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Research article

Antimalarial activities extract of N-hexane, ethyl acetate and ethanol of soursop leaf (*Annona muricata* L) on mice (*Mus musculus*) infected with *Plasmodium berghei*

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Key words: Antimalarial, Soursop Leaf (*Annona muricata* L), Percentage degree of parasitemia.

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Abstract

Objective: The purpose of this study was to determine the ability of soursop leafs extract to inhibit the growth of the *Plasmodium berghei* parasite that causes malaria on mice. **Method:** Soursop leafs were extracted with multilevel maceration using n-hexane, ethyl acetate and ethanol. The extracts obtained were then tested for antimalarial activity in vivo by measuring the number of parasitemia through thin blood smear of mice infected with *Plasmodium berghei*. Antimalarial activities were divided into five treatment groups, namely CMC-Na 1%, chloroquine 10 mg/kg bw, n-hexane, ethyl acetate and ethanol soursop leaf extract at a dose of 150 mg/kg bw orally for 5 days and followed by measurement of percent parasitemia. The test data were analyzed statistically using ANOVA, followed by the Post Hoc Tuckey test with the SPSS program. **Result:** The results of testing the antimalarial activity extract of n-hexane, ethyl acetate and ethanol showed a decrease in parasitemia and a decrease in the smallest parasitemia was ethyl acetate extract by 0.12%, this number was lower than the administration of chloroquine as a positive control of 0.26%. The results of parasitic growth inhibition showed that ethanol extract had a percentage of parasite inhibition of 92.15%, the smallest compared to n-hexane extract of 93.51% and ethyl acetate extract of 99.31%. **Conclusion:** Extract of n-hexane, ethyl acetate and ethanol of soursop leaf (*Annona muricata* L) showed antimalarial activities.

Introduction

Malaria is a tropical parasitic disease that is still a major health problem in the world. The main problem in handling malaria is caused by the increased resistance of *Plasmodium* to malaria drugs that have been use so far and the resistance of vector spreaders to insecticides which has revived malaria outbreaks in some endemic areas. It is estimated that 41% of the world's population lives in areas at high risk of being infected with malaria, especially in tropical and subtropical countries. The incidence of malaria is 350-500 million cases every year, with deaths of more than 1.1 million, the majority of deaths occur in pregnant women and children less than 5 years old. Malaria is the number 4 cause of death in the world after respiratory infections, HIV/AIDS and diarrhea. In Indonesia there are 15 million malaria cases each year and 30,000 of them die. Areas with high clinical cases were reported from eastern Indonesia such as Papua, East Nusa Tenggara, West Papua, Central Sulawesi and Maluku [1].

One of the herbs that is efficacious as an antimalarial is soursop leafs. Soursop leafs contain flavonoids acetogenin. Acetogenin is a flavonoid compound isolated from soursop leafs, which can inhibit the growth of infected parasites into mice. Flavonoids have a mechanism of action with two main targets, namely the membrane formed by the intraeritrocytic malaria parasite, the new permeation pathway by inhibiting the required nutritional transport of parasites and other mechanisms of food vakoula malaria parasites by inhibiting hemoglobin degradation and heme detoxification [2].

Based on previous research, that soursop leaf ethanol extract 150 mg/kg bw per day inoculated with *Plasmodium berghei* significantly increased the level of Interleukin 10 from lymph cells. In addition, the results of the Oreagba study, *et al.* (2013) showed antimalarial activity on mice given ethanol extract of soursop leafs and soursop fruit [3]. Ethanol extract of soursop leafs at a dose of 150 mg/kg bw showed higher inhibitory activity of parasitemia than soursop fruit extract (20 mg/ml).It is necessary to develop research on n-hexane, ethyl acetate and soursop leaf ethanol extracts along with phagocytosis activity on mice infected with *Plasmodium bergei.*

Materials and methods

Preparation of soursop leaf extract

Soursop leafs taken from fresh leafs number four and five from shoots, which were taken at Sei Rotan, Deli Serdang

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Districts, Sumatera Utara. Plant identification was carried out at the Indonesian Institute of Sciences, authentication number No: 038/IPH.1.01/IF.07/1/2018. The leafs are washed, dried, mashed to become simplicia powder and extracted by gradual maceration with n-hexane, ethyl acetate and ethanol. Each extract (n-hexane, ethyl acetate and ethanol) was weighed 150 mg, and then put in mortar, and Na-CMC suspension was added and then crushed evenly. The suspension of soursop leaf ethanol extract was put into a 10 ml volumetric flask, then Na-CMC suspension was added to the flask until the volume limit was reached to obtain a concentration of 15mg/ml.

Phytochemical screening of extract of n-hexane, ethyl acetate and ethanol oursop leaf (*Annona muricata* L)

Phytochemical screening extract of n-hexane, ethyl Acetate and ethanol soursop leaf (*Annona muricata* L) includes examining the chemical secondary metabolites of alkaloids, flavonoids, glycosides, tannins, saponins, triterpenoids, and steroids [4-6].

Animals

Experimental animals used by male mice weighing 20-30 grams were obtained from the Pharmacology Laboratory of the Faculty of Pharmacy, University of Sumatera Utara. Mice were fed standard food and drink sufficiently. The animal trial procedure was a Recommendation Approval of Health Research Ethics, faculty of Mathematics and Natural Sciences, University of Sumatera Utara.

