

## ORIGINAL ARTICLE

### THE EFFECTIVITY OF THE ETHANOL EXTRACT OF EGGPLANT (*SOLANUM MELONGENA L.*) PEELS AS ANTIMALARIAL TO MONOCYTE IN MICE (*MUS MUSCULUS*) INDUCED BY *PLASMODIUM* *BERGHEI*

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**Abstract:** Malaria is a disease caused by Plasmodium that is transmitted to humans by the bite of the female Anopheles mosquito. Malaria can be treated with antimalarial drugs such as chloroquine and artemisinin, but in some endemic areas, it is reported that parasitic resistance to chloroquine was reported so that malaria eradication becomes increasingly difficult. The occurrence of this resistance causes the need for new antimalarial findings. Eggplant (*Solanum melongena L.*) has many secondary metabolites, one of which is solanidine which is toxic to bacteria, fungi, viruses, and protozoa. Eggplant peel was extracted by maceration method using 70% ethanol. The analysis of secondary metabolites from the ethanol extract of eggplant peels used tube test and thin-layer chromatography. The level of parasitemia and the number of monocytes were calculated from blood smear of mice (*Mus musculus*) which were treated with extracts at a dose of 0.075 mg/20 g of body weight; 0.15 mg/20 of body weight; and 0.3 mg/20 g of body weight. 3.744 mg/20 g of body weight Dihydroartemisinin-Piperaquine (DHP) was used as positive control and distilled water was used as the negative control. Ethanol extract of the eggplant peels contains groups of alkaloids, flavonoids, terpenoids, steroids, phenolic, and saponins. 0.075 mg/20 g body weight of ethanol extract of the eggplant peels effectively reduces parasitemia and 0.3 mg/20 g body weight of ethanol extract of the eggplant peels effectively increases the number of monocytes in mice. There is no correlation between an increase in the number of monocytes and a decrease in the level of parasitemia.

**Keywords:** antimalarial, eggplant, monocyte, *Plasmodium berghei*, *Solanum melongena L.*

## INTRODUCTION

Malaria is a disease caused by the *Plasmodium* parasite which is transmitted to humans through the bite of female *Anopheles* mosquitoes. *Plasmodium* carried through mosquito bites will live and multiply in human blood cells. Malaria in humans can be caused by four species of parasites, which are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale*.<sup>1</sup> Malaria can affect all age groups, both men and women. People who have malaria, have clinical symptoms such as fever, chills, weakness, headaches, muscle aches, coughs, nausea, vomiting, and diarrhea.<sup>2</sup>

Nearly half of the world's population is at risk of malaria. The latest WHO data states that at the beginning of 2016, malaria was considered endemic in 91 countries and regions. This number is down from 108 in 2000.<sup>1</sup> Despite progress, malaria still has a detrimental impact on people's health and lives. In 2015, there were 212 million cases of malaria and 429,000 of them died, with the majority of cases occurring in children under 5 years of age.

The number of malaria positive cases in Indonesia decreased from 2011 to 2015.<sup>3</sup> This shows the success of the government's efforts in malaria control programs. In 2015, eastern Indonesia, such as Papua and West Papua, recorded the highest number of malaria cases while Bali and DKI Jakarta were included in the category of malaria-free provinces. Endemic districts/cities in Kalimantan and Sulawesi experienced a decrease in the percentage of malaria cases from 2011-2015 while districts/cities

with moderate endemic levels experienced an increase.<sup>3</sup>

Malaria can be treated by administering antimalarial drugs such as chloroquine and artemisinin, but currently, it is reported in some endemic areas of malaria that there is parasitic resistance to chloroquine, making malaria eradication even more difficult. The occurrence of such resistance has led to the need for new antimalarial discoveries, especially those originating from nature as one of the efforts to explore Indonesia's natural wealth. The discovery can be made through research by isolating active compounds from natural materials that are traditionally used as a cure for malaria or synthesizing compounds with chemical structures similar to chemical compounds that are known to have antimalarial effects.

