

Differences In Counting The Number of Leukocytes Using Cermai Fruit (*Phyllanthus acidus*) Juice and Turk Solution Using The Counting Room Method

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Article Info

Article history:

Received: August 27, 2023

Revised: September 5, 2023

Accepted: September 20, 2023

Keyword:

Cermai Fruit

Count the Number of

Leukocytes

Turk Solution

ABSTRACT

Leukocytes play a role in the body's defense system. Counting the number of leukocytes can be done manually using turk solution. Glacial acetic acid in turk solution functions to lyse erythrocytes but this compound is included in compounds that are carcinogenic. There is a natural ingredient that can lyse blood cells other than leukocytes, namely Cermai fruit. Cermai fruit contains a weak acid, namely citric acid of 39,8% so that it can lyse blood cells other than leukocytes. This study aims to analyze the difference in the number of leukocytes in cermai fruit juice and turk solution. This type of research is experimental with simple random sampling technique. The sample of this research is cermai fruit which will be used to count the number of leukocytes in 10 respondents. The mean number of leukocytes in the control solution was 6.610 cells/mm³, 10% concentration 4.296 cells/mm³, 20% concentration 4.227 cells/mm³, 30% concentration 5.918 cells/mm³ and 100% concentration 5.745 cells/mm³. Data were analyzed using the Kruskal Wallis test and the sig. 0.001 < 0,05 so H₁ is accepted, which means there is a significant difference, so it is continued with the Post Hoc test. Post hoc test showed that there was no significant difference in the results of leukocyte counts using cermai fruit with concentrations of 30% and 100% compared to the control (p > 0,05). Meaning that only the concentrations of 30% and 100% showed the suitability of the number of leukocytes with the control so that these concentrations could be used as an alternative to 3% glacial acetic acid in turksolution.

INTRODUCTION

Leukocytes are blood cells that have an important role in the body's defense or immune system because they can fight bacteria that cause infection, as well as foreign substances that can harm the human body. Turk's solution is an ingredient for manual leukocyte examination with a composition of 1% gentian violet, 3% glacial acetic acid and 100 ml of distilled water. Glacial acetic acid in turk solution functions to lyse erythrocytes and has an acid content with a pH of 2.4, while gentian violet functions as a coloring agent for leukocytes (Dina, 2016). Turk's solution is used as a control solution because it has become a standard for counting

How to cite:

Effendi, A., Nugraha, G., & Santoso, A. (2023). Differences In Counting The Number of Leukocytes Using Cermai Fruit (*Phyllanthus acidus*) Juice and Turk Solution Using The Counting Room Method. *Jurnal Analis Medika Biosains (JAMBS)*, 10(2).

the number of leukocytes using the manual method because the content of glacial acetic acid in Turk's solution can lyse blood cells other than leukocytes (Sari & Nurbidayah, 2021).

According to Umar (2017), the content of glacial acetic acid in turk solution is included in compounds that are dangerous, toxic, and carcinogenic. Therefore, researchers want to find an alternative to glacial acetic acid in turk solution using natural ingredients that are not harmful to the body, namely cermai fruit juice. Cermai fruit (*Phyllanthus acidus*) contains a weak acid, namely citric acid of 39.8% (Ernawati *et al.*, 2021). The high content of citric acid in cermai fruit can lyse blood cells other than leukocytes so that it can be used as a substitute for glacial acetic acid in turk solution.

Based on previous research, there are natural ingredients that have been studied can be used as a substitute for glacial acetic acid in turk solutions, namely tamarind extract with a concentration of 50% (Rahmadhanty *et al.*, 2018) and lime extract with a pH of 2.0 can be used as an alternative to glacial acetic acid 3 % in turk solution to count the number of leukocytes using the manual method (Suba'iyah *et al.*, 2019). From the previous studies that have been carried out, this study aims to prove that a solution of cermai (*Phyllanthus acidus*) juice can replace 3% glacial acetic acid in turk solution to count leukocyte counts using the manual method with an improved neubauer counting chamber.

MATERIALS/METHOD

This study involved 10 respondents and was declared ethically feasible by the health ethics commission at Nahdlatul Ulama University Surabaya with No. 0053/EC/KEPK/UNUSA/2023. This type of research is experimental research using the Simple Random Sampling technique. The tools and materials used include Hemocytometer Improved Neubauer, microscope, blender, knife, measuring cup, beaker glass, dark bottle, cover glass, clean cloth, filter paper, measuring pipette, measuring cup, micropipette and tip, syringe, EDTA tube, tourniquet, petri dishes, cermai fruit, venous blood, glacial acetic acid, 1% gentian violet, distilled water, turk solution (control), cotton, alcohol swabs, plaster, and pH paper.

The clean cermai fruit is cut and separated from the seeds and then mashed using a blender. The cermai fruit is squeezed using a clean cloth. The cermai fruit juice is then filtered using filter paper. Furthermore, the preparation of test solutions with concentrations of 100%, 30%, 20% and 10%. The concentration of cermai fruit juice that has been determined is then measured for pH at each concentration using pH paper. Furthermore, blood dilution was carried out using a solution of cermai fruit juice by sucking the blood sample using a thoma pipette up to a mark of 0.5 or 1. A solution of cermai fruit juice with various concentration variations that had been added 1% gentian violet and distilled water was sucked up to a mark of 11 , homogenized for 2-3 minutes slowly. Then as much as 3-4 drops first discarded, then put 1 drop into the counting chamber through the edge of the cover glass. Incubate for 2-3 minutes in a petri dish containing wet cotton.

