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Noni Leaf Extract (*Morinda citrifolia L.*) as an Immunomodulator Against *Escherichia coli*

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ABSTRACT

In normal circumstances the body can fight exposure to pathogenic microorganisms because of the immune system present in the body, but when the immune system is reduced or inadequate exposure to pathogenic microorganisms can cause various diseases so that the body requires intake to increase immune system activity, one of which is the intake of substances that are immunomodulatory. Noni leaves have active compounds of flavonoids and steroids that can act as immunomodulators. This study aims to determine whether noni leaves can be an immunomodulatory agent against immunoglobulin M (IgM) and what is the optimum dose of noni leaf extract as an immunomodulatory agent against *E. coli* in mice. This study used an experimental method with the hemagglutination test method with adoses used for the extract were 100 mg/200 g BB, 200 mg/200 g BB and 300 mg/200 g BB. Noni leaf extract was given to male DDY mice. Further research data were processed using analysis of variance (ANOVA). The ANOVA statistical results showed that the extract dosage was significantly different and for the optimum dose Duncan post hoc test was used. The results showed that the dose of 300 mg/200 g BB had increased activity of immunoglobulin M (IgM) compared to positive controls, at a dose of 100 mg/200 g BB and 200 mg/200 g BB. The result showed that noni leaf extract could increase the activity of immunoglobulin M (IgM) against E. coli with an optimum dose of 300 mg/200 g BB.

Keywords : Noni Leaf Extract, Immunomodulator, *E. coli*, Immunoglobulin M (IgM)

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INTRODUCTION

Many diseases are caused by exposure to pathogenic microorganisms such as viruses, bacteria, and fungi, which are constantly evolving and developing, making these pathogens resistant to existing medications [1, 2].

Under normal conditions, the body can fight off exposure to pathogenic microorganisms due to the immune system present in the body. However, when the immune system is weakened or inadequate, exposure to pathogenic microorganisms can cause various diseases. Therefore, efforts are needed to maintain the immune system so that it can work optimally to defend against exposure to pathogenic microorganisms. One effort to maintain the immune system is thru the administration of immunomodulators [3, 4].

Immunomodulators are substances that affect the body's biological reactions to foreign substances. The function of the immune system can be stimulated (immunostimulators) or suppressed (immunosuppressants) [5, 6]. Immunostimulants indirectly help reactivate a weakened immune system by boosting the non-specific immune

response. Immunosuppressants are substances that actually suppress the activity of the immune system by interacting at various points within that system [6].

Noni is one of the tropical plants that is quite widely found throughout Indonesia. Noni or Indian mulberry (*Morinda citrifolia L*.) is one of the medicinal plants that has gained popularity in recent years. Noni is widely used as a traditional medicine for various ailments. Some of the benefits of noni include its effects as a chemotherapy agent, antidepressant, hepatoprotective activity, antioxidant, antidislipidemic, antimicrobial, and immunomodulatory [7, 8].

Noni leaves contain active compounds such as anthraquinones, saponins, polyphenols, tannins, triterpenes, alkaloids, flavonoids, terpenoids, and lipid compounds that act like essential oils. There several classes of compounds that can act as immunomodulators, namely carbohydrates, terpenes, steroids, flavonoids, glycoproteins, alkaloids, and several other nitrogen-containing organic compounds [9, 3].

Based on the literature, the author conducted research using noni leaf extract because they wanted to determine if the content in noni fruit extract is the same as that in noni leaf extract, which can act as an immunomodulator in the human body, and what the optimal dose of noni leaf extract is as an immunomodulator. The extraction of active compounds from plants can be done using solvent extraction. The choice of solvent type must consider several factors, including selectivity, extraction ability, toxicity, ease of evaporation, and solvent price. The extraction solvent used is adjusted to the polarity of the desired compound. According to the principle of "like dissolves like," a solvent will tend to dissolve compounds with similar polarity. Polar solvents will dissolve polar compounds, and vise versa. Noni leaves contain several compounds, one of which is flavonoids, which can function as an immunomodulatory [10].