Eggplant (*Solanum melongena* L.) has a good component for health. Empirically, purple eggplant skin has been used by people in the Kubu Raya area to reduce fever in malaria. The antioxidant activity test of the ethanol extract of eggplant shows that eggplants contain antioxidants alkaloids and flavonoids.<sup>4</sup> The content of other eggplants is solanidine which is a secondary metabolite of purple eggplant that is toxic to bacteria, fungi, viruses, and protozoa. Purple eggplants have microorganism activity.<sup>5</sup> Solanaceae plant (*Solanum tuberosum* L. dan *Solanum nigrum*) contains glycoalkaloid which has antimalarial activity.<sup>6</sup> Research on this plant as an antimalarial has also never been done before.

Not yet found publications about the effectiveness of ethanol extract of purple eggplant peels (*Solanum melongena* L.) as an antimalarial induced by *Plasmodium berghei* with test parameters in monocyte levels of mice (*Mus musculus*). *Plasmodium berghei* molecularly showing the equation with *Plasmodium falciparum* so that many antimalarial studies use this type of Plasmodium as a malaria-inducer with mice as its host.<sup>7</sup>

## METHODS

The research conducted is a true experiment design in vivo with a completely randomized design. The sample used in this study was in the form of experimental animals namely mice (*Mus musculus*) Swiss strain as many as 30 mice. The inclusion criteria of the study were healthy male mice with regular activities and aged between 6-8 weeks with a bodyweight of 25-35 grams. The exclusion criteria for the study were sick mice with criteria for injury, anatomical abnormalities of the body, inactivity and not eating or drinking.

Sample mice were divided into six groups, consisting of five mice in each group. Group 1 was given 0.075 mg / 20 gBW of mice extract of purple eggplant skin, group 2 was given 0.15 mg / 20 gBW of mice extract of purple eggplant skin, group 3 was given 0.3 mg / 20 gBW of mice extract of purple eggplant skin, purple 4 were given 3,744 mg / 20 gBW *Dihydro-artemisinin-Piperaquin* (DHP) as positive control, group 5 was given distilled water as negative control, and group 6 was not given intervention (normal control). The treatment is given for 4 days.

The research material was an ethanol extract of purple eggplant skin (*Solanum melongena* L.). The observed data were the number of parasitemia and the number of peripheral blood monocytes. The determination of the dose of purple eggplant peel ethanol extract which effectively reduces the number of parasitemia was obtained from the calculation of the percentage of *Plasmodium berghei* inhibition. The research data were processed using SPSS version 23 with the One Way ANOVA comparative test and Pearson's correlative test.

## RESULTS

In this study, phytochemical screening was conducted qualitatively with the test method of the tube and thin-layer chromatography (TLC). Based on table 1, it can be seen that the ethanol extract of purple eggplant skin contains a group of alkaloid, flavonoid, terpenoid, steroid, phenolic and saponin compounds. This group of secondary metabolites that affect the biological activity of ethanol extract of purple eggplant skin.

**Table 1. Secondary Metabolite Test Results**

| Compound   | Reactor                    | Observation                                    | Result |
|------------|----------------------------|--|--------|
| Alkaloids  | <i>Dragendorff</i>         | Brownish stains appear                         | (+)    |
| Flavonoids | AlCl <sub>3</sub> 5%       | Visible stains that glow under UV light 366 nm | (+)    |
| Terpenoids | <i>Liebermann-Burchard</i> | Brownish red stain                             | (+)    |
| Steroids   | <i>Liebermann-Burchard</i> | A greenish stain appears                       | (+)    |
| Phenolic   | FeCl <sub>3</sub> 1%       | Blackish stains appear                         | (+)    |
| Saponin    | Distilled water            | Formed foam                                    | (+)    |

After giving eggplant skin ethanol extract to mice induced by *Plasmodium berghei*, the mice were tested for parasitemia levels. The level of parasitemia of various groups can be seen in Table 2.

