INTRODUCTION

*Etlingera elatior* (Jack) R.M. Smith is one of the spices native to Indonesia which belongs the Zingiberaceae family. It has been used traditionally used as medicines and flavor enhancers (Farida & Maruzy, 2016; Syarif et al., 2015). *Etlingera elatior* fruit is known as wualae by people in the Konawe region of Southeast Sulawesi as spices. Besides that, it empirically used as traditional medicine as immunomodulator in recovery of typhoid fever in the...
Material used in the study were *E. elatior* Fruit (in local name, wualae), male Wistar rats, inocula *S. aureus* ATCC 25923, Rat IL-6 ELISA Kit (Elabscience®), 96% ethanol (Mercks® (technical grade)), 70% ethanol (Mercks® (technical grade)), methanol (Mercks® (technical grade)), ether (Mercks® (technical grade)), aqua pro injection (Otsu-NS®), 0.5% Na-CMC (Mercks®), 0.9% NaCl (Otsu-NS®), nutrient agar (Merck®), and commercial *Phylantus niruri* extract (Stimuno®).

Sample determination
Determination of *E. elatior* plants was conducted to ensure the validity of the samples used in the study. Determination was conducted by observing the morphological characteristics of *E. elatior* plants based on references and proven at the Researches Center For Biology, Indonesian Institute of Sciences/Lembaga Ilmu Pengetahuan Indonesia, Cibinong, West Java (No. 355/IPH.1.01/II.07/II/2017).

Sample collection, preparation, and extraction
*Etlingera elatior* fruit sample as amount 20 kg was collected in Kalu-kaluku Village, Kodeoha Subdistrict, North Kolaka Regency, Southeast Sulawesi. Collected samples were then continued by wet sorting, separated from the fruit stalks, washed with running water, then cut into smaller sizes, and dried in the sun. After sample was dried, dry sorting were conducted and then powdered with a weight of 2.1 kg obtained. The powder sample was then macerated with 96% ethanol for 3 x 24 hours with a sample : solvent ratio of 1:2. Every 24 hours the solvent was filtered and replaced with a new solvent to obtain the filtrate for the first to the third day. All filtrates were then collected and concentrated using a rotary vacuum evaporator at 50ºC.
The total amount of concentrated extract produced was ±74.6 g with a yield of 3.5%.

**Bacteria**

Staphylococcus aureus was planted into slanted agar and incubated for 24 hours in an incubator at 37°C. Inocula is then suspended with 0.9% NaCl until a turbidity equal to the McFarland 0.5 standard is obtained.

**Experimental animals**

A total of 32 male Wistar rats used were acclimatized for seven days. The acclimatization temperature is 23-25°C in a light/dark cycle for 12/12 hours and is fed with chow pellet diet and access to ad libitum water. All experiments involving these animals were carried out with the approval of the Animal Ethics Committee from Universitas Halu Oleo (No.2739/UN29.20/PPM/2018).

**Experimental design**

Experimental animals were divided into four groups (n = 4) and treated orally once every day for seven days. The division of groups is done based on different treatments as follows:

1. Group I: negative control (0.5% Na CMC)
2. Group II: positive control (Phyllanthus niruri extract/Stimuno®)
3. Group III: E. elatior fruit extract with concentration of 300 mg/kg BW
4. Group IV: E. elatior fruit extract with concentration of 400 mg/kg BW

On the eighth day, each animal was infected with 0.5 mL S. aureus suspension intra peritoneal and left for one hour. Animal blood was then collected with cardiac puncture as much as 3 mL and put in a test tube containing EDTA. The tube was then centrifuged for 15 minutes at 3000 rpm and a temperature of 25°C. Blood plasma was then tested using the ELISA Kit (Elabscience® rat IL-1β and Elabscience® rat IL-6).

**RESULTS AND DISCUSSION**

Administration of extracts in experimental animals is carried out for seven consecutive days orally once per day, which aims to stimulate the immune system of each group of experimental animals. On the eighth day, S. aureus inocula is injected into animals intra peritoneally. Staphylococcus aureus is gram-positive bacteria that can cause infections both in humans and animals. They do not produce protein A, which is an antiphagocyte protein, causing S. aureus unable to avoid phagocytosis of peritoneal macrophages (Hariyanti et al., 2015; Wahyuni et al., 2017). After being injected with a bacterial inocula, all the experimental groups were left for an hour to make the innate immune system work. The innate immune system can be active within the range of 0-12 hours after infection (Abbas et al., 2016).

Macrophages and neutrophils, including the first line of defense in the immune system. Macrophages are able to fight off infections for about an hour before the immune mechanism is mobilized. On this basis, macrophage taking is done one hour after bacterial induction, so it can be seen the extent of the ability of macrophages to conquer bacterial invasion (Chaplin, 2010). Macrophages enter the site of infection are increased and the phagocytic ability of antigens also increases. In addition, levels of IL-1β and IL-6 also increased (Besung et al., 2016). Measurement of plasma IL-1β shows that the highest IL-1β levels is the group IV (concentration of 400 mg/kg BW) (p <0.05) compared to group III (concentration of 300 mg/kg BW) and group II (positive control). The average level of IL-1β in Group II was higher than the concentration of group III (p <0.05). Group I (negative control group) had lower levels of plasma IL-1β value compared to group II but not significant (p >0.05). These results are shown in Figure 1.
Increased levels of IL-1β at concentration of 400 mg/kg BW and IL-6 at concentration of 300 and 400 mg/kg BW are thought to be caused by the presence of flavonoid compounds in E. elatior fruit extracts. According to previous research by Wahyuni et al. (2017), phytochemical screening of E. elatior fruit showed the presence of flavonoid compounds. Flavonoids also accelerate the proliferation and differentiation of macrophages, so that macrophage migration increases (Ginwala et al., 2019).

Increased migration of macrophages to stimuli causes the number of macrophages in the peritoneum to increase. Accelerated macrophage migration occurs due to increased levels of IL-1β and IL-6 produced by monocytes and macrophage cells (Atri et al., 2018). In addition, an increase in the number of T lymphocytes triggers macrophage activation so that bacterial phagocytosis will increase (Abbas et al., 2016).

**CONCLUSION**

*Etlingera elatior* fruit extract increase levels of plasma IL-1β and IL-6 in male Wistar rats after administration concentration of 300 and 400 mg/kg BW. This increased level is associated with an immune response.

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REFERENCES


