

# Antiplasmodial Activity of Ethanolic Extract of *Macaranga Gigantea* Leaf and Its Major Constituent

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## ABSTRACT

**Introduction:** This research main goal is to study the antiplasmodial activity of *Macaranga gigantea* leaf ethanolic extract and its major components on malaria parasites using *ex vivo* model. **Methods:** This study was conducted by extraction of *M. gigantea* leaves using ethanol and isolation of its major constituent. The extract and isolate were tested *ex vivo* on Balb-C mice's blood after i.p. administration of *Plasmodium berghei* strain ANKA. Antiplasmodial activity was observed from mice blood treated by various concentration of either extract or isolate and the parasitaemia percentage were determined by calculating infected blood cell after 24 h of the treatment. It is expressed as decreased of parasitaemia levels and percent of inhibition. Qualitative analysis of active fraction were tested by HPLC method. Chemical structure of isolate were characterized by using UV, IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and MS spectrophotometry. **Results:** *Ex vivo* antiplasmodial study gave the percent inhibition as much as 92.1; 85.7; 64.1; 41.5 and 21.7% at extract concentrations of 300, 100, 30, 10 and 3 µg/mL respectively. The IC<sub>50</sub> values of the extract was 27.1 µg/mL. With respect to the percent of inhibition, at the same concentration, the isolate showed activity as much as 70.2; 62.5; 39.1; 21.7 and 10.8%. The IC<sub>50</sub> value of the isolate was 60.2 µg/mL. At the same concentration with extract and Isolate, Pyrimethamine as positive control gave percent inhibition of 94; 87.5; 44.8; 15.; and 12%, with IC<sub>50</sub> of 31.4 µg/mL. The results showed that major constituent of *M. gigantea* leaves is flavonoid. HPLC analysis using a photo diode-array detector showed that the active fraction have same retention time with that of apigenin as standard. Based on instrumental analysis data and compared with literature, a flavonoid derivate known as apigenin can be said has been isolated. **Conclusion:** It can be concluded that either *M. gigantea* leaves extract or isolated active constituent known as apigenin have potent antiplasmodial property.

**Key words:** *Macaranga gigantea*, *Ex vivo*, *Plasmodium berghei*, Antiplasmodial, Flavonoid.

**Key messages:** The *Macaranga gigantea* was defined as a potential source of natural antimalarial drug candidate. The *M. gigantea* leaf ethanolic extract indicated that they contained phytochemical constituents and showed that major constituent of *M. gigantea* leaves is flavonoid. Active constituent in *M. gigantea* leaves extract were known as apigenin have potent antiplasmodial property.

## INTRODUCTION

Malaria is an infectious disease caused by *Anopheles* mosquitoes bites which transmitted *Plasmodium* parasites. This parasites have a life cycle in mosquito and human hosts.<sup>1</sup> *Plasmodium falciparum* (*P. falciparum*) is the most dangerous type of all parasite.<sup>1,2</sup> Recent researches have shown that eradication of malaria is facing drug resistancy of *Plasmodium*. The protozoans have developed resistance against chemical drugs including chloroquine and pyrimethamine,<sup>3</sup> which revealed the need for developing new classes of medicine to overcome this resistancy problem. The overuse of drugs for prophylaxis, incomplete therapeutic treatments, genetic and metabolic adaptive abilities of the parasites and a massive parasite proliferation are the factors affecting antimalarial resistances.<sup>1</sup> Severe and complicate malaria disease was caused by resistances of mosquitoes and parasites to antimalarial drugs especially to artemisinin derivatives, causing annual mortality of 1-2 million

people. These facts have led to the development and research of new antimalarial compounds from natural materials.

Poor countries are the most vulnerable to malaria and are most dependent on plant resources for malarial infection. Natural products showed a dominant role in the development of drugs to treat human diseases.<sup>4-7</sup> This include the new antimalarial candidate from tropical plant sources.

The treatment of malaria promote the interest in the discovery of new pharmaceuticals with antiplasmodial activity.<sup>7</sup> Antiplasmodial and antimalarial properties from plants mostly due to the presence of terpenes, limonoids, flavonoids, chromones, xanthenes, anthraquinones and related compounds.<sup>2</sup> Many plant species have demonstrated to be effective drugs for the treatment of malaria. Quinine and artemisinin, two main classes of antimalarials agent. A search of medicinal species with antimalarial activity has been developed in our laboratory. Muhaimin in 2018

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and 2019 has put attention on plants that grow wild in tropical Jambi forest which have been used traditionally to cure numerous diseases, especially malaria.<sup>5,6</sup> Former study has reported that the aerial parts and root crude extracts of *Macaranga* genus plant showed antiplasmodial activity and revealed good *in vivo* activity.<sup>7,8</sup>

*Macaranga* is a genus of the family Euphorbiaceae which comprises of about three hundred species. It is present in some parts of the world which include Indonesia, some parts of Africa, Madagascar, Asia, the east coast of Australia and the Pacific islands. The *Macaranga* plants are known to be in the form of shrubs or trees and grow in places with optimum sunlight, secondary forests or forests that have been destroyed.<sup>9</sup> *Macaranga* plants show several bioactivity which include antitumor, anticancer, antimalaria, antimicrobes, cyclooxygenase and antioxidant.<sup>8,9</sup> The plant is also known to have active phytochemicals constituents, especially on its leaves. In continuing phytochemical work on Indonesian *Macaranga* plants, this study is aimed to find a new antimalarial compounds from *M. gigantea*. In this paper, we thoroughly examined the antiplasmodial activity of ethanolic extract *Macaranga gigantea* and the isolated major constituent.

