Test Of the Effectiveness of Moringa (Moringa Oleifera) Leaves on The Number of Leukocytes and Platelets of Male Wistar White Rats (Rattus Norvegicus) Infected with Escherichia Coli

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Abstracts: The research entitled Testing the Effectiveness of Moringa Leaves on the Number of Leukocytes and Platelets of Male Wistar White Rats infected with E.coli, used an experimental design with a Pre Test-Post Test Control Group Design. Where research carries out observations of the control and treatment groups before and after being given a treatment. The research used a purposive sampling technique. The subjects in this study were male white Wistar rats. The number of mice used was 27, which were divided into 3 groups. Data analysis used the Shapiro-Wilk test and One-way Anova test. The results showed that the average leukocyte count of white mice in the control group (K1) was 26.73x103/µL (pre test). Then after treatment (only standard feed) it was 22.98x103/µL (post test). In group (P1), the leukocyte count was 21.38x103/µL (pre test) and after administering the extract at a dose of 150 mg/KgBW/day, the leukocyte count was 16.03x103/µL (post test), for (P2) 20.63x103/µL (pre test), after administering 250 mg/KgBW/day of extract, there was a decrease in the average number of white rat leukocytes and (P2) 13.72x103/µL (post test). The results of examination of the number of platelets (K1) infected with Escherichia coli bacteria and only given standard feed averaged 646.56x103/µL (pre test) and 954.103/ µL (post test). For group P1, the platelet count was 595.6x103/µL (pre test) and after administering Moringa leaf extract at a dose of 150 mg/KgBW/day, the platelet count was 858.8x103/µL (post test). For group P2, the platelet count was 637.1x103/ µL (pre test) and after administering Moringa leaf extract at a dose of 250 mg/KgBW/day 1029.2x103/ µL (post test). The results of the ANOVA test showed that there was a significant difference between the average leukocyte count and platelet count between the groups tested.

Keywords: Moringa Oleifera, Leukocyte Count, Platelet Count, Rattus Norvegicus, Escherichia Coli.

1. INTRODUCTION

Infectious disease is a condition where microorganisms enter the body and can reproduce, and which can cause disease. Infectious diseases are found in many tropical areas, one of which is Indonesia. Escherichia coli (E.coli) is one type of bacteria that is most often found in several bacterial infections. Where E. coli can cause infectious diseases that are often found among the public. E. coli bacteria is a gram-negative bacteria that can reproduce in the body and become normal bacteria in the intestines, but if in abnormal conditions it is pathogenic such as diarrhea, urinary tract infections, pneumonia, wound infections, especially in the abdomen, and meningitis. (Suwito and Andriani, 2018). The existence of E. coli bacteria is often associated with contamination originating from feces, because E. coli is generally a bacteria that lives in the intestines of humans and animals, so the presence of this bacteria in water or food indicates that there is a processing process that has come into contact with it. feces (Rahayu, Nurjanah, and Komalasari, 2018).

A condition that develops as a result of infection can cause abnormalities in various organ systems in the body, one of which is the hematological system. This hematological system functions to distribute nutrients, oxygen and other substances to all organs, so that the body's organs can carry out their functions properly. If hematological function is disturbed, problems will occur in other organs. The pathogenic activity of Escherichia coli is able to lyse the intestinal mucosal wall which can result in bleeding which will affect platelet, leukocyte and erythrocyte cells. Anemia, leukocytosis, leukopenia, and thrombocytopenia are the most common abnormalities that occur in the hematological system during infection (Sari, Wihastuti, and Ardiansyah, 2018)

