

Potential Test of Jungle Water Guava (*Zyzigium jambos* (L.) Alston) Timor as Immunomodulator against Antibody Response of Broiler Chicken (*Gallus domesticus*) In Vivo

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ABSTRAK

Penelitian ini bertujuan untuk mengetahui potensi ekstrak *Syzigium jambos* (L.) Alston) Timor sebagai imunomodulator terhadap respon antibodi ayam broiler (*Gallus domesticus*) secara in vivo. Untuk mengetahui perbedaan imunomodulasi antara tingkat konsentrasi ekstrak *Syzigium jambos* (L.) Alston) ekstrak Timor terhadap respon antibodi ayam broiler (*Gallus domesticus*) secara in vivo. Untuk mengetahui apakah ada perbedaan kemampuan imunomodulasi ekstrak *Syzigium jambos* (L.) Alston) ekstrak Timor antara kelompok kontrol negatif, kelompok eksperimen dan kelompok kontrol positif. Metode penelitian menggunakan rancangan penelitian dengan tiga unit perlakuan yaitu kontrol negatif, perlakuan ekstrak dan kontrol positif mengikuti rancangan pre-test post-test control group design. Data dianalisis secara deskriptif dan inferensial. Kesimpulan dari penelitian ini adalah 1) Ekstrak kulit kayu jambu air hutan Timor terbukti berpotensi sebagai imunomodulator terhadap respon antibodi ayam broiler (*Gallus domesticus*) secara in vivo. 2) Terdapat perbedaan imunomodulasi antar tingkat konsentrasi ekstrak kulit batang jambu air hutan Timor terhadap respon antibodi ayam broiler (*Gallus domesticus*) secara in vivo, terutama pada konsentrasi 25% dengan rata-rata titer antibodi 853,3 sel/ μ l darah, sedangkan pada konsentrasi 50% dan 75% tidak terdapat perbedaan dengan rata-rata titer antibodi 1024 sel/ μ l darah. 3) Terdapat perbedaan kemampuan imunomodulasi antara ekstrak kulit batang jambu air hutan (*Zyzigium jambos* (L.) Alston) kelompok Timor dengan kelompok kontrol negatif, namun "tidak ada" perbedaan dengan kelompok kontrol positif.

Kata Kunci : *Zyzigium jambos* (L.) Alston Timor, ekstrak, imunomodulator, ayam pedaging

ABSTRACT

The study aims to determine the potential of *Syzigium jambos* (L.) Alston) Timor extract as an immunomodulator against the antibody response of broiler chickens (*Gallus domesticus*) in vivo. To determine what is the difference in immunomodulation between concentration levels of *Syzigium jambos* (L.) Alston) Timor extract on the antibody response of broiler chickens (*Gallus domesticus*) in vivo. To determine whether there is a difference in immunomodulating ability of *Syzigium jambos* (L.) Alston) Timor extract between the negative control group, experimental group and positive control group. Methods of the study used a research design with three treatment units, namely negative control, extract treatment and positive control following the pre-test post-test control group design. Data were analyzed descriptively and inferentially. The conclusion of this study is 1) Timor forest water guava bark extract is proven to have the potential as an immunomodulator against the antibody response of broiler chickens (*Gallus domesticus*) in vivo. 2) There are differences in immunomodulation between concentration levels of Timor forest water guava bark extract on the antibody response of broiler chickens (*Gallus domesticus*) in vivo, especially at a concentration of 25% with an average antibody titer of 853.3 cells/ μ l blood, while at concentrations of 50% and 75% there is no difference with an average antibody titer of 1024 cells/ μ l blood. 3) There is a difference in immunomodulation ability between the forest water guava bark extract (*Zyzigium jambos* (L.) Alston) Timor group and the negative control group, but "no" difference with the positive control group.

Keywords : *Zyzigium jambos* (L.) Alston Timor, extract, immunomodulator, Chicken broiler

