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Antibacterial Activity Ethanolic Extract of *Ocimum basilicum* L. Leaves in Inhibiting the Growth of *Escherichia coli* and *Pseudomonas aeruginosa*

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Abstract

Infectious disease is the biggest problem in the world. The problem with this infection is related to antibiotic resistance if its use is not consistent. Utilization of kemangi leaves, which are always used as fresh vegetables, has potential as an antibacterial in inhibiting the growth of *Escherichia coli* and *Pseudomonas aeruginosa* bacteria. This study aims to analyze the inhibition of an ethanol extract of kemangi leaves against *Escherichia coli* and *Pseudomonas aeruginosa*. The Kirby-Bauer method with the disc diffusion method was used to determine the minimum inhibitory diameter and to calculate the activity index value. The test results at a concentration of 500 mg/mL showed activity in the strong category, namely 13.70 ± 0.10 mm (*Escherichia coli*) and 12.93 ± 0.06 mm (*Pseudomonas aeruginosa*), and the minimum inhibitory concentration was at a concentration of 3.125 mg/mL. Conclusion the ethanolic extract of kemangi leaves shows antibacterial activity.

Keywords: *Kemangi leaves, antibacterial, Pseudomonas aeruginosa, Escherichia coli*

Abstrak

Penyakit infeksi merupakan masalah tersebasar didunia. Masalah infeksi ini berkaitan dengan resistensi antibiotik dimana bila penggunaannya tidak patuh dalam pengonsumsiannya. Pemanfaatan daun kemangi yang selalu digunakan sebagai lalapan sebagai sayuran memiliki potensi sebagai antibakteri dalam menghambat pertumbuhan bakteri Escherichia coli dan Pseudomonas aeruginosa. Penelitian ini bertujuan untuk menganalisa daya hambat ekstrak etanol daun kemangi terhadap Escherichia coli dan Pseudomonas aeruginosa. Metode Kirby-Bauer dengan metode difusi cakram digunakan untuk penentuan diameter hambat minimum dan dilakukan perhitungan nilai aktivitas indeks. Hasil pengujian pada konsentrasi 500 mg/mL menunjukkan aktivitas dengan kategori kuat yaitu 13,70 ± 0,10 mm (*Escherichia coli*) dan 12,93 ± 0,06 mm (*Pseudomonas aeruginosa*) dan konsentrasi hambat minimum pada konsentrasi 3.125 mg/mL. Kesimpulan ekstrak etanol daun kemangi menunjukkan aktivitas antibakteri.

Kata kunci: *Daun kemangi, antibakteri, Pseudomonas aeruginosa, Escherichia coli*

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INTRODUCTION

Infectious diseases caused by pathogens are a big problem in the world. Some bacteria are normal flora in the host's body, such as *Escherichia coli*; however, in large numbers, they will cause infectious diseases such as diarrhea and urinary tract infections (UTI)[1].

Other dangerous pathogens, such as *Pseudomonas aeruginosa*, show a high degree of pathogenicity. This bacterium will cause pneumoniae, otitis media, UTI, and other infections[1]. Treatment with antibiotics is always used but has the effect of developing resistance to pathogenic bacteria if non-compliance with the use of antibiotics continuously occurs[2]. Utilization of plants as a natural resource for inhibiting the growth of pathogenic bacteria has the potential to be developed[3,4].

The discovery of antibacterial compounds from plants continues; one example is the use of kemangi leaves, which are typically used as fresh vegetables but have antibacterial potential[5]. Previous studies have proven that the ethanol extract of kemangi leaves has the potential to inhibit the growth of *Staphylococcus aureus*[6]. Based on this description, the researchers were interested in analyzing the antibacterial activity of the ethanol extract of kemangi leaves against Gram-negative pathogenic bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*.

METHODOLOGY

Planing Research

The research was conducted starting with collecting raw materials or samples of kemangi leaves, then extracting them and testing their antibacterial activity in inhibiting the growth of *Escherichia coli* and *Pseudomonas aeruginosa*.

Sampel Preparation

Sampling was carried out purposively without comparing samples from one region to another. Fresh kemangi leaves were purchased from the Jamin Ginting market, washed, dried, and powdered using a blender[1].

Preparation of Kemangi Leaves Ethanol Extract

The ethanol extract of kemangi leaves was prepared using the maceration method with 96% (pa) ethanol as the solvent. As much as 200 g of kemangi leaf powder was soaked in 1500 mL of ethanol for 5 days, then filtered and macerated again for 2 days with 500 mL of ethanol[7]. The extract obtained was concentrated using a rotary vacuum evaporator[7,8].

Preparation of Apparatus and Materials

The glassware used was sterilized using an oven at 170°C for 1 hour[9]. The test medium used was sterilized using an autoclave at 121°C for 15 minutes[10].

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Preparation of Bacterial Stock Cultures

Prepare 1 tube of sterile slanting agar and then streak 1 ose of pure culture of *Escherichia coli* and *Pseudomonas aeruginosa* on the surface of the agar slants for 24 hours at 37°C ± 2°C [11,12].

Preparation of the Various Concentration

A condensed extract of kemangi leaves was weighed at 1 g and then dissolved in 2 mL of DMSO for a concentration of 500 mg/mL. Then it was diluted with various concentrations of 250, 100, 50, 25, 12.5, 6.25, and 3.125 mg/mL[8,11].

Preparation of Bacterial Suspension

One sample of revitalized bacteria was suspended in a test tube containing 10 mL of MHB medium, vortexed, and turbidity was adjusted using 0.5 McFarland standards [7,13].

Antibacterial Activity Test

The method used in this test is the Agar Diffusion Method (Kirby-Bauer)[12]. A total of 0.1 mL of the bacterial suspension was pipetted and placed in a sterile petri dish, and then 15 mL of MHA medium was added[14,15]. Pour into a Petri dish and homogenize; place on the surface of the disc of paper media that contains each concentration, and use chloramphenicol antibiotic disc paper containing 30 mcg. The treatment was carried out over three repetitions[1,9].

Calculation of Index Activity Value

Index activity value is calculated using the following formula: [8]

$$Index\ Activity = \frac{extract\ inhibition\ zone\ diameter}{positive\ control\ inhibition\ zone\ diameter}$$ [16]

Data Analysis

Data is presented in 3 treatments with the average and standard deviation values, and the difference is seen from the control using SPSS v.22 software[9].

RESULT AND DISCUSSION

The results of testing the antibacterial activity of the ethanol extract of kemangi leaves in inhibiting the growth of *Escherichia coli* and *Pseudomonas aeruginosa* showed positive inhibition results. This is indicated by the presence of a clear zone around the disc, which is dripped with a concentrated ethanol extract of kemangi leaves[17,18]. The results of measuring the diameter of the inhibition zone on the two bacteria can be seen in Table 1 and Figure 1.

At Table 1. it can be seen that at a concentration of 500 mg/mL, it showed an inhibition zone of 12.93 ± 0.06 mm (*Pseudomonas aeruginosa*) and 13.70 ± 0.10 mm (*Escherichia coli*).

At a concentration of 500 mg/mL, the inhibition zone was the largest among all concentrations. At the smallest concentration of 3.125 mg/mL, the diameter of the inhibition zone was 6.57 ± 0.15 mm (*Pseudomonas aeruginosa*) and 6.90 ± 0.10 mm (*Escherichia coli*).

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Table 1. Inhibition Result of Ethanolic Extract of *Ocimum basilicum* in inhibiting the growth of *Escherichia coli* and *Pseudomonas aeruginosa*.

No	Concentration	Diameter Inhibition Zones (mm)	
		P. aeruginosa	***E. coli***
1	Negative Control	6,00 ± 0,00	6,00 ± 0,00
2	3,125 mg/mL	6,57 ± 0,15*	6,90 ± 0,10*
3	6,25 mg/mL	7,73 ± 0,06*	7,67 ± 0,15*
4	12,5 mg/mL	8,63 ± 0,15*	8,20 ± 0,20*
5	25 mg/mL	9,00 ± 0,10*	8,93 ± 0,25*
6	50 mg/mL	9,63 ± 0,15*	10,13 ± 0,21*
7	100 mg/mL	10,30 ± 0,10*	11,40 ± 0,26*
8	250 mg/mL	11,73 ± 0,15*	12,63 ± 0,25*
9	500 mg/mL	12,93 ± 0,06*	13,70 ± 0,10*
10	Positive Control	23,17 ± 0,21	25,13 ± 0,15

*Significantly difference with negative and positive control.