To minimize the proliferation of E. coli, plants can be provided which have various health benefits. In Indonesia, there are many plants that provide many health benefits, but not many people know and understand the various benefits of these plants. One of these plants is the Moringa plant. Moringa is a traditional plant that is beneficial for

the community. Moringa (Moringa oleifera) is a herbal plant that is often found among the public, especially in tropical areas, namely in Indonesia. This plant can grow in humid tropical areas or hot dry land, and can survive in less fertile and dry soil. This Moringa plant has various benefits. The part of the Moringa plant that has many benefits is in the leaves. One of the benefits of the Moringa leaf plant is that it is antibacterial. Moringa leaves also have high levels of antioxidants which can be beneficial for health (Tjong, Assa, and Purwanto, 2021). Indonesian people, especially rural residents, have long used moringa as a traditional medicine (Salimi et al., 2019). The existence of the Moringa plant is very easy to find throughout Indonesia (Britany and Sumarni, 2020)

Moringa leaves contain flavonoids, phenols, tannins, saponins, alkaloids (Naufizdihar, Adji, and Billyana, 2022). Moringa leaves have various health benefits which can be used as an antibacterial, infectious disease, preventing ulcers, inflammation, pain, increasing immunity, and also speeding up the wound healing process (Kursia, Aksa, and Nolo, 2018). Apart from that, Moringa leaves can also provide health effects in the form of preventing heart disease, healthy eyes, treating rheumatism, treating internal diseases such as kidney stones and treating cancer (Isnan, 2017).

In Rida et al's research on Moringa leaves as an antibacterial against E. coli bacterial infections, it was explained that Moringa leaf extract has antibacterial activity against the growth of E. Coli (Emelia, Safitri, and Andriyani, 2020). Astawan et al.'s research on the hematological features of white mice (Rattus norvegicus) infected with E. coli showed that there was an increase in the number of leukocytes and a decrease in the number of platelets due to E. coli (Astawan, 2011). Yesi et al.'s research on the effect of giving Moringa leaf extract on hemoglobin levels in male Wistar white rats revealed that there was an influence on hemoglobin levels in rats given Moringa leaf extract (Nurmalasari et al., 2021).

Based on the data above, researchers are interested in conducting research on testing the effectiveness of Moringa oleifera leaf extract on the number of leukocytes and platelets of male white Wistar rats (Rattus norvegicus) infected with Escherichia coli.

2. RESEARCH METHODOLOGY

2.1. Research Design

This research uses an experimental design with Pre-Test- Post Test Control Group Design. Where research carries out observations of the control and treatment groups before and after being given an action.

2.2. Place and Time of Research

This research will be carried out at the Pharmacy Laboratory of the University of North Sumatra. The research will be carried out starting from making the extract, maintaining it to providing treatment to the test animals, then checking the number of leukocytes and platelets, before and after completing the Moringa oliefera (Moringa oliefera) leaf extract treatment. The research will be carried out in May - August 2023

2.3. Research Population

Wistar male white rat (Rattus norvegicus)

2.4. Sample and Determination of Sample Number

1. Sample

White mice that meet the inclusion criteria

2. Sample Size Estimation

Determination of sample size was carried out using the Federer formula:

$$(t-1)\,(n-1) \geq 15$$

Note :

t = Treatment group

n = number of samples for 1 treatment group

$$(t-1)\,(n-1) \geq 15$$

$$(3-1)\,(n-1) \geq 15$$

$$2\,(n-1) \geq 15$$

$$2n - 2 \geq 15$$

$$n \geq 8{,}5 = 9$$

$$n \geq 9$$

$$\text{Sample size} = t \times n$$

$$= 3 \times 9$$

$$= 27 \text{ rats}$$

Correction sample to anticipate 10% drop out/death, the formula is used: n' = n/(1-f)

n’ = sampel koreksi

n = jumlah sampel minimal

f = perkiraan proporsi drop out 10% (0,1)

n’ = 27/ (1-0,1) = 30

Number of spare mice = number of treatment groups x (n'-n)

= 3 x (30 – 27) = 9 mice

The total of 27 mice used were divided into 3 groups, with a reserve of 3 mice for each group, namely given Moringa oleifera leaf extract at a dose of 150 mg/kgBW, 250 mg/kgBW and those only infected with Escherichia coli (as control).

