

# ***In vitro* Test of Macrophage Phagocytic Activity of Extracts and Fractions of Red Dragon Fruit Peel [*Hylocereus polyrhizus* (F.A.C.Weber) Britton and Rose]**

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**ABSTRACT:** The peel of red dragon fruit [*Hylocereus polyrhizus* (F.A.C.Weber) Britton and Rose] can be used to treat various diseases and to improve immune system of body. This study was aimed to investigate the *in vitro* macrophage phagocytic activity of extracts and fractions of red dragon fruit peel (*Hylocereus polyrhizus*). The *in vitro* test was conducted based on the method of Leijh *et al.* The parameters of phagocytic activity were based on the macrophages capacity to phagocytose latex beads using the calculation of phagocytic capacity and phagocytic index. The test results indicated significant difference ( $p < 0.05$ ). The petroleum benzene showed higher phagocytic activity of macrophages than methanol extract of the fruit peel, sediment, and media control (-). The LSD test showed that macrophage phagocytic activity using fractions (500 and 100  $\mu\text{g/ml}$ ) was significantly different from macrophage phagocytic activity using fractions (20  $\mu\text{g/ml}$ ), sediment (500, 100 and 20  $\mu\text{g/ml}$ ), extracts (500, 100 and 20  $\mu\text{g/ml}$ ), and media control.

**Key words :** Red dragon fruit peel, macrophage phagocytosis, immunomodulator.

## **INTRODUCTION**

Immune system is used to protect the body against hazardous substances. Immune system can be improved by immunomodulatory agents. Nature provides various plants that can be used as medicines and compounds. Wagner *et al.*<sup>1</sup> stated that compounds able to modulate system can be derived from plants. The use of plants as immunostimulator is developing now-a-days. Screening results show a few evidences of some plants which have immunomodulatory activity and can be used in clinical practices. So any study on immunostimulator derived from medicinal plants will be very valuable in the future.<sup>2</sup> The peel of red dragon fruit is rich source of natural compounds such as phenolic, flavonoid, carotenoid, anthocyanin, micronutrients and vitamin.<sup>3</sup> The peel can be used as traditional

medicine. The extract of red dragon fruit peel is non-toxic since it did not cause any mortality of a mouse at dose of 48,500 mg/kg b.w.<sup>4,5</sup>

Some compounds which can be immunodulator are polysaccharides, terpenoids, saponins, alkaloids, isoflavonoids, glucosides, tannins, fatty acids, steroids, triterpenes, and flavonoids.<sup>6-8</sup> This study was aimed to investigate the *in vitro* macrophage phagocytic activity of extracts and fractions of red dragon fruit peel (*H. polyrhizus*). Phagocytosis is a process in which cells are involved in the ingestion of pathogenic particles, and due to the capacity of the cells, the pathogens are killed and destroyed.<sup>9</sup> According to Ponske and Indap<sup>10</sup> phagocytosis test is the most frequently used method for screening active substances which can influence immune system.

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## **MATERIALS AND METHODS**

## Materials

The peel of red dragon fruit [*Hylocereus polyrhizus* (F.A.C.Weber) Britton and Rose] obtained from Bantul, Yogyakarta, methanol (technical), aquadest, latex beads (3  $\mu\text{m}$ ), (Sigma, Chem. Co) RPMI (*Roswell Park Memorial Institute*) 1460 (Sigma, Chem. Co), DMSO (Sigma, Chem. Co), PBS (*Phosphate buffered saline*), giemsa 2 (0% b/v), complete medium, coverslip, and 2-4 month old mice Balb/C (20-25 g bw) were used in this experiment.

## Methods

**Extraction.** Samples of red dragon fruit (50 kg) were washed. The peels of the fruit were cut and dried in an oven at 50°C. The dried powder was macerated with methanol for 3  $\times$  24 hours at room temperature. The filtration was performed using Büchner funnel, the materials were macerated twice more by applying the same method. The filtrate was mixed and evaporated using rotavapor until viscous extracts were generated. The extract was blown until getting free from methanol.

**Fractionation of red dragon fruit peel.** Partition fractionation was performed with petroleum benzene solvent. Methanol extracts of the peel (2 g) was added to petroleum benzene (10 ml), mixed with vortex and centrifuged to obtain soluble petroleum benzene fraction and insoluble fraction (sediment). The methanol extract of the peel, soluble fraction, and sediment were tested for their *in vitro* immunomodulatory activity using macrophage phagocytic activity test with latex beads.<sup>11</sup>