I. INTRODUCTION

1. Background

Nutritional needs and balanced nutrition are things that must be met in order to maintain a healthy body (Volkert et al., 2022). Healthy and proper nutrition will affect growth, development, provide energy, protect the body from disease and improve brain intelligence (Zavitsanou & Drigas, 2021). Success in the implementation of national health development relies heavily on how we pay attention to nutrition in the family (Black et al., 2017). Nutrition provides a solid foundation to achieve a healthy life, success in education, and a productive life to achieve a better level of welfare (Chappell & Thorpe, 2022). Healthy and proper nutrition should contain carbohydrates, proteins, fats, vitamins, and minerals (TAŞĞIN, 2017). Protein is one of the important nutrients needed by the body (Phillips et al., 2016). Protein according to its source is divided into two, namely animal and vegetable protein (Mariotti, 2019). One of the foodstuffs that contributes greatly to the need for animal protein is chicken meat. Chicken is currently the largest contributor to animal protein from livestock because chicken meat is very easy to obtain in both modern and traditional markets (Milkias, 2016). The increasing demand for chicken commodities and consumers' desire for chicken meat also make chicken livestock have high economic value (Phibbs et al., 2021). In reality, the chicken farming business does not run easily due to various obstacles faced, such as: inadequate cage quality, improper maintenance management, disease outbreaks and so on. Disease outbreaks in chickens can be caused by bacterial and viral infections; such as tetelo (Newcastle Disease), *Gumboro*, *Colibacillosis*, and many other disease (Dey et al., 2019). Access to synthetic drugs and vaccines is difficult and expensive (Waheed et al., 2016). This makes farmers look for other alternatives that are easier and cheaper. Based on empirical facts from the community, especially in Foho Eka Village, Nanaet Dua Besi Subdistrict, Belu Regency, there are several traditional chicken farmers who experienced crop failure because their chickens were attacked by disease outbreaks. There are other facts according to the results of the researcher's interview with one of the chicken farmer who continue to reap the results of his livestock. He testified that his chickens remained healthy despite the disease outbreak in the surrounding area. He gives the mashed bark of the jungle water guava to his chickens and makes a drink for the chickens so that even in the midst of a disease outbreak, his livestock remains healthy. It is proven that he still earns a large profit from the sale of chickens. Against empirical facts, researchers conducted an exploration to examine the possibility of existing research on this matter. The exploration results show that scientific studies on *Zysigium jambos* (L.) Alston, especially those related to efforts to maintain the health of new chicken livestock, are directed at aspects of its ability as an antibacterial as conducted by (Cock & Cheesman, 2019) showing that *S. jambos* (L.) Alston extract is effective in inhibiting the growth of *Streptococcus* mutants bacteria. Another study by (Cock & Cheesman, 2018) that the leaf extract of *S. jambos* (L.) Alston has the ability as bacteriostatic and bactericidal against *Salmonella pullorum*. Research conducted also by (Uddin et al., 2022) showed that the skin extract of *S. jambos* (L.) Alston against *Staphylococcus aureus* and *E. coli is bacteriolytic*. While from empirical facts *S. jambos* (L.) Alston can function as an immunomodulator.

2. Problem Formulations

In this study there are several problem formulations, which are:

1. What is the potential of *Syzigium jambos* (L.) Alston Timor extract as an immunomodulator against antibody response of broiler chickens (*Gallus domesticus*) in vivo?

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2. What is the difference in immunomodulation between the concentration levels of *Syzigium jambos* (L.) Alston Timor extract on the antibody response of broiler chickens (*Gallus domesticus*) in vivo?
3. Whether there is a difference in the immunomodulating ability of *Syzigium jambos* (L.) Alston Timor extract between the negative control group, the experimental group and the positive control group?

3. Research Objective

The study aims to determine the potential of *Syzigium jambos* (L.) Alston Timor extract as an immunomodulator against the antibody response of broiler chickens (*Gallus domesticus*) in vivo. To determine what is the difference in immunomodulation between concentration levels of *Syzigium jambos* (L.) Alston) Timor extract on the antibody response of broiler chickens (*Gallus domesticus*) in vivo. To determine whether there is a difference in immunomodulating ability of *Syzigium jambos* (L.) Alston) Timor extract between the negative control group, experimental group and positive control group.

4. Research Benefits

The benefits of this study are that we can know the potential of *Syzigium jambos* (L.) Alston Timor extract as an immunomodulator against the antibody response of broiler chickens (*Gallus domesticus*) in vivo. we can know the difference in immunomodulation between the concentration levels of *Syzigium jambos* (L.) Alston Timor extract against the antibody response of broiler chickens (*Gallus domesticus*) in vivo. We can determine the difference in immunomodulating ability of *Syzigium jambos* (L.) Alston Timor extract between negative control group, experimental group and positive control group?

II. RESEARCH METHODS

Place and Time

This research was conducted at the Biology Laboratory of Widya Mandira Kupang Catholic University which is located on Jalan Jend. Achmad Yani No. 50-52 Merdeka Kupang-NTT. The research was conducted from February to June 2023.