2.5. Determination of the Number of Rat Samples

In this research the author used a purposive sampling technique. The subjects in this study were male white Wistar rats. The number of mice used was 27, which were divided into 3 groups. The groups are as follows:

1. The negative control group was infected with Escherichia coli by providing standard feed

2. Treatment group 1 (P1) was infected with Escherichia coli by administering 150 mg/kgBW of Moringa leaf extract.

3. Treatment group 2 (P2) was infected with Escherichia coli by administering 250 mg/kgBW of Moringa leaf extract.

2.6. Inclusion and Drop Out Criteria

1. Inclusion Criteria

- Male white rat Wistar strain (Rattus norvegicus) with a body weight of 150-250 grams

- Mice aged 2-3 months

- There are no anatomical abnormalities

2. Drop Out Criteria

Mice that looked sick, did not move actively, died during treatment

2.7. Tools and Materials

1. Tool

The tools that will be used in this research include a hematology analyzer, measuring cup, blender, rag, container, stirring rod, rotary vacuum evaporator, funnel, filter paper, dropper pipette, test tube, tube, erlenmeyer, test tube rack. , syringes, oral sondes, container boxes, marking labels, digital scales.

2. Material

Moringa leaves (Moringa oleifera), 96% ethanol, Escherichia coli bacteria

2.8. Work Procedures

To get a clear picture of how this research works, it is as follows:

1. Researchers ask for permission by arranging ethical clearance submitted to the educational institution, Faculty of Medicine, HKBP Nommensen University, Medan.

2. Researchers also ask for permission to apply for research in the laboratory which will be submitted to the educational institution, Faculty of Medicine, HKBP Nommensen University, Medan.

3. Preparation of test animals

The animals were adapted for 7 days in the animal house. The 27 test animals used were then grouped into 3 groups. Each group consists of 9 who will then be placed in one cage.

4. Making moringa leaf extract (Moringa oleifera)

Moringa (Moringa oleifera) leaf extract is made using the maceration method using 96% ethanol solvent. Moringa leaves are cleaned of dirt, washed with running water until clean, then sliced thinly and then dried in the sun to dry. Once dry, the Moringa leaves will be ground into powder using a blender. Then the fine Moringa leaves

are macerated by mixing 96% ethanol into a vessel, then tightly closed and left for 3 days, stirring occasionally. Next, the solution from the Moringa leaves is filtered to obtain the filtrate and dregs. Next, the remaining dregs will be soaked again in new ethanol. After that, it will be filtered using a funnel and filter paper and collected. After that, all the filtrate that has been collected will be put into a rotary vacuum evaporator at a temperature of 40℃, then the remaining filtrate is evaporated in a water bath to obtain a thick extract (Jusnita and Syurya, 2019) In this extract a concentration of 100% is used.

5. Preparation of Escherichia coli suspension

Making test bacterial suspensions is done by taking E. coli bacteria using a sterile tube, then placing them in a sterile Nutrient Broth and then incubating at a temperature of 37℃ for 24 hours (Rosmania and Yuniar, 2021).

6. Taking blood samples and reading the results

The male white Wistar rat will be held by its body and then the rat's tail will be stabbed using a syringe to get a blood sample. The blood obtained will be put into a test tube and then the blood that has been obtained will be examined with a hematology analyzer in the laboratory to read the results.

7. Experiment

Step I: 27 mice were used which were divided into 3 groups where each group consisted of 9 mice. 1 control group and 2 treatment groups.

Step II: The mice will be adapted for 7 days and given food and drink before treatment

1) Step III: all groups of mice were infected with Escherichia coli bacteria using an oral probe and left for 4 days until they became infected.

2) Step IV: taking mouse blood to check the number of mouse leukocytes and platelets (pre-test).