Furthermore, the percentage of inhibition of ethanol extract of purple eggplant peel against *Plasmodium berghei* was calculated in various test groups. The percentage inhibition of *Plasmodium berghei* by

purple eggplant skin extract is presented in Table 3.

The next step is to observe the number of monocytes in the test group mice, positive and negative controls that have been induced by *Plasmodium berghei* and normal controls that are not induced by *Plasmodium berghei*. Data on the number of monocytes is presented in Table 4.

**Table 2. Parasitemia Levels**

| Days | Parasitemia Levels (%) |           |            |             |             |
|------|------------------------|-----------|------------|-------------|-------------|
|      | Group 1                | Group 2   | Group 3    | Control (+) | Control (-) |
| 1    | 8,8 ± 5,1              | 8,4 ± 2,2 | 10,4 ± 2,9 | 3,0 ± 1,2   | 6,8 ± 2,6   |
| 2    | 10,8 ± 7,4             | 7,9 ± 2,9 | 10 ± 2,4   | 1,2 ± 0,6   | 10,6 ± 3,4  |
| 3    | 9,5 ± 6,1              | 7,9 ± 3,8 | 11,2 ± 4,6 | 0,7 ± 0,6   | 14,0 ± 5,2  |
| 4    | 4,8 ± 2,9              | 7,9 ± 4,7 | 10,5 ± 3,3 | 0,3 ± 0,2   | 15,5 ± 4,9  |

**Table 3. Inhibition Percentages**

| Sample Test | Inhibition (%) |         |         |             |             |
|-------------|----------------|---------|---------|-------------|-------------|
|             | Group 1        | Group 2 | Group 3 | Control (+) | Control (-) |
| 1           | 88,3           | 32,3    | 0       | 93,8        | 0           |
| 2           | 31,5           | 0       | 0       | 91,7        | 0           |
| 3           | 77,3           | 55,4    | 5,7     | 81,3        | 0           |
| 4           | 0              | 9,9     | 7,4     | 86,7        | 0           |
| 5           | 0              | 0       | 39,3    | 100         | 0           |
| Mean        | 39,4           | 19,5    | 10,5    | 90,7        | 0           |

**Table 4. Monocytes Number**

| Days | Monocytes Number |           |           |             |             |                |
|------|------------------|-----------|-----------|-------------|-------------|----------------|
|      | Group 1          | Group 2   | Group 3   | Control (+) | Control (-) | Normal Control |
| 1    | 5,8 ± 2,8        | 7,2 ± 2,7 | 8 ± 0,7   | 8,4 ± 4,3   | 3 ± 1       | 2,8            |
| 2    | 7,4 ± 0,5        | 8,8 ± 3,5 | 8,8 ± 0,4 | 9 ± 2,3     | 3,8 ± 1,8   | 3              |
| 3    | 7,2 ± 1,3        | 8,2 ± 1,3 | 9 ± 0,7   | 9,2 ± 2,2   | 4,2 ± 1,6   | 3,4            |
| 4    | 6,8 ± 0,8        | 7,8 ± 3,2 | 9,8 ± 0,8 | 9,7 ± 3     | 3,6 ± 1,1   | 3,2            |

## DISCUSSION

### Effect of Ethanol Extract of Purple Eggplant Skin on the Level of Parasitemia

Based on the results of the secondary metabolite test, it is known

that the ethanol extract of the purple eggplant skin contains secondary metabolites, namely alkaloid, flavonoid, terpenoid, steroid, phenolic, and saponin groups (Table

1). In this research, it cannot be explained which groups of alkaloids, flavonoids, terpenoids, steroids, phenolics, and saponins that have antimalarial activity because isolation and activity testing of isolates have not been done. However, in other studies of the anticancer activity of purple eggplant skin extracts, it was found that there were five isolated steroid components in the methanol extract of purple eggplant skin, three of which were from the alkaloid group, namely solasodine, solamargin, and solasodine, and 2 of them were from the glycoside group, namely  $\beta$ -sitosterol-3-O- $\beta$ -D-glucoside and poriferasterol-3-O- $\beta$ -D-glucoside.<sup>8</sup>