Research Design or Model

- 1) Preparing *Syzigium jambos* (L.) Alston Extract
- 2) Extraction of *S. jambos* (L.) Alston bark using maceration and evaporation methods. Concentration of *S. jambos* (L.) Alston extract followed the procedure as stated by Zainab, 2013 using the following formula:

$$\% \text{ Concentration} = \frac{m}{v} \times 100\%$$

Figure 1. formula Concentration of *S. jambos* (L.) Alston extract.

- 2). Preparing the experimental animals

The test animals were fifteen broiler chickens that had adapted for 16 days. Chickens then divided into 5 groups, with each group consisting of 3 chickens.

Materials and Equipment

Materials used in the research are: rotary evaporator, erlenmeyer flask, analytical balance, measuring cup, beaker, oven, incubator, analytical balance, bunsen, dropper pipette, measuring pipette, stirring rod, filter paper, chicken cage, label paper, thermostirer, vortex mixer, oven, magnetic stirrer, aluminum foil, falcon bottle, syringe, incubator, centrifuge, eppendorf, micropipette, analytical balance, microplate, mortar and mortal, forest water guava powder, filter paper, alcohol, distilled water, broiler chickens, EDTA, PBS, RBC, WBC, chicken feed, Nd & IBD vaccines.

Research Stages

The data collection process was carried out following the following steps:

- a. Broiler chicken blood collection. Chicken blood was taken twice, namely when the chickens were 16 days old for the pre-test and 41 days old for the post-test. Broiler blood sampling in each chicken as much as 5 cc through the lateral vein using a 5 cc syringe which is inserted into the EDTA tube. The blood was then stored in a sterilized cold box for the centrifugation process.
- b. Centrifugation of broiler blood. The centrifugation process of each group was carried out carefully. Each group was repeated 3 times.

III. RESULT AND DISCUSSION

The data of this study were analyzed qualitatively inferentially and qualitatively descriptive.

1. Quantitative Inferential Analysis.

The data analysis used is analysis of variance (ANOVA) with its stages.

Table 1. *Quantitative Inferential Analysis*

Source of Variation	DB	JK	KT	F count	F table
					0,01
Treatment					
Error					
Total					

Notes: ** = Significantly affected at F (0.01)

If F count > F table, this indicates that the test extract is significantly capable as an immunomodulator against broiler antibody response.

If the results of the analysis of variance indicate the immunomodulating ability of water guava bark extract on the antibody response of broiler chickens significantly, the analysis is continued with the Least Significant Difference (BNT) test with a significant level of 1%.

$$BNT_{\alpha} = t_{(\alpha, v)} \cdot \sqrt{\frac{2(KT \text{ Galat})}{r}}$$

Figure 1. *Least Significant Difference (BNT) test*

2. Qualitative Descriptive Analysis.

Qualitative Descriptive Analysis is done by collecting antibody titer data, calculating the average of each treatment and then based on the average results of each treatment, qualitative descriptive conclusions are made.

- a) That the extract of forest water guava bark (*Syzigium jambos* (L.) Alston) Timor has the potential as an immunomodulator against the antibody response of broiler chickens (*Gallus domesticus*) in vivo.
- b) That there is a difference in the amount of immunomodulation between the concentration levels of the extract of the bark of forest water guava (*Syzigium*

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jambos (L.) Alston) Timor against the antibody response of broiler chickens (*Gallus domesticus*) in vivo.

c) That there is a difference in immunomodulation ability between the extract of water guava bark (*Syzigium jambos* (L.) Alston) Timor with negative control group and positive control group.

1. The potential of *S. jambos* (L.) Alston Timor bark extract as immunomodulator of the broiler immune system in vivo.

The results of research proving the potential of Timor forest water guava extract as an immunomodulator against the antibody response of broiler chickens (*Gallus domesticus*) in vivo refer to the results of the hem agglutination inhibition test as shown in Figure 2.

