In vitro and In vivo Antiplasmodial of Stem Bark Extract of Garcinia husor

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ABSTRACT

Garcinia husor is one of the folk medicines in Maluku-Indonesia. This species has been used for the treatment of Malaria disease. The phytochemical contents and antiplasmodial activity not reported yet. In this study we evaluated the quantitative phytochemicals, in vitro and in vivo antiplasmodial activity of stem bark ethyl acetate extract. In vitro assay was done using P. falciparum 3D7 strain sensitive of chloroquine. For in vivo analysis, four groups of M. musculus were infected by P. berghei and their parasitemia levels were for 7 days of treatment with ethyl acetate extract; hematological and biochemical parameter were analyzed at the end of experiment. The result showed ethyl acetate extract with the TPC (169.47 mg GAE/100 g ±0.61) and TPC (167.37 mg QE/100 g ±1.05) was active against P. falciparum 3D7 strain (IC50 value of 0.31±0.43 μg/ml). The animal treated with extract showed suppression of parasitemia to 87.57±1.41% compared with the P. berghei infected-mice (negative control), ED50 value of 22.30 mg/kg BW. The dose of extract in 200 mg/kg BW was reduce parasitemia of infected mice with P. berghei more potential. The ethyl acetate of the stem bark G. husor with has antiplasmodial properties and future investigation are necessary to elucidate its mechanism of action.

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1. Introduction

Malaria is a major infectious disease with a high mortality in the developing country (Boampong et al. 2015). Until now, efforts have been made to treat this disease related to the development of alternative antimalarial, due to the emergence of drug-resistant strain. Many plants are used therapeutically in alternative and traditional medicine to treat infectious diseases. New and safe antimalarial drugs are urgently needed and natural products can represent a vast source of leading molecules (Oliveira et al. 2014).

Garcinia species are known to contain phenolic compounds such as xanthones, benzophenones, flavones, and depsidone (Nilar et al. 2005). Phenolic compounds showed that a wide range of biological and pharmacological properties, e.g. antioxidant, anti-inflammatory, antimicrobial, antimalarial, and cytotoxic activities (Minami et al. 1994; Vlientinck et al. 1998; Merza et al. 2006; Cos et al. 2008; Chin et al. 2008; Elfitia et al. 2009). Benzophenones have been reported as anti-HIV, trypanocidal and cytotoxic agents (Gustafson et al. 1992; Vlietinck et al. 1998; Williams et al. 2003; Merza et al. 2006; Cos et al. 2008).

Garcinia husor (locally named “manggustan hutan” in Indonesia) is a medium sized tree occurring in Maluku. In some Maluku communities, the stem bark of G. husor used in the treatment of malaria as endemic area but this has not been scientifically verified. This study was designed to evaluate the antimalarial potential of the ethyl acetate extract stem bark G. husor using Plasmodium falcifarum D37 strain in vitro and in vivo at P. berghel infected mice model.
2. Materials and Methods

2.1. Plant Material
The stem bark of *G. husor* was collected from Allane Forest at Ambon island, Indonesia. This plant was identified and a voucher specimen (No. 52) was deposited at Biology Laboratory of Biology Departement, Pattimura University, Indonesia.

2.2. Preparation of Extract
The stem bark of *G. husor* were dried at room temperature and reduced to coarse powder using a disk mill SMJMA, FFC-15, Shandong Jimo. Dried stem bark powder (500 g) was mixed ethyl acetate and filtered. The solvent was evaporated under reduce pressure (Rotavapor R-210, Buchi, Switzerland) to obtain a solid mass extract of ethyl acetate (13.3% w/w).

2.3. Screening for Secondary Metabolites
The ethyl acetate extract was screened for the presence of phytochemicals using standard procedures described elsewhere (Njoku and Obi 2009; Khan et al. 2011; Tiwari et al. 2011).

2.4. Determination of TPC and TFC
Total phenolic and flavonoid contents were determined by Folin-Ciocalteu’s and aluminium chloride colometric methods, respectively (Singh et al. 2013) following quantification on the basis of standard curve of gallic acid (GAE) and quercetin (QE) equivalent, respectively.

