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Research Paper

Antimalarial activity of *Delonix regia* on mice with *Plasmodium berghei*

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ABSTRACT

Plasmodium falciparum developed drug resistent so their rate of infection become doubled in last two decades. This encourages efforts to continue to explore natural materials that can be used as a new antimalarial drug. This study detected that Flamboyant contain bioactive compounds that can inhibit parasitemia of *Plasmodium berghei* to determine the effectiveness of this by in vivo test. The results of in vivo study of flamboyant extracts tested showed that administration of bark extract could inhibit by 122% and extract the seeds, flowers and leaves only able to inhibit by 87.45%, 75.99% and 78.43% . Group C (with flamboyant bark extract) showed the most potent antimalarial activity compared to the others. Based on the analysis by one way ANOVA, the test group showed a distinct inhibition of parasite growth was statistically significant ($P = 0.00$; $P<0.05$). Thus group C, giving the stem bark extract on mice infected with *Plasmodium berghei* is the most potent therapeutic model.

Keywords: *Plasmodium berghei*; Flamboyant (*Delonix regia*); In vivo test.

INTRODUCTION

Malaria is a very dangerous disease and 1.2% of total human deaths caused by this disease. Many efforts have been made for the treatment of malaria since the year 1820 to the present chloroquine is still used as an effective drug that inhibits the growth of Plasmodium falciparum parasite, but this drug will develop resistance to the parasite, so the use of the drug chloroquine is no longer effective (Saxena, et al., 2003). These drugs cause many side effects such as vertigo, heart and kidney failure. On the other hand, current malaria is still an endemic disease in over 90 countries, mainly in developing countries (Sanchez, et al., 2004). Patients who are infected with malaria in the last 2 decades doubled primarily due to the emergence of strains of Plasmodium falciparum malaria resistant to available drugs, especially chloroquine and its derivatives (Trape, et al., 2002).

This encourages efforts to continue to explore natural materials that can be used as a new antimalarial drug. Based on research that has been reported, to date the secondary metabolites that are as antimalarial is derived from the group of alkaloids, terpenoids, flavonoids, limonoids, calkon, peptides, Santon and coumarin. The

compounds were obtained from the isolation of living things including plants, bacteria and fungi (Kaur, et al., 2009). Also reported that of these chemicals have been tested activeness in vitro and in vivo in experimental animals. Perez et. al. (1997) states that the global spread of malaria parasites that are resistant to several drugs available is a major health problem that it takes effort to get the source material of new antimalarial drugs. Drug discovery from natural ingredients based on the selection of sample reports or scientific journals of bioactivity testing of a plant. Besides the selection of the sample is also based on the traditional use of plant specific information. Usually the source of information is an herbalist or from ordinary people who use medicinal plants.

Based on information from the community in East Nusa Tenggara region Indonesia, the family *Leguminosae* plants such as Delonix regia having antimalarial activity. Given the kinship between plants in one family the possibility of having almost the same chemical components and also have biological activities similar as well (Edward, et al., 1993). Based on the conducted research on the potential of Delonix regia in lowering the level of parasitemia that were inoculated with *Plasmodium sp*. Delonix regia is one type of plant that may inhibit the malaria parasite that infects humans (Carter and Diggs, 1977).

MATERIALS AND METHODS

***Extraction and isolation of compounds in Delonix regia*:** Parts of flamboyant (*Delonix regia*) (bark, leaves, fruits, seeds and flowers) were obtained from BALITRO Bogor which identified by BALITO on May 2010. The parts of famboyant are separated and cleaned of debris. Once clean the chopped herbs and dried, then ground and weighed. Extracting material extraction is done by using a maceration. Leaves, bark and flowers taken simplicia made by drying at room temperature (30°C) and not exposed to direct sunlight. Dry matter is made of powder until smooth and filtered with a specific filter to obtain a homogeneous powder. Simplicia already homogeneous extraction is then done by maceration rise. Maceration is done by soaking and stirring of 250g powder simplicia in 500ml solvent (hexane, chloroform, methanol, ethanol and water) for 48h so that the active substances within the cell cavity will dissolve and because concentration difference, the active substance will be pushed out of the cell. After that the solution is slowly filtered and the solvent is kept above the powder so that the powder remains submerged and the resulting filtrate. Furthermore, the filtrate was concentrated with an evaporator until the solvent runs out. The extract is then stored in dark bottles (Depkes, 1986).

***Phytochemical Screening*:** Phytochemical screening conducted to determine the chemical components found in plants using the method of Cuilei (1982). The components identified include alkaloids, flavonoids, tannins, quinones and terpenes.