**Isolation and culture of macrophages.** The experimental animals were anesthetized with chloroform and dissected. The mouse was put on dissection tray. Its abdominal skin was opened and cleaned with alcohol (70%). RPMI solvent (10 ml) was injected into peritoneal socket, left for 3 minutes and rolled slowly. The peritoneal liquid was removed from the socket by pushing the inner organ with two fingers; the tissue was aspirated with a hypodermic syringe, choosing a non fatty part and far from intestine. The aspirative syringe was put into beaker

glass filled with ice. The suspension was then put into centrifuge tube. The aspirate was centrifuged at 1.200 rpm 4°C for 10 minutes. The supernatant was removed and complete medium (3 ml) was added on the generated pellet. Number of cells was counted using hemocytometer and resuspended with complete medium for cell suspension with solidity of  $2.5 \times 10^6$  ml. The calculated cell suspensions were cultured on plates which had been added with round coverslips. Each well was 200  $\mu\text{l}$  ( $5 \times 10^5$ ). The cultures were incubated in an incubator (CO<sub>2</sub> 5%) at 37°C for 30 minutes. Complete medium (1mL/well) was added and reincubated for 2 hours. The cells were washed twice with RPMI and then added with complete medium (1 ml/well), and the incubation was continued until 2 hours.

**Test of macrophage phagocytic activity with latex beads.** Non specific *in vitro* test of phagocytic capacity was performed with latex beads (3  $\mu\text{m}$ ). The latex beads were suspended in PBS to get concentration of  $2.2 \times 10^7$ /ml. Isolate was added into RPMI media (400  $\mu\text{l}$ ) with some concentrations using solvent control media DMSO (0.0025%). The macrophages cultured a day before were washed and incubated for 60 minutes at 37°C (CO<sub>2</sub> 5%). The cells were then washed three times with PBS to remove particles which were not phagocytosed; they were dried at room temperature and fixed with absolute methanol. After drying the coverslips were stained with Giemsa 20% b/v for 30 minutes. The percentage of cell phagocytosing latex particles was counted from 100 cells, observed under light microscope (400x). The treatments were performed three times.<sup>11</sup>

**Data analysis.** The homogeneity and distribution of data result were analyzed using Shapiro-Wilk test. If the data were normally distributed and homogeneous, the analysis would be continued using one-way analysis of variance (ANOVA) SPSS 22 *for windows* and LSD analysis at the 95% level of confidence to see the differences among the treatments.

## RESULTS AND DISCUSSION

**Sample preparation.** The sample used was the peel of red dragon fruit (*Hylocereus polyrhizus* (F.A.C.Weber) Britton and Rose) obtained from Bantul, Yogyakarta, Indonesia. The total amount of fruit used was 30.29 kg. From this, the peel generated was 6.79 kg or 23.683 % of the total fruit weight. The sample was chosen from the culture place for its variance type, growth location, similar age, and clear harvest time. The peels of the fruit were collected and processed into simplisia form through some stages, which were wet sorted, washed, dried, dry sorted and stored. The weight of obtained simplisia was 518.68 gm.

**Extraction and fractionation.** The extraction of the peel was performed using maceration method with methanol. The method was implemented for its good capacity to extract compounds and prevent decomposition of compounds which are unstable toward heating. The repetition of maceration process was an effort to get extracts maximally. The diffusion of liquid into the plant cell containing active compounds led to different osmotic pressure inside and outside the cell; the compounds were released due to the pressure. The viscous methanol extract (2 g) was partitioned with petroleum ether. Soluble fraction and insoluble fraction (sediment) were generated.

**Test of macrophage phagocytic activity.** Macrophage is the main phagocytic cells which can counter pathogens through phagocytosis mechanism; it is important for response of both inherited immune system and adaptive immune system.<sup>12</sup> *In vitro* test of macrophage phagocytic activity was performed. The phagocytic capacity of macrophages was measured by observing the ability of macrophages to ingest latex, calculating the percentage of macrophages ingesting latex in each treatment and their phagocytic index. The treatments were performed three times.<sup>11</sup>

$$\text{Phagocytic index} = \frac{\text{Number of phagocytosed latex}}{\text{Number of active macrophages}}$$

$$\text{Phagocytic capacity} = \frac{\text{No. of phagocytosed macrophages}}{\text{Number of calculated macrophages}}$$

Data of potential immunomodulator was analyzed based on the improved cellular immune response signified with the increased phagocytic capacity of macrophages toward latex beads compared with solvent control. The results of the percentage of macrophages phagocytosing latex are shown in figure 1.