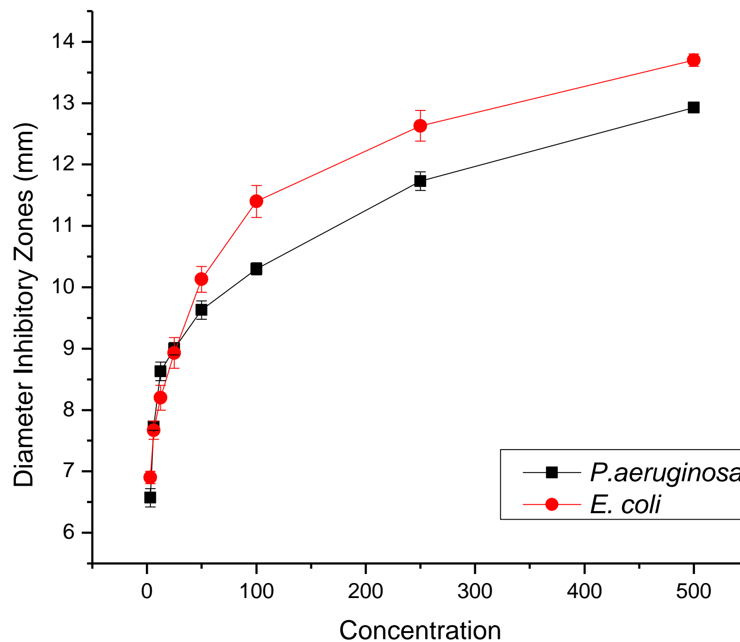

Figure 1. Graph of diameter inhibitory zones versus concentration against *Pseudomonas aeruginosa* and *Echerichia coli*

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Table 2. Activity Index Value

No	Concentration	Activity Index	
		P. aeruginosa	*E. coli*
1	Negative Control	0,26 ± 0,00	0,24 ± 0,00
2	3,125 mg/mL	0,28 ± 0,07	0,27 ± 0,06
3	6,25 mg/mL	0,33 ± 0,02	0,31 ± 0,10
4	12,5 mg/mL	0,37 ± 0,07	0,33 ± 0,13
5	25 mg/mL	0,39 ± 0,04	0,36 ± 0,16
6	50 mg/mL	0,42 ± 0,07	0,40 ± 0,13
7	100 mg/mL	0,44 ± 0,06	0,45 ± 0,17
8	250 mg/mL	0,51 ± 0,05	0,50 ± 0,16
9	500 mg/mL	0,56 ± 0,02	0,55 ± 0,06

The formation of this inhibition zone is due to the diffusion power from high concentrations to lower concentrations[19]. In this case, the movement of the active substance or secondary metabolite compounds from the disc paper moves and diffuses into the medium so as to provide a zone of inhibition around the disc paper[20,21].

Table 2 shows the value of the index activity calculation results. Index activity is a comparison between the diameter of the inhibition zone of each concentration and that of the positive control[22]. If the index activity value is close to 1, then the test concentration activity is close to the activity of the positive control[23]. In Table 2, it can be seen that at the largest concentration of 500 mg/mL, it shows an activity index value of 0.56 ± 0.02 (*Pseudomonas aeruginosa*) and 0.55 ± 0.06 (*Escherichia coli*). This value means that *Pseudomonas aeruginosa* has an activity of 56% of the test concentration against the positive control, while *Escherichia coli* shows an activity of 55% of the test concentration against the positive control of chloramphenicol.

Based on the results of phytochemical screening by Angga Nugraha et al. (2022)[6], positive results were observed for the presence of secondary metabolites in the form of alkaloids, flavonoids, steroids, saponins, tannins, and glycosides in kemangi leaf extract. This is consistent with the findings of Angga Nugraha et al. (2022), who tested the antibacterial activity of the ethanol extract of kemangi leaves against Staphylococcus aureus at a concentration of 500 mg/mL and found an inhibition zone diameter of 11.93 ± 0.25 mm.[6].

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The mechanism of secondary metabolites as antibacterials, namely tannins, works by inhibiting the reverse transcriptase and DNA topoisomerase enzymes so that bacterial cells cannot form[24,25]. Likewise, flavonoids function by forming complex compounds with extracellular and dissolving proteins, which then disrupt the bacterial cell membrane and lead to the release of intracellular chemicals[26,27]. It is supported by saponin chemicals and has a method of action involving the reduction of surface tension, which results in enhanced permeability or cell leakage and the release of intracellular substances[28]. Likewise, other secondary metabolites synergize in providing antibacterial effects.

CONCLUSION

Strong antibacterial activity was demonstrated by the ethanolic extract of kemangi leaves, which had an activity index value of 55–56% against the positive control.

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Formulation of Hand Sanitizer Gel from Chitosan Shell of Fresh Water Lobster (*Cherax Quadricarinatus*)

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Abstract

Certain aberrant disorders impair the operation of a portion or the entire body. Infectious diseases can be spread by touching a person's hands or after contact with inanimate objects contaminated with bacteria, which can cause a variety of ailments, including diarrhoea. Using hand sanitizer as the primary deterrent is one of the methods to avoid this transmission. This time, the primary element is a natural substance, such as chitosan. Chitosan can be extracted from the shells of freshwater crayfish and utilized as an antibacterial agent in hand sanitizer formulations. This project intends to produce chitosan from crab shells. Crustacean chitosan can be utilized as an active ingredient in hand sanitizer gel. The production of chitosan begins with the demineralization, deproteination, and deacetylation processes. The resulting chitosan was then used to make hand sanitizer gels with 1, 2, and 3% concentrations. Organoleptic, homogeneity, pH, dispersion, and viscosity tests were then conducted on the gel preparation. The results revealed that the hand sanitizer preparations subjected to organoleptic tests on F1, F2, and F3 did not exhibit any changes in odor, shape, or color; the homogeneity test revealed the absence of coarse grains; the pH obtained on average was within the skin pH range; and the spreadability test revealed that the average was in the range of 5-7 cm, indicating that the formula satisfies the specifications.

Keywords: *Chitosan, Hand sanitizer Gel, Irritant*

Abstrak

Gangguan menyimpang tertentu merusak operasi sebagian atau seluruh tubuh. Penyakit menular dapat menyebar dengan menyentuh tangan seseorang atau setelah kontak dengan benda mati yang terkontaminasi bakteri, yang dapat menyebabkan berbagai penyakit, termasuk diare. Menggunakan hand sanitizer sebagai pencegah utama adalah salah satu cara untuk menghindari penularan ini. Kali ini, unsur utamanya adalah bahan alami, seperti kitosan. Chitosan dapat diekstraksi dari cangkang lobster air tawar dan dimanfaatkan sebagai agen antibakteri dalam formulasi pembersih tangan. Proyek ini bermaksud untuk memproduksi kitosan dari cangkang kepiting. Crustacea chitosan dapat dimanfaatkan sebagai bahan aktif gel hand sanitizer. Pembuatan kitosan diawali dengan proses demineralisasi, deproteinasi, dan deasetilasi. Kitosan yang dihasilkan kemudian digunakan untuk membuat gel hand sanitizer dengan konsentrasi 1, 2, dan 3%. Selanjutnya dilakukan uji organoleptik, homogenitas, pH, dispersi, dan viskositas terhadap sediaan gel. Hasil penelitian menunjukkan bahwa sediaan hand sanitizer yang diuji organoleptik pada F1, F2, dan F3 tidak menunjukkan adanya perubahan bau, bentuk, maupun warna; uji homogenitas mengungkapkan tidak adanya butiran kasar; pH yang diperoleh rata-rata berada dalam rentang pH kulit; dan uji daya sebar menunjukkan bahwa rata-rata berada pada kisaran 5-7 cm, yang menunjukkan bahwa formula tersebut memenuhi spesifikasi.

Kata Kunci: *Kitosan, Gel Handsanitizer, Iritasi*

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INTRODUCTION

Indonesia is abundant with natural resources, both in terms of marine and plant wealth. We can see this from various developments at this time, especially at this time when Indonesia has experienced a pandemic. At this time, we are required to do activities at home, always be hygienic, and not travel to break the chain of transmission and stop its spread. With the government urging us to always wash our hands and use hand sanitizer, we are obliged to provide hand sanitizer because it is simpler and more practical. However, the main ingredient (alcohol) has side effects that irritate and dry the skin. Therefore, the researchers decided to examine chitosan from crayfish as the main ingredient where the shells of freshwater crayfish can produce chitosan, which can be antibacterial[1].

Hand sanitizer contains compounds that can kill bacteria or viruses in the hands, such as Staphylococcus aureus bacteria, usually from alcohol. This hand sanitizer product is also increasingly diverse in its composition and the carrier substance and has been widely used in the community[2].

Hand sanitizer, if used excessively and continuously, can be dangerous and irritate, causing a burning feeling on the skin. Because the basic ingredient of antiseptics, in general, is alcohol, Several studies using natural materials include those using clove flowers[3], basil leaves[4], red betel leaves[5], and so on. One of the efforts to reduce the use of these chemicals in hand sanitizer products is to innovate hand sanitizer products using natural ingredients that have antibacterial properties, namely chitosan from the shells of crayfish (*Cherax quandricarinatus*).