2.5. Antimalarial Assays

2.5.1. In Vitro Test
The antimalarial activity of the extract was determined by the procedure described by Widyawaruyanti et al. 2007. In brief, extract dissolved in DMSO (10⁻² mol L⁻¹) and kept at -20°C until used. The malarial parasite *P. falciparum* 3D7 clone was propagated in a 24-well culture plate in the presence of a wide range of concentrations of each compound. The growth of the parasite was monitored by making a blood smear fixed with MeOH and stained with Geimsa stain.

\[
\% \text{growth} = \% \text{paristemia} - DO \\
\% \text{inhibition} = 100\% - (\frac{Xu}{Xk} \times 100\%)
\]

Where:
- DO :% growth at 0 o’clock
- Xu :% growth at solution test
- Xk :% growth at negative control

Based on percent inhibition data, an analysis was conducted between the concentration of the test and the percent inhibition using probit log analysis to determine the IC₅₀ value or concentration of the extract which could inhibit parasite growth by 50%.

2.5.2. In Vivo Tests
Animals and husbandry *M. musculus* weighing 20-24 g were obtained from Biology Laboratory Pattimura University, Indonesia. The animal were housed in stainless steel cages (34 x 47 x 18 cm) with soft wood shaving as bedding, fed with normal commercial pellet diet given water *ad libitum* and maintained under ambient laboratory conditions. All procedures and techniques used in these studies were in accordance with the National Institute of health Guidelines for the Care and Use of Laboratory Animals (NRC 1996). All ethical protocols used for the study were approved by the Departemental Ethics Committe.

2.5.2.1. Animals
Four-week-old *M. Musculus* from the Zoology Laboratory of Pattimura University were used in the experiments. Prior to the experimental produces, the animals were kept at 22±2°C on a 12 h dark-light, with balanced feed and water *ad libitum* for 15 days. All experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care adopted by the Animal Use Ethics committe of Airlangga University.

2.5.2.2. Acute Toxicity Evaluation
The acute toxicity assay was performed following the guidelines of The Organization for Economic Cooperation and Development (OCDE)-423/2001. When previous studies have assessed the acute toxicity of a known sample, the initial dose used can be the maximum dose without side effects. *G. husor* was tested in three groups of three animals each that were analyzed every 30 min during the first 4 h, periodically for 24 h and daily until the 14th day. Several parameters such as general activity, irritability, contortion, ataxia, tremors, convulsion, piloerection, hypothermia, breathing, cyanosis, hyperemia, and death were analyzed.

2.5.2.3. Induction of the Experimental Malaria Model
The malaria model was induced using the *P. berghei* ANKA (PbA) line. For the inoculation of the experimental groups, a blood drop from infected animals was collected from the terminal portion
of the tail to analyze parasite density. The blood content of animals with a parasite density higher than 10% in the ascent phase was collected by cardiac puncture using an insulin syringe washed with the anticoagulant agent EDTA. To guarantee uniform parasitemia in the different experimental groups, animals were infected intraperitoneally with a standardized inoculum of approximately 10^6 parasitized erythrocytes in 100 μl PBS.

2.5.2.4. Determination of Parasite Desity and Parasitemic Suppression

Parasites were investigated in mouse tail blood smears fixed in absolute methanol for 2 min and stained with 10% Giemsa for 15 min. Percent parasitemia was determined in each animal as the ratio between the quantity of parasitized erythrocytes and the total quantity of cells (500 cells per smear):

\[
\% \text{ parasitemia} = \frac{\text{quantity of parasitized erythrocytes}}{500}
\]

post-inoculation of BALB/c mice with PbA line. The model proposed by Girma et al. (2015) was used to determine the suppression potential of oleoresin against the parasitemia rate of P. berghei-infected mice.

2.5.2.5. Experimental Design

Mice were randomly divided into four groups of 3 animals each: Group 1 (G1), untreated P. berghei-infected mice; Groups G2, G3, and G4, P. berghei-infected mice treated orally with ethyl acetate G. husor stem bark extract, 10, 100, and 200 mg/kg/day, respectively. Extract was suspended with 5% CMC-Na as vehicle. All treatments began on the 4th day after P. berghei inoculation, when parasite density was first determined to confirm infection. For the collection biological material at the end of treatment, animals were kept on their habitual diet without fasting due to the fragility of the malarial state.

2.5.2.6. Statistical Analysis

Data were analyzed statistically with Microsoft Excel 2010 and SPSS v22. Result were analyzed by ANOVA followed by LSD if significant difference. p value <0.05 was considered statistically significant. All data were expressed as mean ± SD (triplicate measurement).