In an antimalarial study which isolated various glycoalkaloid compounds from the *Solanaceae* plant, it was found that chanonine and solamargine had greater antimalarial activity than solanine and solasonine.<sup>6</sup> Chanonine and solamargine are glycoalkaloids which have chacotriose carbohydrate chain while solanine and solasonine have solatriose carbohydrate chain, so it can be concluded that glycoalkaloids with chacotriose carbohydrate chain have higher antimalarial activity than solatriose. Based on these studies it is known that antimalarial activity in glycoalkaloid compounds depends on nonspecific carbohydrate interactions.<sup>6</sup>

The carbohydrate chain of glycoalkaloid plays a role in the binding of sugar molecules that bind to cell membrane receptors that cause the formation and insertion of sterol complexes into the plasma membrane of *Plasmodium* resulting in disruption and loss of membrane integrity.<sup>6</sup>

Antimalarial activity of purple eggplant skin ethanol extract can be seen from the average level of parasitemia in groups 1, 2, and 3, which experienced a decrease in the level of parasitemia on the fourth day of treatment compared to the level of parasitemia on the first day. Besides, it was also seen from the comparison with the negative control group that experienced an increase in the level of parasitemia during 4 days of treatment (Table 2). Statistical analysis on the fourth day also showed that there were significant differences between the positive control group and groups 1, 2 and 3, as well as between the negative control group and groups 1 and 2 (Post hoc,  $p < 0.05$ ).

The effective dose of purple eggplant peel ethanol extract which can reduce the level of parasitemia can be determined by calculating the percentage of inhibition against *Plasmodium* (Table 3). The percentage inhibition is the ability of each dose to inhibit the growth of *Plasmodium berghei* in mice. The percentage of inhibition of the positive control group was higher than that of all treatment groups, but group 1 was more effective in inhibiting *Plasmodium* compared to 2 and 3 seen from the results of the calculation of the higher percentage of inhibition.

#### **Effect of Ethanol Extract from Purple Eggplant Skin on Monocyte Amount**

The observation of monocyte counts showed that the ethanol extract of purple eggplant skin has the ability as an immunomodulator by increasing the number of mice monocytes

induced by *Plasmodium berghei*. The number of monocytes in groups 1, 2, and 3 was higher than the negative control group with group 3 which had the most stable increase compared to groups 1 and 2.

Monocytes are one of the effector cells that play a role in eliminating parasites through the mechanism of phagocytosis in the nonspecific immune system, meaning that monocytes will play a role in the early times of parasitic infections.<sup>9</sup> The protective immune response against intracellular microorganisms is cellular immunity. The first immune response that occurs is the destruction of *Plasmodium* phagocytosis by macrophages and other phagocytic cells activated by cytokines produced by T lymphocytes. T lymphocytes will respond to antigens from *Plasmodium* that are phagocytized by macrophages that bind to MHC class II molecules to the cell surface. Furthermore, monocytes will produce IL-4 which then activates B cells to produce immunoglobulins which play a role in the process of opsonization, which increases phagocytic activity. The intracellular parasite will stimulate monocytes to produce IL-12 which will activate NK cells, which then secrete IFN- $\gamma$  which will activate macrophages.<sup>10</sup>