Antimalarial activities

Antimalarial activities were divided into five treatment groups, namely CMC-Na 1% as negative control, chloroquine 10 mg/kg bw as positive control, n-hexane, ethyl acetate and ethanol soursop leaf extract at a dose of 150 mg/kg bw orally for 5 days and followed by measurement of percent parasitemia. The test data were analyzed statistically using ANOVA, followed by the Post Hoc Tuckey test with the SPSS program.

Measurement the number of parasitemia

Plasmodium used was *Plasmodium berghei*, Method in Malariae Research, namely the technique of measuring the degree of parasitemia by initially making thin blood smear with Giemsa staining [7].

$$\text{Percent degree of parasitemia} = \frac{\text{The number of erythrocytes was infected}}{\text{1000 erythrocytes}} \times 100\%$$

Percentage of inhibition = (Xk-Xu/Xk) x 100 %

Explanation:
Xu = % growth in the test solution
Xk = % growth in negative controls

Results and discussion

The plants used were soursop leaves which have been identified as "Herbarium Bogoriense" in the field of Botanical Biology Research Center, Bogor Indonesian Institute of Sciences, with another name (*Annona muricata* L), Annonaceae tribe. The results of phytochemical screening on simplicia and extract of soursop leaf can be seen in table 1.

Measurement the number of parasitemia

The test results on the calculation of the number of parasitemia in 1000 erythrocyte cells can be seen in table 2.

Table 2 showed that extract of n-hexane, ethyl acetate and soursop leaf ethanol (dose 150 mg / kb bb) starting day 1 to day 5 experienced a decrease in percentage of parasitemia. This was directly proportional to the administration of chloroquine and inversely proportional to the treatment of CMC-Na.

Based on the percentage results of parasitemia, the percentage value of the inhibition of *Plasmodium berghei* was obtained in each treatment. The results of the calculation of the average value of the percentage of inhibitory power on each treatment can be seen in Table 3.

Table 1. Results of phytochemical screening on simplicia and extract of soursop leaf.

Sr. No.	Secondary Metabolite	Results			
		Simplicia	N-hexane extract	Ethyl acetate extract	Ethanol extract
1	Saponin	+	-	-	+
2	Tannin	+	-	+	+
3	Flavonoids	+	-	+	+
4	Triterpenoid / Steroids	+	+	+	+
5	Glycosides	+	-	+	+
6	Alkaloids	+	-	-	+

(+) = Contains a class of compounds
(-) = Did not contain a class of compounds

Table 2. Percentage of parasitemia of soursop leaf extract.

Sr. No.	Treatment	% Parasitemia				
		Day 1	Day 2	Day 3	Day 4	Day 5
1	n-hexane extract	21.06	15.82	7.80	2.94	1.14
2	Ethyl Acetate extract	16.08	8.76	1.38	0.54	0.12
3	Ethanol extract	13.74	10.34	5.22	3.08	1.38
4	Positive Control	16.42	11.20	2.38	0.98	0.26
5	Negative Control	10.02	11.17	13.85	15.04	17.58

Table 3. Percentage of the inhibitory power of soursop leaf extract.

Sr. No.	Treatment	% Inhibitory				
		Day 1	Day 2	Day 3	Day 4	Day 5
1.	n-hexane extract	-110.1	-41.63	43.68	80.45	93.51
2.	Ethyl Acetate extract	-60.47	21.57	90.03	96.41	99.31
3.	Ethanol extract	-37.12	-7.43	62.31	79.52	92.15
4.	Positive Control	-63.87	-0.27	82.81	93.48	98.52
5.	Negative Control	10.02	11.17	13.85	15.04	17.58

Table 3 showed that the highest percentage of inhibitory power on day 5 was ethyl acetate extract at a dose of 150 mg / kg bw at 99.31%. This value was above the value of the positive control inhibitory power given chloroquine suspension 10 mg / kg bw at 98.52%.

Based on the percentage of inhibitory power the growth of parasites obtained 92.15% - 99.31% (Table 3) it can be concluded that the soursop leaf extract has very good potential in inhibiting parasite growth. Pouplin *et al.* (2007) say an extract was said to have anti*Plasmodium* properties if it can provide parasitic inhibition of more than 30% [8].

Inhibition of *Plasmodium berghei* growth in this study was suspected because the compounds contained in soursop leaf contain flavonoid compounds. Flavonoid compounds have a mechanism of action with two main targets, namely the membrane formed by the intraeritrocytic malaria parasite namely the new permeation pathway by inhibiting the required nutrient transport of the parasite and other mechanisms of food vakoula malaria parasites namely by inhibiting hemoglobin degradation and detoxification of heme [9]. In addition, in a manner in vitro of flavonoid compounds have been shown to be a strong inhibitor of lipid peroxidation, capturing oxygen or nitrogen compounds, inhibiting damage to hem of protein and binding of metal ions [10].

Conclusion

Extract of n-hexane, ethyl acetate and ethanol of soursop leaf (*Annona muricata* L) showed antimalarial activities. Ethyl acetate extract was the highest potential as an antimalarial activity.

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