3) Step V: Moringa leaf extract (Moringa oleifera) was given to treatment group 1 (P1) at a dose of 150 ml/kgBW/day and treatment group 2 (P2) at a dose of 250 ml/kgBW/day for 7 days.

4) Step VI: after 7 days of treatment, the number of rat leukocytes and platelets was checked (post-test).

3. DATA ANALYSIS

The data resulting from this research were analyzed using a computer. The data were analyzed using a normality test using the Shapiro-Wilk test, then using the One-way Anova test to determine the average difference between the group of mice given Moringa leaf extract at a dose of 150 ml, the group of mice given Moringa leaf extract at a dose of 250 ml and the negative control. which was not given Moringa leaf extract.

4. RESULTS AND DISCUSSION

4.1. Research Result

In this study, the research samples were male white rats of the Wistar strain (Rattus norvegicus) infected with Escherichia coli. There were 27 rats used, male white Wistar rats, which were divided into 3 groups, each group consisting of 9 rats. 1 control group and 2 treatment groups.

The groups are as follows:

The negative control group (K1) was infected with Escherichia coli with standard feeding

Treatment group 1 (P1) was infected with Escherichia coli by administering 150 mg/kgBW of Moringa leaf extract.

Treatment group 2 (P2) was infected with Escherichia coli by administering 250 mg/kgBW of Moringa leaf extract.

The duration of treatment for the samples was up to 18 days by taking blood from mice to check the number of leukocytes and platelets (pre test and post test).

a. Validity test

Validity testing in research is a step to ensure that the instruments or methods used in research measure concepts or variables accurately and precisely. In research on the effectiveness test of Moringa leaves (Moringa oleifera) on the number of leukocytes and number of platelets of male Wistar white rats (Rattus norvegicus) infected with Escherichia coli, to ensure that the results of the research are actual phenomena and the results can be used as a reference for further research related to the research. about leukocyte count and platelet count.

Table 1. Mouse Leukocyte Count

Rata- rata Pengukuran Jumlah Lekosit Tikus Putih								
PRE TEST								
	K1			**P1**			**P2**	
MIN	21.12			15.17			13.15	
MAX		32.00			27.00			24.13
R	26.73			21.38			20.63	
POST TEST								
MIN	16.62			16.42			10.74	
MAX		27.87			20.96			20.04
R	22.98			16.03			13.72	

In table 1 it can be seen that the average leukocyte count of white mice in group K1 was higher than groups P1 and P2 after being infected with E. coli, however after treatment there was a decrease in the number of leukocytes, but still above normal values in the three groups. Normal value of white Wistar rat leukocyte count: 2,000 - 10,000/µL (Astawan et al., 2011)

The average leukocyte count of white mice in the control group (K1) was 26.73x103/µL (pre test). Then after treatment (only standard feed) it was 22.98x103/µL (post test). There was a decrease in the number of leukocytes in the control group (K1). In groups P1 and P2 before treatment with Moringa leaf extract, it was found that (P1) 21.38x103/µL and (P2) 20.63x103/µL (pre test), after giving the extract there was a decrease in the average number of white rat leukocytes (P1) 16.03x103/ µL and (P2) 13.72x103/µL (post test). The highest reduction was seen in group P2 (extract dose 250 ml/kgBW/day).

Tabel 2. Sample Normality Test

Case Processing Summary						
	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Pre	9	100.0%	0	0.0%	9	100.0%
Past	9	100.0%	0	0.0%	9	100.0%

The data in table 2 above shows the results of the normality test analysis using the Shapiro-Wilk test for two different groups of data, namely pre-test and post-test.

The results of the Shapiro-Wilk test show that all data in the pre-test and post-test groups have a normal distribution considering that the p value is less than 0.05. This indicates that the data in the two groups is normally distributed.

