Effect of Sarang Banua (*Clerodendrumfragrans* Vent Willd) Leaves Extract on Serum Globulin Levels of Rabbit (*Oryctolagus cuniculus*)

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**Abstract.** Sarang banua plants are found in Simalungun, North Sumatra, Indonesia, which have been used by the community as traditional medicinal plants and is a type of *Clerodendrumfragrans* Vent Willd, including the family Verbanaceae. The study design was completely randomized design (CRD) with five treatments namely 1% Na-CMC (K1, negative control), bovine serum antigen (K2), ethanol extract of sarang banua leaf 200 mg/kg BW (K3), ethanol extract of sarang banua leaf 200 mg/kg BW with bovine serum antigen (K4) and ethanol extract of sarang banua leaf 150 mg/kg BW with bovine serum antigen (K5). The results showed that administration of ethanol extract of sarang banua leaf (*Clerodendrumfragrans* Vent Willd) significantly affected serum globulin levels of rabbits (*Oryctolagus cuniculus*). The ethanol extract of sarang banua leaves 200 mg/kg BW with bovine serum antigen (K4) produced the highest serum globulin levels of rabbits (12.91 mg/mL).

1. Introduction

Sarang banua is one of traditional medicinal plants found in Raya Usang village, Simalungun district, North Sumatra, Indonesia. This plant is typically shrubs and grows to around 2.5-3.0 m high, it has whitish pink flowers and the flower petals is reddish purple in color and 10-15 mm in length, the leaves are simple and opposite, oval and its length 6-25 cm and 2-25 cm wide and the edges of the leaves are wavy as shown in Figure 1. Based on the herbarium determination carried out by the “Herbarium Bogoriense” Botanical Field of the Biology Research Center-LIPI Bogor, the plant is classified as a type of the *Clerodendrumfragrans* Vent Willd which is a division of the Verbanaceae family and has been stored as herbarium form “Herbarium Bogoriense” in June 2017.
The results of the phytochemical analysis show that the ethanol extract of the sarang banua leaves includes several types of secondary metabolites such as alkaloids, triterpenoids, flavonoids, saponins, tannins and quinines [1]. Several studies have shown that plant extracts containing secondary metabolites such as alkaloids, flavonoids, tannins and saponins can increase the formation of immunoglobulin proteins (antibodies) as humoral immune responses in animal studies such as rabbits and mice [2, 3].

Based on the above, the writer is interested in discussing the potential of immunostimulatory bioactivity from secondary metabolites of the ethanol extract of Clerodendrumfragrans Vent Willd leaves.

2. Materials and Methods

2.1. Collection of plant sample
A total of 10.0 kg sarang banua (C. fragrans Vent Willd) fresh leaves were obtained from Raya Usang village, Dolok Masihul sub-district, Simalungun district, North Sumatera, Indonesia. The leaves were properly washed with bi-distilled water to remove unwanted materials, drained and then air dried away from direct sunlight. The dried leaves were mechanically pulverized into fine powder and weighed (1.25 kg).

2.2. Preparation of ethanol extract
A total of 1000.0 g of simplicia powder of the C. fragrans leaves were macerated with ethanol solvent lasted for 48 hours with three times solvent additions, filtered and the filtrate obtained was concentrated in a rotary evaporator vacuum to obtain concentrated ethanol extracts. Finally, the concentrated ethanol extracts of the C. fragrans leaves were weighed.

2.3. Experimental animals
The experimental animal used was 15 male white rabbits of New Zealand strain (Oryctolagus cuniculus) with the criteria of being 22-52 weeks old, body weight (BW) ± 2 kg, and in a healthy condition.

The experimental animals were acclimatized for 7 days and placed in clean disinfected cages. Rabbits were fed with vegetables (water spinach and carrots) and water. Cage cleaning is done regularly to maintain the cleanliness and health of rabbits.

2.4. Experimental design
The rabbits in the groups, K1, K2, K3, K4 and K5 were treated as follows;
K1 rabbits: were given 1% Na-CMC.
K2 rabbits: were given bovine serum antigen.
K3 rabbits: were given ethanol extract of sarang banua leaves 200 mg/kg BW.
K4 rabbits: were given ethanol extract of sarang banua leaves 200 mg/kg BW and bovine serum antigen.

Figure 1. C.fragrans Vent Willd

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K5 rabbits: were given ethanol extract of sarang banua leaves 150 mg/kg BW and bovine serum antigen.

Groups K1, K2, K3, K4 and K5 rabbits were treated for a period of thirty (30) days

2.5. Administration of extracts

The ethanol extract of sarang banua leaves was made from each mixture of 420.0 mg (for the K4 rabbits group) and 315.0 mg (for the K5 rabbits group) ethanol extract of the sarang banua leaves with 20 mL Na-CMC 1%. Then the ethanol extract of the sarang banua leaves was obtained with a concentration of 21.0 mg/mL and 15.75 mg/mL.

2.6. Determination of globulin levels

The concentration of rabbit serum globulin was calculated by using the Bradford method [4] and Simorangkir [5]. Rabbit serum globulin levels in this study were tested using the Bradford method. In this method, rabbit serum globulin levels are determined based on their absorbance using a UV-vis spectrophotometer at a wavelength of 595 nm. Measurement of rabbit serum globulin was carried out by repeating 3 times for each treatment group.

