

## **The effect of ethanol extract *Coleus ambonicus* L on antibody titer of white rats (*Rattus norvegicus*) trough SRBC as antigen**

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**Abstract.** This study aimed to determine the effect of ethanol extract of leaves bangunbangun (*Coleus ambonicus* L) antibody titer as humoral immunity and body weight in Wistar strain rats by using the sheep red blood cells (SRBC) as antigen. This study used 24 male white rats were randomly divided into four treatment groups consisting of six rats. The first group was given distilled water as a control, the second group was given 500 g / kg ethanol extract bangunbangun (EEC), the third group was given 500 g / kg EEC SRBC, and the fourth group was given distilled water+SRBC. EEC was administered orally every day for thirty days. SRBC administered intraperitoneally of 0.1 ml on day eight and day fifteenth. On day 31, blood was collected by decapitation, and collected in a tube that has been coated with an anticoagulant, and centrifuged to obtain serum. Antibody titers were measured by haemagglutination method. Weight gain is obtained by calculating the difference in initial weight and final weight of research. The data obtained were tabulated and analyzed by ANOVA and LSD test. Results of data analysis showed that administration of EC for 30 days in rats can increase the antibody titer significantly, and administration of EC for 30 days in rats didnt influence the gain of body weight.

**Keywords:** *Coleus ambonicus* L, antibody titer, SRBC

### **Introduction**

Immunostimulant are substances that can increase the body's resistance to infections . The content of the bangunbangun leaves that serve as immunostimulant namely vitamin C ( Santosa and Hertiana, 2005), flavonoid ( Suhirman and Christina, 2000) in particular classes of flavonols (Purwantari et al, 2011). Benefits wake - up leaves as immunostimulatory will be assessed by measuring immunoglobulin that acts as an antibody to kill the antigen enters the body. In bangunbangun leaves , there are also vitamin C , vitamin B1, vitamin B12 , beta carotene, niacin , carvacrol , calcium , fatty acids , oxalic acid, and fiber. The compounds have the potential for a variety of biological activities , including functioning as immunostimulan (Santosa and Hertiana, 2005). As immunostimulatory Sunita et al ( 2010) have reported the results of his research. Bangunbangun leaves or Torbangun (*Coleus amboinicus* Lour ), is known to have many benefits, as an antipyretic, analgesic, wound medicine, cough medicine, and thrush (Depkes RI, 2005), antioxidant, antitumor, anticancer, and hypotensive (Duke, 2000). Usually drugs that have multifunction have receptors on the target organ limforetikular systems that perform immune functions . Immunomodulator is a way to restore and repair the compromised immune system and suppress excessive immune function ( Bellanti and Kadlec 1993). Bio- active substances suspected bangunbangun leaves immunostimulan are flavonoids, steroids, and polyphenols. Flavonoids are antioxidants and prevent the oxidation of low density lipoprotein ( LDL ) and lowers the risk of atherosclerosis. Antioxidants

contained in food can stimulate cellular immunity and help prevent oxidative damage to cellular components. Various vitamins contained in the bangunbangun such as vitamin C, B12, and beta carotene can function in enhancing immunity in the body.

Of some of the results of research and opinion on the research conducted on the effects of administration of immunoglobulin response leaves immunostimulan bangunbangun as the test animals. As antigen in this study are given SRBC (Sheep Red Blood Cells)

### **Materials and Methods**

This research was conducted over three months in the Laboratory of Biology, State University of Medan as the maintenance and treatment of experimental animals, Pharmacy and Laboratory Medicine, North Sumatra University as a place making Bangunbangun leaf extract and antibody titer analysis. Animals used in this study were male white rats (*Rattus norvegicus*) three-month-old Wistar strain weighing 140-180 g. Rats were obtained from the laboratory of faculty of Pharmacy, University of North Sumatra and bred in the laboratory animal cages Laboratory of Biology Department, State University of Medan. Commercial rat pellet feed derived from farm store. Bangunbangun leaves (*Coleus ambonicus* L.) in planting themselves in the land around the animal enclosures. Sheep red blood cells (SRBC) as antigen obtained from the Veterinary Laboratories Medan, North Sumatra. ethanol 96%, K<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, NaCl, distilled water to make PBS

### **Animal acclimation**

This study used 24 male rats (*Rattus norvegicus*) three-month-old Wistar strain weighing 140-180 g. Rats were placed in plastic cages measuring 40 x 25 x 20 cm, at the top of the cage is equipped with a wire cover. Each cage is filled with chaff as the base and then placed three rats per cage. Food and beverages provided ad libitum, acclimation is done for 7 days.