Next, to find out the average difference between groups of mice that were given Moringa leaf extract at a dose of 150 ml, groups of mice that were given Moringa leaf extract at a dose of 250 ml and negative controls that were not given Moringa leaf extract. So the One-way Anova test was carried out

Table 3. Anova test for mouse leukocytes

Uji *One-way ANOVA* Total of leukocyte					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	223.535	2	111.768	8.557	.001
Within Groups	352.644	27	13.061		
Total	576.179	29			

Based on table 3, the results of the ANOVA test show that there is a significant difference between the average number of leukocytes between the groups tested. The p value is low (0.01). The results of statistical tests showed that giving Moringa oleifera leaf extract had a significant effect (P<0.05) on reducing the number of white rat leukocytes.

4.2. Platelets

Table 4. Total of Rat platelet

Total Average Measurement of White Rat Platelet								
PRE TEST								
	K1			**P1**			**P2**	
MIN	100			121			543	
MAX		1.084			879			786
R	646.8			595.6			637.1	
POST TEST								
MIN	415			453			838	
MAX		1.297			1.366			1.297
R	954.6			858.8			1029.2	

Table 4. the results of examination of the number of platelets (K1) infected with Escherichia coli bacteria and given standard feed but not treated with an average of 646.56x103/µL (pre test) and 954,103/ µL (post test). For group P1, the platelet count was 595.6x103/µL (pre test) and after administering Moringa leaf extract at a dose of 150 mg/KgBW/day, the platelet count was 858.8x103/µL (post test). For group P2, the platelet count was 637.1x103/ µL (pre test) and after administering Moringa leaf extract at a dose of 250 mg/KgBW/day 1029.2x103/ µL (post test).

Table 5. Total Platelet Normality Test

Case Processing Summary						
	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Pre	9	100.0%	0	0.0%	9	100.0%
Past	9	100.0%	0	0.0%	9	100.0%

The data in table 5 above shows the results of the normality test analysis using the Shapiro-Wilk test for two different groups of data, namely pre-test and post-test. The results of the Shapiro-Wilk test show that all data in the pre-test and past-test groups have a normal distribution considering The p value is less than 0.05. This indicates that the data in the two groups is normally distributed.

Table 6 One-way Anova Test for Total Platelet

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	468.605	2	234.303	15.824	<.001
Within Groups	399.789	27	14.807		
Total	868.394	27			

Based on table 6, the results of the ANOVA test show that there is a significant difference between the average number of leukocytes between the groups tested. The low p value (0.01) indicates that there is an effect of Moringa leaf extract on increasing the number of platelets.

DISCUSSION

In research on the effect of Moringa oleifera leaf extract on the number of white wistar rats (Rattus Norvegicus) infected with Escherichia coli, they showed signs such as weakness, the rats' feces became soft and liquefied and even bled (Rosmania and Yuniar, 2021)

Table 1 shows that the control group which was only infected with bacteria and given standard feed but received no treatment showed a significant increase in the number of leukocytes with an average of 26.73x103/µl (pretest), while the post test was 22.98x103/µL. In the treatment group (P1) with a dose of 150ml/kgBW with an average result of 21.38x103/µl (prestest) and posttest 16.03x103/µL. In the treatment group (P2) with a dose of 250 ml/KgBW 20.63x103/µl (pretest) and posttest 13.72x103/µL. This shows that in the three groups there was a decrease in the number of leukocytes after treatment (Sugihartini, Jannah, and Yuwono, 2020)

The decrease in the number of leukocytes is because Moringa leaves contain alkaloids, saponins and flavonoids which function as antibacterials and can inhibit the growth of Escherichia coli bacteria. The flavonoid content of Moringa leaves can destroy the cytoplasmic membrane by creating metabolites which function to damage bacterial enzymes (Oktaviani et al., 2020; Sugihartini, Jannah, and Yuwono, 2020)

Table 2 shows the results of the leukocyte normality test in the three groups. This shows that all data in the pre-test and post-test groups have a normal distribution considering that the p value is <0.05.

