Ethanolic Extract of *Xestospongia Sp.* Induces CD$^{4+}$ and CD$^{14}$ Cells Levels on Wistar Male Rat Infected with *Staphylococcus aureus*

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**Abstract**

Immunomodulator is a substance that increases or suppresses the immune response through the certain mechanism. The marine sponge *Xestospongia sp.* has immunomodulatory activity by increasing phagocytic activity. In addition, the phagocytic activity is affected by CD$^{4+}$ and CD$^{14}$ cells levels. Thus, this study aims to investigate the effect of *Xestospongia sp.* extract toward CD$^{4+}$ and CD$^{14}$ cells level in model rat. Animals were divided into 4 groups (n=5) and treated for 7 days, as follow: Group I (Ethanolic extract of *Xestospongia sp.* dose of 300 mg/KgBW); Group II (Ethanolic extract of *Xestospongia sp.* dose of 400 mg/KgBW); Group III (*Phyllanthus niruri* extract); and Group IV (0.5% Na CMC). On day 8, animals were intraperitoneally infected to *Staphylococcus aureus* and the blood was collected by cardiac puncture and assayed with ELISA kit CD4 (elabscience®) and ELISA kit CD14 (elabscience®). Ethanolic extract of *Xestospongia sp.* provided high levels of both CD$^{4+}$ and CD$^{14}$ cells (group II) compared to baseline (group IV) (p<0.05). Group I provided similar activity to group III (p>0.05) and group II provided significant activity with higher levels of CD$^{4+}$ and CD$^{14}$ cells compared to group III (p<0.05). In conclusion, both doses of *Xestospongia sp.* extract showed immunomodulatory activity by increasing CD$^{4+}$ and CD$^{14}$ cells levels, yet dose of 400 mg/KgBW provides the higher immunomodulatory activity.

**Keywords:** *Xestospongia sp.;* immunomodulatory; CD$^{4+}$ Cells; CD$^{14}$ Cells

**Introduction**

Immune systems act by body survival and protection against antigen that arise due to various materials from the environment.$^{1}$ Body’s immune systems are able to identify and eliminate microorganisms that potentially harm to the body to prevent infections causing organs damage.$^{2}$

WHO (World Health Organization) stated that immune system disorder is the biggest problem in the worldwide. Immune system disorders are divided into primary and secondary disorders that caused by environmental factors, drugs and radiation$^{3}$ resulted in high sensitivity to infection, thus, immunomodulators can be used to increase the immune system as an alternative to control against infection.

Immunomodulator is a substance that changes activity of immune system, either

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by increasing or suppressing the immune response through certain mechanism. Their works in two ways, the stimulatory effect (increasing the immune response) or vice versa, the effect of suppression effect (suppress the immune response).\textsuperscript{1,2,4}

The use of terrestrial products such as marine sponge can be utilized in discovering novel drug such as immunomodulator agents. Marine sponges exhibit abundant biopharmacological activity such as antibacterial, antiviral, antifungal, antimalarial, antitumor, immunomodulator, and cardiovascular activity.\textsuperscript{5} *Xestospongia* sp. reported has activity as immunomodulator by stimulating phagocytosis examined by its specific phagocytic activity.\textsuperscript{6}

Immune system involves phagocytic cells such as macrophages that play important role in the body’s immune system against pathogens. One of the main roles of macrophages is phagocytosis, which aims to eliminate antigens, damaged or dead cells, and pathogenic bacteria such as CD\textsuperscript{4+} and CD\textsuperscript{14} cells. CD\textsuperscript{4+} cells are antigen expres cells in the subset of thymocytes and T cell inflammatory cells (about 2/3 peripheral T cells), monocytes, and macrophages.\textsuperscript{7,8} CD\textsuperscript{4+} cells are type of white blood cells or lymphocytes which are important as part of the immune system. CD\textsuperscript{4} or CD\textsuperscript{4+} cells are referred to as T helper or T4 cells that activate Th1 cells thus activate macrophages in eliminating pathogens.\textsuperscript{9}

In other hand, CD\textsuperscript{14} is Pattern Recognition Receptors (PRR) which enhance nonspecific immune responses to infections by increasing the sensitivity of immune cells to components of bacteria including lipopolysaccharide (LPS), lipoprotein and lipoteichoic acid (LTA). CD\textsuperscript{14} delivers the bacterial component to various TLR (Toll-Like Receptors) on the surface membrane and induces immune cell activation.\textsuperscript{11} Binding bacteria by the CD\textsuperscript{14} receptor activate phagocytosis.\textsuperscript{12}

Thus, this study aims to examine the activity of ethanolic extract of *Xestospongia* sp. to phagocytosis activity observed from the increase in CD\textsuperscript{4+} and CD\textsuperscript{14} levels of rats models-treated with extracts and induced with *Staphylococcus aureus*.

**Methods**

**Sample Preparation and Extraction**

Sample collected from Soropia, Konawe Regency, Southeast Sulawesi at the depth of 3 m. Sample collected (5.3 Kg) was sorted and cleaned under running water. The sample was determined in Faculty of Fisheries and Marine Sciences of Halu Oleo University with document number 008b/UN29.112.1.1/pp/2018. Then, sample was chopped into pieces. The sample was macerated for 3 days with 96% ethanol. Filtrate collected was then concentrated and obtained 205.28 g of the concentrated extract (3.87%).