3. Results

3.1. Preliminary Phytochemical of Ethyl Acetate Extract

The qualitative and quantitative phytochemical of ethyl acetate extract was prepared from G. husor stem bark. The results of preliminary tests of different phytochemicals extract revealed the presence of alkaloid, terpenoids, flavonoid, tannins, and phenolics (Table 1).

The quantitative of phytochemical by determined of TPC (Total phenolic content) and TFC (Total flavonoid content) from extracts. The amount of total phenolic compounds present in extract was determined from linear regression equation of calibrate curve, \( y = 0.0045x + 0.0481 \) (R2=0.9581) and expressed as Gallic acid equivalent in mg/ml of extract. Table 1 depicts the result of assessment of total phenolic content in the tested extracts. It is found that, stem bark of G. husor (p<0.05) displayed high contents of total phenolics in aqueous extracts showed 169.47 mg GAE/100 g ±0.61 mg GAE/100 g.

The total flavonoid in extract was determined from linear regression equation of calibration curve obtained from the different concentration of quercetin \( y=0.009x + 0.0161 \) (R2=0.983) and the results was expressed as Gallic acid equivalent in mg/ml of extract (Table 1). However, extract demonstrated considerably high amount of flavonoids in stem bark G. husor with 167.37 mg QE/100 g ±1.05 (Table 1).

Table 1. Qualitative and quantitative phytochemical of ethyl acetate of G. husor stem bark extract

<table>
<thead>
<tr>
<th>Secondary metabolites tested</th>
<th>Ethyl acetate extract</th>
</tr>
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<tbody>
<tr>
<td>Qualitative of phytochemical</td>
<td></td>
</tr>
<tr>
<td>Alkaloïds</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Phenolics</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Quantitative of phytochemical</td>
<td></td>
</tr>
<tr>
<td>Total phenolic content</td>
<td>169.47 mg GAE/100 g ±0.61^a</td>
</tr>
<tr>
<td>Total flavonoid content</td>
<td>167.37 mg QE/100 g ±1.05^b</td>
</tr>
</tbody>
</table>

^a Results are expressed as gallic acid equivalents//100 g of extract
^b Results are expressed as quercetin equivalents//100 g of extract

Value were performed in triplicated and represented as mean ± SD. Level of significance: (p<0.001)
3.2. *In Vitro* Antimalarial Activity

The *in vitro* assay, antimalarial activity with the treatment of *G. husor* stem bark ethyl acetate extract showed a decrease in parasite percentage of growth according to increased concentration, 0.01 μg/ml (3.28%); 0.1 μg/ml (2.54%); 1 μg/ml (1.74%); 10 μg/ml (1.01%); 100 μg/ml (0.36%) (Figure 1a). Thus, the inhibition of parasite growth increased as the concentration increased, 0.01 μg/ml (22.89%); 0.1 μg/ml (40.25%); 1 μg/ml (59.04%); 10 μg/ml (76.29%); 100 μg/ml (91.55%) (Figure 1b). Chloroquine as a positive control showed 100% inhibition at 100 μg/ml concentration and the lowest inhibition of 59.09%, this is equivalent to inhibition of the extract at 1 μg/ml of concentration. Extract exhibiting IC₅₀ value ≤10 μg/ml was considered to be active. Those exhibiting IC₅₀ value in the range 10 to ≤10 μg/ml were considered moderately active. Extract exhibiting IC₅₀ value ≥10 μg/ml was considered in active (Bagavan *et al.* 2011; Bunyong *et al.* 2014; Lima *et al.* 2015). Extract ethyl acetate potent inhibitory activity, with an IC₅₀ value of 0.31 μg/ml less than that antimalarial drug, chloroquine (IC₅₀ 0.006 μg/ml). The inhibitory activity of these extract supports the traditional use of the dried stem bark of *G. husor* as an antimalarial drug.

3.3. *In Vivo* Antimalarial Activity

3.3.1. Acute Toxicity Evaluation

The administration of the maximum dose of ethyl acetate *G. husor* stem bark extract did not promote any changes such as loss of the hair or color, tremors, increased salivation, diarrhoea, lethargy, increased or reduced sleep, pain or suffering, or weight loss.

