maximal percent parasitemia (75.39%). Percent suppression showed 6.76 ± 20.97 in IHD group and 9.29 ± 3.98 in ILD group. There was no significantly difference in suppressive percentage parasitemia of both groups when compared to PC group.

Ring stage, the data showed 3.47±0.55 on Day 1 and increased to maximal 31.91±1.57 on Day 6. After that ring stage of PC group decreased to 17.96±1.47 on Day 7 and the last mice died on Day 7 with 20.29 percent. For IHD group, ring stage percentage on Day 1 was 3.16±0.92 increased continuously to 27.96±2.85 on Day 6. On Day 7, ring stage percentage reduced to 16.58±5.92. The last mice from IHD group died on Day 7 with maximal percentage ring stage was 34.3%. For ILD group, percentage ring stage showed 2.19±0.35 on Day 1 increased to maximal ring stage on Day 5 with 27.98±1.42 and reduced to 24.50±2.66 on Day 6. On Day 7, percent ring stage of IHD group reduced to 24.02±8.87 and the last mice died in Day 7. Moreover, showed suppressive percentage ring stage 44.94 ± 2.78 in IHD group. There was significantly difference at $p=0.001$ compared to PC group. For suppressive percentage ring stage in ILD group was 34.43 ± 5.01 that presented significantly difference at $p\leq0.001$ compared to PC group.

Trophozoite stage, the result of percentage trophozoite stage in PC group on Day 1 was 0.02±0.02 increased continuously to maximal percentage at 32.14±8.27 on Day 7. The last mice in PC group died in Day7 with 46.58 percent trophozoite stage. For percentage trophozoite stage of IHD group, the data showed 0.00±0.00 on Day1 but after that day parasitemia began increasing to 1.45±0.20 on Day 3. On Day 7, the data of IHD group presented maximal percent trophozoite stage was 28.37±3.06. After that the last mice in IHD group died in Day 7 with 37 percentage trophozoite stage. Next, the result of percentage trophozoite stage from ILD group Day 1 was 0.00±0.00 then increased continuously to 24.64±1.83 on Day 7. The last mice in ILD group died in Day7 with 26.69 percent trophozoite stage. There were not significantly different in suppressive percentage parasitemia.

Schizont stage, the percentage schizont stage data of PC group were not found in Day 1. On Day 2, percentage schizont stage 0.05±0.03 presented in this day and increased continuously to maximum 12.15±4.65 on Day 7. The last mice in this group died in Day 7 with 21.14 percent schizont stage. Then, the percentage schizont stage data of IHD

group was not showed in Day 1 and Day 2 but after that there was 0.03±0.03 on Day 3. On Day 4, IHD group showed 1.83±0.74 percent schizont stage and increased continuously to maximal schizont at 11.69±1.32 on Day 7 and the last mice in IHD group died in Day 7 with 14.79 percentage schizont stage. Next, the result of percentage schizont stage

Table 2 The data of effective of *A. racemosus* root ethanol extract on anti-malarial activity (Mean±SEM)

Groups	Pre-PCV	Post-PCV	%Suppression			
			All stage	Ring	Trophozoite	Schizont
NC	77.19 ± 2.12	86.38 ± 1.80	-	-	-	-
PC	67.76 ± 2.36*	16.91 ± 3.01*	0.00	0.00	0.00	0.00
HD	86.03 ± 0.98*,**	69.53 ± 3.29*,**	-	-	-	-
LD	87.50 ± 1.21*,**	70.39 ± 2.76*,**	-	-	-	-
IHD	90.57 ± 1.54*,**	27.05 ± 4.61*	6.76 ± 20.97	44.94 ± 2.78**	-6.19±28.42	-94.68±78.32
ILD	90.05 ± 2.01*,**	23.05 ± 3.37*	9.29 ± 3.98	34.43 ± 5.01**	-14.39±16.35	-180.64±28.64

Table 3 The data of morphological and histopathological liver tissue (Mean±SEM)

Groups	%Relative weight	Width (cm)	Length (cm)	Diameter of each cells (µm)			
				Hepatocyte	Hepatic sinusoid	Kupffer cell	%Hemozoin
NC	3.82 ± 0.14	1.88 ± 0.49	2.46 ± 0.10	14.09±0.20	4.38±0.19	5.45±0.06	-
PC	6.26 ± 0.18*	2.50 ± 0.00*	3.14 ± 0.30	13.09±0.32	8.86±0.42*	5.09±0.16	6.34±0.24
HD	4.86 ± 0.08*,**	3.00 ± 0.00**	2.52 ± 0.08	15.90±0.45*,**	4.61±0.20**	5.86±0.97**	-
LD	4.65 ± 0.16*,**	2.06 ± 0.20	2.60 ± 0.19	15.25±0.23**	4.90±0.19**	6.03±0.99*,**	-
IHD	6.50 ± 0.12*	2.60 ± 0.10*	3.20 ± 0.20	13.61±0.30	9.38±0.38*	5.36±0.83	4.57±0.34**
ILD	6.28 ± 0.27*	2.60 ± 0.10*	2.80 ± 0.12	13.53±0.38	9.09±0.28*	5.31±0.15	3.83±0.57**

* The mean difference is significant at p-value < 0.05 compared to NC group (N)

** The mean difference is significant at p-value < 0.05 compared to PC group (P)

not found in ILD group in Day 1 and Day 2 but showed 0.13±0.03 on Day 3. Percent schizont stage of ILD group increased continuously to maximal 6.20±1.31 at Day 7 and the last mice in ILD group died with 6.76 percent schizont stage in Day 7. There were not suppressive percentage parasitemia. All data of parasite stage presented in Table 2.

