

Effect Of *Aegle marmelos* on IgM And IgG Antibody Titers in *Rattus norvegicus*

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ABSTRACT

Aegle marmelos is a traditional plant with many therapeutic benefits; for example, it is used as an immunomodulatory agent. Literature studies reveal many functional and bioactive compounds, such as alkaloids, phenolics, coumarins, flavonoids, terpenoids, and other antioxidants. Based on its chemical content, *Aegle marmelos* could be an immunomodulatory agent because it can stimulate the production of antibodies against foreign antigens, increasing IgM and IgG. This study aims to determine the effect of *Aegle marmelos* filtrate on IgM and IgG titers in *Rattus norvegicus* induced (sheep red blood cells) SRBC 2%. In this study, 30 rats were divided into five groups: negative control received standard diets, positive control received SRBC 2% injection, P1 received SRBC 2% + *Aegle marmelos* filtrate 25%, P2 received SRBC 2% + *Aegle marmelos* filtrate 50%, and P3 received SRBC 2% + *Aegle marmelos* filtrate 75%. The results showed that administration of *Aegle marmelos* filtrate could increase IgM titers at the highest dose of 75%, although it was not statistically significant. Meanwhile, *Aegle marmelos* filtrate does not affect IgG titers in SRBC 2% compared to treatment groups.

Keywords: *Aegle marmelos*; Immunoglobulin M; Immunoglobulin G.

ABSTRAK

Aegle marmelos merupakan salah satu tanaman tradisional yang memiliki banyak manfaat terapeutik salah satunya sebagai agen imunomodulator. Studi literatur mengungkapkan banyak senyawa fungsional dan bioaktif seperti alkaloid, fenolik, kumarin, flavonoid, terpenoid dan antioksidan lainnya. Berdasarkan kandungan kimianya ada kemungkinan *Aegle marmelos* dapat menjadi agen imunomodulator yang baik karena dapat meningkatkan produksi antibodi untuk melawan antigen asing sehingga terjadi peningkatan pada IgM dan IgG. Penelitian ini bertujuan untuk mengetahui pengaruh pemberian filtrat *Aegle marmelos* pada *Rattus norvegicus* terhadap titer IgM dan IgG. Dalam penelitian ini digunakan sebanyak 30 tikus yang dibagi kedalam 5 kelompok yakni kontrol negatif mendapat pakan standart, kontrol positif mendapat injeksi sel darah merah domba (SDMD) 2%, P1 mendapatkan SDMD 2% dan filtrat *A.marmelos* 25%, P2 mendapatkan SRBC2% dan filtrat *A.marmelos* 50% dan P3 mendapatkan SDMD 2% dan filtrat

A.marmelos 75%. Hasil penelitian menunjukkan bahwa pemberian filtrat *Aegle marmelos* pada *Rattus norvegicus* berpengaruh dalam meningkatkan titer IgM pada dosis tertinggi yaitu 75% meskipun secara statistik tidak signifikan. Sedangkan, tidak ada pengaruh pemberian filtrat *Aegle marmelos* pada *Rattus norvegicus* terhadap titer IgG pada kelompok yang diberi SDMD 2% dengan kelompok perlakuan.

Kata Kunci: *Aegle marmelos*; Immunoglobulin M; Immunoglobulin G

INTRODUCTION

The immune system was first discovered because of the desire to prevent the spread of disease and develop better treatments for the sick (McComb *et al.*, 2019). The first antibody formed in the primary immune response is Immunoglobulin M (IgM), produced when the antigen first enters the body. IgM will appear on the fifth day of infection. However, follicular B cells undergo isotype switching upon antigen exposure, producing IgG (Sathe & Cusick, 2022). Antibody production can be seen by examining agglutination obtained from red blood cells taken from various components of microorganisms, such as bacterial or animal proteins, using the hemagglutination method, which is the clumping of red blood cells caused by antibodies to antigens on the surface of red blood cells (Agustina *et al.*, 2022). Disease manifestations due to pathogens depend on the ability of the immune system to achieve disease resistance. An alternative that can be given to optimize the work of the immune system

is to use herbal plants (Paul *et al.*, 2020). Several studies have revealed the beneficial application of herbs as natural additives, which have the potential to induce immune responses and increase resistance to disease or pathogen infection (Wangkahart *et al.*, 2022). The efficacy of herbal plants in helping to prevent various diseases has long been studied globally, but information related to how strong the research evidence regarding the potential of herbal plants in improving the body's immune system is still limited (Suciady *et al.*, 2021).