## MATERIALS AND METHODS

### Collection of plant materials

Fresh leaves of *Macaranga gigantea* were collected in the month of January, 2018 from Mendalo (Jambi) and identified by a taxonomist from the Department of Biology, Faculty of Mathematics and Natural Sciences of Padjadjaran University, where voucher specimen (No. 029/HB/05/2018) was deposited. The fresh leaves were washed thoroughly to remove dirt and soil, then dried and stored at room temperature. They were grinded and then kept in closed container and stored at room temperature until they will be used for the next process. Information about the plant, the location and date of collection were stated in the Department of Biology, Faculty of Mathematics and Natural Sciences of Padjadjaran University.

### Chemicals and reagents

Quercetin dehydrate, gallic acid, anhydrous sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), aluminum trichloride ( $\text{AlCl}_3$ ), potassium acetate ( $\text{CH}_3\text{COOK}$ ), sodium acetate ( $\text{CH}_3\text{COONa}$ ), ferric chloride hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ), Folin-Ciocalteu reagent, Dragendorff's reagent, mercuric chloride, potassium iodide, apigenin and iodine were purchased from Sigma-Aldrich Chemie GmbH, Steinheim, Germany. Ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ), methanol ( $\text{CH}_3\text{OH}$ ), hydrochloric acid (HCl), sulfuric acid ( $\text{H}_2\text{SO}_4$ ), chloroform ( $\text{CHCl}_3$ ), ammonia ( $\text{NH}_3$ ), glacial acetic acid, sodium hydroxide (NaOH), acetonitrile, acetic acid, orthophosphoric acid and potassium peroxodisulfate were bought from Merck Chemicals GmbH, Darmstadt, Germany. The chemicals used were of good quantity and quality standard and do not require further purification.

### Extraction

Dried *M. gigantea* leaves (10 kg) were grinded and extracted three times with 12.5 L of ethanol, (24 h each) by maceration technique. The macerate was then concentrated, evaporated and dried in a vacuum at 60°C using a rotary evaporator (buchi rotavapor R-205). The yield value was as much as 14.8% (w/w). The dry extract was stored in refrigerator at 4°C until when it will be used.<sup>5,10</sup>

### Phytochemical screening

Phytochemical screenings of the extract and isolate were performed to estimate the presence of its chemical constituents such as alkaloid, flavonoid, saponin, triterpenoid, steroid, tanins, glycosides and phenolic.<sup>7</sup>

### Isolation and purification

The ethanol extract was fractionated by vacuum liquid chromatography using combination of eluent with increasing polarity to give fractions. Silica gel has been used as stationary phase and varying ratios of n-hexane, ethyl acetate and water in increasing polarities. The column was packed with activated silica gel (70-230 mesh size) slurry with constant tapping. The crude extract was dissolved in little amount of ethanol, mixed with silica gel and then loaded onto the column. Five fractions were obtained, and were crystallized with benzene. Flavonoid was identified from the second and third fraction and subjected to further identification. Characterization of apigenin were conducted by confirming the melting point using Gallenkamp Melting Point Apparatus. The apigenin structure was confirmed with UV and IR Spectrophotometer (Perkin Elmer), <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were recorded on a Bruker Avance III 500 MHz Digital NMR spectrometer, using  $\text{CD}_3\text{OD}$  as a solvent and mass spectra of apigenin determined by Shimadzu HRMS-EI. All data compared with literature and previous data.

HPLC analysis was carried out with Shimadzu Japan HPLC system using UV detector with autosampler controller. Data collection and analysis were performed using LC solution. Separation was performed on C18G column (250 mm × 4.6 mm i.d., 5µm) at wavelength of 356 nm. The mobile phase used were consisted of methanol: acetonitrile: acetic acid: orthophosphoric acid: water (40: 20: 0.05: 0.05: 40). The flow rate was 0.6 mL min<sup>-1</sup>.

### Plasmodium and animal

The test for plasmodium used in this research was *P. berghei* from Eijkman Institute for Molecular Biology, Jakarta. *Ex vivo* study of antiplasmodium activity was conducted using both adult male and female (50:50%) mice of BALB/c species weighed 20 to 30 g at age 2 – 3 months. Plasmodium were obtained and cultivated in Eijkman Institute for Molecular Biology, Jakarta. For *ex vivo* antiplasmodial assay, the mice were separated into 3 groups each consist of 5 mice. Acute toxicity study used mice of both sexes which were divided into 8 groups and were fasted overnight. The experimental procedures relating to the animals were authorized by Ethical committee, No. 1332/UN6. KEP/EC/2018 from Padjadjaran University. Animals were kept in a temperature-controlled room. They were fed *ad libitum* and were acclimatized for at least 1 week prior the experiments.