The number of leukocyte cells was counted using a microscope with 400x magnification on 4 large squares of leukocytes. The leukocyte cells obtained were calculated using the formula: Number of Leukocytes = $N \times P \times KV$ (Nugraha, 2017). The calculation of the number of leukocytes in this study was repeated 3 times to avoid biased results and subjectivity. The data obtained from the data

collection process were then analyzed with the Kruskal Wallis test and continued with the Man Whitney post hoc test.

RESULTS AND DISCUSSION

Table 1 Descriptive Analysis Results Count the number of leukocytes

Treatment Group	N	Minimum Amount (cell/mm³)	Maximum Amount (cell/mm³)	Average (cell/mm³)	SD (cell/mm³)
Control	10	4.325	10.250	6.610	1.583
Concentration 10%	10	3.600	5.325	4.296	633
Concentration 20%	10	3.600	5.475	4.227	682
Concentration 30%	10	4.325	9.625	5.918	1.528
Concentration 100%	10	3.675	8.650	5.745	1.521

Table 1 shows the average number of leukocytes in each group. Comparison of the results of the average leukocyte count using juice Cermai fruit with a concentration of 30% and 100% of the control was obtained the results are not much different. While the average leukocyte count is at concentrations of 10% and 20% showed results that were far below control.

Table 2 Kruskal Wallis Test Results

Variable	Kruskal Wallis test
Leukocyte Count	0,001

Based on table 2 it is known that the p-value with (α) 0.05, the result is 0.001 <0.05. The results show that H1 is accepted, it can be concluded that there is a significant difference significantly between the number of leukocytes in the treatment group with control group (turk), it is necessary to carry out further tests (Post Hoc) to find out the differences between groups and for find out the treatment groups that have similarity significant.

Table 3 Man Whitney Post Hoc Test Results

Treatment Group	Treatment Group	Post Hoc Test
	Concentration 10%	0,001
	Concentration 20%	0,004
Control	Concentration 30%	0,096
	Concentration 100%	0,130

Based on table 3 that the groups have differences significantly to the control is the concentration of 10% and concentration 20% with a p-value <0.05. While in the treatment group with 30% concentration and 100% concentration showed a significant similarity to the control with a p-value >0.05. So it can be seen that the

concentration that can be used to count the number of leukocytes using cermai fruit juice is a concentration of 30% and 100%.

Based on the results of observations under the microscope, the appearance at a concentration of 10% showed round leukocytes with an unstained center nucleus and visible dirt due to sediment from cermai fruit juice that was missed during the filtering process, many erythrocyte cells were seen that were not lysed and filled each field of view so that only a few leukocytes were seen. This causes the number of leukocytes at a concentration of 10% far from control. Microscopic observation of leukocytes with cermai fruit juice with a concentration of 20%, showed round-shaped leukocytes with an unstained center nucleus, visible dirt and many erythrocyte cells that were not lysed and filled each field of view. This causes the number of leukocytes at a concentration of 20% far from control.

Microscopic observation of leukocytes with cermai fruit juice with a concentration of 30% showed round-shaped leukocytes with unstained central nuclei, dirt and erythrocyte cells that were not lysed, but not as much as the concentrations of 10% and 20%. At a concentration of 30%, the leukocyte cells were spread evenly enough to obtain an average number of leukocytes close to the control. Microscopic observation of leukocytes with cermai fruit juice concentration of 100% showed that leukocytes were round in shape with an unstained nucleus in the middle, dirt was visible and erythrocyte cells were not lysed but leukocyte cells were spread evenly enough to obtain an average number of leukocytes close to control.

Although in this study concentrations of 10%, 20%, 30% and 100% blood cells other than leukocytes could not be completely lysed, at concentrations of 30% and 100% the distribution of leukocyte cells was quite even and not covered by blood cells other than leukocytes, so the average leukocyte results were close to the control. In examining the number of leukocytes using a cermai water solution it looks a bit cloudy but leukocyte cells can still be seen but not as clear as in the control, this is because the juice of the cermai fruit contains other compounds besides citric acid and no specific extraction of the compound needed is carried out (Salman *et al.*, 2021). So it can be seen that the concentration that can be used to count the number of leukocytes using cermai fruit juice is a concentration of 30% and 100%.

The results of this study are supported by research conducted by Habibuddin, (2021) on starfruit juice at a concentration of 30% can be used to count the number of leukocytes using the improved Neubauer method. Cermai fruit contains citric acid which is a weak acid compound that is high enough so that it can be used as an alternative to 3% glacial acetic acid in Turk solution to count leukocyte counts manually using an improved Neubauer counting chamber (Susantyo *et al.*, 2020).

CONCLUSIONS

Based on the results of the study of differences in the number of leukocyte counts Using Cermai fruit juice (*Phyllanthus acidus*) and Turk's solution with Counting Chamber method, it can be concluded that cermai fruit juice at a concentration of 30% and 100% can be used as a substitute for 3% glacial acetic acid in Turk solution, while at a concentration of 10% and 20% it cannot be used as a substitute for 3% glacial acetic acid in Turk solution. It is recommended that further research should be carried out filtered repeatedly to minimize the presence

of impurities which are still left behind so as to facilitate the calculation of the amount leukocytes.

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