The average percentage of normal monocytes in Swiss strain mice is 0-5%.<sup>11</sup> In this study, the number of monocytes in the negative control group increased until the third day, then decreased on the fourth and fifth days, but still within the normal range but in the treated group. ethanol extract of purple eggplant skin has increased the number of monocytes

above the normal range with a trend that tends to increase for 5 days. This increase can occur due to the content of secondary metabolites found in the ethanol extract of purple eggplant skin, especially flavonoids which act as immunostimulants, which can improve cellular and humoral immune responses to malaria infections.<sup>12</sup> The content of secondary metabolites that act as immunostimulants can increase IFN- $\gamma$  levels and phagocytic ability of macrophages. Flavonoids can increase IL-2 and IL-4. IL-2 will stimulate T cell growth and increase IFN- $\gamma$  secretion. IFN- $\gamma$  in addition to activating monocytes and macrophages also increases IL-12 levels. IL-12 and IL-4 will stimulate the production of immunoglobulins which will indirectly increase the activity of macrophages, monocytes, neutrophils, in phagocytizing the *Plasmodium* parasite.<sup>13</sup>

The role of immunostimulant from purple eggplant skin ethanol extract was proven when compared to the negative control group which increased only until the third day then decreased on the fourth and fifth day, and was still in the normal range. The best doses as immunostimulants are group 3 (0.3 mg / 20 gBW mice), seen from an increase in the number of monocytes which are more stable than group 1 (0.075 mg / 20 gBW mice) and 2 (0.15 mg / 20 gBW mice), and almost equal the average number of positive control group monocytes (Table 4). In addition, the statistical analysis also found a significant difference between group 3 and the negative control group from the first day to the fourth day. However, the immunostimulant role

of purple eggplant skin ethanol extract has not been able to match the immunostimulatory effect of the positive control group treated with DHP drugs.

### **Correlation between Parasitemia Level and Monocyte Count**

SPSS analysis of the correlation test showed there was no direct relationship between the decrease in the level of parasitemia and an increase in the number of monocytes after 4 days of treatment. Infection by *Plasmodium berghei* will induce the immune system starting from the physical defense, innate natural defense, and finally, the defense obtained. Incoming parasites will soon be faced by the nonspecific immune system to eliminate or inhibit the development of parasites in the body. Effector cells that play a role in eliminating these include neutrophils, monocytes, and macrophages through the mechanism of phagocytosis. A continuous and increasing infection of *Plasmodium berghei* will activate the specific immune system played by T lymphocytes (cellular immunity) and B lymphocytes (humoral immunity). T lymphocytes are divided into two, namely helper T cells (CD4) and cytotoxic T cells (CD8). Helper T cells (Th) are further divided into Th-1 cells for cellular immune response and Th-2 for humoral immune responses. Th-1 cells will produce proinflammatory cytokines, namely IL-2, IFN- $\gamma$ , TNF- $\alpha$  which then stimulates more phagocytic cells and macrophages.<sup>12</sup> Therefore, an increase in the number of macrophages and phagocyte cells, one of which is monocytes, is expected to reduce the

level of parasitemia by phagocytosing the parasite.

However, the results of the statistical analysis of the correlation test showed that there was no relationship between the decrease in the level of parasitemia with an increase in the number of monocytes that occurred during the 4 days of treatment. This means that the decrease in the level of parasitemia that occurs after the administration of purple eggplant skin ethanol extract is not influenced by an increase in the number of monocytes. The decrease in the level of parasitemia that occurs is the effect of the secondary metabolite content found in the ethanol extract of purple eggplant skin, especially solasodine, solamargin, and solasodine which damage the parasitic cell membrane.<sup>6</sup> The increase in the number of monocytes is also an effect of the ethanol extract of purple eggplant skin containing flavonoids which act as immunostimulants rather than influenced by the level of parasitemia.

### **CONCLUSIONS**

Ethanol extract of purple eggplant skin (*Solanum melongena* L.) has antimalarial activity (with an effective dose of 0.075 mg / 20 gBW) and immunomodulators in increasing the number of monocytes with an effective dose of 0.3 mg / 20 gBW. There is no relationship between the decrease in the level of parasitemia with an increase in the number of monocytes of mice treated with purple eggplant skin ethanol extract (*Solanum melongena* L.) after being induced by *Plasmodium berghei*.

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