Body weight is one of indicator that shows the state of malaria disease. Generally, in the *P. berghei*-infected state, the body weight of the mice will decrease as the number of infected red blood cells increases. Thus, by observing the weight changes of the mice, it is hoped to know the relationship with the change of parasitemia number in mice. In Table 2, *P. berghei* infection caused significant (*p<0.05*) decrease in the body weight-gain in the mice relative normal of all groups. All tested doses of the aqueous extract significantly protected the mice from body weight loss. Thus it can be said that the extract is safe and effective as an antimalarial and does not significantly influence body weight loss of mice (Fentahun *et al.* 2017).

3.3.2. Quantitative Analysis of the Evaluation of Parasitemia

Quantitative analysis of number of parasites in blood samples collected on 4th day post-inoculation showed level growth of parasite in the mean percentage of G1, infected only (48.3±0.007%) higher than G2 (27.66±0.06%), G3 (21.66±0.01%), G4 (Growth of parasite (%))

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Changes in body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected only (G1)</td>
<td>20.17±0.05</td>
<td>19.84±0.11 -0.32±0.06</td>
</tr>
<tr>
<td>Infected + extract dose 10 mg/kg BW (G2)</td>
<td>20.58±0.39</td>
<td>20.42±0.47 -0.16±0.10</td>
</tr>
<tr>
<td>Infected + extract dose 100 mg/kg BW (G3)</td>
<td>21.25±0.12</td>
<td>21.20±0.11 -0.05±0.02</td>
</tr>
<tr>
<td>Infected + extract dose 200 mg/kg BW(G4)</td>
<td>21.93±0.29</td>
<td>21.92±0.36 -0.01±0.01</td>
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</table>

Values are given as mean ± SD (n=3)

*Significantly different from normal (*p<0.05*).
Effects of extract on *P. berghei* infected *M. musculus*. The results showed that an average percentage of inhibition against *P. berghei* of the infected only group (G1) and the infected treatment extract group (G2, G3, and G4) where the increased of dose treatment decrease percent growth of parasite. This indicates higher percentage of inhibition G4 (87.37%) than G2 and G3. The G1 as negative control showed that higher growth of parasite (48.30±8.17%) and can not inhibit of parasite. So it can be said that ethyl acetate *G. husor* stem bark extract has good antimalarial activity because at a dose of 200 mg/kg BW showed percentage of inhibition ≥50% (Munoz et al. 2000).

Extract with percentage of inhibition ≥50% an in vivo assay at a dose of 100 mg/kg BW was classified as having excellent anti *Plasmodium* activity (Bantie et al. 2014; De Villiers et al. 2017). Based on this classification, the ethyl acetate *G. husor* stem bark extract have excellent antiplasmodial because at a dose of 100 mg/kg BW has been able to inhibit the growth parasite of *P. berghei* ≥50%. Furthermore, probit analysis obtained ED₅₀ value ethyl acetate *G. husor* stem bark extract of 22, 30 mg/kg BW.

The results of change level parasitemia by ANOVA test and further analysis LSD showed that a significant difference in the mean percentage of decrease of parasitemia, p<0.05 G1 (0.01±0.07%), G2 (0.10±0.06%), G3 (0.24±0.01), G4 (0.34±0.01%). The result showed that parasitemia was reduced in all treated groups in relation to untreated *P. berghei*-infected mice (G1) (Figure 3). The infected untreated extract (G1) was significantly different with the doses of 10 (G2), 100 (G3), and 200 mg/kg BW (G4), the dose 10 mg/kg BW (G2) was not significantly different with the dose of 100 mg/kg BW (G3), but the dose of 100 mg/kg BW (G3) was significantly different with a dose of 200 mg/kg BW (G4).

Malaria infection also causes renal damage due to high parasitemia, when parasitized erythrocytes become hard, with reduced deformability and changes conformation. This increases blood viscosity, slowing and microcirculation and altering renal blood flow. Additionally, the cytoadherence of infected erythrocytes alters the local circulation, aggravating the renal injury. Our result showed that treatment with extract may contribute to an increase in the microcirculation, reducing erythrocyte cytoadherence, with a significant decrease of parasitemia.

The level of parasitemia is obtained from thin blood preparation by counting the number of infected cells *P. berghei* (ring-shaped trophozoid, advanced stage trophozoid, and schizont) in 1000 erythrocytes. Erythrocytes containing old trophozoites and schizons have obviously coarse points (Maurer's point) spread over two-thirds of the erythrocytes. The more trophozoites and schizons, the higher the level of parasitemia (Figure 4).

