Immunostimulatory Activity from Pirdot Leaves Ethanol Extract (*Saurauia vulcani Korth.*) in Rats (*Rattus norvegicus*)

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Abstract. Erythrocyte, lymphocyte quantities and spleen histology were observed to investigate the immunostimulatory effect Pirdor leaves ethanol extract (*Saurauia vulcani Korth.*) (EES) in white rats (*Rattus norvegicus* L.)-induced with sheep red blood cell (SRBC) as antigen. Experimental design used in this study was Complete Randomized Design. Twenty four rats were classified into four groups: a control group fed with distilled water (G0), a group treated with sheep red blood cell (SRBC) (G1), a group treated with ethanol extract of pirdot leaves (G2), and a group treated with ethanol extract of pirdot leaves + SRBC (G3) for 31 days. Blood was withdrawn Then the red blood cell in white rats were sized by using ABX micros 60. Spleen of white rats were taken and stained by using haematoxylin and eosin to visualize lymphoid follicles. Data were analyzed by one-way ANOVA at 5% significance level. Significant differences were observed in total erythrocytes and spleen histological image which showed a prospect of EES as immunostimulant.

1. Introduction
Immunocompromisation is a dynamic physiological condition which sometimes becomes a gateway to variety of diseases caused by bacterial infection, virus, or other specific antigens in human body. The improvement of human immunity on humans is achieved through vaccination and drug supplementation. Medicinal plants can effectively boost the human immune system [1]. Several researchers have reported the utilization of certain medicinal plant species in enhancing immune responses. The enhancement of serum IgG increased immune response in rats through administration of *Camelia murbei* tea in the fourth week of treatment [2]. Increasing red and white blood cells also indicated as a humoral immune response in rats after administration of *Nyctanthes arbor-tritii* (Oleacea) ethanolic extract [3].

*Saurauia vulcani* Korth or known as Pirdot, is one of medicinal plants from Actinidiaceae family. It has been used tradionally by Batakinese to lower cholesterol and blood glucose level, to prevent cancer and may act as wound healing. Sitorus [4] summarized that Pirdot leaves contained few secondary metabolites, e.g. flavonoid, glycoside, saponins, tannins, and steroids/triterpenoids. The phytochemical constituents have properties in lowering blood glucose level in alloxan-induced
diabetic mice for ten days. In recent interview with local people of North Tapanuli stated that Pirdor leaves may also improve human immune system.

Immunostimulatory assay has been used to investigate a few of immune system parameters by detecting humoral and cellular constituent activations [5]. Golindo and Hosokawa [6] reported immunostimulants as chemical agents which activated leukocytes and stimulated innate humoral and cellular effectively by interacting directly with the activated cells. The immunostimulants act as activator of human defense mechanisms against pathogens. Ortuno et al. [7] studied immunostimulant properties as biocompatible, biodegradable, cost effective, and environmental-friendly chemicals.

Ethanolic extract of Pirdot leaves (Saurauia vulcani, Korth.) or EES are utilized in this research to obtain the evidence of immunostimulatory activities of bioactive compound. Sheep Red Blood Cell (SRBC) is used to stimulate conformation of specific antibody as antigen. Babae et al., [1], administered 1% SRBC suspension as antigen in rats. They reported that 1% SRBC suspension exhibit high antigenic properties from immunized rat and formed a detectable antibody with precipitation reaction. Antigen injection was delivered through intraperitoneal region in order to expose a high immune response to its maximum capacity. Data on erythrocyte and lymphocyte is collected to analyze immunostimulatory regulation in rats (Rattus norvegicus L.), which previously administered with EES (Saurauia vulcani Korth.). Histological image of spleen is other parameter in this study to represent immune regulation in rats.

2. Method

2.1. Preparation of Animals
Twenty four white rats (Rattus norvegicus) of 3 month ages were taken from Laboratory of Pharmacy, Institut Teknologi Bandung, with an average weight of 150-200 g and acclimatized at 24 ± 27°C for a week in Laboratory of Pharmacology, Universitas Padjajaran. Subsequently, rats were treated for 30 days and fed with feeds and water ad libitum.

2.2. Preparation of Ethanolic extract of Pirdot leaves (EES)
Fresh Pirdot leaves (Saurauia vulcani Korth.) were collected from North Tapanuli (North Sumatera, Indonesia) and washed with tap water. Six kilograms of leaves were air-dried. Dried Pirdot leaves were further dried in an oven until constant weight. Approximately, 500 g of dried Pirdot leaves were crushed using blender until powder form, which were later put in two receptacles, i.e. each 250 g and added to distilled ethanol (1875 mL/container). Pirdot powder were soaked for 5 d and stirred twice daily. Filtrates were separated by using filter paper to collect 2.1 L with further addition of ethanol to achieve 3 L solution and left for 6 days. Subsequently, Pirdot powders were filtered and filtrates were concentrated by using a rotary-evaporator and settled on water bath for 4 d. This process yielded 51 g of crude ethanolic extract. Ethanol extract pirdot leaves were administered orally in white rats 500 mg/kg for 30 days.