Hand sanitizer gel is a gel-shaped preparation used to reduce or inhibit the growth of microorganisms. Where the active ingredient is chitosan from snail shells, it contains a positively charged polycation that can suppress and inhibit bacterial growth. The use of easy and practical hand sanitizer gel is increasingly in demand by the public; most hand antiseptic gel products use alcohol as an antibacterial[5]. The use of chemicals in topical preparations has harmful side effects and can irritate the skin. People who care about their health are increasingly turning to hand sanitizer gel preparations containing antiseptics as a convenient and easy way to maintain their health and hand hygiene. The public utilizes gel preparations due to their high aesthetic value, specifically that they are translucent, easily distributed when applied to the skin without pressure, provide a cooling feeling, do not leave markings on the skin,

and are simple to use.

Chitosan is a derivative of chitin. Chitosan is the result of the deacetylation of chitin using a strong base and includes amino polysaccharides. Several studies have isolated chitosan, including from the shells of feather clams, where the degree of deacetylation was 90%[6]. Chitosan is widely used in the cosmetic field and has been applied as a humectant, thickening agent, stabilizer, and moisturizer. Chitosan has also been used as a preservative in seafood[7].

The researcher chose chitosan as the main ingredient in the hand sanitizer because, based on previous research, chitosan is an antibacterial. Chitosan has the advantage that it can be used as an ingredient for skin care because it has a positive electric charge and can penetrate the skin. Chitosan also functions as a moisturizer on the skin.

Based on the description above, the properties of snail shell chitosan have antibacterial and bactericidal properties, so it becomes an opportunity to be used as a hand sanitizer preparation as a preventive effort during the Corona pandemic this year.

METHODOLOGY

Samples were taken from the Central Aceh district. The sampling method was done on purpose because the sample was taken from a single area, and there was no comparison with other regions.

Sample Processing

The lobster shells are separated from the meat, the shells are washed clean, the shells are dried using an oven, the shells are crushed with a blender, then sieved, and fine powder is obtained.

Chitosan Isolation

Demineralisasi

In a ratio of 1:15 (w/v) between the solvent and the sample, 1.5 M HCl solution was added to the sample in a glass beaker. The mixture was then heated at 60 to 70°C for four hours with magnetic stirring at 50 revolutions per minute. The residue was separated, dried, and then weighed in a desiccator after cooling[8].

Deproteinasi

Transferring the demineralization residue to a new glass beaker, a 1:10 (w/v) ratio of a 3.5 percent NaOH solution was placed between the solvent and the sample. The mixture was then heated at 60 to 70°C for four hours using a magnetic stirrer at 50 revolutions per minute. After being dried, the residue is cooled in a desiccator and then weighed[9].

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Depigmentation Process

Chitin was placed in a glass beaker, followed by the addition of 60% NaOH in a 1:20 (w/v) ratio, and then the mixture was heated at 100°C - 110°C for four hours with magnetic stirring at 50 rpm. Before being weighed, the residue is refrigerated in a desiccator after it has been dried. The extracted chitosan from each sample was then evaluated qualitatively [10].

Chitin Deacetylation Process

Chitin was placed in a glass beaker, followed by the addition of 60% NaOH in a 1:20 (w/v) ratio, and then the mixture was heated at 100°C-110°C for 4 hours with magnetic stirring at 50 rpm. Before being weighed, the residue is refrigerated in a desiccator after it has been dried. The extracted chitosan from each sample was then evaluated qualitatively[11].

Hand Sanitizer Gel Formulation

The formula for hand sanitaizer gel can be seen in table 1.

Procedure for making hand sanitizer gel

CMC-Na was developed by sprinkling it over water in a mortar, letting it swell for 30 minutes, and then grinding it. Chitosan in several concentrations was dissolved in 20 ml of 4% acetic acid and ground until homogeneous. Added propylene glycol and methylparaben, dissolved in hot propylene glycol, and ground them homogeneously to form a gel base[12]. The solution was added little by little to the base gel. Distilled water was added until the gel reached 100 ml, and the fragrance was added.

Table 1. Hand sanitizer gel formula

Ingredients	The concentration of ingredients in the formula (%) w/v			
	F_0	F_1	F_2	F_3
Chitosan	-	1	2	3
Na CMC	2.5	2.5	2.5	2.5
PEG	2	2	2	2
Metil paraben	0.02	0.02	0.02	0.02
Essens Lemon (gtt)	3	3	3	3
Aquadest ad	100	100	100	100

Evaluation of Hand Sanitizer Gel Preparations

Organoleptic Test

The organoleptic test was carried out by direct observation (visually) in the form of color, smell, and shape of the gel preparation[13].

Homogeneity Test

The observation of homogeneity was carried out by smearing the gel preparation on a piece of glass. This test was conducted to determine the homogeneity of the active ingredient

and other additives in the preparation[14].

pH test

The pH is measured with a pH meter, which is calibrated with distilled water before being dried. After weighing and dissolving the preparation in 100 cc of distilled water, the electrode is dipped in the solution until a consistent pH is reached. pH 4.5–6.5 is the acceptable range for the pH value of the formulation (normal pH on the skin) [15].

Spreadability Test

A total of 0.5 g of the preparation was weighed, placed on a watch glass, covered with another watch glass, and then given a load of 50 g. A ruler is used to measure spread power. The requirements for the spreadability test that meet the requirements are 5-7 cm[16].

Irritation test

On the back of the volunteer's hand, the prepared formulas (FI, FII, and FIII) were applied to determine the irritancy of the gel preparations using the patch test procedure. This experiment was conducted on 20 volunteers using the positive control, which consisted of pure extract without a mixing of other gel-forming substances, and then the resulting symptoms were observed. If irritation develops, a skin reaction will occur after the preparation is applied to the skin[17].

RESULT AND DISCUSSION

Chitosan Isolation

Figure 1. Results of crayfish shell chitosan

In this case, the first step is the demineralization process. The purpose of this process is to remove or separate the inorganic salts present in the lobster shells. Next is the deproteination stage.

Deproteination Stage, The goal is to separate the protein in the lobster shell using 3.5% NaOH with a ratio of 1:10[18]. Then do the deacetylation step. Deacetylation stage, namely by adding 60% NaOH with a ratio of 20:1 ($^{v}/_{w}$) and putting it into the extractor at 80°C for 1 hour. After chilling, it is filtered, and the solid obtained is neutralized with distilled water. The solid was then dried in an extractor without a solution at 80°C for 24 hours, and the chitosan was ready for analysis. The yield obtained in the deacetylation process was filtered and washed with distilled water until the pH was neutral, then dried to obtain a solid product in the form of chitosan. This is to break the acetyl group with the

nitrogen atom found in chitin to produce an amine, a group found in chitosan.

Evaluation of Hand Sanitizer Gel Preparations

This gel's primary ingredient is chitosan, which functions as an antibacterial agent. Additional ingredients include Na-CMC, methylparaben, propylene glycol, distilled water, and fragrance. Four formulas were used to create this gel: the first formula was made without the addition of chitosan, also known as "blank," the second formula was made with the addition of chitosan at a concentration of 1%, the third formula was made with the addition of chitosan at a concentration of 2%, and the final formula was made with the addition of chitosan at a concentration of 3%. After the gel was created, a physical evaluation was conducted with the test parameters, including organoleptic observations, homogeneity, pH measurements, viscosity tests, and 0th to 3rd week observations.

Organoleptic Test

The results of the organoleptic evaluation of the hand sanitizer gel made from chitosan lobster shells were quite favorable; based on the smell, shape, and color of the gel preparation, there was no change from week 0 to week 3 in the preparation's smell, shape, or color. The aroma is the consequence of the addition of lemon perfume. In contrast, Blank has a faint odor of chitosan because no fragrance was applied. The turbidity of each hand sanitizer gel preparation is caused by the addition of chitosan; the higher the concentration of chitosan used, the more turbid the color of the hand sanitizer gel preparation. And the gel form is thick because the texture of the gel preparation is thick.

Homogeneity Test

Results for Hand Sanitizer Gel Preparations from tests conducted; it was determined that the gel preparation did not form coarse grains; it can be concluded that the gel hand sanitizer preparation is physically homogeneous and the gel ingredients used in the formulation are fully dissolved and mixed.

Spreadability of Hand Sanitizer Gel Preparations

The results of the testing of the spreading power of the hand sanitizer gel preparations can be seen in the table below. So it can be concluded that the gel preparation has good spreadability, which is between 5-7 cm from the spreading power test, which can be seen in Table 2.

pH measurement

The pH test is carried out to measure the preparation's pH (acidity degree) and determine whether the preparation meets the pH requirements according to the skin pH, namely 4–8[19]. The pH observations were carried out every

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week for three weeks to produce a stable gel because it did not change during storage, as seen in Table 3.

Table 2. Spreadability test

Formulas	Spreading power (cm)
Blank	6
1%	6.2
2%	5.4
3%	6

Table 3. pH test

Formulas	Average pH	
	Week -0	Week-3
Blank	6.0	5.5
1 %	6.7	6.7
2 %	6.2	6.3
3 %	5.7	5.7

From the table above, the resulting preparations are acidic, and a too acidic pH can cause skin irritation. Meanwhile, if the pH is too alkaline, it causes scaly skin. The test was carried out using a pH meter, whose resulting pH has a higher accuracy than a universal pH[20].

