EFFECTIVENESS OF FINGER LEAF INFUSION *(Psophocarpus tetragonolobus (L.) DC)* ON THE MALARIA MODEL OF RATS *(Rattus norvegicus)*.

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ABSTRACT

Winged bean is a type of plant that is used as traditional medicine. This study aims to determine the effectiveness of winged bean leaf infusion *(Psophocarpus tetragonolobus (L.) DC)* against white rat *(Rattus norvegicus)* model of malaria. The method used in this research is completely randomized design with 4 treatment groups and 3 replications consisting of a control group without being given an infusion (P1), a group that was given an infusion of concentrations: 31.25 mg/mL (P2), 62.5 mg/mL (P3), and 125 mg/mL (P4). This study used 20 male rats, of which 5 rats serving as donor mice. Donor mice were infected with *Plasmodium berghei* and left until the parasitemia percentage reached > 20%. Then 4 groups of model mice were infected with *Plasmodium berghei*. Observations were made for 7 days, starting from day 0 (before treatment), 4 days during treatment and 2 days after treatment. The percentage of parasitemia was calculated starting from the day before administration of winged bean leaf infusion (IDK) until the 7th day. The results of this study indicate that IDK can inhibit parasite growth by reducing the level of parasitemia along with increasing concentrations, namely (P2) of 73.78%; (P3) of 89.33%; and (P4) of 93.69%. It can be concluded has potential in inhibiting the growth of *Plasmodium berghei* in the rat model of malaria with an effective concentration of 31.25 mg/mL.

Keywords: infused, flavonoid, plasmodium berghei, parasitemia

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INTRODUCTION

Malaria is a disease caused by the Plasmodium parasite which is transmitted through Anopheles mosquito. *Plasmodium* sp. As a malaria parasite, it has a complex life cycle in its host's body, starting from the entry of sporozoites through mosquito bites followed by the growth of the parasite in liver cells. With the completion of the parasite cycle in the liver, many merozoites will form which will invade the red blood cells. Plasmodium life in red blood cells is a stage that is responsible for the emergence of various clinical, pathological, and immunological abnormalities in the sufferer's body (Hutapea, 2009). *Plasmodium berghei* is a hemoproteozoa that causes malaria in rodents (Harjianto, 2000), especially small rodents. *Plasmodium berghei* is widely used in developmental biology research. Molecularly, *Plasmodium berghei* has similar properties with plasmodium group that infects humans. With the availability of *in vitro* culture technology and purification at the stages of the life cycle, knowledge of the genome structure and its arrangement, then
various research models may be carried out including manipulation of the host so that the immunological changes that occur during malaria infection can be studied (Thomas 1983). Until now, malaria is still a public health problem, especially in tropical countries, including in Indonesia. According to WHO, around 3.2 billion people are at risk of malaria in 97 countries in 2013 and cases of disease are estimated at 198 million cases (range: 124,000,000-283,000,000) (WHO, 2016). The incidence of malaria in Indonesia is in the third highest position. Based on the Annual Parasite Incidence (API) value, around 17% of Indonesia's population lives in high malaria transmission areas (API > 5‰) and 44% in low malaria transmission areas (API < 5‰). Number positive cases of malaria in 2012 reached 417,819 cases, but in 2013 the number of cases decreased to 343,527 cases (Hasyimi et al., 2015). The eastern part of Indonesia is included in the stratification of high malaria with the highest percentage of cases, which is around 70% (Ditjen, 2009; Fathiyah, 2013) and Maluku is one of the provinces that has a high prevalence of 10.7% (Balitbangkes, 2019).