2.3. Preparation of Sheep Red Blood Cell
Sheep blood antigens were taken from Lembang Laboratory of Veterinary, Bandung, Indonesia. Sheep blood was withdrawn from neck vein 5 mL and washed by using PBS with pH 7.4 then centrifuged with 200 rpm for 15 min. Centrifugation was performed three times. The result of centrifugation was stored in freezer at -4°C.

2.4. Collection of Blood and Spleen
The white rats were euthanized after 30 d of treatment. Blood was collected from neck vein of rats. Red blood cells were measured by using ABX micros 60. Surgery of all group treatments were conducted to remove the spleens. Spleens were stained by using hematoxyline and eosin to visualize lymphoid follicles.
2.5. Data Analysis

Experimental design used in this study was Complete Randomized Design. Twenty four rats were classified into 4 groups: a control group fed with distilled water (G0), a group treated with sheep red blood cell (SRBC) (G1), a group treated with ethanol extract pirdot leaves (EES) (G2), and a group treated with ethanol extract pirdot leaves (EES) + SRBC (G3). Data were analyzed by using SPSS version 23.

3. Results and Discussions

The results will be discussed in sub-sections, i.e. effect of pirdor leaves ethanolic extracts to erythrocyte and lymphocyte quantities and histological changes to spleens.

3.1. The effect of ethanol extract of Pirdot leaves on quantity of erythrocyte

(G2) (8.24 ± 0.51), high value was also showed on a group treated with ethanol extract pirdot leaves (EES) + SRBC (G3) (7.16 ± 0.82), then a control group with aquades (G0) (6.76 ± 0.82), and the lowest value was seen on a group treated with sheep red blood cell (SRBC) (G1) (6.39 ± 0.82). Table 1 was described increasing erythrocyte value on a group treated with ethanol extract pirdot leaves (EES) (G2) and a group treated with ethanol extract pirdot leaves (EES) + SRBC (G3).

| No. | Treatment | mean± SD (million) |
|-----|-----------|--------------------|
| 1   | Aquades (G0) | 6.76 ± 1.03        |
| 2   | SRBC (G1)   | 6.39 ± 0.82        |
| 3   | 500 mg/kg bw ethanol extract pirdot leaves (EES) (G2) | 8.24 ± 0.51        |
| 4   | 500 mg/kg bw ethanol extract pirdot leaves (EES) + SRBC (G3) | 7.16 ± 0.82        |

Note: p>0.05; SD = standar of deviation

Erythrocyte is one of blood components which has biconcave disc shape to maximise volume ratio of diffusion [8]. The flexibility of them make their way through in vessel. Hemoglobin, contains protein (globin) and iron (heme), bind oxygen and carbon dioxide. Iron has a main role on enzymatic functioning of immunostimulant in cell [9]. Therefore, increasing total erythrocyte value is related to an innate immune respon. According to Smith and Mangkoewidjojo [10], the value of erythrocyte in rats are 7.200.000 – 9.000.000.

On this study, administration of EES (G2) and EES+SRBC (G3) tend to be elevating the value of erythrocyte in rats (8.24 ± 0.51 dan 7.16 ± 0.82). The increased of its indicates ethanol extract of *Saurauia vulcani*, Korth give an impact on immunostimulant activity. Guyton and Hall [11] reported it participates directly on immune response againts pathogen invansion. The process of immune response will be induced by releasing molecule oxidants to activate innate immune cells. It is called erythrophagocytosis. A heme-binding protein on bacterial and iron regulated will induce platelet aggregation [12]. Sheep red blood cell (SRBC) as antigen would decrease total erythrocyte value on this research. In a treated with EES+SRBC, supplementation of pirdot can significantly preserve it. The table 1 showed the group treated with ethanol extract pirdot leaves (EES) (8.24 ± 0.51 x 10 6 cell/µl) has the highest of erythrocyte value which pirdot give a contribution to increase total erythrocyte in immunostimulant activity. The total erythrocyte value of a group treated with EES+SRBC (G3) (7.16 ± 0.82 x 10 6 sel/µl ) is higher than a control group with aquades (G0) and it is in normal value 7.1 x 10 6 / µl [13].
3.2. The effect of ethanol extract of Pirdot leaves on quantity of lymphocyte

Quantity of lymphocyte in rats was done at 31 days treated. Average of its value was seen on table 2. The group treated with ethanol extract pirdot leaves (EES) (G2) has the highest lymphocyte value and the control group with aquades (G0) has the lowest lymphocyte value. Then the lymphocyte value on a group treated with sheep red blood cell (SRBC) (G1) is higher than control group and is also lower than a group treated with ethanol extract pirdot leaves (EES) + SRBC (G3). There is a statistically significant differences seen in Table 2. Lymphocyte is one of part of white blood cells that has a main role on immune response. It help the body against antigen such as viruses, cancer cells, and bacteria invasion. Lymphocytes contain natural killer cells (cell-mediated, cytotoxic innate immunity), T cells (cell-mediated, cytotoxic adaptive immunity), and B cells (humoral, antibody) [10].