Escherichia coli bacteria are members of the normal intestinal flora. *E. coli* becomes a pathogen if the number of these bacteria in the digestive tract increases or if they are outside the intestines. E. coli produces enterotoxins that cause some cases of diarrhea. *E. coli* associated with enteropathogenic strains produces enterotoxins in epithelial cells. This *E. coli* bacteria will be injected into mice as a source of infection or antigen for the mice. This study also aims to examine the activity of Immunoglobulin M (IgM) produced by the noni leaf extract as an immunomodulator using a hemagglutination test. A hemagglutination test was performed because the equipment and materials are relatively easy to obtain and affordable, yet it provides reasonably accurate results [4].

MATERIALS AND METHODS

The materials used in this study were noni leaves (Morinda citrifolia L.), distilled water, nutrient agar media, physiological NaCl, *E. coli* bacterial suspension, 96% ethanol solvent, 0,5% CMC, and 0,5% erythrocyte suspension.

The tools used in this study are Autoclave, stirring rod, blue tip, bulb pipette, Bunsen burner, Petri dish, Erlenmeyer flask, evaporator, beaker, measuring cylinder, grinder, incubator, micropipette, analytical balance, circular loop, inoculation needle, oven, dropper, measuring pipette, volumetric pipette, rotary evaporator, centrifuge, spatula, spirit lamp, syringe, test tube, serological tube, and yellow tip.

The dried noni leaves are then ground using a grinder. Weighed 200 grams of finely ground noni leaves, which were then macerated with 96% ethanol solvent for 48 hours. The maceration results were filtered and then evaporated using a rotary evaporator until a thick extract was obtained at a temperature of 45-50°C. The thick extract was then subjected to phytochemical testing to determine the content of the noni leaves.

Next, the test animals were treated. Test animals were divided into five groups: negative control, positive control, and test groups. Each group consisted of five mice, resulting in a total of 15 mice used. Group one, serving as the positive control, was only given EDM orally at a dose of 100 mg/200 g body weight. Group two, serving as the negative control, received no treatment. Groups three to five, serving as the experimental groups, were immunized with noni leaf extract (EDM). Before treatment, the mice were marked using yellow dye from picric acid with the following codes: plain number one, upper right number two, upper left number three, lower right number four, lower left number five, and tail number six. The mice underwent a seven-day adaptation process, during which they were only given food. On the eighth day, or the first day after the adaptation period, the mice were weighed and given noni leaf extract according to their respective doses: the third group received a dose of EDM 100 mg/200 g BW, the fourth group received a dose of EDM 200 mg/200 g BW, and the fifth group received a dose of EDM 300 mg/200 g BW. They were then re-incubated for 20 days. On day 21, mice in groups of three to five were infected with an *E. coli* bacterial suspension injected thru the tail. On day 25, the mice were dissected and blood was taken to make serum for hemagglutination testing.

The prepared serum was then used for hemagglutination testing. Eight series of serological tubes were prepared, seven tubes for testing and one tube as an erythrocyte control. The dilution series were 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, and the last tube was the erythrocyte control. To the first tube, 300 µL of

Physiological NaCl was added; tubes two thru seven were filled with 200 µL of Physiological NaCl, and tube eight was filled with 400 µL of Physiological NaCl. To the first tube, 100 µL of mouse serum was added, and the mixture was vortexed until homogeneous. 200 µL was taken from the first tube and transferred to the second tube, which was then vortexed until homogeneous. Such treatment continued until the seventh tube. From tube seven, 200 µL was taken and discarded. Added 200 µL of physiological NaCl to tubes one thru eight. Added 400 µL of 0,5% erythrocyte suspension to all tubes, and homogenized. Stored in an incubator at 37°C for 45 minutes. Then hemagglutination was observed visually by the formation of ring-like circles at the bottom of the tube.