Aegle marmelos (maja plant) comes from the Rutaceae family, one of the traditional and nutrient-rich plants. This plant has many therapeutic benefits, one of which is as an immunomodulatory agent (Savita *et al.*, 2021). Literature studies reveal the presence of many functional and bioactive compounds, such as carotenoids, phenolics, alkaloids, coumarins, flavonoids, terpenoids, and other antioxidants in *Aegle marmelos* fruit extracts. Based on its

chemical content profile, *Aegle marmelos* may be a good source of immunomodulatory agents. However, few biological studies show the plant's immunostimulatory role. Previous studies by Patel & Asdaq (2010) showed that the methanol extract of *Aegle marmelos* fruit has immunomodulatory activity in experimental mice models of cellular and humoral immunity (Govinda & Asdaq, 2011; Patel & Asdaq, 2010). However, previous studies were limited to hemagglutination testing at only one time, 14 days after administering *Aegle marmelos* ethanol extract, to determine the ability to form antibodies. So, the novelty of this study is that researchers injected SRBC 2% twice to see the ability of *Aegle marmelos* filtrate to stimulate IgM production as the first antigen response and IgG production as the second antigen response.

METHODS

The study is true experimental with Post-Test Only Control Group Design. *Rattus norvegicus* has obtained ethical permission from the Ethical Feasibility of Research and Health Faculty of Dentistry, Universitas Airlangga (No.538/HRECC.FODM/V/2023).

Tools and Materials

The equipment used in this study included measuring pipettes, micropipettes, blue tips,

yellow tips, test tubes, 1mL and 3mL syringes, beaker glass, incubator, centrifuge, test tube rack, analytical balance, blender, sterile gauze, measuring cups, animal scales, and Petri dishes. The materials used in this study are phosphate buffer saline (PBS) solution pH 7, EDTA 4%, *A. marmelos* fruit filtrate, *Rattus norvegicus*, distilled water, 70% alcohol, cotton, plaster, and SRBC 2%.

Preparation of 25%, 50%, and 75% *A. marmelos* Filtrate

Aegle marmelos used in this study were obtained at Jalan Parengan Pinggir Kali, RT 18 RW 04 Kraton Krian Village Sidoarjo, and have gone through the process of being determined by UPT Laboratorium Herbal Materia Medika Batu (No. 067/260/102.20/2023). *A. marmelos* filtrate is prepared by pureeing the clean pulp using a blender. Then, filtering is done to obtain the fruit filtrate. The concentration to be made is 25% by mixing 25 mL of filtrate with 75 mL of distilled water, 50% by mixing 50 mL of filtrate with 50 mL of distilled water, 75% by mixing 75 mL of filtrate with 25 mL of distilled water.

Treatment of Animal Tests

Male *Rattus norvegicus* Wistar strain species weighing 150-200 grams that have been adapted for one week. A total of 30 white rats were grouped into five groups,

each group consisting of 6 rats. The first group was a negative control (K-), which was not treated only given standard feed. As a positive control (K+), the second group was only given an intraperitoneal injection of 2% sheep red blood cells (SRBC 2%). The third to fifth groups (P1, P2, and P3) were the treatment groups given SRBC 2% on the first day. The next day, *A. marmelos* filtrate with different concentration variations of 25%, 50%, and 75%, respectively. One week after administering *A. marmelos* filtrate, rat blood was taken to check the IgM titer using the hemagglutination method. The injection of SRBC 2% was again carried out in the rats of the positive control group, the third to fifth groups with the same treatment as before. Five days after the second SRBC 2% injection, blood was retaken for examination of IgG titer using the hemagglutination method.

Hemagglutination Test

The serum that has been obtained is heated in a water bath at 56°C for 30 minutes. Serum dilution is done in multiples of two, namely $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, $\frac{1}{256}$, $\frac{1}{512}$, $\frac{1}{1024}$ and so on. The diluted serum is then incubated for one hour at room temperature, and hemagglutination will be formed. The observation is based on the spread of SRBC

at the bottom of the tube, which can be seen uprightly.

Data Analysis

Titer data was statistically processed using the Kruskal Wallis test After being transformed with $2 \log(\text{titer})+1$ (Indrisari, 2018).