Table 2. The total of lymphocyte in rats treated

| No  | Treatment                              | Mean±SD (%) |
|-----|----------------------------------------|-------------|
| 1.  | Aquades (G0)                           | 69.17 ± 4.96a |
| 2.  | SRBC (G1)                              | 74.00 ± 2.92ab |
| 3.  | 500 mg/kg bw ethanol extract pirdot leaves (EES) (G2) | 77.67 ± 4.55c |
| 4.  | 500 mg/kg bw ethanol extract pirdot leaves (EES) + SRBC (G3) | 74.83 ± 3.19ab |

Note; a,b,p<0.05.

Studies reported that plant extracts have different immunostimulant activity on human Lymphocyte [14]. Nayak [15] reported Morinda citrifolia increased the phagocytic activity of neutrophils. T-cells and B-cells are important lymphocyte part to stimulate of immune function [16]. According to Smith and Mangkoewidjio [10], the normal value of lymphocyte is 55-85% in rats. Data of treatment showed the average of total lymphocyte is in range of normal value. Furthermore, when the administration of effect of pirdot extract compared with others group of rats treated, the group treated with ethanol extract pirdot leaves (EES) (G2) was significantly the highest of lymphocyte in this treatment. It was indicated that pirdot extract affected significantly on enhancement of lymphocyte value, 77.67 % of G2, compared with the control group with aquades (G0) which lymphocyte value had 69.17%. Increasing of lymphocyte was found in a group treated with ethanol extract pirdot leaves (EES) + SRBC (G3) which is significant higher than a group treated with sheep red blood cell (SRBC) (G1). According to Darzi [14] antigen is of significant importance for decreasing immune response. Our study revealed pirdot extract stimulated lymphocyte in G3 which SRBC as antigen decreased lymphocyte in treatment. It was caused the lymphocyte vaue of G3 lower than G2 and higher than a group treated with sheep red blood cell (SRBC) (G1).

Extract of Saurauia vulcani, Korth has contributed on enhancement of T cell and B cells which ethanol extract pirdot elevated lymphocyte value in this treatment. Our studies investigated the administration of ethanol extract pirdot has a main role to increase lymphocyte value in rats treated. Therefore, it was tend to be a good immunostimulant. Pirdot leaves contain vitamin B that has a good function to stimulate immune response in increasing of lymphocyte value. Flavonoid of pirdot leaves investigated for their antiseptic, anticancer antioxidant potential. It induced on macrophage activities to enhance to lymphocyte value. B cells made antibodies when sheep red blood cell as antigen contacted to immune system. Thus, the value of lymphocyte of a group treated ethanol extract pirdot leaves (EES) + SRBC (G3) is lower than a group treated ethanol extract pirdot leaves (EES) (G2).
3.3. The effect of ethanol extract of Pirdot leaves on spleen rats

The histology of the spleen can be seen in Figure 1. There were various forms of histology of various spleen from several treatment groups using ethanol extract of pirdot leaves [13].

![Figure 1](image_url)

- **Figure 1.** G₀, Lymphoid follicle in a group control (A. Lymphoid follicle, B. Centrum germinativum); G₁, Lymphoid follicle in a group treated SRBC (A. Lymphoid follicle, B. Centrum germinativum); G₂, Lymphoid follicle in a group treated EES (A. Lymphoid follicle, B. Centrum germinativum); G₃, Lymphoid follicle in a group treated EES + SRBC (A. Lymphoid follicle, B. Centrum germinativum).

The spleen is part of the lymphatic system to protect body against infections. It contains lymphocytes and macrophages (kind of white blood cell) [11]. Flavonoid of pirdot has stimulated immunostimulant activity in rats treated. Flavonoid is a source of energy to elevate immune response in cells [17-20]. It stimulated cytokine and innate mediator immune cell such as IL-2 in activation of T cells. Flavonoid has also a role main to maintain chromatin during cleavage of cells [4]. Ethanol extract pirdot was significantly given an effect of spleen in microscopic anatomy of rats treated. Thus, Figure 3 showed a good lymphoid follicle in a group treated ethanol extract Pirdot (EES) (G2).

4. Conclusions

*Saurauia vulcani* Korth. leaves have a potential immunostimulant activity which the parameter showed the enhancement of erthrocyte value and lymphocyte. It was also obtained a good histologic spleen.

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