![Figure 2](image_url)

**Figure 2.** (a) Growth of parasite (%) and (b) inhibition parasite (%) in *M. musculus*. Each bar represents the mean ± SD level parasitemia of animal belonging to G1 (of *P. berghei* infected-mice), G2 to G4 (aqueous *G. husor* stem bark extract 10, 100, and 200 kg/BW), respectively. Significantly different (p<0.001) in level parasitemia of G1 compared to G2-G4.
4. Discussion

During the prospection of new substances with antimalarial potential an important feature is required: the substances must present reduced IC\textsubscript{50} value assays, minimizing the lack of drug specificity and side effect (de Souza et al. 2017). Extract have IC\textsubscript{50} value ≤ to \textit{P. falcifarum} can be continued to isolation of active compounds in medicinal plant (Oliveira et al. 2014). Ethyl acetate \textit{G. husor} stem bark extract showed reduced IC\textsubscript{50} value (0.31 μg/ml) against \textit{P. falcifarum} 3D7 strain. Moreover, it is expected that a potent antimalarial agent (Katusno et al. 2015) and be continue \textit{in vivo} antimalaria assay.

The present results shown a low toxicity of ethyl acetate extract even at doses higher than used in the study. The absence of clinical features of toxicity or weight variation is important, mainly because in the malaria state the animal can present symptoms belonging to both states such as pelage change, lethargy and weight loss which could interfere which the results.

The observation that no death caused by an oral highest dose of 200 mg/kg body weight of ethyl acetate extract of the \textit{G. husor} stem bark could imply the safety of the plant to be used in the treatment of Malaria as also suggested in Akele et al. 2013 and Murithi et al. 2014. The acute toxicity result of the present study suggested that
the oral extract at the highest dose (200 mg/kg BW) and included in the category of practically non-toxic (OECD 2008). The experimental determination of acute toxicity at the extract dose of up to 2000 mg/kg body weight of mice may justify the use of this plant for malaria treatment.

In vivo antiplasmodial activity can be classified as moderate, good and very good if an extract displayed a respective percent parasite suppression equal to or greater 50% at doses of 500, 250, and 100 mg/kg body weight per day (Munoz et al. 2000; Deharo et al. 2001). Based on this classification, the extract of G. husor are considered to exhibit good antiplasmodial activity, with dose dependent inhibition against P. berghei infection in mice.

Malaria is an inflammatory cytokine-driven disease that often results in mortality due to sequestered parasitized red cells preventing sufficient oxygen access to where it is needed together with mitochondria inability to generate enough adenosine triphosphate to maintain normal cellular function (Clarck et al. 2010). P. berghei parasite is used in predicting treatment outcomes of any suspected antimalarial agent due to its high sensitivity to chloroquine, making it the appropriate parasite for this study (Boampong et al. 2015). In this study, three doses of the ethyl acetate stem bark extracts of G. husor exerted curative antimalarial activities against the blood stage of P. berghei. Ethyl acetate extract give high effect for reduce level of parasitemia in dose 100 mg/kgBW 24±0.01% and 87.37±1.41% inhibition of parasite of P. berghey–infected mice. This result showed that effect ethyl acetate extract lower than treated orally dose 100 mg/kg BW with artemisinin were 54±0.14%; 95%, respectively (de Souza et al. 2017). This implies that the active principles for the observed antimalarial effects are present in ethyl acetate extract. It is proposed that the presence of alkaloids, triterpenoids, flavonoids, phenolics, and tannins could be responsible for the observed curative antiplasmodial activities of the extract but further experiments are needed to confirm this hypothesis. Some alkaloids that act against malaria parasites inhibit protein synthesis by intercalating with DNA of the parasite (Okonk et al. 2008; Christian et al. 2012). In addition, flavonoids and tannins have been suggested to act as primary antioxidants or free radical scavengers that can counteract the oxidative damage induced by the malaria parasite (Gautam et al. 2011; Kayani et al. 2016). Numerous studies have implicated oxidative stress in several pathological conditions such as stroke, diabetes, Parkinson’s disease and so on. Apart from their detrimental effects, free radicals are also involved in cellular signaling and as carriers for iron requirement needed by parasites for survival in a host. Iron is a crucial nutrient for survival and replication of microorganisms such as Plasmodium parasites. Thus, the ability of an antioxidant to chelate/sequester iron needed by the Plasmodium parasite will adversely affect the survival of the parasite in a host. Flavonoids in particular have the ability to chelate iron and they have been suggested that such compounds with the ability to sequester iron should be employed in treatment of infections (Chandrashekar et al. 2010). Ethyl acetate extract have high total flavonoid content, which possibly contributed to the antiplasmodial effect observed in the mice. Some terpenoids attack the broadest age range of parasites, from the tiniest rings that have recently invaded erythrocytes to more mature stages of parasites such as developing trophozoites and schizonts. Their relatively broad stage–specificity of action has been reported to extend to the ability to impede development of gametocytes (Yoo et al. 2008; Coma-cros et al. 2018). The data provide evidence that stem bark extracts of G. husor antiplasmodial activity which may partly be mediated by its phytochemical compounds.