In table 3, the ANOVA test shows that there is a significant difference between the average number of leukocytes between the groups tested. The results of this study show that the statistical results obtained are a p value of 0.000 ($p<0.05$), so it can be concluded that there is a significant difference in the average number of leukocytes in each group. Group P2 was more effective than group P1 in reducing leukocytes in white mice infected with Escherichia coli. This result is different from research conducted by Astawan, et al which used probiotics on mice infected with Escherichia coli but failed to reduce the leukocyte levels of the mice (Marhaeni, 2021)

In table 4. results of examination of the number of platelets (K1) infected with Escherichia coli bacteria and only given standard feed with an average of 646.56x103/µL (pre test) and 954,103/ µL (post test). For group P1, the platelet count was 595.6x103/µL (pre test) and the platelet count after administering Moringa leaf extract at a dose of 150 mg/KgBW/day was found to be 858.8x103/µL (post test). For group P2, the platelet count was 637.1x103/ µL (pre test) and after administering the extract 250 mg/KgBW/day the average platelet count was 1029.2x103/ µL (post test). Moringa leaves have anti-inflammatory activity. If inflammation is not controlled, it will disrupt the body's homeostatic balance, developing into chronic inflammation and causing tissue damage. The anti-inflammatory activity of Moringa leaves is related to the phytochemical compounds contained, namely alkaloids, flavonoids and tannins (Sugihartini, Jannah, and Yuwono, 2020)

Based on table 5, it shows the results of the normality test for examining platelet counts in the three groups. This shows that all data in the pre test and post test groups have a normal distribution with a p value < 0.05.

Table 6 shows that the results of the One-way Anova statistical test obtained $p < 0.05$ so it can show that there is a difference in the average number of platelets in each group. (P2) has the highest mean platelet count compared to (K1) and (P1). This is in line with Astawan et al.'s research that white mice (Rattus norvegicus) infected with Escherichia coli experienced a decrease in the number of platelets (Saputra, Arfi, and Yulian, 2020; Naushad, 2022)

The difference in the number of platelets occurs because Moringa leaves contain flavonoids, phenols, tannins, saponins, alkaloids and steroids which function as antibacterials and can inhibit the growth of Escherichia coli

bacteria (Sugihartini, Jannah, and Yuwono, 2020). The flavonoid content of Moringa leaves has antibacterial activity. namely by inhibiting nucleic acid synthesis from bacteria, inhibiting cell membrane function and inhibiting energy metabolism. The phenol content has antibacterial activity by denaturing cell proteins. The tannin content has antibacterial activity by inactivating bacterial cell adhesins, inactivating enzymes, and disrupting protein transport in the inner layers of cells. The saponin content has antibacterial activity by reducing the surface tension of bacterial cell walls and damaging the permeability of cell membranes which greatly disrupts the survival of bacteria. The alkaloid content has antibacterial activity which can occur by interfering with the peptidoglycan components in bacterial cells so that the cell wall layer is not perfect, which will then cause the death of the bacterial cell. This is in line with research by Emelia R., et al regarding Moringa oleifera leaves as an antibacterial against Escherichia coli bacterial infections, explaining that Moringa leaf extract has antibacterial activity against the growth of Escherichia coli (Emelia, Safitri, and Andriyani, 2020)

Apart from that, Moringa leaves have anti-inflammatory activity. If inflammation is not controlled, it will disrupt the body's homeostatic balance, developing into chronic inflammation and causing tissue damage. The anti-inflammatory activity of Moringa leaves is related to the phytochemical compounds contained, namely alkaloids, flavonoids and tannins. Moringa leaves have an anti-inflammatory role with the mechanism of inhibiting the lipoxygenase enzyme which functions in the formation of leukotrienes which causes the metabolism of arachidonic acid to be inhibited resulting in a reduction in prostaglandin production. Inhibition of inflammatory mediators will also inhibit the inflammatory proliferation process (Nurrahman and Mariyam, 2019)