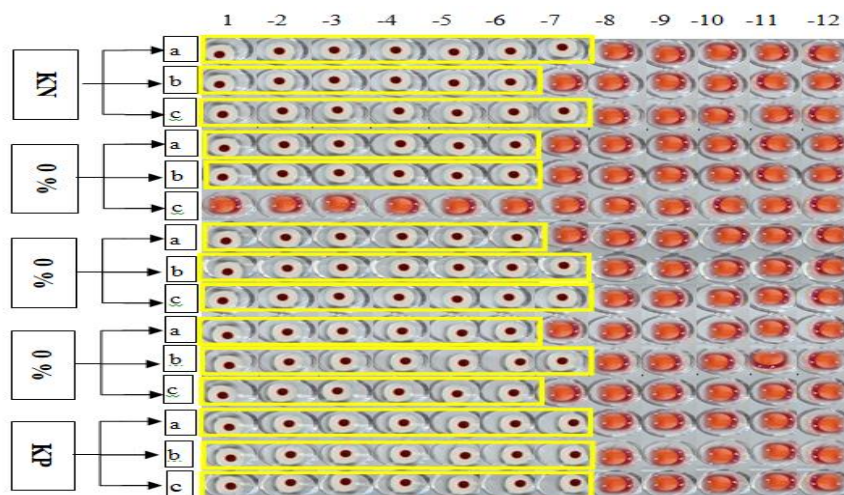


Figure 2. Indicator of broiler antibody titer results before treatment

Table 2. Antibody titer data of broiler chickens before treatment with extract of *Syzigium jambos* (L.) Alston

No	Treatment	Titer Antibody (cells/ μ l of blood)			Total	Average
		Repetition 1	Repetition 2	Repetition 3		
1	0	64	32	64	160	53.3
2	0%	32	32	0	64	21.3
3	0%	32	64	64	160	53.3
4	0%	32	64	32	128	42.7
5	0%	64	64	64	192	64.0
Total					704	234.7

The table above shows that the total antibody titer of broiler chickens before treatment is as follows: one chicken was 0, six chickens were 32 cells/ μ l blood, and eight chickens were 64 cells/ μ l blood Figure 5. Indicator of broiler antibody titer results after treatment

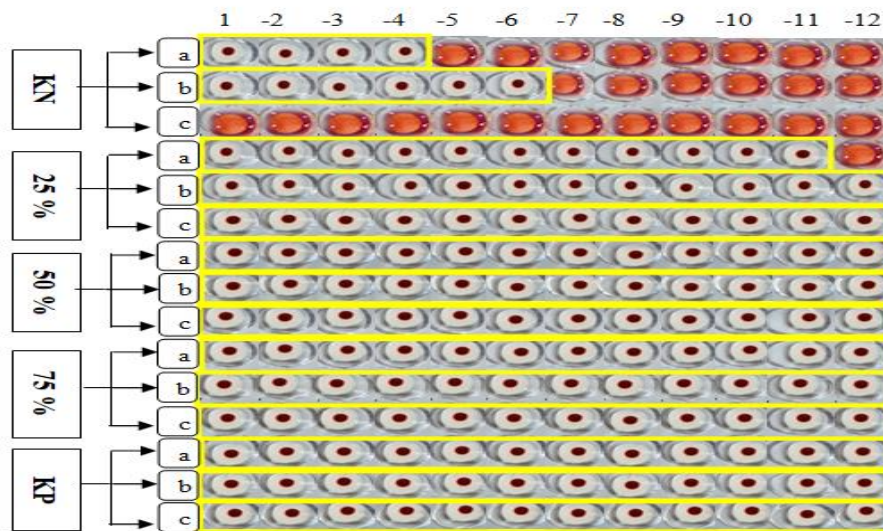


Figure 3. Indicator of broiler antibody titer results after treatment

Figure above is a serological test after being treated, it appears that the antibody titer of broiler chickens ranges from 0-12. Antibody titer results of broiler chickens after treatment with extracts of *S. jambos* (L.) Alston Timor at concentrations of 25%, 50% and 75% as seen on table below. The result shows that the number of antibody titers of broiler chickens after being treated is as follows: in the negative control replicate 1 amounted to 4 cells/ μ l of blood, replicate 2 amounted to 16 cells/ μ l of blood, and replicate 3 amounted to 0. In the treatment of 25% concentration replicate 1 amounted to 512 cells/ μ l of blood, replicate 2 and 3 amounted to 1024 cells/ μ l of blood. In the treatment of 50% concentration is the same as the 75% concentration and positive control which amounted to 1024 cells/ μ l of blood in each blood sample.

IV. KESIMPULAN

Analysis of variance data showed F count 40.230 greater than F table 10.92 means that the extract of the stem bark of water guava (*S. jambos* (L.) Alston) Timor proved to have potential as an immunomodulator indicated by the agglutination of erythrocytes by leukocytes; 2) The results of BNT further analysis at a significant level of 0.01 showed that 25% concentration = 846.6 significantly different from the negative control: 6.7; significantly different from the concentration of 50%, 75% and positive control: 1017.3. The 50% concentration: 1017.3 was significantly different from the negative control: 6.7 and 25% concentration: 846.6; but not significantly different from 75% concentration and positive control. 3) There is a difference in immunomodulating ability between the forest water guava bark extract (*Syzigium jambos* (L.) Alston) Timor group and the negative control group, but there is no difference with the positive.

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