**Animals**

Animals were acclimatized for 7 days under controlled temperature (20-21ºC), relative humidity maintained (45-70%), 12 h light/dark cycle, and free access to food and water ad libitum. The animals were conducted in accordance with ethical clearance issued by Komisi Etik Penelitian Kesehatan of Halu Oleo University (No: 2739/UN29.20/PPM/2018)

**Bacteria**

Inocula *Staphylococcus aureus* was suspended in 0.9% NaCl equivalent with 0.5 Mc Farland turbidity (1.5x108 Colony Forming Unit (CFU)/mL).

**Immunomodulatory Activity in vivo**

Animals used in the study were divided into
4 groups (n=5), which were treated for 7 days with 5 mL of treatment orally in one daily dose, as follow:
Group I : ethanolic extract of *Xestospongia sp.* dose of 300 mg/KgBw
Group II : ethanolic extract of *Xestospongia sp.* dose of 400 mg/KgBw
Group III : as positive control, *Phylantus niruri* extract (Stimuno®) dose of 1.064 mg
Group IV : as a control, 0.5% Na CMC (used as baseline)

On day eight, each animal was infected intraperitoneally to 0.5 mL of *Staphylococcus aureus*, and left for 1 hour. After that, 3 mL of blood was collected intra cardial and put in EDTA-tube. Collected blood was centrifuged 3000 rpm for 15 minutes. Blood assayed with ELISA kit CD\(^4\) (elabscience®) and ELISA kit CD\(^14\) (elabscience®).

**Results and Discussion**

Immunomodulator is a substance that enhance immune response with various mechanisms. Both CD\(^4\) and CD\(^14\) cells are activators of macrophages that involved in phagocytosis activity. The CD\(^4+\) cells expressed on surface of lymphocytes and the CD\(^14\) cells are expressed on surface of macrophages membranes. The CD\(^4+\) cells stimulating Th1 cell, thus activating macrophages to eliminate pathogens. In other hand, macrophages are expressing CD\(^14\) on the surface of membrane, named mCD\(^14\). As the cell is activated, the mCD\(^14\) decay into a membrane-free form (dissolved CD\(^14\), sCD\(^14\)). The sCD\(^14\) is a marker of activation of macrophages and monocytes, thus the more macrophages and monocytes are activated, the higher dissolved CD\(^14\) levels in the circulation.\(^13-15\) Rats induced with ethanolic extract of *Xestospongia sp.* and positive control (*Phylantus niruri* extract), extract of *Xestospongia sp.* demonstrated expected results in increasing CD\(^4\) and CD\(^14\) cell levels.

According to Figure 1, all groups was significant to group IV as normal control

![Figure 1. Increasing levels of CD4 in administration of ethanolic extract of Xestospongia sp. (n=5, Group I: of Xestospongia sp. dose of 300 mg/KgBw; Group II: Xestospongia sp. dose of 400 mg/KgBw; Group III: Phylantus niruri extract (Stimuno®); Group IV: 0.5% Na CMC)](image-url)
Figure 1. UV-Vis Spectrum of fraction 2 (Isolated compound) Leaves

Figure 2. Increasing levels of CD14 in administration of ethanolic extract of Xestospongia sp. (n=5, Group I: of Xestospongia sp. dose of 300 mg/KgBw; Group II: Xestospongia sp. dose of 400 mg/KgBw; Group III: Phylantus niruri extract (Stimuno®); Group IV: 0.5% Na CMC)

The high levels of CD4+ cell as well CD14 cells are supporting previous study that ethanolic extract of Xestospongia sp. increases phagocytic activity possibly affected by flavonoids contain in it characterized by increased levels of CD4+ and CD14 cells, although the exact mechanisms are not well understood.

According with results conducted at Figure 2, group II with 45.85±19.69 ng/mL provided high increasing levels of CD14 compared to group I with 18.03±1.13 ng/mL, although both group was significant to group IV with 9.93±0.74 ng/mL as normal control (p<0.05). Group II was significant to group III as positive control (Phylantus niruri extract) with 2.796±0.56 ng/mL (p<0.05). Group I and Group III had similar activity in increasing CD4 levels.

Previous study showed that ethanolic extract of Xestospongia sp. has activity as immunomodulator by increasing phagocytic activity of macrophages. Flavonoids contained in extract are suspected responsible in increasing phagocytic activity by activating Th1 cell assisted with T cell helper (CD4+ cell). The high levels of CD4+ cell as well CD14 cells are supporting previous study that ethanolic extract of Xestospongia sp. increases phagocytic activity possibly affected by flavonoids contain in it characterized by increased levels of CD4+ and CD14 cells, although the exact mechanisms are not well understood.

**Conclusion**

Ethanolic extract of Xestospongia sp. might have immunomodulatory activity by increasing CD4+ and CD14 levels in rats which is responsible for enhancing immune system. Dose of 300 mg/KgBw is considered the lowest effective dose by knowing the immunomodulatory activity of ethanolic extract of Xestospongia sp. These results can be formulated into dosage form and used as immunomodulator.

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Conflict of Interest
None declared

References