### Ex vivo erythrocytic-antiplasmodial assay

To study the *ex vivo* antiplasmodial activity of ethanolic leaves extract of *M. gigantea* and its isolated flavonoid constituent, various concentrations of samples (300, 100, 30, 10 and 3) µg/mL were prepared. The samples were tested *ex vivo* on Balb-C mice blood which was infected by *P. berghei* strain ANKA. Plasmodium infection was deposited in mice by IP administration of donor mouse blood containing about  $1 \times 10^6$  parasites and let infected for 24 h. Blood samples were collected from the tail of each mouse. Samples of extract and isolate at 300, 100, 30, 10 and 3 µg/mL were introduced onto infected mice blood and incubated for another 24 h. Antiplasmodial activity were observed by calculating the parasitaemia percentage either from extract or isolated flavonoid treatments. The results were compared with negative control groups treated with dimethylsulfoxide (DMSO) as solvent and positive control groups treated with standard drugs (pyremethamine) in same concentrations (300, 100, 30, 10 and 3 µg/mL). The antiplasmodial activity expressed as decreased levels of parasitaemia and percent of inhibition. Every treatments were repeated three times. In the 24<sup>th</sup> h, blood treated by samples were stained with Giemsa's stain which usually used to determine parasitized erythrocytes.<sup>11,12</sup>

### Acute toxicity of the extract

Study on lethal dose (LD<sub>50</sub>) of the ethanolic leaf extract of *M gigantea* was determine in mice using the slight modified method described by Lorke.<sup>13</sup> A different dose of crude extract was suspended in 2% PGA in water and were administered to rats in the treatment groups by the oral route. Experimental Design were as follow:

Group control : 2%PGA suspension in water

Group I: dose 1: extract (3 g/kg)

Group II: dose 2: extract (6 g/kg)

Group III: dose 3: extract (9 g/kg)

Group IV: dose 4: extract (12 g/kg)

Group V: dose 5: extract (15 g/kg)

Group VI: dose 6: extract (18 g/kg)

Group VII: dose 7: extract (21 g/kg)

The mice per cage were administered by extract extract p.o. and were observed for parameters of toxicity and mortality for the first 4 h and 24 h. The signs of toxicity and mortality mice were also observed at regular

intervals for 24 h, 48 h and 72 h respectively. At the end of experiment the mice were killed by CO<sub>2</sub> euthanasia.

### RESULTS

The phytochemical screening of crude ethanolic extract of *M. gigantea* leaf revealed the presence of some secondary metabolites such as alkaloids, steroids, flavonoids, phenolics and tanins (Table 1). These phytochemical compounds are known to be responsible for some medicinal activity which in this present study is antiplasmodial activity. The screening for the potential antimalarial properties of the phytochemicals exist in ethanolic extract of *M. Gigantea* against *P. berghei* will become a basis to the next assay on determination of antimalarial activity.

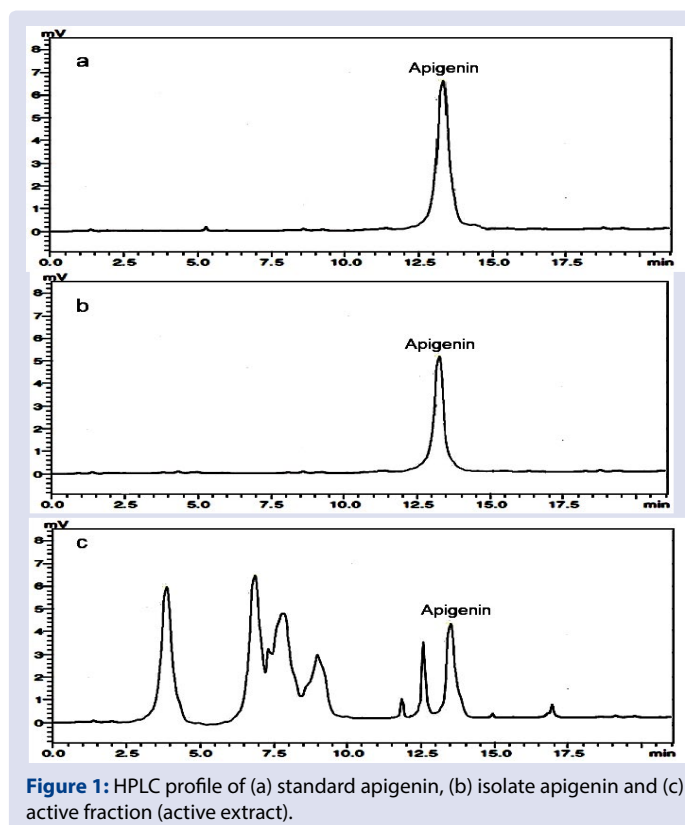
### Qualitative analysis of high performance liquid chromatography (HPLC) profiling

The HPLC crude extract profile of *M. gigantea* showed many peaks at the retention time between 3 to 17.5 min (Figure 1c). It could be seen that the peak spectrum of apigenin appeared at 13.25 min (Figure 1b) which in line to the HPLC profiling of standard apigenin (Figure 1a).