RESULTS AND DISCUSSION

IgM Titer Test Result

The examination of IgM titer by hemagglutination method carried out on day six after injection of SRBC 2% and administration of *Aegle marmelos* filtrate aims to determine whether the administration of filtrate from *Aegle marmelos* fruit can trigger the production of IgM antibody titer. The results of the calculation of IgM titer after being transformed in $2 \log(\text{titer})+1$ show that in the negative control group is 0, no IgM is formed during this study. The mean IgM titer in the positive control group was 2.49; the 25% filtrate group was 1.65; the 50% filtrate group was 1.88; and the 75% filtrate group was 2.85. The distribution of IgM titer

of each sample after transformation is shown in Figure 1.

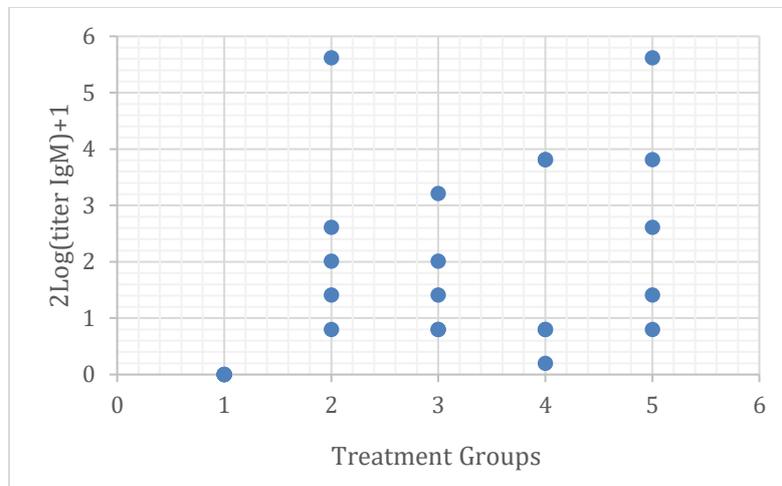


Figure 1. Distribution of converted hemagglutination titers (IgM) in all groups.

Treatment group 1 = negative control; 2 = positive control; 3 = 25% filtrate; 4 = 50% filtrate; 5 = 75% filtrate.

The results of the Kruskal-Wallis test obtained a p-value of $0.012 < 0.05$, which means that there is an effect of giving *Aegle marmelos* filtrate on the production of IgM antibody titers as evidenced by the formation of a higher hemagglutination titer in the group given the filtrate with the negative control group which showed negative results in the hemagglutination test. Mann-Whitney further test showed a significant difference ($p < 0.05$) in group K- with K+, P1, P2, and P3. However, there was no difference between the K+ and all treatment groups ($p > 0.05$).

Perkasa *et al.* (2016) indicate that IgM measurement is done within 5 to 7 days. Within this period, IgM is maximized in the

blood compared to other antibodies. In line with this study, IgM titers were formed in the K+ and treatment groups compared to the K- groups. Although statistically insignificant, IgM production was found to be the highest in the P3 group, as evidenced by an increase in IgM titer of 0.36 (14.4%) compared to the K+ group; this indicates that the administration of *Aegle marmelos* filtrate at a dose of 75% can stimulate the formation of IgM antibody titers in *Rattus norvegicus*.

The group with a 25% filtrate dose had a lower mean IgM titer than the other treatment groups. It could be due to the filtrate's lack of ability to trigger IgM production after the first infection.

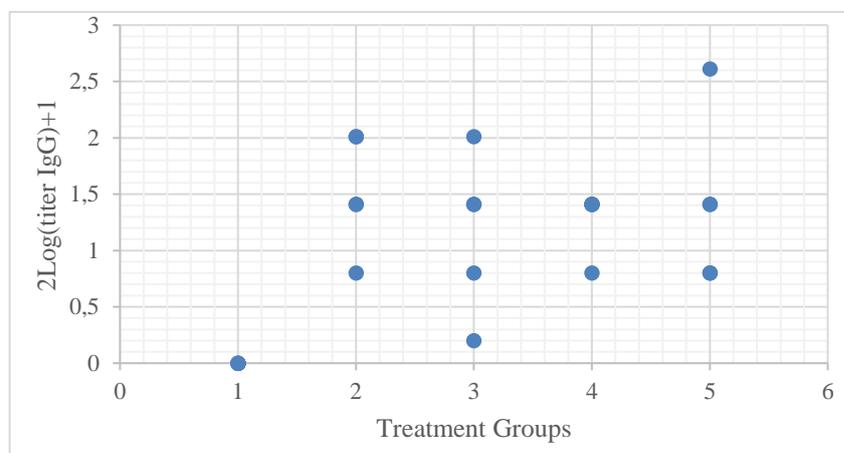
However, no adverse reaction occurred in the low-dose group. The highest IgM titer production in the filtrate dosing with a concentration of 75% is likely due to the highest the dose is given, the more it will trigger antibody production so that the bond between the antigen and antibody is also higher (Perkasa et al., 2016). According to Savita *et al.*, (2021) there is flavonoid content in *Aegle marmelos*. Flavonoid content can increase the activity of T helper cells, which have immunomodulatory activity against the specific immune system (Indrisari, 2018). Researchers argue that the higher the dose of filtrate given, the higher the flavonoid content, which is directly proportional to its immunomodulatory activity.

