Effect of saponin-Pods Extract Acacia (*Acacia mangium*) to Hematocrit, Hemoglobin at Tilapia (*Oreochromis niloticus*)

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Abstract—Problem of aquaculture shrimp, one of which is wild fish, competitor. Usually to eliminate the use of saponin from tea seed. saponin to kill fish competitors, (tilapia) are entered when filling water. Saponin to kill fish competitors, (tilapia) are entered when filling water. Saponin used from tea seed meal is imported. Indonesia is rich in biological resources of plants / substances that secondary metabolites naturally untapped optimal. The study was conducted in a laboratory scale with a completely randomized factorial design, using a series of six concentrations and three replicates for each species. LC50 analysis using probit analysis. Unfortunately, there was no scientific information for it. It has been done a laboratory work to study chemical compounds of pod Acacia (Acacia mangium) by phytochemical screening and measure those quantitatively. The result of these were that pod Acacia (Acacia mangium) contain alkaloid, flavonoid, tanin, saponin, steroid, and terpenoid. The results of this study indicate LC50-96h values for Pod Acacia mangium at salinity 0, 5, 10 and 15 promil is 5.487 ppm, 4.313 ppm, 3.985 ppm and 2.944. Value hemoglobin at 0promil at conentration saponin-pod Acacia 0ppm, 5ppm, 10ppm dan 15ppm consecutive is 11.353±0.63)g/dL,10.607±0.18g/dL, 9.860±0.36 g/dL and 8.80±0.60. Value hemoglobin at 5promil at conentration saponin-pod Acacia 0ppm, 5ppm, 10ppm dan 15ppm consecutive is 8.86±1.02 g/dL,7.76±0.943 g/dL, 7.42±0.71 g/dL and 4.68±0.53g/dL. Value hemoglobin at 10 promil at conentration saponin-pod Acacia 0ppm, 5ppm, 10ppm dan 15ppm consecutive is 7.34±1.95 g/dL,6.07±0.755 g/dL, 5.23±1.34 g/dL and 3.23±035 g/dL. Value hemoglobin at 15promil at conentration saponin-pod Acacia 0ppm, 5ppm, 10ppm dan 15ppm consecutive is 8.570±0.478 g/dL,5.18±0.876 g/dL, 3.42±0386 g/dL and 3.817±0.355g/dL. Value hematocrit at 0promil at conentration saponin-pod Acacia 0ppm, 5ppm, 10ppm dan 15ppm consecutive is 35.033±3.32g/dL, 31.2±0.90 g/dL, 29.367±0.97 g/dL and26.60±1.345. Value hematocrit at 5promil at conentration saponin-pod Acacia 0ppm, 5ppm, 10ppm dan 15ppm consecutive is 25.633±1.24 g/dL, 23.90±2.68 g/dL, 18.367±2.554 g/dL and 12.867±2.38 g/dL. Value hematocrit at 10 promil at conentration saponin-pod Acacia 0ppm, 5ppm, 10ppm dan 15ppm consecutive is 21.567±6.134 g/dL,18.567±1.48 g/dL,14.767±3.46 g/dL and 10.53±3.38 g/dL. Value hematocrit at 15promil at conentration saponin-pod Acacia 0ppm, 5ppm, 10ppm dan 15ppm consecutive is 26.100±1.42 g/dL, 15.667±2.79 g/dL,9.933± 2.04 g/dL and 11.933±1.19 g/dL. Saponin of acacia may lower hematocrit and hemoglobin an indication of damage to the red blood cells occurs hemolysis

Keywords—pod Acacia mangiuim, saponin, hematocrit, hemoglobin

INTRODUCTION

Problem of aquaculture shrimp, one of which is wild fish, competitor. Usually to eliminate the use of saponin from tea seed. Saponin to kill fish competitors, (tilapia) are entered when filling water. Saponin used from tea seed meal is imported. Indonesia is rich in biological resources of plants / substances that secondary metabolites naturally untapped optimal.

Saponin effects reported very toxic to fish for damaging the respiratory epithelium [1]. The fish will also provide stress response to exposure saponin in water [2]. Hematocrit is the percentage of red cells in the blood volume of fish. The results of the examination of hematocrit can be used as a benchmark to determine the state of health of the fish, hematocrit values less than 22% indicates the occurrence of anemia. Changes in environmental conditions or environmental pollution will cause hematocrit values decreased as a result of the stress response in fish [3].

The classical definition of saponins of saponins is based on their surface activity and many saponin have es, give stable foams in water, show hmolytic activity have beter taste [4]. saponin is one class of compounds glycosides, steroids and their structure and specificity triterpenoid, its has colloidal solutions form in water and foamy like soap, if mixture was shaken [5].

Biological effects of saponins are erythrocyte hemolysis, the effect of red blood cells and the level of cholesterol in the liver, the effects on growth, the effect of bloating in ruminants, penghambataan activity of smooth muscle, inhibits the enzyme, the effect on nutrient absorption [6].

Blood is composed of two main components, namely blood cells and blood plasma. The composition contained in the blood is water. One cubic millimeter of blood contains about 5 million fish red corpuscle called leukocytes and 200,000 to 300,000 platelets called thrombocytes. Another component is the mineral salts and dissolved organic substances [7].

MATERIAL AND METHODS

a. Collection and identification of plant materials

Acacia mangium were collected only pod the plant, from arif rachman hakim street -Surabaya, Growth centre location. The pod of Acacia mangium were air-dried and choise only pod without seed and partikel of dust and ground into uniform powder using milling machine.

b. Phytochemical screening

Chemical test were carried out on the etanol extract and on the powdered specimens using standard procedures to identify the constituents as described by [8]. 0.5 g of the dried powdered samples was boiled in 20 ml of water in test tube and thenfiltered. A few drops of 0.1% ferric chloride was added and observed for browrish green or a blue-black colouration.