The problem of resistance is one of the causes of high cases of malaria. Widespread resistance Plasmodium as a causative agent of malaria to antimalarial drugs will make it more difficult to eradicate malaria (Oliaro, 2019), and in the future more expensive drugs will be needed. As efforts to find an ideal malaria vaccine have not been successful, research activities aimed at discovering new drugs remain the main means (Burke, 2003; Widyawaruyanti, 2011; Wijayanti, 2003), including knowledge-based research and traditional medicine using natural ingredients. One way is to use ingredients in the form of plant ingredients as traditional medicine. The use of plants or plant parts for malaria drugs has been known and used for thousands of years and is still being developed until now, because it produces quite good effects. Based on empirical experience, people in one area in Maluku use winged bean leaves as a traditional medicinal ingredient. Since 1975, winged bean has been predicted to be very promising in the future as a biological ingredient with high economic value and has a myriad of benefits, including as a herbal medicine. Winged bean is a vine belonging to the Fabaceae (Leguminosae) tribe. Winged bean is also a fast growing annual plant with vines reaching 2-4 m in length. All parts of the winged bean plant (except the stem), namely leaves, flowers, young pods, seeds (fresh or dry) and tubers, can be consumed. Therefore, scientists call this plant a supermarket on the stalk (Handayani, 2013).

Winged bean contains several active compounds that have potential as traditional medicines. From the results of the phytochemical test it turned out that winged bean contains flavonoids, saponins, polyphenols, steroids and terpenoids (Hanum, 1997). Furthermore, according to Nurmla (2018), the active compounds of winged bean plants, especially winged bean leaves and seeds, contain saponins, flavonoids and tannins. This content is also similar to the content of active compounds found in plants used to treat malaria. In addition, winged bean also contains various important minerals such as calcium, zinc, sodium, potassium, magnesium, phosphorus, and iron (Handayani, 2013). The traditional use of medicinal plants can be in the form of a decoction, but on the one hand the method of boiling can also affect the content of the active substance or certain substances in the plant can be damaged by boiling at high temperatures (Amoo, 2006). Another way that can be used to obtain water extracts from plants at a certain temperature with easier methods and equipment is the infusion technique (Dewi, 1997).

METHODS

This study used a completely randomized design with 4 treatments and 3 replications, used 20 rats consisting of a control group (P1) and a group Winged bean leaf infusion (IDK) with a concentration of 31.25 mg/mL (P2); The IDK group had a concentration of 62.5 mg/mL (P3); and the IDK group with a concentration of 125 mg/mL (P4).

Materials

The tools used in the study were: rat cages with dimensions LxWxH = 40 x 30 x 15 cm, analytical balance, stove, infusion pan, electron microscope, hand counter, refrigerator, EDTA tube, syringe, measuring cup, Erlenmeyer, glass object, scratch slides, gastric sonde, mixing spoon, spatula, volume pipette, thermometer, and digital camera. The materials used in this study were winged bean leaves, experimental animals in the form of male white rats (Rattus norvegicus), Plasmodium berghei culture, giemsa dye, methanol, immersion oil, alcohol, ketamine, distilled water, white rat feed, filter paper, and tissue.

Procedures

The process of inoculation of infection in donor rats. Plasmodium berghei was infected intraperitoneally (ip) as many as 107 parasites in 0.2 ml of blood/rat, in 5 donor rats. After 4-5 days, the infected mice were taken daily by cutting the tip of the tail, then preparations were made and then the parasitemia rate was examined with a microscope. If the parasitemia rate is >20%, then blood is taken from the heart using a syringe after the rats are anesthetized with ketamine. Blood obtained from the heart is collected in a tube.
containing the anticoagulant EDTA. All the blood is mixed and diluted with Saline Phosphate Buffer (PBS) and then infected to the test animals. Each rat was injected ip0.1 ml (Moll et al, 2008; Intan, 2017).

Making winged bean leaf infusion. The finely chopped leaves are then dried. The dried leaves were weighed as much as 25 grams, then put in the infusion container (container 1) and added 100 ml of distilled water. After that, put enough water in container 2. Then place container 1 into container 2 and place it on the stove. Bring to a boil until it reaches 90°C and sustain up to 15 minutes. The infusion of winged bean leaves is allowed to cool then filtered using a flannel cloth. Thus the initial concentration is taken from 25%, namely 25 grams of winged bean in 100 ml of water in container 1 so that the initial concentration can be 250 mg/mL. Then, multilevel dilution was carried out to 125 mg/mL; 62.5mg/mL; 31.25mg/mL.