Viscosity Test

The viscosity was measured using a Brookfield viscometer with an L3 spindle speed of 20 revolutions per minute. The viscosity was measured twice at the beginning and after three weeks of storage at room temperature. Based on the results of the study, it can be concluded that hand sanitizer gel meets the criteria for a good gel, which are between 2,000 and 4,000 cps. As shown in Table 4.

Table 4. Viscosity test results

Formulas	Viscosity (cP)	
	Week–0	Week–3
F0	2680	3890
F1	2170	3584
F2	2020	3450
F3	2110	3300

Irritation Test on Volunteers

Based on observations of the hand sanitizer gel irritation test with chitosan lobster shells on several volunteers. In this observation, no volunteers experienced or felt the effects of irritation on the skin. At the time of testing, the effects noticed were an itching effect on the skin, the skin becoming rough, and the skin becoming dry. So it can be concluded that this preparation is good and safe.

CONCLUSION

Based on the research results of hand sanitizer gel formulations containing chitosan, it can be concluded that all formulas meet the physical stability test criteria, are stable in storage, and do not irritate the skin, so it is safe to use.

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Comparison of Examination Results of Acid-Fast Bacilli (AFB) Ziehl Neelsen (ZN) Method on BTA Preparations that are Directly Stained with Delayed Preparations

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Abstract

Tuberculosis is a direct infectious disease caused by the bacterium Mycobacterium tuberculosis. Most tuberculosis germs attack the lungs, which is known as Pulmonary Tuberculosis (pulmonary TB), but can also affect other organs, which is called extrapulmonary TB. Pulmonary TB germs in the form of rods, have special properties, namely acid resistance to staining. The purpose of this study was to determine the results of examination of acid fast bacilli (AFB) by the Ziehl Neelsen method on smear preparations which were immediately stained and left to stand for three days. The research was conducted using a descriptive approach with a sample of 10 people using a purposive sampling technique. In this study, staining was carried out twice, namely direct staining and delayed staining for 3 days and examined under a microscope. The results showed that there was no difference between the preparedness that was immediately colored and the one that was postponed for three days.

Keywords: *Tuberculosis, Phlegm, BTA*

Abstrak

Tuberkulosis adalah penyakit menular langsung yang disebabkan oleh bakteri Mycobacterium tuberculosis. Sebagian besar kuman tuberkulosis menyerang paru-paru yang dikenal dengan Tuberkulosis Paru (TB Paru), tetapi dapat juga menyerang organ lain yang disebut TB ekstra paru. Kuman TBC Paru yang berbentuk batang, memiliki sifat khusus yaitu tahan asam terhadap pewarnaan. Tujuan penelitian ini adalah untuk mengetahui hasil pemeriksaan basil tahan asam (BTA) dengan metode Ziehl Neelsen pada sediaan apusan yang langsung diwarnai dan didiamkan selama tiga hari. Penelitian dilakukan dengan menggunakan pendekatan deskriptif dengan sampel sebanyak 10 orang dengan menggunakan teknik purposive sampling. Pada penelitian ini pewarnaan dilakukan dua kali yaitu pewarnaan langsung dan pewarnaan tertunda selama 3 hari dan diperiksa di bawah mikroskop. Hasil penelitian menunjukkan bahwa tidak ada perbedaan antara kesiapsiagaan yang langsung diwarnai dengan yang ditunda selama tiga hari.

Kata kunci: *Tuberculosis, Dahak, BTA*

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INTRODUCTION

Tuberculosis is a direct infectious disease caused by the bacterium *Mycobacterium tuberculosis*. Most tuberculosis germs attack the lungs, which is known as pulmonary tuberculosis (pulmonary TB), but can also affect other organs of the body, called extrapulmonary TB. Pulmonary TB germs are rod-

shaped, have special properties, which are resistant to acid on staining. Therefore it is also known as acid fast bacilli (BTA). Lung TB germs die quickly in direct sunlight, but can survive for several hours in a dark and damp place. In the body's tissues, these germs can be dormant, sleeping for a long time for several years[1]. In Indonesia, pulmonary TB is the fifth disease in the world (India, China, South Africa, Nigeria and Indonesia). Based on the TB Global Report in 2010, the prevalence of pulmonary TB cases in Indonesia nationally in 2010 was 285 per 100,000 population, the TB mortality rate has fallen to 27 per 100,000 population[2]. Every year there are 660,000 cases in Indonesia, of which 61,000 die. The majority of pulmonary TB sufferers are of productive age, so the economic burden is quite large. Another challenge is the increase in multi-drug resistant (MDR-TB) cases and pulmonary TB-HIV co-infection [3].

From the 2010 WHO Global Report, obtained data on pulmonary TB in Indonesia, the total of all pulmonary TB cases in 2009 was 294,731 cases, of which 169,213 were cases of new smear-positive pulmonary TB, 108,616 cases of smear-negative pulmonary TB, 11,215 extra pulmonary TB cases, 3,709 pulmonary TB cases Relapse and 1,978 cases of re-treatment outside of relapse cases. Pulmonary TB is not a new disease in Indonesia, but until now it is still a major health problem. It is estimated that the number of pulmonary TB cases in Indonesia accounts for around 5.8 percent of the total number of pulmonary TB in the world. The prevalence of tuberculosis in Indonesia is 281 cases per 100,000 population with a treatment success rate of 90.3%. This number decreased compared to 2010 of 289 per 100,000 population[4]. On March 3 2014, the Stop TB Partnership Forum for the Southeast Asia, West Pacific and East Mediterranean Regions was held in Jakarta. The forum involved 100 participants from 13 countries consisting of national TB program managers, national stop TB partnerships, and related NGOs [5].

The forum stated that although the prevalence has decreased significantly in recent years, the number of tuberculosis sufferers in Indonesia is still relatively high. In fact, currently the number of TB sufferers in Indonesia is ranked fourth in the world. Indonesia has the fourth highest number of TB sufferers after China, India and South Africa. The prevalence of TB in Indonesia in 2013 was 297 per 100,000 population with new cases reaching 460,000 cases every year. Thus, the total cases until 2013 reached around 800,000 - 900,000 cases. In South Sulawesi, the number of tuberculosis (TB) sufferers is still

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high. Based on data from the Provincial Health Office, in 2011, there were 8,939 cases of this infectious disease. This figure has increased significantly compared to the previous year which only had 7,783 cases. In the city of Makassar, the number of suspected TB cases is around 13,701, BTA(+) is around 1,737 cases, relapses are around 92 cases[6]. Takalar Regency was ranked first in the number of cases with a growth in TB sufferers above 109%, followed by Pare-pare 79%, Pinrang 75%, followed by Makassar 70% and the lowest was Luwu Regency 33% and Jeneponto 36%.

Until now, the tuberculosis revention program using the DOTS (Directly Observed Treatment shortcourse) strategy has not been able to support all health centers. In the prevention of tuberculosis, examination of microscopic preparations of Acid Resistant Basil (BTA) from sputum specimens is a key component for establishing a diagnosis as well as evaluating and following up treatment. Therefore the results of smear microscopic examination must be precise and thorough. In order to guarantee the quality of the results of microscopic examination of AFB microscopic preparations, quality assurance activities must be carried out in various aspects regularly and continuously. Specimens were collected in wide-mouthed sputum pots of 6 cm or more in cross-section.

METHODOLOGY

The research that will be carried out is research with a descriptive approach to find out the results of microscopic examination of BTA (acid fast bacilli) between preparations which after fixation are immediately stained with preparations that are delayed for several days. The location has been carried out at the Laboratory of Bhayangkara Hospital, where the research was carried out in June 2022. The first thing to do, namely: 1. Sample preparation. Samples in the form of sputum from all patients who came to check at Bhayangkara Hospital were then collected in a wide-mouthed pot with a transparent lid, colorless, not easily broken and leaky. 2. The tools used in this research are: Microscope, staining rack, object glass, spirit lamp, and one or stick 3. The materials used in this research are The patient's phlegm, Carbol fuchsin, Methylene Blue, Aquadest, 3% HCl-Alcohol, and Oil immersion. 4. How it works. 1. Manufacture of smear preparations. The patient's identification number is written on the glass object using a permanent marker or on a sticker placed on the

back of the preparation. Next, select and take part of the purulent phlegm using an ose or stick with the tip flattened. To flatten the preparation, small spirals were made during the semi-dry smear using a sharp stick so that the distribution of leukocytes was more even and the reading area was more homogeneous. The use that has been used is dipped in a bottle of disinfectant sand, then burned until the ose is smoldering. When using a stick, immediately throw it into a bottle filled with disinfectant. Furthermore, the preparations were dried in air and then fixed by passing 3 times through the flame of a spirit lamp. The preparations were then dried on the shelf and avoided being exposed to direct sunlight. 2. Ziehl-Neelsen method of staining. The preparation is placed on the staining rack with the smear facing upwards, each preparation is approximately 1 finger apart. The entire surface of the preparation was flooded with carbol fuchsin. The preparation is heated from below using a spirit flame on the preparation until steam comes out (do not boil) then allowed to stand for 5 minutes. After that, it was rinsed carefully with running water and then flooded with alcoholic acid until no red carbol fuchsin color was visible. Furthermore, the surface of the preparation was flooded with methylene blue for 10-20 seconds, then rinsed with running water, and then dried on a drying rack. 3. Smear reading. One drop of immersion oil is placed on the surface of the preparation, then the 100 x objective lens is carefully rotated over the smear. The focus is adjusted carefully until the cells are visible. BTA is visible in the form of rods, bright red on a blue background without any traces of fuchsin dye. BTA found established the diagnosis of TB and the number of BTA found indicated the number of germs in the TB patient. The results of this report refer to the scale of the International Union Against Tuberculosis and Lung Diseases (IUATLD) and the World Health Organization. The research results are presented in tabular form and then presented.[6]