Test for saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorousley for stable persistent froth. The frothingwas mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emultion.

Test for flavonoids: Thee methodes were used to determine the presence eat for flavonoids: Three methods were used to determine the presence of flavonoids in the plant sample [9,8]. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H2S04. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 of ethyl over a steam

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bath for 3 min. was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for steriods: Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H2S04. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids (Salkowski test): Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 m) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids. Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Saponin determination:

The method used was that of [10]. The samples were ground and 20 g of each were put into a conical flask and 100 cm3 of 20% aqueous ethanol were a were heated over a hot water bath for 4 h with continous strirring at about 55oC. The mixture was filtered and the residu re-extract ed with another 200 ml 20% etanol. The combined extracts were reduced to 40 bout 90oC. The concentrate was tran sferred into a 250 ml separatory funnel and 20mdiethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether la was discarded. The purification process was repeated.

60 ml of n-butanol was added. The combined n-butanol extracts werewashed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation the samples were dried in the oven to a constant weight; the saponin contens was calculated as percentage.

c. Method of *mikrohematokrit*

In the method of micro blood sample (capillary blood, EDTA blood, heparin blood or blood-potassium-ammonium oxalate) is inserted in the capillary tube which has a length of 75 mm with a diameter of 1 mm. Capillary tubes are used there are 2 kinds, ie containing heparin (marked red) for capillary blood sample (direct), and without anticoagulant (marked blue) for blood EDTA / heparin / ammonium-potassium-oxalate [11].

RESULT AND DISCUSION

The present study carried out on the plant samples revealed the presence of medicinally active constituent

Qualitative analysis some Phytochemican frPods of Acacia phytokimia screening results, positive contain alkaloids, flavonoids, tannins, saponins, steroids, triterpenes (Preliminary Test). Determination of saponin on pod Acacia mangium (procentage crude) is 3.32%.

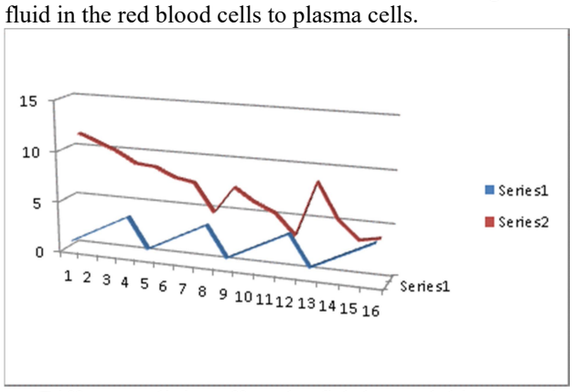
Homogeneity and normality test result that the data hemoglobin and hematocrit, including homogeneous and normality.

Salinity increased to trent the hemoglobin tilapia decreased. Also in hematokrit too. hemoglobin decreased due to a red blood cell hemolysis. Hemoglobin values decreased with increasing salinity. This is caused by damage to red blood cells, or hemolysis. This occurs in hematocrit values were also down. Hematocrit values were down for grains through lysis of red blood cells. In the centrifuges is not obtained real separation between the red blood cells to plasma. Plasma tend to be colored red due to the mixing of the fluid in the red blood cells to plasma cells.

Table 1. Effect Salinity and saponin dose on hemoglobin and hematocrit values in blood tilapia (*Oreochromis niloticus).*

Salinity	dose saponin (ppm)	Hemoglobin ± SD	Hematokrit ± SD
0‰	0	11.353±0.63129c,n	35.033±3.32466c,z
	5	10.607±0.18475c,m	31.200±0.90000c,y
	10	9.860±0.36166c,l	29.367±0.97125c,x
	15	8.800±0.60000c,k	26.600±1.34536c,x
5‰	0	8.630±1.02235b,n	25.633±1.24231b,z
	5	7.760±0.94319b,m	23.900±2.68514b,y
	10	7.420±0.70873b,l	18.367±2.55408b,x
	15	4.683±0.52937b,k	12.867±2.38607b,x
10‰	0	7.343±1.95132a,n	21.567±6.13379a,z
	5	6.073±0.75507a,m	18.567±1.48436a,y
	10	5.229±1.34263a,l	14.767±3.45881a,x
	15	3.230±0.03464a,k	10.533±3.38280a,x
15‰	0	8.570±0.47823a,n	26.100±1.41774a,z
	5	5.183±0.87649a,m	15.667±2.79702a,y
	10	3.420±0.38626a,l	9.933±2.04042a,x
	15	3.817±0.35473a,k	11.933±1.19304a,x

Hemoglobin values decreased with increasing salinity. This is caused by damage to red blood cells, or hemolysis. This occurs in hematocrit values were also down. Hematocrit values were down for grains through lysis of red blood cells. In the centrifuges is not obtained real separation between the red blood cells to plasma. Plasma tend to be colored red due to the mixing of the fluid in the red blood cells to plasma cells.



Different salinity affect the fish hemoglobin, saponin concentration in fish media influence on the content of hemoglobin. At salinity higher hemoglobin level lowers the fish.

Hemoglobin

			Subset			
	Kons_saponin	N	1	2	3	4
Tukey HSD[a]	4	12	5.1325			
	3	12		6.4823		
	2	12			7.4058	
	1	12				8.9742
	Sig.		1.000	1.000	1.000	1.000
Duncan[a]	4	12	5.1325			
	3	12		6.4823		
	2	12			7.4058	
	1	12				8.9742
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .693.
a. Uses Harmonic Mean Sample Size = 12.000.

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