**Table 1:** Phytochemical screening of ethanolic leaf extract of *Macaranga gigantea*.

Secondary metabolites	Results
Alkaloid	+
Flavonoids	+++
Terpenoids	-
Phenolic	+
Steroids	+
Tanins	+
Saponins	-
Glycosides	-

+: Presence; -: Absence



**Figure 1:** HPLC profile of (a) standard apigenin, (b) isolate apigenin and (c) active fraction (active extract).

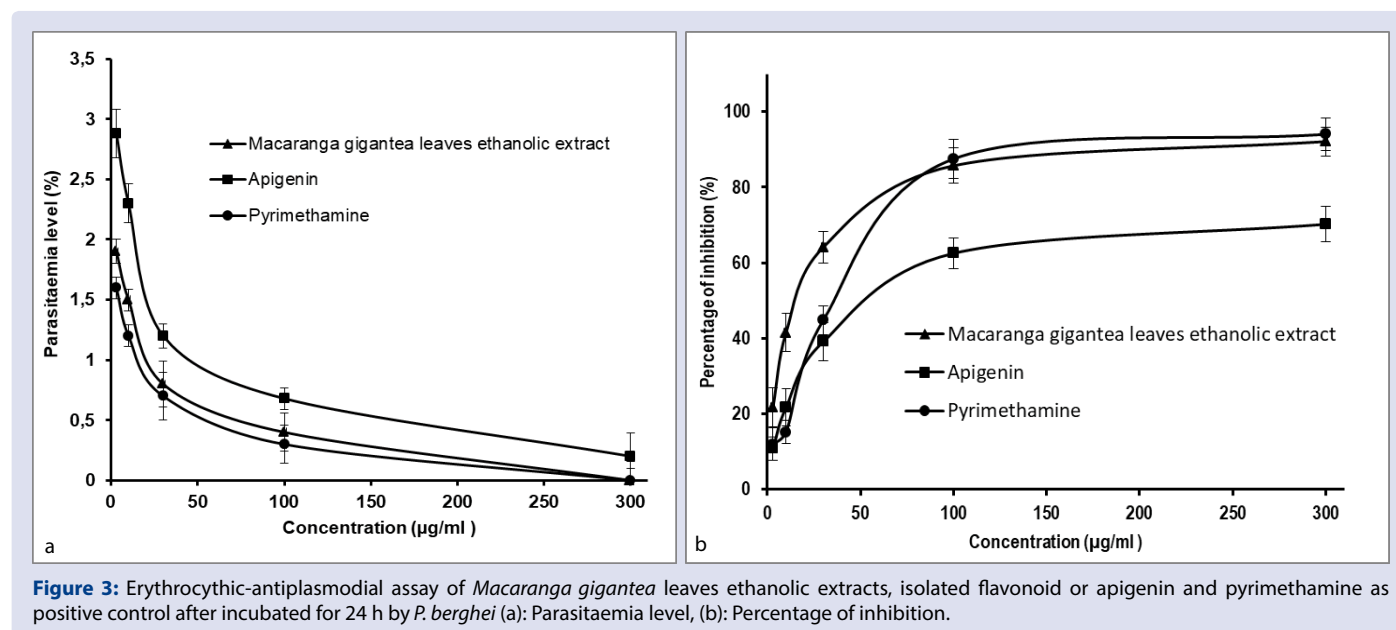
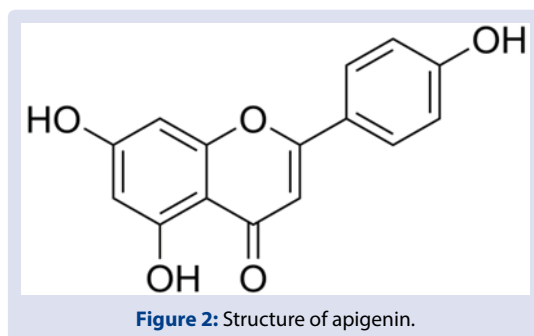
The peak spectrum showed similar pattern between the active fraction, isolate and standard apigenin peak spectrum at 13.25 min.

The flavonoid was collected as an important component when the crude extract was further extracted. Its structure was made clear by spectroscopic methods which include UV, IR,  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  and MS. The isolated flavonoid was a yellow crystalline substance with a melting point of (344-345) $^\circ\text{C}$ . The UV spectra in  $\text{CH}_3\text{OH}$  (ethanol) made it visible that the absorbance at  $\lambda_{\text{max}}$  were (log  $\epsilon$ ) 212 (3.21), 271 (1.82) and 327 (2.06) nm. The infrared spectra showed a sharp aromatic C-H stretching vibration at 3400  $\text{cm}^{-1}$ , aliphatic C-H stretching vibration at 2875  $\text{cm}^{-1}$  and 2925  $\text{cm}^{-1}$ . The aromatic C-H bending vibration also showed in finger print 835  $\text{cm}^{-1}$ , 779  $\text{cm}^{-1}$  and 655  $\text{cm}^{-1}$ . These vibrating regions indicate the substituted aromatic functional groups or compounds. The sharp C=O stretching vibration was at 1615  $\text{cm}^{-1}$ . Sharp aromatic C=C stretching vibration were also shown at 1535  $\text{cm}^{-1}$  and 1450  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra showed  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ ):  $\delta$  12.976 (1-H,s,H-17), 11.214 (1-H,s,H-18), 9.995 (1-H,s,H-19), 8.114 (2H,d,H-5,H-3), 7.216 (2H,d,H-2,H-6), 7.015 (1H,s,H-15), 6.865 (1-H,s,H-13), 15.992 (1H,s,H-16);  $^{13}\text{C-NMR}$  ( $\text{CD}_3\text{OD}$ ):  $\delta$  183.2 (s,C-4),163.7 (s,C-5),162.5 (s,C2), 160.9 (s,C-4), 160.3 (s,C-9), 156.8 (s,C-7), 129.1 (d,C-2' and C-6'), 119.7 (s,C1), 114.6 (d,C-3' and C-5'), 105.1 (s,C-10), 104.2 (d,C-3), 97.4 (d,C-6), 92.9 (d,C-8). This isolated flavonoid, showed ion peak at  $m/z$  269.8 [M] $^+$  in the EIMS spectrum, which correlates to the molecular formula  $\text{C}_{15}\text{H}_{10}\text{O}_5$ . On the basis of phytochemical screening results, the spectrum data of UV, IR,  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , EIMS and by the literature comparism, the main components from the isolated ethanolic leaves extract of *M. gigantea* could be inferred to be Apigenin, as seen formerly in literatures