RESULT AND DISCUSSION

AFB sputum examination was carried out by collecting 3 sputum samples which were taken sequentially in two days of visits. The sample is called the SPS sample (in the morning-in time). Sputum sampling for TB examination is carried out by: S (When): sputum is collected into a sterile container (sputum pot) when the patient comes for the first visit. On

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discharge, the patient will be given an empty sputum pot to fill in a second sputum sample the following day. F (Morning): on the second day, you must collect the sputum in the morning, at home after waking up. The pot must be brought and handed over to the officer at the health facility. S (when): You will be asked to collect the third phlegm in the last pot when you come to hand over the morning phlegm at the health facility. The phlegm collected should be thick, cloudy, and sticky mucus that is expelled from the lungs. Not from the nose or saliva from the mouth[7]. There are several methods of collecting phlegm that you can do: Coughing hard: the patient will be asked to take a deep breath, hold it for 5 seconds, and exhale slowly. After that, take a few deep breaths and try to cough hard. This step will draw phlegm from the lungs to collect in the mouth. Sputum induction: for patients who cannot cough up phlegm, sputum production can be stimulated by administering saline solution via a nebulizer. Bronchoscopy: another way to collect sputum is by bronchoscopy. You will usually be given an anesthetic. later. The bronchoscope will be inserted through the nose or mouth into the throat until it reaches the lungs. Sputum contained in the lungs will be aspirated into a bronchoscope tube and collected in a sterile pot. Gastric aspiration: this method is used on sputum swallowed by the patient. Usually this action is performed on patients who are unconscious or children. Examination of sputum samples The samples that have been collected will then be analyzed in the laboratory. There are two types of sputum examination in the AFB test, including: AFB staining test In this sputum test, the sample will be smeared on a glass object to be colored, heated, and doused with an acid solution. Under a microscope, laboratory workers will observe the color changes in the sample. Test results are usually available after 1-2 days. Sputum culture in this sputum test for tuberculosis, the sample will be put into a special medium that supports the cultivation of acid-fast bacteria. A positive result from this culture of acid-fast bacteria can confirm a diagnosis of TB or another infection. However, it takes 6-8 weeks for bacteria to grow in sufficient numbers to detect infection. Diagnosis of TB disease by examination of Basil Acid Resistant (AFB). In principle, this examination is done by looking at germs *Mycobacterium tuberculosis* as a cause of tuberculosis directly under the microscope. In in this examination sputum is used as a specimen because sputum is a a good medium for the growth and life of TB germs,

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so that the germs TB germs will collect in the phlegm[8].

Based on the results of examination of acid fast bacilli (AFB) by the ziehl neelsen (ZN) method on smear preparations which were immediately stained and which were postponed for three days as many as 10 samples which were carried out at the Laboratory of Laburan Baji Hospital Makassar on 08 to 11 August 2022 obtained results as in the table below.

Tabel 1. Results of Examination of Acid Resistant Basil (AFB) Ziehl Neelsen (ZN) Method on AFB preparations which were immediately stained and which were delayed for three days.

No	Sample Code	Check up Result		Desription
		Direct Preparations	Delay 3 Days	
1	A1	+++	+++	Same
	B1	+++	+++	Same
	C1	+++	+++	Same
2	A2	-	-	Same
	B2	+	+	Same
	C2	+	+	Same
3	A3	+++	+++	Same
	B3	+++	+++	Same
	C3	+++	+++	Same
4	A4	+++	+++	Same
	B4	+++	+++	Same
	C4	+++	+++	Same
5	A5	-	-	Same
	B5	+	+	Same
	C5	+	+	Same
6	A6	-	-	Same
	B6	++	++	Same
	C6	++	++	Same
7	A7	+++	+++	Same
	B7	+++	+++	Same
	C7	+++	+++	Same
8	A8	-	-	Same
	B8	+	+	Same
	C8	+	+	Same
9	A9	-	-	Same
	B9	++	++	Same
	C9	++	++	Same
10	A10	+++	+++	Same
	B10	+++	+++	Same
	C10	+++	+++	Same

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Examination of Acid-Fast Bacteria or AFB or is one of the examinations carried out to establish a diagnosis of Tuberculosis (TB), this examination is carried out to detect the presence of bacteria that cause tuberculosis, especially pulmonary tuberculosis. Tuberculosis bacteria can survive in an acidic environment, so the examination is called acid-fast bacteria examination

In the prevention of tuberculosis, examination of microscopic preparations of Acid Resistant Basil (AFB) from sputum specimens is a key component for establishing a diagnosis as well as evaluating and following up treatment. Therefore the results of smear microscopic examination must be precise and thorough. In order to guarantee the quality of the results of microscopic examination of AFB microscopic preparations, quality assurance activities must be carried out in various aspects regularly and continuously. Specimens were collected in sputum pots with wide mouths with a cross section of 6 cm or more with screw caps that were not easily broken or leaked. The diagnosis of tuberculosis is established by examining three sputum specimens, namely every morning – every hour (SPS). This means that when a suspected tuberculosis comes to visit for the first time it is called when, when he comes home the suspect brings a pot to collect the second phlegm called morning phlegm which is taken immediately after waking up. When the suspect comes to bring the morning phlegm, the phlegm is taken again, which is called phlegm when. After the phlegm has been taken, preparations are made immediately. All preparations that have been fixed are immediately stored in the preparation box to avoid the risk of breaking. Then the preparations were sent to the Microscopic Referral Health Center (PRM) for a pulmonary TB laboratory for sputum examination[9].

Microscopic examination of sputum is efficient, easy, cheap, specific, sensitive and can be carried out in all laboratory units of health care facilities that have microscopes and trained TB microscopists. Good sputum is collected in a transparent pot, volume 3-5 ml, mucoid thickness and the color is yellowish green (purulent). The results of the macroscopic study of sputum samples for direct AFB examination and 24 hours delay there are some physical differences. 1. Viscosity, initially thick sputum (purulent, mukopurulent), after being stored 24 hours at room temperature space (25°C) becomes dilute. Watery sputum can occurs because the room temperature tends to warm (25°C) within 24 hours can make decreased

sputum consistency. Warm temperatures can causing the granules to rupture sputum compound, so the liquid will come out of granules, thereby appearing more watery[10]. The condition of watery sputum means that the quality is decreasing. Watery sputum will be difficult to make preparations AFB, because the results of the preparation will be thin, sometimes difficult average and in conclusion the preparation is not good. The Ministry of Health of the Republic of Indonesia, 2017 states that preparations that are called good must meet 6 criteria The standard is a purulent/mucopurulent specimen, good coloring, clean, good thickness, size 2x3 cm and evenness >80%. 2 Smell of sputum stored 3 days on room temperature smells sharp/ pungent, different with a characteristic fresh sputum odor. Changes in the smell of sputum caused by: growth of spoilage microbes and possibilities mold. Sputum is also a source of nutrition for other microbes besides *Mycobacterium tuberculosis*, so it is very possible if left on room temperature can be grown by other microbes such as mold and other spoilage bacteria. That smell stinging can interfere with the manufacturing process preparation, namely to the officer who makes it AFB preparations. The presence of fungi and bacteria / other microbes can interfere with microscopic examination especially in result reading. Mold or other microbes can cover it AFB contained in the preparation. Results of preparation reading can be negative or false positive. The quality of sputum determines the outcome smear preparation readings, false negative or positive results counterfeit can lead to incorrect treatment or untreated which eventually becomes a source of transmission in community [10]. Of the 10 direct sputum samples and sputum in store for 3 days at 25°C (temp room) by microscopic examination, was found sample with the same result. calculation results, preparations originating from Sputum samples stored for 3 days at 25°C require a longer time even in the manufacture of preparations, because of the quality of the sputum dilute and produce a thin preparation which means the quality of the test sample and the thickness is not good but the inspection results remain the same. Research conducted by Maria (2011) obtained result that the direct method and concentration method can be used for the diagnosis of pulmonary TB, but for concentration method it can increase the number of AFB because concentration collecting method AFB and damaging bacteria aside from AFB. Based on this on This research was

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conducted by method concentration. AFB examination method concentration can increase the number discovery of AFB, because on the method AFB concentrations were collected so that the AFB coverage figure will be increase. This is because AFB microscopic examination method concentration requires volume relatively large number of sputum specimens viz about 2-4 ml of phlegm so as to find AFB in more sputum easy, this is important for TB cases lungs with Mycobacterium counts little tuberculosis. But this thing becomes difficult to do when the amount small sputum specimens were obtained or less than 2 ml. If found thing like that, then inspection AFB microscopy direct method can be done.