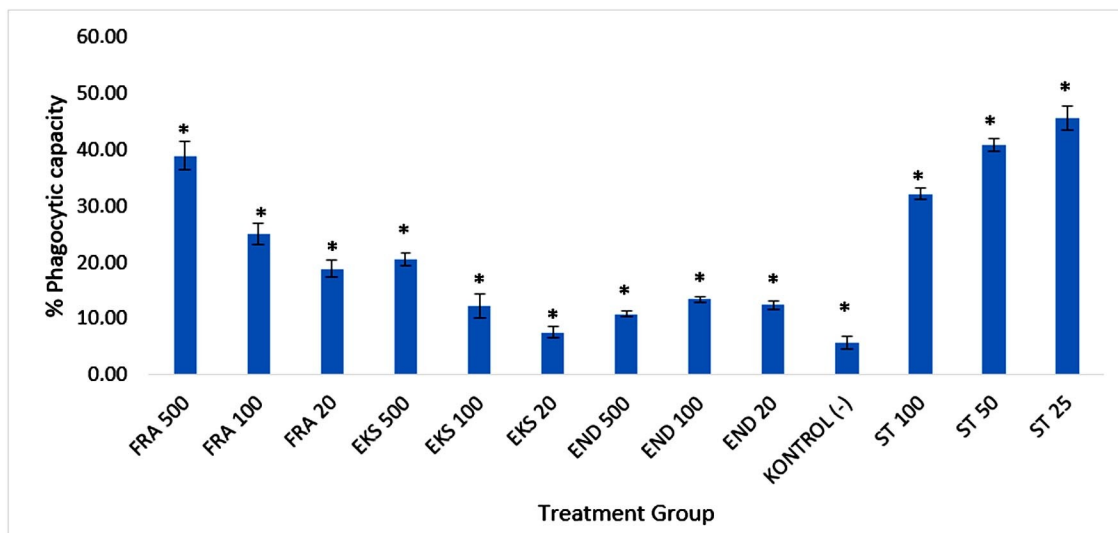


Figure. 1 Capacity phagocytic of extracts and fractions of red dragon fruit peel. The value is mean ± SD, n = 5, a = 0.05. \* showed a significant difference (p < 0.05) between the treatment group and the control group.

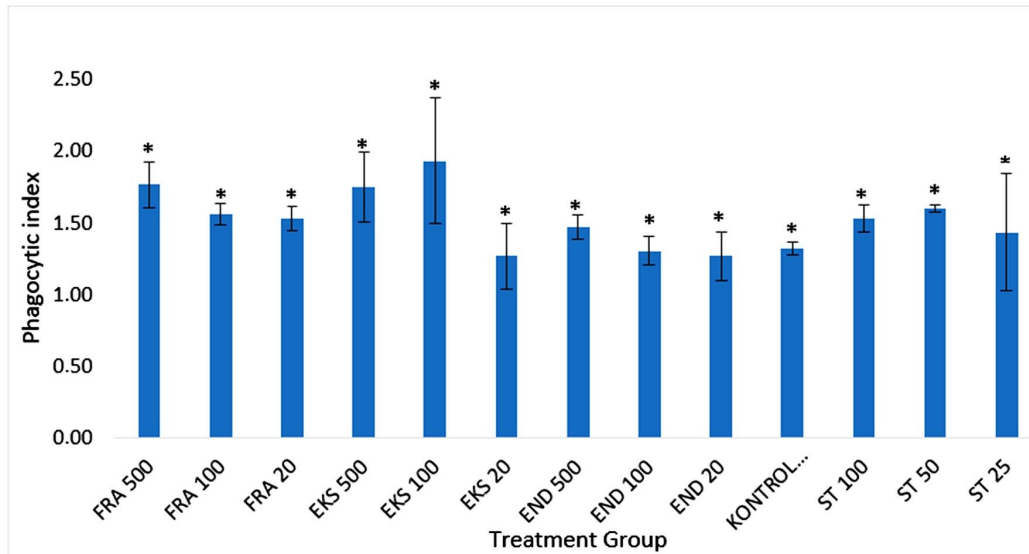


Figure. 2 Index phagocytic of extracts and fractions of red dragon fruit peel. The value is mean  $\pm$  SD, n = 5,  $\alpha$  = 0.05. \* showed a significant difference ( $P < 0.05$ ) between the treatment group and the control group.

Macrophage stimulation toward latex phagocytosis was identified by either the sticking or ingesting latex particle into macrophage cell. The addition of compounds (sample) with higher concentration through *in vitro* method was able to reduce the number of macrophages phagocytosing latex. The results of macrophage phagocytic activity and ANNOVA indicated significant difference of KF (Kelompok perlakuan) (treatment group) and IF (Indeks Fagositosis) (Phagocytic index) parameters at the 95% level of confidence (Figures 1 and 2). Soluble fraction showed higher phagocytic activity of macrophages than methanol extract of the fruit peel, sediment and media control (-). The LSD test showed that macrophage phagocytic activity using fractions (500 and 100  $\mu\text{g/ml}$ ) was significantly different from macrophage phagocytic activity using fractions (20  $\mu\text{g/ml}$ ), sediment (500, 100 and 20  $\mu\text{g/ml}$ ), extracts (500, 100 and 20  $\mu\text{g/ml}$ ), and media control (-).

## CONCLUSION

The *in vitro* test revealed that fractions of partitioned methanol extract of red dragon fruit peel with petroleum benzene were able to improve macrophage phagocytic activity

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