### **Preparation of Ethanol Extract Bangunbangun Leaf**

A total of 8 kg of fresh leaves are dried using an oven with a temperature of 400 to be completely dried or brittle like crackers. 500 g of dried leaves in a blender until pulverized powder form. The powder was placed in 2 containers each 250 grams of 95% ethanol and added that already distilled some 2000 ml / container. Leaves soaked and stirred for 5 days once a day. Leaves filtered using filter paper and added up to 3 liters of ethanol, allowed to stand for 2 days and again filtered. Extract obtained was concentrated by using a rotary evaporator and dried using a water bath to get dried ethanol extract.

### **Preparation of Sheep Red Blood Cells (SRBC)**

Preparation of SRBC is done at the Department of Animal Husbandry Medan . Sheep blood was taken and added to 5ml as much as 5 ml alcever with a ratio of 1:1 , alceverfunction as receptors , that bind with other cells and preservatives . Blood was washed with a solution with a pH of 7.4 koloner piluent then centrifuged at a speed of 200 rpm selma 15 minutes . Results of centrifuges in the exhaust section and filled it again with koloner piluent to 40 ml , then centrifuged again at 200 rpm and the speed for 15 minutes . Washing with the centrifuge process is done 3 times . Results of centrifuges in store in the refrigerator - 4°C.

### **Determination of Dose Treatment Ethanol Extract Bangunbangun ( EEB) on Rats**

Dose of EEB to rats at 500 mg / kg ( Jose et al. , 2005 , Patel, 2011 ). EEB was dissolved in 1 % CMC ( Patel , 2011) . Made solution is a 10% solution. EEB was given orally every day in for 30 days

### **Research Design**

This study is experimental , in its design used Completely Randomized Design (CRD) with four treatment were each given six replications . This study used 24 white rats (*Rattus norvegicus*) aged 3 months with an average weight of 150-200 grams and adapted for 7 days . Subsequently rats were divided into 4 groups , each consisting of 6 rats . Each group nurtured and fed and watered *ad libitum* . Future maintenance and provision of treatment carried out for 30 days . Group I (C) as a control were given distilled water orally every day . Group II (EEB) were given 500 mg EEB / kg without SRBC , groups III were given 500 mg (EEB + SRBC), groups IV were given 0.1 ml SRBC. SRBC was given on day 8 and 15 treatments.

### **Humoral Antibody Titer Determination**

On day - 31 treatment , blood was collected via neck decapitation, housed in a tube that has been coated with EDTA as anticoagulant , then centrifuge for 10 minutes at 6000 rpm. Serum was transferred into a serum tube that has been labeled according to treatment. After the serum was collected dekomplementasi the heat tubes containing serum over a waterbath at a temperature of 56°C for 30 minutes . It aims to prevent lysis of the SRBC would obscure hemagglutination, further dilution storied mikroplate. In the 2nd column mikroplate added 50 mL PBS until the 12th column , while the first column is left blank. 100 mL serum that had been inserted into the first column didekomplementasikan . Further take 50 mL serum from the first column to the second column and then enter into the third column and homogenkan then onwards until the twelfth column . After dilution to 50 mL final series exhaust last serum dilution . Thus obtained 12 serum dilution series with

multiples of two: 1/1 , 1/2 , 1/4 , 1/8 , 1/16 , 1/32 , 1/64 , 1/128 , 1/256 , 1/1024 , and 1 / in 2048 . Antibody titer reading of the reaction products is done by direct observation with the eye by measuring the antibody concentration by titration multilevel retailing and arithmetically converted into the formula  $2\log (\text{titer}) + 1$  ( Farhath S , Vijaya and Vimal , 2013)

### The Rats Weight Measurements

Measurement of rats body weight done each day before feeding with Tanika scales . Weight reported in this study was change in weight from the beginning to the end of the study .