(Figure 2), but more adequate identification is still needed. Apigenin is a flavone derivative from the genera of *Macaranga*.<sup>14</sup>

Treating the mice with ethanolic extract and isolated flavonoid (apigenin) reduced the parasitaemia level of infected erythrocytic in respect to the dose taken (30, 100 and 300  $\mu\text{g}/\text{mL}$ ) (Figure 3). The results were compared with the positive control (pyrimethamine) and negative control dimethyl sulphoxide (DMSO). The data showed that ethanolic extract of *M. gigantea* leaves brought about the reduction of parasitaemia percentage caused by *P. berghei* with percentage of inhibition as much as 92.1%, 85.7%, 64.1%, 41.5% and 21.7% at concentrations of (300, 100, 30, 10 and 3)  $\mu\text{g}/\text{mL}$  respectively while  $\text{IC}_{50}$  was 27.1  $\mu\text{g}/\text{mL}$  (Figure 3b, EE). This could be attributed that ethanol extracts contained bioactive compounds which acts on the plasmodial parasites. The major ingredients known as alkaloids, terpenoids and flavonoids is attributed to the antimicrobial activity in plants.<sup>15</sup>

The results from the antiplasmodial activity study have shown that ethanolic extract of *M. gigantea* leaves are more active than the isolated flavonoid and almost the same with Pyrimethamine (Figure 3). This result can be explained that therapeutic action of many medicinal plant are based on either its individual or synergistic effect of each constituents. Therefore, use of herbals in therapy is effective due to synergistic therapeutic action of each compound present. The isolation, characterization, structural elucidation of active biochemical compound of plant including steroids, alkaloids, terpenoids, polyphenols, flavonoids, glycosides, polysaccharides, terpenoids, etc are more to identify particular phytochemical responsible for specific therapeutic action.





Reduction of parasitaemia level caused by extract treatment was comparable to that of the standard drug, pyrimethamine. Based on the data, it can be stated that ethanolic extract of *M. gigantea* leaves, is a promising agent to be developed as a source of antimalarial agent.

Parasitaemia level treated by different concentration of extract investigated from first day to days five continues to increase (Figure 4). This means that the ethanol extract of *M. gigantea* can inhibit the growth of *P. berghei* in mice. Similarly, effect of apigenin isolated from *M. gigantea* on parasitaemia level at 5 days of investigation was also continues to increase in faster manner (Figure 5). Compared to extract and isolated apigenin from *M. gigantea*, samples treated by Pyrimetamine as standard drug revealed lower parasitaemia level (Figure 6). Infected blood sample without treatment and using DMSO as solvent which is assumed as negative control showed highest parasitaemia level (Figure 7) which is in line with estimated result due to absence of antiplasmodial activity. In accordance with the study conducted by Peters (1970), it can be seen that the antispasmodial activity extract to plasmodium can be calculated based on the percentage of fourth day parasitaemia.<sup>16</sup>

Antiplasmodial activity can also be studied from the inhibition of plasmodium life cycle. The blood stage is responsible for the pathology associated with malaria. The malarial infection is initiated by sporozoites which injected by mosquito bites. Sporozoites spread to

circulatory system and invade hepatocytes.<sup>16</sup> The early trophozoite is often referred as 'ring form' because of its morphology.

After 24<sup>th</sup> h, blood treated by samples which then were stained with Giemsa's stain were captured to determined how the extract and isolated apigenin affected parasitized plasmodium life cycle. *M. gigantea* leaves extract has activity in hindering the development of ring stage to the schizonts stage (Figure 8). The schizonts stage formation was not found at 300 µg/mL of extract which can be assumed that the life cycle of plasmodium has been affected since the schizonts has been destroyed. But, at lower concentration, trophozoite and schizonts still survived with defect related to morphological situations.

Treating the bloods sample by 300 µg/mL isolated apigenin and investigated the plasmodium life cycle after 24 h gave the results that the schizonts can survived with defect and several are destroyed. Lower concentration of apigenin treatment on blood samples resulted in unaffected life cycle of plasmodium (Figure 9).

### Acute toxicity test

There was no mortality observed in mice after oral administration of the methanol extract even at doses higher than 21 g/kg signifying that the oral LD<sub>50</sub> was more than 21 g/kg. Thus the experimental doses used (3, 6, 9, 12,15, 18 and 21 g/kg) were within safe margin.