Administration of winged bean leaf infusion on rat models. Winged bean leaf infusion (IDK) was given once a day after the rats were infected orally using a gastric tube for 4 consecutive days. Observation of the level of parasitemia was carried out until day 6, namely observation day 0 (D0), observation day 1 (D1); 2nd day (D2); 3rd day (D3); and the 4th day (D4), and 2 days after giving IDK, namely the 5th day (D5); and the 6th day (D6).

Data Analysis
Data on parasitemia growth and inhibition of parasitemia by winged bean leaf infusion were observed for 7 days. The data obtained were analyzed using ANOVA with a 95% confidence level. If there is a significant difference, then the BNT test will be continued to find out the differences in each treatment as well as a probit analysis to be able to determine the value of the ED50.

DISCUSSION RESULT
The results of the study can be explained based on the occurrence of parasite growth, parasite inhibition, average percentage of parasitemia after winged bean leaf infusion (IDK) therapy.

1. Parasite Growth
Winged bean leaf infusion therapy (IDK) can significantly reduce the growth of parasitemia parasites (p <0.05) compared to the control group (P1). The higher the concentration of the extract given, the higher the response that occurs in reducing the growth of parasitemia. But statistically, there was no significant difference between P2, P3 and P4.

![Figure 1. Effect of IDK concentration on average parasite growth.](image)

In general, the group treated with winged bean leaf infusion (IDK) was average parasite growth was lower when compared to the control group. The lowest parasite growth occurred in the 125mg/mL infusion concentration group of 0.042%, while the highest parasite growth was in the control group of 0.665%. Test results the effect between groups with a 95% confidence level indicates that IDK can suppress the growth of Plasmodium berghei.

2. Inhibition of Parasites
IDK administration had a significant effect (p<0.05) on inhibiting the growth of Plasmodium berghei. In general, IDK which was given to all groups P2, P3, and P4, was able to inhibit the growth of Plasmodium berghei, and the greatest inhibition of 93.69% occurred at a concentration of 125 mg/mL(P4). Furthermore, at a concentration of 62.5 mg/mL (P3) there was an inhibition of 89.33% and at a concentration of 31.25
mg/mL (P2) there was an inhibition of 82.26%. Meanwhile for the control group (P1) there was no inhibition of *Plasmodium berghei* growth.

![Figure 2. Effect of IDK Concentration on *Plasmodium berghei* Growth Inhibition](image)

3. **Average Percentage of Parasitemia Model Rats Treated with IDK with Different Concentrations**

The average percentage of *Plasmodium berghei* parasitemia in model rats that were not treated with IDK or the control group (P1), and in malaria rats treated with IDK concentration was 31.5 mg/mL (P2); concentration 62.5mg/mL (P3); concentration of 125mg/mL (P4), starting from day 0 (D0) to day 4 (D4) and Day 5 (D5) to day 6 (D6), can be seen in the following table:

Table 1. Average Percentage of Parasitemia in Model Rats Given Winged Bean Infusion (IDK)

<table>
<thead>
<tr>
<th>Day</th>
<th>Measurement Parasitemia</th>
<th>Infusion Concentration Winged bean leaves (<em>Psophocarpus tetragonolobus</em> L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1</td>
<td>P2</td>
</tr>
<tr>
<td>D0</td>
<td>2.24</td>
<td>2.14</td>
</tr>
<tr>
<td>D1</td>
<td>2.60</td>
<td>3.06</td>
</tr>
<tr>
<td>D2</td>
<td>3.32</td>
<td>3.54</td>
</tr>
<tr>
<td>D3</td>
<td>4.00</td>
<td>3.93</td>
</tr>
<tr>
<td>D4</td>
<td>4.94</td>
<td>4.00</td>
</tr>
<tr>
<td>D5</td>
<td>5.69</td>
<td>3.46</td>
</tr>
<tr>
<td>D6</td>
<td>6.25</td>
<td>2.8</td>
</tr>
</tbody>
</table>

(X ±SD) 4.15±1.50 3.28±0.75 2.89±0.96

Description: Superscripts with the same letters are not significantly different (p>0.05)