There is a change in the consistency of sputum will complicate the laboratory personnel in make good preparations. which preparation must meet 6 standard criteria including purulent specimens or mucopurulent, good staining, clean, good thickness, appropriate size and evenness good (> 80%). Laboratory staff expected to understand the procedure good specimen management for support diagnostic accuracy. specimen sputum for AFB examination is recommended immediately after sampling. This is done to minimize error results are not as good as positive or false negatives it can cause error in decision making patient treatment [11].

In general, TB disease in Indonesia is now experiencing increase from year to year along with the increase in HIV cases [12] so that prevention efforts are needed and countermeasures. Prevention efforts that can be done include: provide health counseling about the importance of checking sputum early on if there are symptoms of tuberculosis, maintain personal hygiene and the environment. And for countermeasures can be done by providing medical treatment intensive.

CONCLUSION

Based on the results of research that has been carried out on 10 sputum samples examined immediately and which was suspended for 3 days from room temperature samples in the Bhayangkara Hospital, Makassar City. in June 2022, then got concluded that: Sputum samples examined directly macroscopically, the viscosity is mucoid (not watery), yellowish green color (purulent), sputum characteristic odor. Sputum samples suspended for 3 days at temperature room is the viscosity begins to decrease (melting), so it becomes runny. The color is dull yellowish, the smell is sharper than

sputum immediately check and some of them have mold contamination. However, there was no difference in the results of the examination of the preparations that were immediately colored and those that were delayed for three days at room temperature. The presence of fungi and bacteria / other microbes can interfere with microscopic examination especially in result reading. Mold or other microbes can cover it BTA contained in the preparation. Results of preparation reading can be negative or false positive.

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Inhibition of Apoptosis of Liver Cells of Mice Infected With *Plasmodium berghei* Through The Expression of Caspase-3 Using *Sargassum duplicatum* Extract

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Abstract

The process of apoptosis is an integrated process between external and internal factors involving several enzymes (Caspase-9, -8, -7, -6, -3) that act as major players in the process of apoptosis. This research aims to determine the potential of brown algae methanol extract Sargassum duplicatum against inhibition of apoptosis of liver cells of mice infected with Plasmodium berghei through the expression of caspase-3. Mice weighing 20–30 grams in Plasmodium berghei infection as much as 0.1 ml per head and left until the percent of parasitemia reaches 1-5%. Then mice (Mus musculus) were given methanol extract of Sargassum duplicatum seaweed at a dose of 1 gr / 100 ml, 10 gr / 100 ml, 100, gr / 100 ml, and 200 gr / 100 ml for 4 consecutive days and observed until day 6. After that, a histological preparation was made with immunohistochemistry staining to see the expression of caspase-3. The results of the observations will be analyzed descriptively. The results showed that Sargassum duplicatum methanol extract was able to inhibit liver cell apoptosis in mice infected with Plasmodium berghei. The decrease in Caspase-3 expression in this study is thought to be caused because the brown algae Sargassum duplicatum contains flavonoid compounds, tannins, and saponins which can reduce the pro-inflammatory cytokine caspase-3 through the role of NF-kB which is a transcription factor that plays a role in stimulating and coordinating innate and adaptive immune responses.

***Keywords**: Malaria, Caspase-3, Sargassum duplicatum, Plasmodium.*

Abstrak

Proses apoptosis merupakan suatu proses yang terintegrasi antara faktor eksternal dan internal yang melibatkan sejumlah enzim (Caspase-9, -8, -7, -6, -3) yang berperan sebagai pemain utama dalam proses apoptosis. Penelitian ini bertujuan untuk mengetahui potensi ekstrak metanol alga cokelat Sargassum duplicatum terhadap penghambatan apoptosis sel hati mencit terinfeksi Plasmodium berghei melalui ekspresi caspase-3. Mencit dengan berat badan 20 – 30 gram di infeksi Plasmodium berghei sebanyak 0,1 ml per ekor dan dibiarkan sampai persen parasitemia mencapai 1-5%. Kemudian mencit (Mus musculus) diberi ekstrak methanol rumput laut Sargassum duplicatum dengan dosis 1 gr/100 ml, 10 gr/100 ml, 100 gr/100 ml dan 200 gr/100 ml selama 4 hari berturut-turut dan diamati sampai hari ke 6. Setelah itu dibuat preparat histologi dengan pewarnaan immunohistokimia untuk melihat ekspresi caspase-3. Hasil pengamatan akan dianalisis secara deskriptif. Hasil penelitian menunjukkan bahwa ekstrak methanol Sargassum duplicatum mampu menghambat apoptosis sel hati pada mencit terinfeksi Plasmodium berghei. Terjadinya penurunan ekspresi Caspase-3 dalam penelitian ini diduga disebabkan karena alga cokelat Sargassum duplicatum mengandung senyawa flavonoid, tannin dan saponin yang dapat menurunkan sitokin proinflamasi caspase-3 melalui peranan NF-kB yang merupakan faktor transkripsi yang berperan dalam merangsang dan mengkoordinasi respon imun innate dan adaptif.

Kata kunci: Malaria, Caspase-3, Sargassum duplicatum, Plasmodium.

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INTRODUCTION

The existence of Plasmodium in the body will stimulate the immune system, especially the Reticulo Endothelial System (RES) by activating T lymphocyte cells, macrophages, and various cytokines such as Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-1 (IL-1), causing induction of the release of Reactive Oxygen Intermediate (ROI) and Reactive Nitrogen Intermediate (RNI) oxygen metabolites that will react to form Nitric Oxide (NO) through the activation of inducible Nitric Oxide Synthase (iNOS / NOS / NOS type2) which plays a role in eliminating parasites, but due to its non-specific nature, it can cause pathological abnormalities[1].

The increase in free radicals due to malaria pathogenesis will cause various damages to body organs, including the liver, kidneys, and lungs. In liver cells, TNF-α will cause protein synthesis in the acute phase, and in muscle cells will cause catabolism and in some cells will cause apoptosis. Apoptosis is programmed cell death which is an important process in the normal regulation of homeostasis, this process generates a balance in the number of cells of a certain tissue through the elimination of damaged cells and physiological proliferation and thus maintains normal tissue function.[2,3]

The process of apoptosis is an integrated process between external and internal factors involving several enzymes (Caspase-9, -8, -7, -6, -3) that act as major players in the process of apoptosis where Caspase-3 is the caspase executor in cell apoptosis. This mechanism includes two pathways, namely: the extrinsic pathway, and the intrinsic pathway.[4]

The widely reported cases of resistance further enhance the progressiveness of studies to find new antimalarial drugs. Plants used as antimalarial drugs so far are mostly from land plants, while plants derived from the sea such as seaweed (algae) have not received much attention.[5,6] Currently, *Sargassum duplicatum* has not been optimally utilized,[7] even though *Sargassum duplicatum* is very useful, for example in the fields of health, microbiology, enzymology, and ecotoxicology.[8] Sargassum contains steroid compounds, alkaloids, phenols, flavonoids, saponins, and tannins.[9] The role of flavonoid compounds in inhibiting the growth of malaria parasites has been proven in some antimalarial medicinal plants.[10] The presence of antioxidants in the brown algae *Sargassum duplicatum* can increase the immune system in the form of cytokines, inhibiting the occurrence of cell apoptosis, namely by neutralizing free radicals so that macrophage activity in the body will be reduced.

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METHODOLOGY

Types of Research

The type of research used in this study is an experimental method (laboratory experiment).

Tools and Materials

The tools used are an Erlenmeyer measuring 1000 ml piece of Erlenmeyer measuring 500 ml, measuring cup, filter paper, a set of glassware, electronic scales, blender, rotavapor, mice confinement container, analytical balance, syringe, object glass container, scratch slide, electron microscope, tube centrifuge, hearing, sonde tool, volume pipette, mortar, spatula, test tube, vaporizer and digital camera, paraffin box, object glass, microtome, Micropipette.

While the ingredients used are brown algae, methanol, *Plasmodium berghei* culture, mice, CMC Na 0.5%, alceiver, aluminum foil, tissue, cotton, immersion oil and detergent, alcohol (30%, 50%, 70%, 80%, 90%, 100%), Albumin Glycerin, Caspase 3 antibody, secondary antibody labeled biotin, chloroform, DAB (3,3 diaminobenzidine), etellan, formalin 4%, FBS, Hematoksilin, H_2O_2 %, paraffin (I, II, III), PBS (Phosphate Buffer Saline), SA-HRP Peroxidase (Strep Avidin-Hesoradish Peroxidase), Sterile Aquades, and Xylol I, II, III.