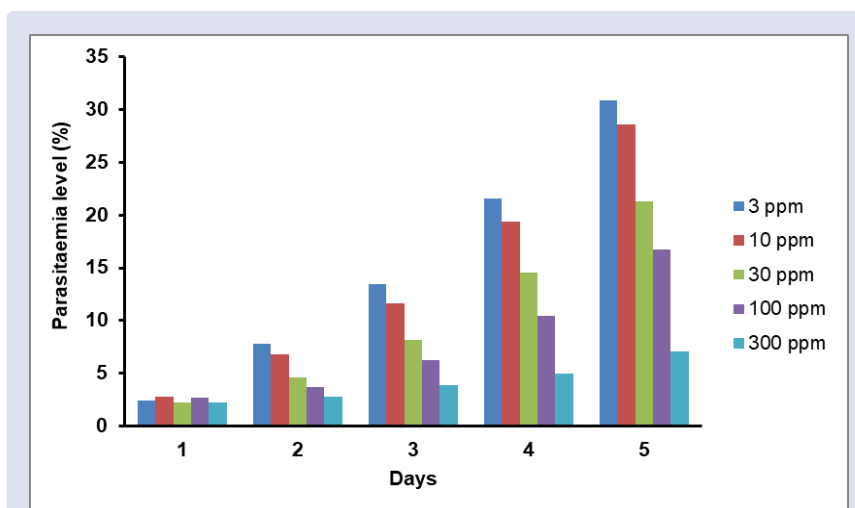


Figure 4: Effect of *Macaranga gigantea* ethanolic extract on parasitaemia level at 5 days of investigation.

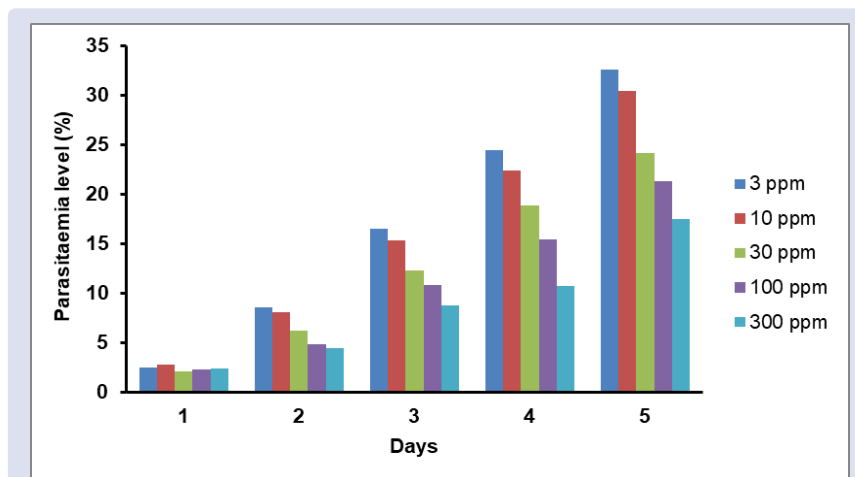
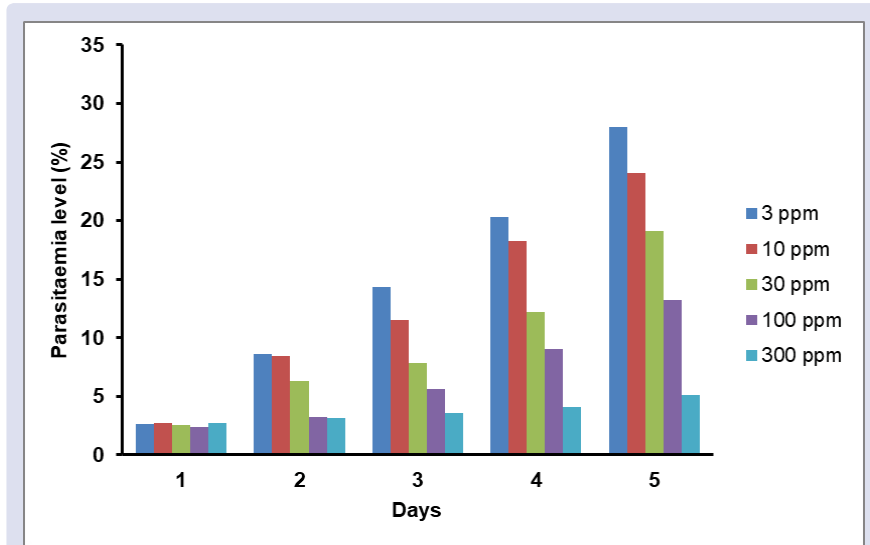
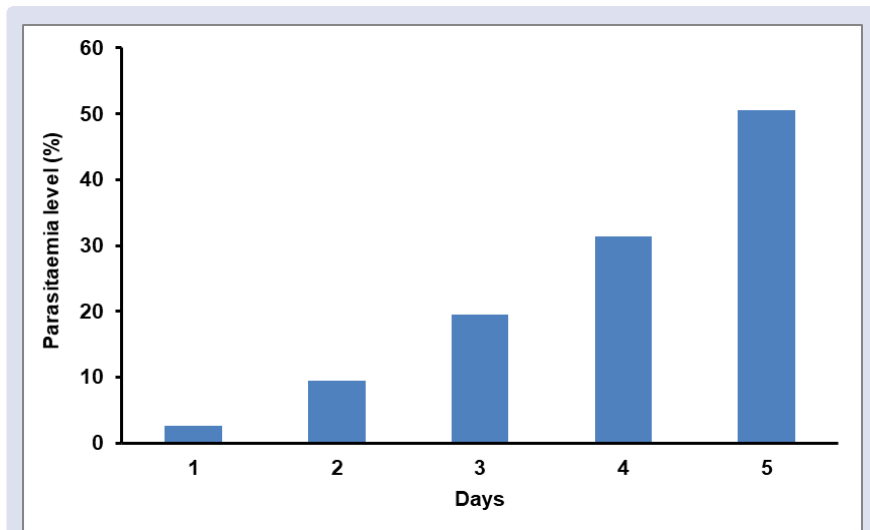