Identification of alkaloids, done by way of extract added with 10ml of chloroform. Then added 10ml of ammonia 0.005 N and placed into a separating funnel. Then add 10 drops of 2 N sulfuric acid, shaken gently and allowed to form a layer of acid and chloroform. The next layer is taken acid and separated in a test tube. Into this solution was added 1 drop of Meyer's reagent. The presence of alkaloids is indicated by the formation of white smoke or mist to produce a white precipitate.

***Effectiveness in-vivo test of bioactive Delonix regia*:** Mice used were male white mice (*Mus musculus*) from DDY strain aged 2-3 months with body weight between 20-30g. Before treatment, the animal was acclimatized prior to drug administration worm (1 day) were given antibiotics for 3 consecutive days. Feed and drinking water

provided adibitum. Mice inoculated with 0.2ml of a suspension of *P. berghei* intra-peritoneal then incubated for 48 hours. The next day mice from tail vein blood taken to determine parasite infection in mice. Mice that had been positive for the parasite P. berghei are grouped into 5 treatment groups at day 0 (D0).

Giving the plant stem bark extract of Cempedak on mice based on data from previous studies that showed that the use of a dose of 100mg/kg once daily administration for 4 days can inhibit the growth of P. berghei amounting to 76.70% (Widyawaruyanti, 2007). Usual dose of chloroquine use in a day is 8mg/kg human. Conversion factor of 70kg man with a BB to BB mice with 20g is equal to 0.0026. So that the mice used for dose = 8mg/kg x 70kg x 0.0026 = 0.728 human mg/20kg body weight of mice = 72.8mg/kg body weight of mice.

***In vivo testing of antimalarial activity*:** Antimalarial activity assay is performed referring to Peter's standard methods Test (4-Days suppressive test) (Philipson, 1991). Parasite-infected mice were divided into six groups, each group consisted of three mice,each treated group:

1- One negative control group (untreated group): given a solution of 2% DMSO in 0.5% CMC Na D0-D2

2- Five groups of test:

Group A: Administered the test solution flamboyant leaf extract 72.8mg/kg
Group B: Administered the test solution flamboyant fruit peel extract 72.8mg/kg
Group C: Administered the test solution flamboyant bark extract 72.8mg/kg
Group D: Administered the test solution flamboyant seed extract 72.8mg/kg
Group E: Administered the test solution flamboyant flower extract 72.8mg/kg

- Provision of test materials on mice carried out if the existing growth of parasites (± 1%).
- D0 is the first day of manufacture of thin blood smear before giving the test and treatment materials. D1 was observed on day-2, D2 is the observation on day-3, D3 was observed on day 4 onwards.
- Each day mice were observed until day 7 (D0 - D6).

RESULTS

***Phytochemical screening of Flamboyant extract*:** Bark, leaves, fruits, seeds and flowers of *Delonix regia* were obtained from BALITRO Bogor, separated and cleaned of debris. After the clean parts of the plant is cut into small pieces and dried, then ground using a blender and weighed each of part 250g (Table 1).

In-vivo test: Observations of flamboyant extract (fruit peel, leaf, bark, seeds and flowers) for 6 days in mice infected with *P.berghei* shown in Table 2 and Figure 1.

DISSCUSSION

The results of phytochemical screening of the flamboyant plants indicate the presence of many kinds of compounds. Phytochemical test results in Table 1 shows that the bark and fruit peel extract have alkaloid compounds very strong intensity and the leaf extract has a strong intensity. Whereas flavonoids compounds found in flowers with a very strong intensity. Phenolic compounds with strong intensity found in the bark and flower and there is sufficien intensity on leaves.

Observations parasitaemia one day after administration of test substance, followed up on day 6 shown that administration of the plant extract showed inhibition

of parasitemia flamboyant compared to negative control. However, if you look at table 2, that the skin of the fruit and bark provide most of the parasitic resistance, respectively by 117% and 122%. This shows that the skin of fruit and bark contain a flamboyant effective to inhibit the parasite. From the figure 1. shows that each extract is under negative control charts.

On day 2, the parasite begins to infect the blood of mice demonstrated by a high percentage parasitaemia in the negative control. Each dose of the extract gave inhibitory power on the growth of Plasmodium berghei indicated that varies with the percentage of parasitemia lower than controls. Giving flamboyant extract at a dose of 72.8mg/kg body weight have an influence on the inhibition of parasite growth but from the fifth part of the plant extracts flamboyant, extract from the bark and fruit peel is much higher effect in inhibiting the growth of the parasite.

On the third day, groups A and C give effect to the inhibition of parasite significantly compared with groups B, D and E. On the fourth day, group A has a power resistor on the growth of Plasmodium berghei, but still lower than the inhibitory power given by chloroquine. Of Plasmodium berghei growth charts for the fourth day, fifth and sixth day of observation, the number of parasites tends to increase the control treatment, as Figure 3. In all groups there was an increase in early infection is relatively slow but parasitemia on day 4 there was an increase parasitemia.