Research Design

This research used a complete randomized design with 4 treatments and 3 repeats. The division of groups can be seen as follows:

1. Group I : Mice were given Plasmodium berghei infection but not treated.
2. Group II: Mice infected with Plasmodium berghei and given Sargassum duplicatum extract at a dose of 10 mg/kgBB.
3. Group III: Mice infected with Plasmodium berghei and given Sargassum duplicatum extract at a dose of 100 mg/kgBB.
4. Group IV: Mice infected with Plasmodium berghei and given Sargassum duplicatum extract at a dose of 200 mg/kgBB.

Work Procedure

Extraction

Sargassum duplicatum is taken and dried to air at room temperature. After drying, it is mashed with a blender (grinder) and the powder that has been smooth is weighed. Furthermore, extraction is carried out using the maceration method. A total of 100 grams of *Sargassum duplicatum* powder was put into Erlenmeyer with a size of 1000 ml, input methanol 250 ml and homogenized then left for 24 hours. After that, it is filtered and applied using a rotary evaporator so that a concentrated extract is obtained.

Testing the effectiveness of anti-malarial in vivo

Mice tried to be infected with as much as 200 μl of blood from donor mice and then observed the level of

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parasitemia. After the percentage of parasitemia (1-5%) is known, it is continually testing the effectiveness of malaria from the extract according to the predetermined dosage. The treatment was carried out for 4 consecutive days and observations were carried out until the 6th day (D0-D6). After that, hepatectomy is performed to take the liver organs of mice.

Making Liver Histology Preparations

Preparation of liver tissue is carried out by procedures according to Suntoro:[11]

1. Liver that has been fixed with Formalin 4 % washed with equates for 5 minutes, dehydrated in stratified alcohol ranging from 30 %, 50 %, 70 %, 80 %, 90 %, and 100% each for 5 minutes.
2. The remaining alcohol is cleaned by a clearing process, and the liver is soaked in Xylol I and Xylol II for 5 minutes each.
3. Infiltration process, liver organs are inserted in Paraffin I, Paraffin II, and Paraffin at III 60°C, each for 45 minutes.
4. In The process of embedding or planting, the liver is put in a Paraffin box for 15 minutes. Then sectioning or slicing is carried out through boiling in Paraffin blocks for a while then cutting with microtome with a thickness of 6 microns.
5. After taking the slicing results, the object glass is smeared with Albumin Glycerin so that the slicing results can stick to the object glass then placed on a hotplate with a temperature of 40 °C which aims to stretch the sliced results and melt paraffin on the object glass.

Observation of Caspase-3 Expression by Immunohistochemistry Method

Observation of Caspase-3 expression was carried out using immunohistochemistry painting methods according to Larasati:[12]

1. Hepatic histology preparations were washed with PBS (Phosphate Buffer Saline) pH 7.4 for 3 minutes, 3 times.
2. To remove endogenous perioxide, 3% H2O2 is used for 20 minutes. Next, the preparations are re-washed with PBS pH 7.4 for 3 minutes, 3 times.
3. The preparations were dripped with Caspase-3 primary antibodies, then incubated at 4°C for one night. After that the preparation is washed with PBS pH 7.4 for 3 minutes, 3 times.
4. Next, drip the preparation with a secondary antibody labeled AP (Alkaline Phosphatase) 1: 2500 anti-IgGgG AP Labelled) and then incubated at room temperature for one hour. After that, it is washed with PBS pH 7.4 for 3 minutes, 3 times.
5. Drops of preparations with Peroxidase Strep Avidin-Hesoradish Peroxidase (SA-HRP) and incubates at room temperature for 60 minutes. The preparations were then washed back with PBS pH 7.4 for 3 minutes, 3 times.

6. Chromogen is administered by dripping with a solution of 3,3-diaminobenzidine (DAB), and incubated at room temperature for 20 minutes. Then washed with equates for 5 minutes, 3 times.
7. The next process is counterstain, using hamatoksilin incubated at room temperature for 20 minutes. Then dripped with tap water, and washed equates to 5 minutes, 3 times.
8. Dehydration is carried out with a stratified alcohol solution of 70%, 80%, 90%, and 100% and absolute alcohol I and II for 1 minute each.
9. Clearing is done with xylol I and xylol II for 3 minutes each, then the preparations are dried.
10. Mounting using an entellan, then covered with a glass object, labeled with the name of the preparation is then observed under a microscope.
11. If Caspase-3 is detected, it will be brown.

Data Analysis

Caspase-3 expressions will be analyzed descriptively.

RESULTS AND DISCUSSION

Photomicrograph of liver cells of mice (*Mus musculus*) with Immunohistochemistry staining can be seen in Figure 1.

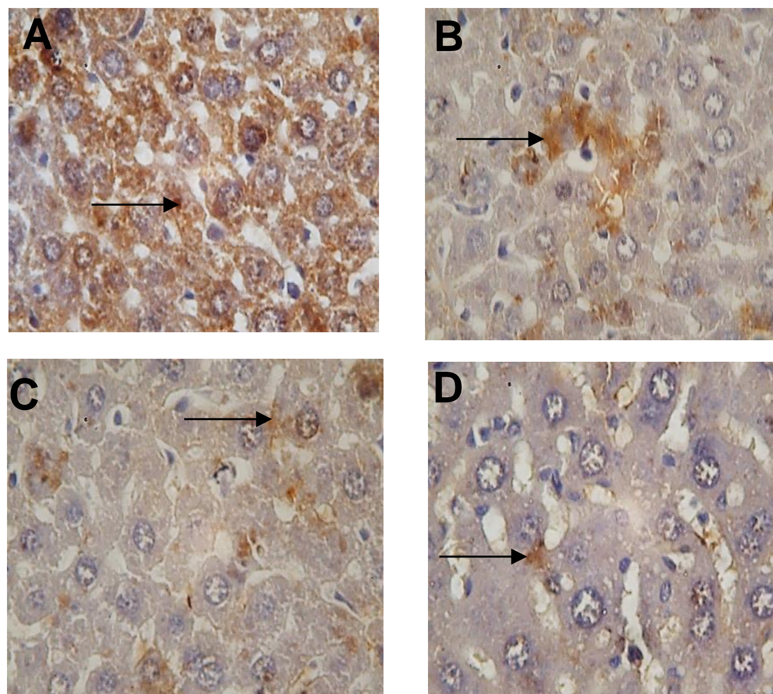

Figure 1. Photomicrograph of liver cells of mice by immunohistochemistry staining method. (A) group I, (B) group II, (C) group III, and (D) group IV.

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Based on the results that have been obtained, it can be explained that Plasmodium berghei infection in mice can cause apoptosis of liver cells. This is seen through the expression of Caspase-3 on liver cells when animals are infected with Plasmodium berghei (Figure 1A). Caspase-3 can be activated also through intrinsic pathways that can lead to the activation of caspase and the induction of apoptosis through the mitochondria. In mitochondria, apoptosis occurs through a potential reduction in the inner mitochondrial membrane and the release of a number of small molecules including apoptosis-inducing factor (AIF), endonuclease G, SMAC/DIABLO, HtrA2/Omi, and cytochrome c (Cyt c).[13] Under normal circumstances, cyt c is located in the space between the inner and outer mitochondrial membranes where it functions in oxidative phosphorylation. However, once released it will immediately join in the proteolytic activation of caspase-3 by caspase-9. The event led to the gathering of a multiprotein complex that activates caspase called 'apoptosome'. Apoptotic protease activating factor-1 (Apaf-1) forms the principal component of the apoptosome.[14] Atroph-1 is a protein that plays a role in the activation of caspase so that it is bound to cyt c, which is related to procaspase-9 through the breakdown of the caspase recruitment domain (CARD) and activates it. Caspase-9 can then reproduce procaspase-3, activating it through enzymatic breakdown to form caspase-3, which can then initiate a cascade of apoptosis involving caspase-2, -3, -6, and -7 effectors which ultimately results in DNA fragmentation.[3] This event is also followed by the translocation process of phosphatidylserine to the outer plasma membrane which will facilitate the introduction of apoptotic cells by macrophages, resulting in the clearance of dead cells.

Administration of *Sargassum duplicatum* extract can inhibit apoptosis of liver cells of mice infected with *Plasmodium berghei.* This can be seen by the large number of cells expressing caspase-3 in the negative control group (infected with *Plasmodium bergehi* but not given extract *Sargassum dulicatum*) when compared to the positive control group, the mice group infected with *Plasmodium bergehi* and given methanol brown algae Sargassum duplicatum dose 10 mg/kg BB (Figure 1B), 100 mg/kg BB (Figure 1C) and 200 mg/kg BB (Figure 1D).