Other factors that influence the number of leukocytes and the number of platelets are environmental conditions, age, and nutritional content of feed. Nutritional factors (protein) have a very important role in the process of forming leukocytes and platelets because protein is one of the components of the formation and development of blood cells (haematopoeisis). (Mutiarahmi, Hartady, and Lesmana, 2021; Upa, Saroyo, & Katili, 2017)

CONCLUSION

After analyzing the data and findings explained in the points above, the researcher wants to draw conclusions from this research. The conclusion that can be obtained is that Escherichia coli infection in male Wistar white rats (Rattus norvegicus) affects the number of leukocytes and platelets in male Wistar white rats. Administration of Moringa oleifera leaf extract at a dose of 150 ml/kgBW and 250 ml/kgBW, had an effect on reducing the number of leukocytes and increasing the number of platelets in male Wistar white rats infected with Escherichia coli in the pre-test and post-test. There was a significant difference in the average number of leukocytes and platelets in each group ($p < 0.05$). Giving moringa leaf extract (Moringa oleifera) can act as an antibiotic.

REFERENCES

[1] Astawan M, Wresdiyati T, Arief II, Suhesti E. (2011). Gambaran hematologi tikus putih (Rattus norvegicus) yang diinfeksi Escherichia coli enteropatogenik dan diberikan probiotik. Media Peternak, 34(1), 7–13.

[2] Britany, M.N. and Sumarni, L. (2020). Pembuatan Teh Herbal Dari Daun Kelor Untuk Meningkatkan Daya Tahan Tubuh Selama Pandemi Covid-19 Di Kecamatan Limo. Pros Semin Nas Pengabdi Masy LPPM UMJ. 2020;1–6.

[3] Emelia R, Safitri, D. D, and Andriyani, H. (2020). Daun kelor (Moringa oleifera) sebagai antibakteri terhadap infeksi bakteri Escherichia coli. INFOKES-Politeknik Piksi Ganesha, 44–50.

[4] Isnan W, M N. (2017). Ragam Manfaat Tanaman Kelor (Moringa oleifera Lamk) Bagi Masyarakat. Info Tek EBONI, 14(1), 63–75.

[5] Jusnita, N. and Syurya, W. (2019). Karakterisasi Nanoemulsi Ekstrak Daun Kelor (Moringa oleifera Lamk.). J Sains Farm Klin, 6(1), 16–24.

[6] Kursia, S., Aksa, R., and Nolo, M. M. (2018). Potensi Antibakteri Isolat Jamur Endofit dari Daun Kelor (Moringa oleifera Lam.). Pharmauho J Farm Sains, dan Kesehat, 4(1), 30–3.

[7] Marhaeni, L. S. (2021). Daun Kelor (Moringa oleifera) Sebagai Sumber Pangan Fungsional dan Antioksidan. J Agrisia, 13(2), 40–53.

[8] Mutiarahmi, C. N., Hartady, T., and Lesmana, R. (2021). Use of Mice as Experimental Animals in Laboratories that Refer to the Principles of Animal Welfare : A literatur Review. Indonesia Medicus Veternus, 10(1), 134-145

[9] Naufizdihar, N. A., Adji, A. S., and Billyana Y. (2022). Potensi Ekstraksi Moringa oleifera Untuk Mengatasi Gastroenteritis Bakteri. JIMKI: Jurnal Ilmiah Mahasiswa Kedokteran Indonesia, 9(3), 54–63.