The data collected is the highest dilution that still produces hemagglutination results, also known as the titer. These titer results will be transformed into titer numbers using the formula [2 Log(titer) +1] for data analysis..

RESULTS AND DISCUSSION

The purpose of testing the Immunoglobulin M (IgM) activity of noni leaf ethanol extract is to determine the ability of noni leaf extract as an immunomodulator caused by the compounds contained in the noni leaf, by observing the agglutination of mouse blood against the dilution levels that occur in the hemagglutination test, and then calculating the dilution titer.

The process of extracting noni leaves with the polar solvent absolute ethanol 96% aims to extract the substances contained in noni leaves that can enhance antibody activity, namely flavonoids. Phytochemical test results proved that noni leaves contain flavonoids that can be used as an immunomodulator. The extraction results were administered orally to mice according to the dosage for each group on the eighth day after a seven-day adaptation period for the mice. The volume of mengkudu leaf extract administered to the mice varied according to each mouse's body weight, but with the same dose concentration. After being given the noni leaf extract, the mice will be incubated for 20 days, with the aim of the noni leaf extract increasing antibody activity in the mice's bodies. On day 21, the mice were infected with *E. coli*, whose presence had been previously confirmed by the IMViC test. This infection process aimed to produce IgM in the mice's bodies, which had been previously enhanced with noni leaf extract. When the body is infected, it responds by producing antibodies. On day 25, the mice were dissected and their blood was drawn to make serum as needed for the hemagglutination test. Immunoglobulin M (IgM) measurements are taken within a timeframe of 5 to 7 days. When an antigen first enters the body, the antibodies released within 5-7 days are IgM. During this time, IgM is at its peak in the blood compared to other antibodies.

The hemagglutination method test is the binding that occurs between 1% goat red blood cells (GRBC 1%), which in this study were replaced with 0,5% O blood group red blood cell suspension as the antigen with the antibody, causing visible agglutination or clumping. Hemagglutination occurs due to cross-linking between red blood cells and antibodies. The antibody that binds to 0,5% red blood cells is immunoglobulin M (IgM). Determining the hemagglutination antibody titer aims to establish the response humoral against 0,5% red blood cells, as evidenced by an increase in mouse antibody titers.

The results of hemagglutination can be seen visually. Here is a graph of the average antibody titer in mice from Figure 1. Based on Figure 1, it can be seen that as the dose of noni leaf extract increases, the resulting agglutination titer also increases, which proves a better increase in immunoglobulin M (IgM) activity. The immunoglobulin M (IgM) titer from the blood serum of mice given noni leaf extract (*Morinda citrifolia L.*) experienced the highest increase at a dose of 300 mg/200 g body weight in the first and second repetitions. However, in the fourth repetition, there was a drastic decrease in titer compared to the other repetitions.

This is because the immune reaction is highly determined by the balance between the amount of antigen and antibody. When the amount of antigen or antibody is imbalanced (one is excessive), the immune reaction will be disrupted.

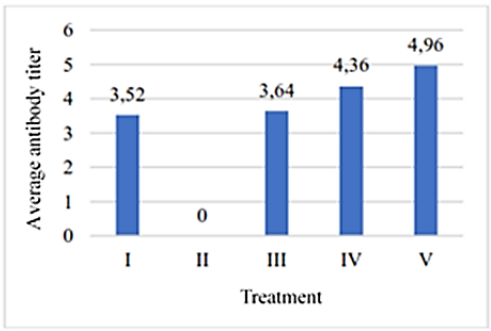

Figure 1. Effect of Noni Leaf Extract Concentration on Antibody Titer

CONCLUSIONS

Based on the research conducted on the sample and hypothesis testing using one-way analysis of variance (one-way Anova) and Duncan's post hoc test, it can be concluded that noni leaf extract can be an immunomodulator or increase antibody activity, with the optimal dose of noni leaf extract as an immunomodulator being 300 mg/200 g body weight.

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