### Results and Discussion

#### Effect of Ethanol Extract Bangunbangun leaves (*Coleus amboinicus* Lour) Against Rats Humoral Antibody Titer

Table 1. The average antibody titer and change body weight of rats

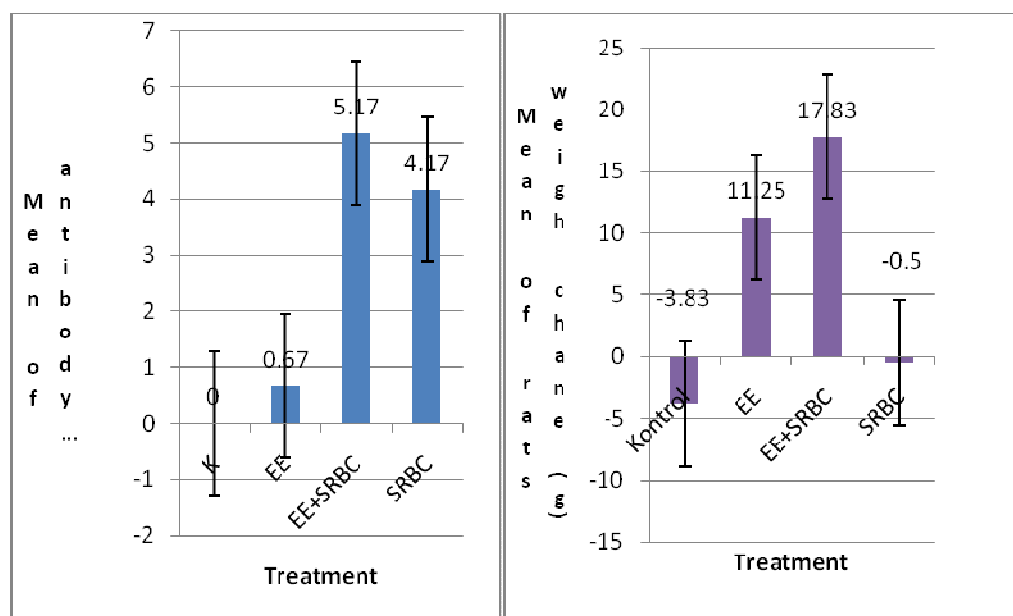
No	Treatment	Parameters	
		Antibody Titers	Weight difference (g)
1	Control	0	-3,83±18.03
2	EEB	0,67	11,25±34.66
3	EEB+SRBC	5,16**	17,83±20.80
4	SRBC	4,16*	-0,5±16.10

Antibody humoral titers (Table 1) was higher in rats fed ethanol extract leaf bangunbangun + SRBC. Whereas in rats that were not given the extract antibodies did not even formed. EE showed imunostimulannya activity with the formation of antibodies in the body of rats. The immunostimulatory activity formed more if granting EE bangunbangun leaves with SRBC as antigen delivery that did "challenged". Antibody titers in rats treated SRBC + EE significantly different (Table 2)

Table 2. List of variance analysis of rat antibody titers

The diversity of sources	Db	JK	KT	F hitung	F tabel	
					0,05	0,01
Treatment	3	1452	484	29,69**	3,10	4,94
Error	20	326	16,3			
Total	23	1778				

Increased antibody titers in mice the effect of leaf immunostilulan wake - up given to the mice . Flavonoids contained in the leaves and proferasi bangunbangun involved in B cell differentiation into plasma cells that are producing antibodies. One of the contents of the wake - up is particularly flavonoids flavonols . Flavonols are the highest contents of flavonoids. Effects of flavonoids on immune system is very complex and the real role as immunostimulan (Carlo et al. 1999). Flavonols immunostimulan potential as being able to increase the production of IL2 (interleukin) is involved in the activation and proliferation of T cells (Dewi et al , 2003). Immunodulator effects observed in the wake of the serial dilution agglutination in serum (containing antibodies) SRBC was added back in microtiter wells . Antibody titers in this study is higher than antibody titers on research conducted by Koul and Khosa (2013). The researchers reported with administration of 30 mg/kg BW ethanol *Melissa officinalis* extract in mice formed antibodies titer 2:49 ± 0:21. Body weight of rats during the study with no significant change. Mice treated SRBC EE and EE + a growing body of research from day 1 to day 31. While in the control treatment and were given SRBC alone lost weight at the end of the study.



**A**

**B**

Figure 1. Mean antibody titer mice (A), and Mean weight change (B) from day 1 to day 30 study. (C = control; EE = ethanol extract of Bangunbangun ; SRBC = sheep red blood cells)

### Conclusion

From these results it can be concluded that (1) . Ethanol extract of Bangunbangun leaf significantly increased antibody titers in rats, (2) Ethanol extract of Bangunbangun leaf did not significantly affect the increase in body weight of rats.

### Suggestion

- 1 . Further tests need to be conducted to determine the response of immunoglobulin G and immunoglobulin M as the impact of giving Ethanol leaf bangunbangun extract in rats
- 2 . Study of other parameters such as Lysozim , Leukocyte cell differentiation and other immunostimulatory parameters also need to be done

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