[10] Naushad H. (2022). Leukocyre count (WBC) [Internet]. Medscape. 2022. Tersedia pada: https://emedicine.medscape.com/article/2054452-overview

[11] Oppusunggu, R. ., Zahara, R. ., & ., M. (2023). The Effect of Giving Mitekor (Moringa Leaf Noodles) On Overcoming Stunting in School Children in Medan Tuntungan District, Medan . International Journal of Membrane Science and Technology, 10(3), 2750-2754. https://doi.org/10.15379/ijmst.v10i3.2054

[12] Nurmalasari, Y., Rafie, R., Warganegara, E., and Wahyuni, L. D. (2021). Pengaruh pemberian ekstrak daun kelor (Moringa oleifera) terhadap kadar hemoglobin pada tikus putih (Rattus norvegicus) galur wistar jantan. Jurnal Medika Malahayati, 5(2), 91–101. DOI: https://doi.org/10.33024/jmm.v5i2.4155

[13] Nurrahman, N. and Mariyam, M. (2019). Status Hematologi, Kadar IgG dan IgA Tikus yang Mengonsumsi berbagai Variasi Jumlah Tempe Kedelai Hitam. agriTECH, 39(3), 215-221. DOI: https://doi.org/10.22146/agritech.26118

[14] Oktaviani, D. J., Widiyastuti, S., Maharani, D. A., Amalia, A. N., Ishak, A. M., and Zuhrotun. (2020). Hubungan aktivitas fisik terhadap jumlah trombosit dalam darah mahasiswa shift D 2016 fakultas farmasi universitas padjadjaran. Farmaka, 18(1), 1– 15.

[15] Rahayu, W. P., Nurjanah, S., and Komalasari, E. (2018). Escherichia coli : Patogenesis, Analisis, dan Kajian Risiko. Bogor: IPB Press.

[16] Rosmania, R. and Yuniar, Y. (2021). Pengaruh waktu penyimpanan inokulum Escherichia coli dan Staphylococcus aureus Pada suhu dingin terhadap jumlah sel bakteri di Laboratorium Mikrobiologi. J Penelit Sains, 23(3):117-124. DOI: https://doi.org/10.56064/jps.v23i3.624

[17] Salimi, Y., Bialangi, N., Abdulkadir, W., and Parulian, B. R. (2019). Senyawa Triterpenoid dari Ekstrak N-heksana Daun Kelor (Moringa oleifera Lamk.) dan Uji Aktivitas Antibakteri Terhadap Staphylococcus aureus dan Escherichia coli. Indo J Chem Res, 7(1):32–40.

[18] Sari, E. K., Wihastuti, T. A., and Ardiansyah, W. (2018). Probiotik meningkatkan konsentrasi hemoglobin pada tikus putih diinduksi lipopolisakarida Esh. Maj Kesehat, 5(1):18–25.

[19] Saputra, A., Arfi, F., and Yulian, M. (2020). Literatur Review: Analisis Fitokimia Dan Manfaat Ekstrak Daun Kelor (Moringa oleifera). Amina, 2(3), 114–119. doi: https://doi.org/10.22373/amina.v2i3.1220

[20] Sugihartini, N., Jannah, S., and Yuwono, T. (2020). Formulasi Gel Ekstrak Daun Kelor (Moringa oleifera Lamk) Sebagai Sediaan Antiinflamasi. Pharm Sci Res, 7(1), 9–16

[21] Suwito, W. and Andriani, A. (2018). Uji Toksisitas Escherichia coli Asal Daging Terhadap Sel Vero. Jurnal Biologi Tropis, 18(2), 230–234.

[22] Tjong, A, Assa, Y. A, and Purwanto, D. S. (2021). Kandungan Antioksidan Pada Daun Kelor (Moringa oleifera) dan Potensi Sebagai Penurun Kadar Kolesterol Darah. e- Biomedik, 9(2):248–254. DOI: https://doi.org/10.35790/ebm.9.2.2021.33452

[23] Upa, F. T., Saroyo, S., & Katili, D. Y. (2017). Komposisi pakan tikus ekor putih (maxomys hellwandii) di kandang. Jurnal Ilmiah Sains, 17(1), 7–12. https://doi.org/10.35799/jis.17.1.2017.14900

DOI: https://doi.org/10.15379/ijmst.v10i4.2288