IDK : Infusion of winged bean leaves
P1 : Control
P2 : Treatment IDK concentration 31.5 mg/mL
P3 : Treatment concentration of IDK 62.5mg/mL
P4 : Treatment IDK concentration 125mg/mL
D0 : The day before administration of winged bean leaf infusion, but only infected with *Plasmodium berghei*
D1-D4 : IDK giving day
D5-D6 : Giving IDK is stopped but observations of parasitemia are still being carried out

The research data in Table 1 shows that giving IDK has an effect on inhibiting the percentage of *Plasmodium berghei parasitemia*. Inhibition occurred since the administration of IDK concentration of 31.25 mg/mL (P2) with an average percentage of parasitemia of 3.28 then decreased also in the treatment with a concentration of 62.5 (P3) to 2.89 and in the P4 treatment it did not decrease when compared to P3 , but different from the control (P1), which had an average percentage of parasitemia of 4.15. This can be explained by Figure 3 below:
The histogram above clarifies the condition of the percentage of parasitemia calculated in the blood of model rats, namely in the P1 treatment (control) it experienced an increase starting from day 1 (D1) increasing to day 4 (D4) and continuing to increase on day 5 (D5). There was an increase starting from day 1 (D1) to day 6 (D6). Average percentage of parasitemia of white rats (Rattus norvegicus) after IDK administration in the three groups (P2, P3, and P4) from day 1 to day 4 (D1-D4) showed an increase, namely D0 of 2.15 ± 0.15, D1 of 2.85 ± 0.55, D2 of 3.3 ± 0.60, D3 of 3.5 ± 0.81, D4 of 3.74 ± 1.07. But a very significant difference in the percentage of parasitemia occurred on day 5 (D5) and day 6 (D6), namely D5 was 3.61 ± 1.43, and D6 was 3.49 ± 1.76. Thus it can be explained that from day 1 to day 4 of IDK administration, the increase in percent parasitemia was not statistically significantly different from day 0. Giving IDK on day 4 to day 6 experienced a significant difference when compared to Day 0. While between treatments, it can be explained that there was a significant difference between P2, P3, and P4, when compared with P1, but not significant when compared between P2, P3, and P4.

DISCUSSION

The results showed that the infusion of winged bean leaves given to the P2, P3, and P4 groups of mice had an effect on the growth of Plasmodium berghei and a decrease in the percentage of parasitemia, when compared to the P1 group. The growth of Plasmodium berghei for the group given IDK seemed to decrease with increasing concentrations given. The lowest parasite growth of 0.042% occurred at a concentration of 125mg/mL IDK and the greatest inhibition of 93.69% also occurred at a concentration of 125mg/mL. Statistically, IDK administration had a significant (p<0.05) effect on parasite growth compared to the control group. Likewise with the percentage of parasitemia, which can be explained that between treatments P1, with P2, P3, P4, there were significant differences but not significantly different between treatments P2, P3, and P4. This shows that through the concentration given, the active compounds contained in IDK are able to restrain the growth rate of the parasite. This is in line with the results of a study conducted by (Kabiru et al, 2012), which administered a dose of 100 mg/kg of methanol extract of Eucalyptus camadulensis leaves had the potential to inhibit parasite growth by more than 50%. The same thing was said by (Santoso, 1993), that significant parasite inhibition against control (p <0.05), means that it has potential activity as an antimalarial.