In treatment A, B, C, D and E parasites tends to increase but relatively lower compared with controls. The extract of flowers, leaves and seeds is possible still contains a mixture of various active compounds that have not known for certain activities. In addition, these extracts also contain various impurities which participated in the extract is dissolved, including chlorophyll. Chlorophyll is thought to interfere with the activity of the active compounds contained in the extract.

Based on the analysis by ANOVA ($\alpha = 0.05$) as in Table 3, there were significant differences between groups. Of the five test groups showed the test group C gives the most significant difference when compared with test groups A, B, D, and E. While among the test groups A, B, D, and E did not give significant differences to the malaria parasite growth inhibition at 0.95 confidence level ($\alpha = 0.05$). While the test results of Tukey test and homogenous subset as Table 4 are used to test group that has a real difference and average differences are not significantly different.

From the results of this study indicate that administration of extract of bark and fruit peel has a highly effective, each amounting to 122% and 117% compared with the other flamboyant extracts. From Table 2 shows that the fruit peel extract had the highest inhibition compared to the other extracts. if it is associated with the test results phytochemical extracts, the compound has the strongest intensity in the fruit peel is an alkaloid, so it can be concluded that the alkaloid in fruit peel can inhibit of parasitemia growth Plasmodium berghei in mice.

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Table-1: Phytochemical screening of bioactive compounds in flamboyant.

	Bark	Leaf	Fruit peel	Seed	Flower
Alkaloid	++++	+++	++++	+	+
Flavonoid	++	+++	++	+	++++
Terpen	++	+	+	+	+
Phenolic	+++	++	+	+	+++

- \+ means weak intensity; ++ means sufficient intensity; +++ means strong intensity; ++++ very strong intensity.

Table-2: Percentage growth and inhibition of parasitemia to extract flamboyant.

Flamboyant extract	% Growth	% Inhibition
Control	17.62	
Fruit peel (A)	-3	117
Leaf (B)	3.8	78.43
Bark peel (C)	-3.93	122
Seed (D)	2.21	87.45
Flowers (E)	4.23	75.99

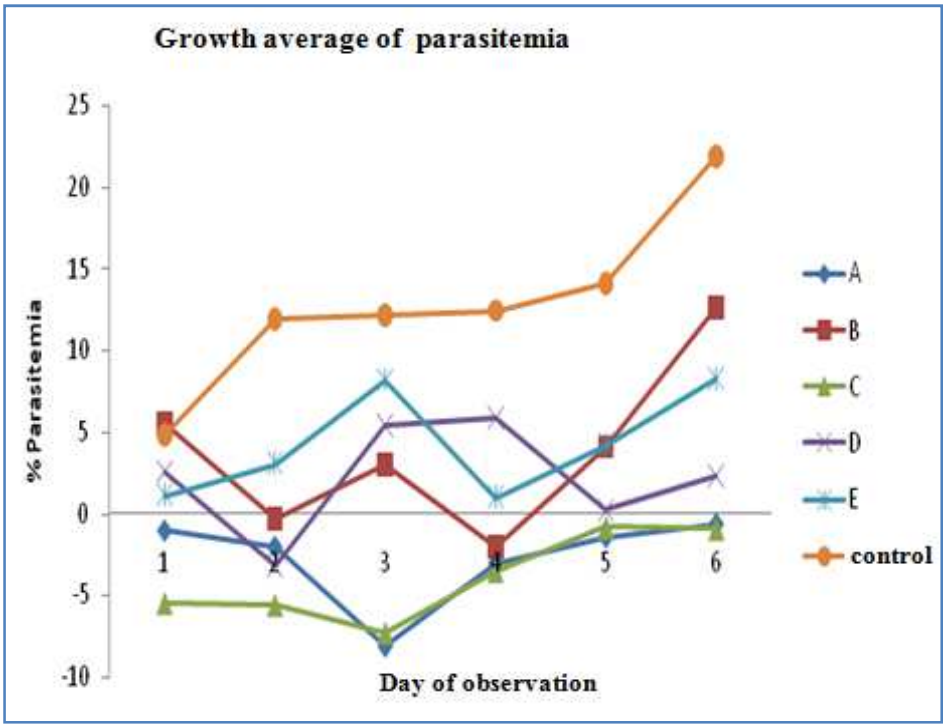

Figure-1: Graph average percent growth of various groups parasite test.

- D0-D6: Day 1 to day 7 observation
- Group A: administered the test solution flamboyant fruit peel extract 72.8mg/kg
- Group B: given test solution flamboyant fruit leaf extract 72.8mg/kg
- Group C: administered the test solution flamboyant bark extract 72.8mg/kg
- Group D: administered the test solution flamboyant seed extract 72.8mg/kg
- Group E: given test solution flamboyant flower extract 72.8mg/kg