IgG Titer Test Result

IgG titer examination was carried out on day 11 by Hemagglutination method after being injected with the second SRBC 2% and given *Aegle marmelos* filtrate. This test aims to see whether *Aegle marmelos* filtrate can trigger the production of IgG antibody titers in secondary infections. The results of the calculation of IgG titer after being transformed in $2 \log(\text{titer})+1$ showed that in the negative control group, it was 0. The mean IgG titer in the positive control group was 1.52; the 25% filtrate group was 1.16; the 50% filtrate group was 1.28; and the 75% filtrate group was 1.41. The distribution of the IgG titer of each sample after transformation is shown in Figure 2.

Image 2. Distribution of converted hemagglutination titers (IgG) in all groups.

Treatment group 1 = negative control; 2 = positive control; 3 = 25% filtrate; 4 = 50% filtrate; 5 = 75% filtrate.



The Kruskal-Wallis test results obtained a p-value of $0.010 < 0.05$, which means that giving *Aegle marmelos* filtrate affects the production of IgG antibody titers. Mann-Whitney further test showed a significant difference ($p < 0.05$) in group K- with K+, P1, P2, and P3. However, there was no difference between the K+ and all treatment groups ($p > 0.05$).

In testing the IgG titer, the K+ group still has the highest mean antibody titer compared to the treatment groups. It occurs because the K+ group has produced more elevated antibodies in the primary immune response, and antibodies continue to fight secondary infections due to second exposure to SRBC (Restuati & Gultom, 2013). The inability of *Aegle marmelos* filtrate to increase IgG production in groups P1, P2, and P3 could be due to secondary metabolites, such as terpenoids, that inhibit lymphocyte proliferation. In vivo studies show that the administration of limonene contained in terpenoids can suppress the production of Th1 and Th2 mediated by CD4+ and CD8+ T cells (Hikmah & Triastuti, 2022; Lappas & Lappas, 2012). The Th1 subset response induces activated macrophages and IgG class turnover (Breyscher, 2014). Researchers suspect that this terpenoid content causes the administration of *Aegle marmelos* filtrate in groups P1, P2, and P3

to be unable to increase IgG production due to the suppression of the immune system (immunosuppressant). However, the shortcoming of this study is that reuse should have tested not test the content of secondary metabolites contained in *Aegle marmelos* fruit.

In addition, technical errors during the study may inevitably lead to misinterpretation. The researcher believes that the lack of secondary immune response is due to the reduced antigenic properties of the SRBC used. In this study, the SRBC used was SRBC that had been stored in the freezer for more than ten days. The researcher's assumption is also reinforced by Oktari & Mulyati's research (2022) that red blood cells are best used when they do not pass through a storage period of more than four days because it can cause contamination so that there will be two populations of antigens, namely antigens contained in the blood and foreign antigens that stimulate antigen-antibody reactions on the part of antibodies contained in the sample against foreign antigens so that mixed reactions occur can cause false negative and false positive responses (Oktari & Mulyati, 2022).

CONCLUSION

The administration of *Aegle marmelos* filtrate to *Rattus norvegicus* affected the

production of IgM titers at the highest dose of 75%, although it was not statistically significant. Further research is needed to determine the immunomodulatory effects of *Aegle marmelos* on long-term use.