It is known that winged bean leaves and seeds contain saponins, flavonoids and tannins (Nurmala et al, 2018). In addition, the results of examining the content of active compounds in winged bean fruit also explained that besides saponins, flavonoids and tannins, there are also steroids or terpenoids, triterpenoids (Hanu, 1997). Active compounds such as flavonoids can act as antimalarials (Priangga et al, 2013) and their role in inhibiting the growth of malaria parasites has been proven in several antimalarial medicinal plants. Flavonoids are a group of phenols potential as an antioxidant, antibacterial, antibiotic, anti-inflammatory, antiviral, anti-inflammatory and able to protect cell structure, increases the effectiveness of vitamin C, prevents bone loss and has bioactivity as a drug (Parubak, 2013). Flavonoids contain 15 carbon atoms arranged in a C6-C3-C6 configuration, namely two aromatic rings connected by three carbon units (Sabandono, 2006). In some cases, flavonoids can act directly as antibiotics by interfering with the function of microorganisms such as bacteria and viruses (Dwayana et al, 2010). Based on the results of the study, there were eight prenylated flavonoid compounds (Artoindonesin E-1, Artoindonesinan Z-4,
Artoindocyanin Z-5, Gemicalkon A, Gemicalkon B, Moracalkon A, Norartokarpanon, and Dihidrororin) isolated from the Artocarpus altillis plant, which have potential as antimalarials. From the results of research (Parubak, 2013), regarding the antimalarial activity test on Morakalkon A and ME2 compounds and 3 other flavonoid compounds, which were isolated from the dichloromethane extract of A. champeden, namely cyclohexenon, artoindonesianin A2 and in Plasmodium falciparum 3D7 culture in vitro showed that these compounds these compounds were able to inhibit the growth of parasites, with an IC50 of 0.28 each; 0.35; 0.08; 0.49; and 0.53µg/ml. not only inhibiting but the results of parasite morphology analysis after incubation with these compounds, showed changes in parasite morphology in the form of trophozoites located outside the erythrocytes, swollen food vacuoles and black hemoglobin and slower growth of the parasite. Likewise with the results of the study (Bilia, 2002), which also showed that other polyphenolic compounds besides tannins, namely the flavonoid group, actually had the ability to inhibit heme polymerization. In its mechanism, flavonoid compounds synergize with artemisinin from the Artemisia plant by increasing the binding ability of artemisinin to heme which causes the formation of artemisinin peroxide which has antimalarial effects. Thus, the results of this study indicate that flavonoid compounds have potent antimalarial activity through inhibition of hemoglobin degradation and heme detoxification and other mechanisms that are not yet known.

Apart from flavonoid compounds, other compounds contained in winged bean leaves, such as tannins, steroids or terpenoids, triterpenoids, are also efficacious as antimalarials (Andayani, 2009). Several previous studies reported that these compounds have antimalarial potential. The antimalarial activity of these compounds can occur through several inhibitory mechanisms including inhibiting heme polymerization. Saponins, tannins, steroids or triterpenoids, and quinones, which are present in 70% ethanol extract of sambung leaves, were reported to have antimalarial activity (Septiana et al, 2017). The qualitative test results showed that keluwih leaf extract contains many terpene compounds. The artemisinin component (currently the most potent antimalarial drug), is a terpene compound isolated from the Artemisia annua plant. The active compounds in keluwih leaves are thought to play an important role as antimalarials (Priangga et al, 2013). Then, the triterpenoid bioactive compounds extracted from the plant Diospyros rubra Lec. can function as antimalarials. Triterpenoid compounds extracted from the leaves of the Erythrina variegata plant are known to have antimalarial activity. The hexane extract of the leaves of the Azadirachta indica A. Juss plant, was able to inhibit the growth of the Plasmodium berghei parasite by 78.35%. It turns out that these fractions contain triterpenoid, steroid and phenolic compounds which are effective as antimalarials (Sucilestari et al, 2013). According to Pratiwi (2007), an extract is said to have positive potential antimalarial activity if it can reduce parasitemia by 30% or more. Based on the research results obtained, winged bean leaf infusion is an extract that has potential as an anti-malarial because it has the ability to inhibit Plasmodium berghei parasites greater than 30%, namely above 80%. Therefore, the effect of reducing parasitemia from winged bean leaf extract is thought to be the result of the activity of flavonoid compounds or other compounds contained in winged bean.

CONCLUSION

Based on the results of the study it can be concluded that winged bean leaf infusion (IDK) was able to suppress parasitemia growth by 93.69% and an average growth rate of 0.042%, with an effective concentration of 31.25 mg/mL. Therefore, winged bean leaf infusion (IDK) has the potential to be developed as an alternative raw material for antimalarial drugs.

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