In the qualitative and quantitative analysis of this plant studied, some of these secondary metabolites were present and saponin was absent. It has been reported that several plant constituents, vis; flavonoid, tannins, xanthone, phenolic, and triterpenoid posses protein-binding and enzim-inhibiting properties (Selvanayagam and Ghanavendhan 1995; Fale et al. 2012; Oluwatosin et al. 2014). The likely mechanism of action of this extract contain flavanoid may be inhibition of key pathognic enzymes of the parasite since extract is known to interference with enzymes sistem (Okunji et al. 2007; Penduka et al. 2011). Alkaloids are important phytochemicals, that are said to be pharmacologically active. Quinine, an alkaloid, is popular for is antimalarial activity against the malaria parasite (Bankole et al. 2016). Triterpen, 3b-hydroxy-23-oxo-9,16-lanostadien-26-oic acid or garcihombronane D from G. celebica showed a selective activity against P. falcifarum (Elfita et al. 2009).

Pearson’s correlation coefficient (r) showed high and positive correlation between phenolic content in extract with antimalarial activity (Table 3). In the
The present study, TPC and TFC to be analytical markers since they were the major components of the test sample. Similarly, phenolic were have confirmed its antimalarial activity. On the other hand, due to the complexity of the sample, it is difficult to state that molecule is responsible for the antimalarial potential of extract, with a synergistic effect probably playing an important role in the activity.

The previous studies reported that phenolic components from *Garcinia* genus have antimalarial potential i.e. α and β-mangostin (*G. mangostana*), 1,5-dihidrokisi-3,6-dimetoksi-2,7-diprenilantan isosantoximol (*G. griffithii*), kolaviron (*G. cola*) (Mahabusarakham et al. 2006; Oluwatusin et al. 2014).

Malaria infection also causes renal damage due to high parasitemia, when parasitized erythrocytes become hard, with reduced deformability and changes conformation. This increases blood viscosity, slowing and microcirculation and altering renal blood flow. Additionally, the cytoadherence of infected erythrocytes alters the local circulation, aggravating the renal injury. Our result showed that treatment with extract may contribute to an increase in the microcirculation, reducing erythrocyte cytoadherence, with a significant decrease of parasitemia.

The present study revealed that ethyl acetate stem bark *G. husor* extract effective herb with significant antimalarial in vitro test antimalasmodium against *P. falcifarum* 3D7 strain and in vivo test in *M. musculus* infected *P. berghei* (ANKA) strain. The biological activities observed in the study could be attributed to the higher phenolic and flavonoid contents of the extract. The result obtained in this study demonstrated that the ethyl acetate of *G. husor* stem bark extract potent antimalarial activity. However, this appears to the first report of antimalarial properties of stem bark *G. husor* extract in vitro and in vivo research. The result of this study support the traditional claim of the plant in the use for malaria treatment, and effort are currently underway to identify the bioactive components of the plant and toxicological effects in animals.

<table>
<thead>
<tr>
<th>TPC</th>
<th>TFC</th>
<th>APf</th>
<th>APb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>0.888*</td>
<td>0.904*</td>
<td>0.747*</td>
</tr>
<tr>
<td>TFC</td>
<td></td>
<td>0.999*</td>
<td>0.968*</td>
</tr>
<tr>
<td>APf</td>
<td></td>
<td></td>
<td>0.959*</td>
</tr>
<tr>
<td>APb</td>
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</table>

Table 3. Pearson's correlation coefficient (r) of TPC, TFC, APf, and APb.

The overall, result showed that ethyl acetate of *G. husor* stem bark in vitro and in vivo has good activities as antipalsmodial. Furthermore that can be used as antimalarial drugs alternative or as a combination with antimalarials other.

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