REFERENCES

- Agustina, R., Iswahyudi, I., Samsul, E., Febrianie, N. L., Prabowo, W. C., Arifuddin, M., Kuncoro, H., Narsa, A. C., Azminah, A., Alam, G., Mun'im, A., & Ahmad, I. (2022). Aktivitas Hemaglutinasi dan Identifikasi Ekstrak Berbasis Green Solvent dari Akar Tapak Dara (*Vinca rosea*) dan Daun Kadamba (*Mitragyna speciosa* Korth. Havil) Menggunakan FTIR-Kemometrik. *Jurnal Sains Dan Kesehatan*, 4(5), 511–522.
- Breyscher, P. A. (2014). On the Mechanism Determining the Th1/Th2 Phenotype of an Immune Response, and its Pertinence to Strategies for the Prevention, and Treatment, of Certain Infectious Diseases. *Scand J Immunol*, 6(79), 361–376.
- Govinda, H. V., & Asdaq, S. M. B. (2011). Immunomodulatory potential of methanol extract of *Aegle marmelos* in animals. *Indian Journal of Pharmaceutical Sciences*, 73(2), 235–240.
- Hikmah, U., & Triastuti, A. (2022). Mechanism and Immunomodulator bioactive Compounds of *Phyllanthus niruri* (meniran). *Jurnal Ilmiah Farmasi*, 2(18), 205–218.
- Indrisari, M. (2018). Uji Efek Ekstrak Etanol Daun Jarak Pagar (*Jatropha curcas* L.) Terhadap Titer Immunoglobulin M (IgM) Dan Immunoglobulin G (IgG) Pada Tikus Putih Jantan (*Rattus norvegicus*). *Akademi Kebangsaan Farmasi*.
- Lappas, C., & Lappas, N. (2012). D-Limonene modulates T lymphocyte activity and viability. *Cell Immunology*, 1(279), 30–41.
- McComb, S., Thiriot, A., Akache, B., Krishnan, L., & Stark, F. (2019). Introduction to Immune System. In *Immunoproteomics: Methods and Protocols, Methods in Molecular Biology* (pp. 1–24). Springer.
- Oktari, A., & Mulyati, L. (2022). Pengaruh Waktu Dan Suhu Penyimpanan Sampel Darah Terhadap Hasil Pemeriksaan Uji Silang Serasi (Cross Match). *Journal of*

- Indonesian Medical Laboratory and Science (JoIMedLabS)*, 3(2), 133–145.
- Patel, P., & Asdaq, S. M. B. (2010). Immunomodulatory activity of methanolic fruit extract of *Aegle marmelos* in experimental animals. *Saudi Pharmaceutical Journal*, 18(3), 161–165.
- Paul, S., Hmar, E. B., & Sharma, H. K. (2020). Strengthening Immunity with Immunostimulants: A Review. *Current Trend in Pharmaceutical Research*, 7(1), 35–63.
- Perkasa, I. F. R., Fitriani, V. Y., & Masruhim, M. A. (2016). Aktivitas Immunoglobulin M (IgM) ekstrak buah mengkudu (*Morinda citrifolia* L.) terhadap tikus putih (*Rattus Norvegiens*). *Jurnal Sains Dan Kesehatan*, 1(6), 321–326.
- Restuati, M., & Gultom, E. S. (2013). Pengaruh Ekstrak Daun Sirsak (*Annona muricata*) Sebagai Immunostimulan Terhadap Titer Antibodi dan Leukosit Pada Tikus Putih (*Rattus norvegicus*). *JurnalSiantika*, 3(11), 119–125.
- Sathe, A., & Cusick, J. K. (2022). *Biochemistry, Immunoglobulin M*. StatPearls Publishing.
- Savita, , Singh, A. P., & Singh, A. P. (2021). *Aegle marmelos* (L.) (Bael): A Systematic Review. *Journal of Drug Delivery and Therapeutics*, 11(3-S), 131–136.
- Suciady, M., Meiliana, M., & Hendryanti, D. N. (2021). Studi Literatur: Potensi Tanaman Herbal Indonesia sebagai Immunostimulan dan Anti-stress untuk Pencegahan Covid-19 Berbasis Evidence-based Analysis. *Praxis : Jurnal Sains, Teknologi, Masyarakat Dan Jejaring*, 4(1), 90.
- Wangkahart, E., Wachiraamonloes, S., Lee, P.-T., Subramani, P. A., Qi, Z., & Wang, B. (2022). Impacts of *Aegle marmelos* fruit extract as a medicinal herb on growth performance, antioxidant and immune responses, digestive enzymes, and disease resistance against *Streptococcus agalactiae* in Nile tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology*, 120, 402–410.