4. Morphology and histopathology change in liver mice resulted from *A. racemosus* root ethanol extract

Morphology, color of liver, Normal control group (Figure 1A) showed reddish brown color, high dose AE (Figure1B) and low dose AE (Figure1C) group showed reddish brown color not different from normal control group. In contrast, parasite control group (Figure1D) showed brown color, parasite high dose AE (Figure1E), and parasite low dose AE (Figure1F) groups black color. Liver width and length showed in Table 3. In addition, hepatocytes, Kupffer cells, Hepatic sinusoids and hemozoin were observed and measured under LM. The data showed in Table 3.

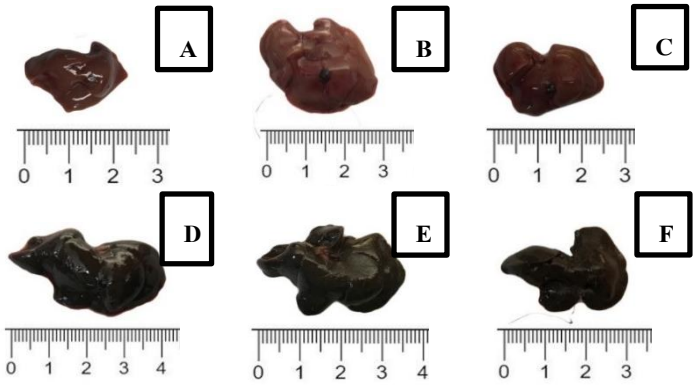

Figure 1 Morphology of mice livers; A: Normal control, B: High dose AE, C: Low dose AE, D: Parasite control, E: Parasite high dose AE, F: Parasite low dose AE.

Discussion and Conclusions

Asparagus racemosus have an antioxidant capacity which important role in scavenging free radicals. Several researchers founded the potential of antioxidant property. Previous studies reported positive results for steroids, phytosterols, carbohydrates, tannins, anthraquinones, saponins, glycosides and flavonoids. (Agrawal et al., 2008) This study *A. racemosus* ethanol extract was investigated by ORAC assay to assess the antioxidant capacity. From the obtained result in this study, the ORAC value showed 18,385 µM TE/100 g. The antioxidant capacity was compared with standard Trolox. Thus, *A. racemosus* extract have an antioxidant capacity which important role in scavenging free radicals.

During intra-erythrocytic parasite stage, malarial parasite produces the redox active by-products, free haem and H_2O_2. There are exposed to increased ROS formation through the Fenton reaction. (Rahbari et al., 2017) Both FP and H_2O_2 are toxic molecules that the parasite needs to destroy or neutralize. However, the parasite also supplies antioxidant moieties to the host and possesses an efficient enzymatic antioxidant defense system. In this study founded that the ethanolic extract of *A. racemosus* giving anti-malarial activity in infected mice. It presented mild suppression on ring stage at 44.94% under high dosage (750 mg/kg) and 34.43% under low dosage (250 mg/kg). Previous study, the major ingredients known to occur in most medicinal

plants are the alkaloids, terpenoids and flavonoids whose presences is attributed to the antimicrobial activity in plants. The root extracts contain alkaloids, and the well-known antimalarial, plant origin e.g. quinine, are alkaloids. However, it is worth noting that the plant materials that contain alkaloids also contained terpenoids. This is noteworthy because the newer antimalarial compounds, such as quinghaosu, which is already in clinical use, and the quassinoids, are terpenoids. (Nostro et al., 2000) This would be attributed to the fact that ethanolic extract of *A. racemosus* contained many of the bioactive compounds from the plant materials which acts on the plasmodial parasites.

Furthermore, the liver is an important organ involved during the hepatic stage of the malaria parasite's life cycle where malaria sporozoites develop into merozoites. The merozoites are then released into the circulation and enter the erythrocytic stage. In the erythrocytic stage, parasitized red blood cells (PRBCs) become sequestered in small blood vessels. The degraded hemozoin pigment is then engulfed by local tissue macrophages, such as Kupffer cells and alveolar macrophages. Common histopathological findings of the liver in *P. falciparum* malaria include reactive Kupffer cells, retention of hemozoin pigment and minimal PRBC sequestration. (Whitten et al., 2011), (Rupani et al., 2009) From the study of K. Vasant et al. founded that hyperplastic Kupffer cells and portal tract inflammation are two main features found in the liver tissues of severe *P. falciparum* malaria cases. In this study, most cells in NC group showed normal hepatocytes similar to HD and LD. In hepatic sinusoids appeared clear area and lined by Kupffer cells without any pigment inside in NC, HD and LD. In contrast, infected groups showed the enlargement of hepatic sinusoids. In Kupffer cells, NC groups showed normal characteristic similar to HD and LD groups but the diameter of all groups was not different. The accumulation of hemozoin in IHD and ILD groups showed reducing of percent hemozoin when compared to PC significantly difference. Therefore, *A. Racemosus* extract have not the hepatoprotective effect from malaria parasite but it was able to reduce percent hemozoin because hemozoins are released during trophozoite and schizont stage. When the extract presented the suppression on ring stage, it resulted in decreasing of percent hemozoin as well.

Conclusion, the fact that *A. racemosus* had an anti-oxidant property which are important role in antimalarial activity. This may be explained by synergistic effects of the constituents of the crude extracts or presences of prodrugs. *A. racemosus* root ethanolic extract at 750 mg/kg seems to be a potential drug candidate for malaria treatment to suppress ring stage in erythrocytic stages and decrease percentage of hemozoin in liver. However, it is necessary to carry out detailed phytochemical studies to identify the active constituents in this plant.

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