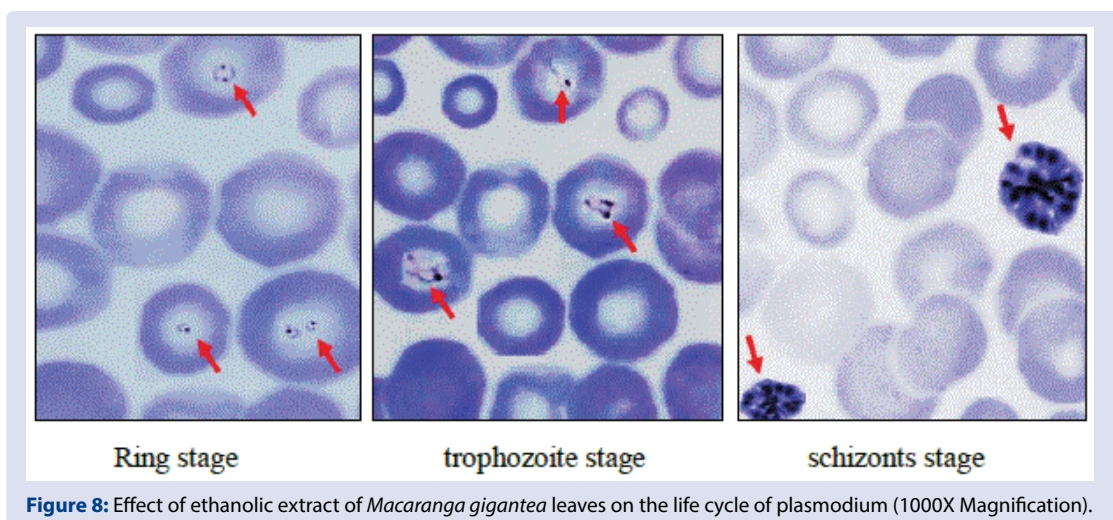
Figure 5: Effect of apigenin isolated from *Macaranga gigantea* on parasitaemia level at 5 days of investigation.



**Figure 6:** Effect of Pyrimethamine on parasitaemia level at 5 days of investigation.



**Figure 7:** Parasitaemia level of sample without treatment at 5 days of investigation.



**Figure 8:** Effect of ethanolic extract of *Macaranga gigantea* leaves on the life cycle of plasmodium (1000X Magnification).

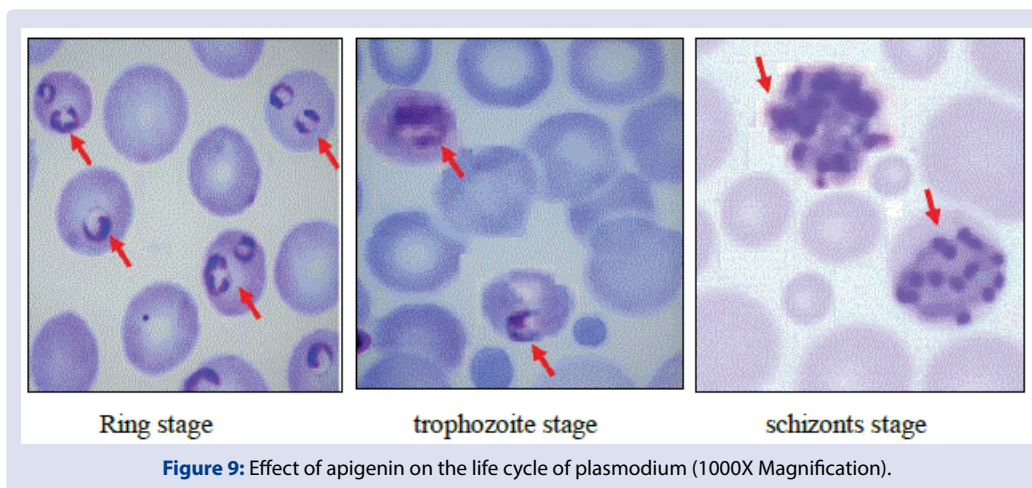


Figure 9: Effect of apigenin on the life cycle of plasmodium (1000X Magnification).

## DISCUSSION

To investigate components from *Macaranga gigantea*, the HPLC analysis was performed. The similarity with phytochemical screening results of crude extract which was raising flavonoid as major constituent was in line with HPLC results which mainly showed many peaks with same retention time with apigenin. This major components was responsible to give antiplasmodial activity. The flavonoid were founded both in extract and isolated active fraction which may be become explanation for strong and potent antiplasmodial activity against *P. berghei*.

Isolated flavonoid demonstrated antiplasmodial activity with percent inhibition of 70.2%, 62.5%, 39.1%, 21.7% and 10.8% at concentrations of (300, 100, 30, 10 and 3)  $\mu\text{g}/\text{mL}$  respectively with  $\text{IC}_{50}$  of 60.2  $\mu\text{g}/\text{ml}$  (Figure 3b). Pyrimethamine as a positive control gave the percent inhibition of 94%, 87.5%, 44.8%, 15.2% and 12% at the same concentration with  $\text{IC}_{50}$  of 31.4  $\mu\text{g}/\text{ml}$  as compared to negative control (Figure 3b). The negative control (DMSO) has no activity.