2.7. Statistical analysis

The data analysis method used in this research is ANOVA. This data analysis technique is used to determine the effect of the treatment given on the value of observations (response) also find out significant differences in the value of observations as a result of giving different treatments to the research object.

3. Results and Discussion

The effects of oral administration of ethanol extracts of Clerodendrum fragrans Vent Willd leaves on serum globulin of experimental rabbits are as described in Table 1 and Figure 2.

Table 1. Globulin (mg/mL) serum levels in rabbits after treatment

| Treatment | Globulin Serum Levels (mg/mL) | Average |
|-----------|-------------------------------|---------|
|           | Repetition 1 | Repetition 2 | Repetition 3 |       |
| K1        | 7.19          | 7.71         | 8.28         | 7.73 ± 0.55 |
| K2        | 17.19         | 17.96        | 18.76        | 17.97± 0.78 |
| K3        | 10.2          | 12.48        | 14.78        | 12.49 ± 2.29 |
| K4        | 18.52         | 22.78        | 20.63        | 20.64 ± 2.13 |
| K5        | 18.16         | 19.98        | 19.10        | 19.08 ± 0.91 |

Treatment :
K1 : rabbits were given 1% Na-CMC.
K2 : rabbits were given bovine serum antigen.
K3 : rabbits were given ethanol extract of sarang banua leaves 200 mg/kg BW.
K4 : rabbits were given ethanol extract of sarang banua leaves 200 mg/kg BW and bovine serum antigen.
K5 : rabbits were given ethanol extract of sarang banua leaves 150 mg/kg BW and bovine serum antigen.
Figure 2. Serum globulin levels of rabbits

Treatment
K1 : rabbits were given 1% Na-CMC.
K2 : rabbits were given bovine serum antigen.
K3 : rabbits were given ethanol extract of sarang banua leaves 200 mg/kg BW.
K4 : rabbits were given ethanol extract of sarang banua leaves 200 mg/kg BW and bovine serum antigen.
K5 : rabbits were given ethanol extract of sarang banua leaves 150 mg/kg BW and bovine serum antigen.

From the results of this study, the implementation of bovine antigens as much as 1 mL per 2 weeks (2 times) for 28 days (K2) gave an average yield of rabbit serum globulin of 17.97 mg/mL higher than without the administration of bovine antigens (K1) with an average globulin level of 7.73 mg/mL. The results showed that the study sarang banua extract at a dose of 200 mg/kg BW with bovine antigen (K4) gave an average globulin level (20.64 mg/mL) higher than that of the sarang banua extract at a dose 150 mg/kg BW (K5) with an average globulin level of 19.08 mg/mL. The administration of ethanol extract of sarang banua leaves at a dose of 200 mg/kg BW and bovine serum antigen (K4) gives an average yield of globulin levels (20.64 mg/mL) higher than using ethanol extract of sarang banua leaf and only bovine serum antigen (K2) is given with an average globulin content of 17.97 mg/mL. Giving ethanol extract of sarang banua leaves at a dose of 200 mg/kg BW without antigen (K3) gives an average globulin level (12.49 mg/mL) higher than the use without ethanol extract of sarang banua leaves and can only be used as Na-CMC 1% (K1) with an average globulin level of 7.73 mg/mL.

From the data above supports bovine antigens and a combination of bovine antigens with ethanol extract of sarang banua leaves can significantly increase rabbit globulin levels. This is caused by the rabbit's immune system producing more antibodies to fight bovine antigen infections. In this case bovine antigens are foreign substances to the body of rabbits because bovine antigens have different protein codes from rabbit proteins. Antibodies are also called immunoglobulins which are components of globulin. Increased levels of immunoglobulin cause overall globulin levels to increase. Giving ethanol extract of sarang banua leaves at a dose of 200 mg/BW (K4) can increase serum globulin levels in rabbits compared to without giving ethanol extract (K2). Increased rabbit serum globulin shows the humoral immune response of rabbits to the ethanol extract of sarang banua leaves. This shows that the ethanol extract of sarang banua leaves has immunostimulatory activity. Increased immunostimulatory activity can be caused in the presence of flavonoids or saponins that add uphumoral response, by stimulating macrophages and B-lymphocytes the subset involved in antibody synthesis [6]. Flavonoids and saponins found in the ethanol extract of sarang banua leaves [1] are likely to cause an increase in rabbit humoral immune response in the form of increased synthesis of antibodies or globulins by B-lymphocytes. Triphala (Terminalia chebula, Terminalia belerica and Emblica officinalis) plants can also improve the immune system through increased neutrophil function which is a cellular immune response [7, 8]. From the results of research that has been done, sarang banua (Clerodendrumfragrans) Vent Willd) plants have the potential as an alternative natural immunostimulant material.
4. Conclusions

Ethanol extract of sarang banua (Clerodendrumfragrans Vent Willd) leaf significantly affected the increase in serum globulin levels of rabbits (Oryctolagus cuniculus). The ethanol extract of sarang banua leaves with a dose of 200 mg/kg BW and bovine serum antigen gave the highest levels of rabbit globulin (12.91 mg/mL).

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