Some secondary metabolites of plants such as alkaloids, flavonoids and triterpenoids have been proven to have antiplasmodial activity.<sup>8,9</sup> Former research proved that Terpenoids have an important role as antimalarial activity.<sup>17</sup> It inhibit the growth of the plasmodium parasite in ring to trophozoites form and inhibited nutrient intake by inhibiting the permeation pathway.<sup>17</sup> Alkaloids has also potent antimalarial activity in some parts of Apocynaceae plants. Flavonoids was also has been proved to give inhibition percentage of Plasmodium hence act as antiplasmodial. Flavonoids inhibit the influx of L-glutamine and myoinositol into infected red blood cells.<sup>17</sup> Quinone derivative compounds Diospyrin, from a genus Diospyros acts as antimalarial activity of this genus. Study on *Ochrosia akkeringae* and *Tabernaemontana pandacaqui*, indicated that anthraquinone from this plant was responsible in producing the potent antimalarial activity by killing the parasite through various mechanisms, resulting from aldehyde at C-2.<sup>13,17</sup> Although many study has proved the role of many phytochemicals in inhibiting plasmodium growth, further studies are required to evaluate its mechanisms. Mechanisms of antiplasmodial activity may be due to antioxidation and free radical scavenging, immunomodulatory, intercalation in DNA, inhibition of protein synthesis, interference with the invasion of new RBCs by parasites, or by any other mechanisms.<sup>18,19</sup>

In present study, the isolated flavonoid from *M. gigantea* can be assumed to be responsible for its antiplasmodial activity. In cases where the extract is more responsive than the isolate, it suggests that lack of cross-resistance with chloroquine from isolate was probably due to fewer mode of action of compounds of isolate compared with those

present in the extracts. This kind of extract indicates to have potential of solving the problem of multi-drug resistance. The bioactive produced from this extract could be used to be developed as cheap anti-malarial drugs.

Antioxidant activity of some plants particularly those which have high flavonoids content, have been proved to have schizonticidal activity due to its ability in regulating cellular signalling pathway.<sup>7,8</sup> Quercetin as flavonoid derivate is an example of such compound responsible for antiplasmodial activity in some plants.<sup>7,8</sup> Free radicals levels used as characteristics of malaria disease are connected to chronic malaria. Extracts containing phenol and flavonoids with antioxidant activity are very potent compounds to be used.<sup>6,8</sup> Other study revealed that flavonoids applied the antiplasmodial activity by mechanism of nucleic acid base pairing parasite chelation.<sup>3,8</sup> Hence, flavonoid as an important component of ethanolic leaves extract of *M. gigantea* may give contribution to the antiplasmodial activity.

Trophozoite enlargement is accompanied with metabolism including the ingestion of host cytoplasm. The intracellular parasite replicate and known as exoerythrocytic schizogony called as schizont stage,<sup>20-22</sup> as the end of the trophic period and manifested by multiple rounds of nuclear division without cytokinesis.<sup>20</sup> At 0 h, the growth of parasites is domineering at the ring stage. Parasites continue to grow into trophozoite and schizonts stages at 24 h, but by using a microscope it's observed that the parasitic morphological situations look unhealthy and parasites look like dead cells (Figure 8). At extract concentrations higher than 30 $\mu\text{g}/\text{ml}$  a static ring still neither grew nor looked unhealthy. Conclusions can be made that ethanolic extracts of *M. Gigantea* leaves have activity on plasmodium life cycle.

According to the reduction in the parasitaemia levels, percentage of inhibition and inhibition activity on plasmodium growth, a conclusion can be drawn that the ethanolic extracts and isolated flavonoid (apigenin) which is an essential component of *M. gigantea* leaves have antiplasmodial activity. Present study indicated that ethanolic extract of *M. gigantea* leaves potent to be antimalarial agents.<sup>18,19</sup> The data was in line with previous investigation on this genus which showed that its crude extracts and compounds offered bioactivity including anticancer,<sup>23,24</sup> antioxidant,<sup>25</sup> antimicrobial,<sup>26</sup> anti-inflammatory<sup>27</sup> and other different types of biological activities.<sup>28</sup>

In the acute toxicity study, a maximum dose of 21 g/kg to either male and female mice has been determined. The results showed that at given dose neither signs of toxicity nor mortality during the 14 days of experiment were observed. This results suggested that no toxic effect

from the extract on the vital organs. According to toxicity criteria and based on the result observed in mice, the extract can be concluded as non toxic.

In conclusion, accumulating evidence suggests that products derived from *M gigantea* extract hold potential as antiplasmodial which makes it a potent source of natural antimalarial agent.

## CONCLUSION

Based on the decreasing parasitaemia levels and percentage of inhibition and inhibition activity on plasmodium growth, it is observed that either ethanolic extracts of *Macaranga gigantea* leaves and apigenin as flavonoid derivate isolated from the extract reveled antiplasmodial activity. The results of this study indicated that *Macaranga gigantea* is a potent source of natural antimalarial agent.

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## CONFLICTS OF INTEREST

No conflicts of interest is associated with this work.

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