The decrease in Caspase-3 expression in this research was caused because *Sargassum duplicatum* contains flavonoid compounds, tannins, and saponins which are thought to reduce the pro-inflammatory cytokine caspase-3 through the role of NF-kB which is a transcription factor that plays a role in stimulating and coordinating innate and adaptive immune responses. The mechanism of decreasing NF-kB activation includes

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inhibiting the translocation of NF-kB into the nucleus, inhibiting Ik-B phosphorylation, inhibiting the activation of genes that encode NF-kB transactivation and Ik-B degradation, or through the obstruction of the Ik-B degradation process by the proteasome. The decrease in NF-kB activation results in a decrease in NF-kB signaling in immune cells, thus regulating the decrease in TNF-α.[15]

Flavonoid compounds (prenylated stilbene) from Artocarpus integer have in vitro antimalarial activity in Plasmodium falciparum.[16] Flavonoid activity was also reported in studies conducted on plants. This plant is known as the basic ingredient of the malaria drug artemisinin with a lactone sesquiterpene content that is active against drugs resistant to Plasmodium falciparum. The results of research on several flavonoid compounds of this plant are known that flavonoids can increase the reaction of artemisinin to hemin and have antimalarial activity in vitro potential.[17]

CONCLUSION

Based on the results that have been obtained, it can be concluded that the methanol extract of brown algae *Sargassum duplicatum* can potentially inhibit liver cell apoptosis through the expression of Caspase-3.

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Organeleptic Test for Fortified Packaging Milk by Utilizing Calcium from Egg Shell Waste

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Abstract

In terms of national needs, the necessity for eggs reached 4,742,240 tons employing massive impact on the production of egg shell waste. Nevertheless, packaging milk released in the market contains low calcium and unsatisfies the nutritional needs of adult calcium, namely 1000 mg/day. Therefore, it is necessary to add waste of calcium-rich egg shells so the nutritional needs of calcium can be fulfilled and reduces the egg shell waste at the same time. The research method used is combination of experimental methods, interviews, and literature study. The data is analyzed by using descriptive statistics and non-parametric statistical tests to draw conclusions. Based on the data obtained, the best variation is the milk control with a total value of 580 points, 33 points different from 2:1 variation. The giving of egg shell waste reduces the milk aroma which is preferable for people who dislike its aroma. Through non-parametric statistical tests, it was investigated that there was no significant difference in adding egg shell flour to make fortified milk except for egg shell control. Therefore, egg shell flour can be used as additional material to enrich the calcium, to reduce the milk aroma and the amount of organic waste in Indonesia.

***Keywords**: Calcium, Egg shell waste, Fortified milk, Non parametric statistics, Packaging milk*

Abstrak

Dari sisi kebutuhan nasional, kebutuhan telur mencapai 4.742.240 ton berdampak besar pada produksi limbah cangkang telur. Namun demikian, susu kemasan yang beredar di pasaran mengandung kalsium yang rendah dan belum memenuhi kebutuhan gizi kalsium orang dewasa yaitu 1000 mg/hari. Oleh karena itu, perlu dilakukan penambahan limbah cangkang telur yang kaya kalsium agar kebutuhan nutrisi kalsium dapat terpenuhi sekaligus mengurangi limbah cangkang telur. Metode penelitian yang digunakan adalah kombinasi metode eksperimen, wawancara, dan studi literatur. Data dianalisis dengan menggunakan statistik deskriptif dan uji statistik non parametrik untuk menarik kesimpulan. Berdasarkan data yang diperoleh, variasi terbaik adalah susu kontrol dengan total nilai 580 poin, berbeda 33 poin dari variasi 2:1. Pemberian limbah cangkang telur mengurangi aroma susu yang disukai oleh masyarakat yang tidak menyukai aromanya. Melalui uji statistik non parametrik diketahui bahwa tidak ada perbedaan yang signifikan dalam penambahan tepung cangkang telur untuk membuat susu fortifikasi kecuali pada kontrol cangkang telur. Oleh karena itu tepung cangkang telur dapat digunakan sebagai bahan tambahan untuk memperkaya kalsium, mengurangi aroma susu dan jumlah sampah organik di Indonesia.

***Kata kunci**: Kalsium, Limbah Cangkang Telur, Statistik Non-Paerametrik, Susu Kemasan, Susu Terfortifikasi,*

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INTRODUCTION

Environmental pollution is the entry or inclusion of living things, energy substances, and or other components into the environment or changes in the environmental order by human activities or by natural processes so that the quality of the environment drops to a certain level which causes the environment to become less or unable to function anymore[1]. Environmental pollution has impact on health, aesthetics, economic losses, and disruption of ecosystems naturally[2]. Environmental pollution is generally caused by waste from the community, both organic and non-organic waste. One of the wastes that has the potential to pollute the environment is egg shells with the main composition of $CaCO_3$ which will become waste and can cause pollution due to microbial activity in the environment[3].

Eggs are an important source of protein for humans in everyday life. With various processes such as boiling, frying and steaming, eggs become a simple food that is quite delicious[4]. In terms of national needs, the need for eggs in Indonesia currently reaches 4,742,240 tons which has an impact on the large amount of eggshell waste production. So far, eggshell waste has not been used optimally. The eggshell is only used as a handicraft product. Whereas 97% of the calcium content in eggshells has the potential as an additional ingredient extracted for food minerals[5]. Egg shells are also composed of 94% calcium carbonate, 1% magnesium carbonate, 1% calcium phosphate and 4% organic matter, especially protein[6]. The composition of egg shells in general consists of 1.6% water and 98.4% dry matter, namely 95.1% minerals and 3.3% protein[7].

Calcium is one of the essential minerals that has an important role in the body, namely as a major component in forming bones and teeth[8]. Calcium needed every day ranges from 1200 mg / day for ages 10-18 years, 1100 mg / day for the 18-29 year age group, while for those over 29 years it's 1000 mg / day . One source of calcium that is generally used by Indonesians comes from cow's milk. However, in general Indonesian people experience lactose intolerance (digestive disorders). Cow's milk contains a lot of lactose so that generally Indonesian people cannot meet their calcium needs from cow's milk so it is necessary to look for other alternative drinks[9]. Meanwhile, other packaged milk circulating in the market contains relatively low calcium and tends to have insufficient calcium nutritional needs.

In addition, one of the most vulnerable health problems in Indonesia is cholesterol and calcium

deficiency. The data states that the level of cholesterol sufferers in Indonesia is 70%. The number of people suffering from calcium deficiency disease in Indonesia is much greater than the latest data from the Ministry of Health, which set a figure of 19.7% of the entire population. Therefore, we need a food industry that provides foods with low cholesterol levels with high calcium levels[10].To meet calcium needs, it is necessary to add egg shell waste which is rich in calcium so that the nutritional needs of calcium can be met.

Therefore, this research will conduct an experiment entitled "Organeleptic Test of Fortified Packaged Milk Using Calcium from Eggshell Waste". The eggshell flour will be combined with packaged flour to get the best variety in order to get the best fortified milk in terms of taste, aroma and color. This research aims to investigate the effect of eggshell waste in terms of aroma, color and taste in packaged milk mixtures and to investigate the optimal variation between egg shell waste and packaged milk in order to obtain the best milk formulation rich in calcium in terms of aroma, color and taste.

METHODOLOGY

Research Type

This type of research is a type of quantitative experimental research using a combination of experimental methods, observation and literature studies. The research began with a literature study of egg shell waste and made fortified milk using the internet and continued with observations to obtain judgement of respondents.. With the combination of these methods, maximum results will be obtained both quantitatively and qualitatively.

Tools and Materials

Tools which have been used within this research are beaker glass, funnel, tablespoons, tissue, filter paper, measuring cup, ordinary glass, strainer, blender, strainer, spatula, pan, erlenmeyer, basin. For the material, this research requires many materials like egg shell waste, packaging milk powder and water.

Research Variables

The independent variable is the variable that affects or causes the change or the emergence of the dependent variable[11]. The independent variables in this study were egg shell waste and packaged flour which were varied based on composition. Variations were made as much:

1. Egg shell : packaged milk (1: 1)
2. Egg shell : packaged milk (1: 2)
3. Egg shell : packaged milk (2: 1)
4. Egg shell flour control
5. Packaged milk control

The dependent variable is the variable that is affected or that is the result, because of the independent variable[11]. The dependent variable in this study is the milk which are seen based on the taste, aroma and texture of milk.

Research Steps

This research was conducted in three stages, the first stage was the preparation stage including egg shell waste to form egg sheel flour. The second stage is the stage of mixing step of packaging milk powder and egg shell waste or another name fortified milk. The third stage is the testing phase in the form of interviews with fortified milk. The research stages are described as follows.

Preparation phase

The details of the preparation stages are described as follows: First, egg shell wastes are collected and all white membrans are surely removed. Next, all egg shell waste are washed, rinsed and later boiled about 30 minutes. Afterthat, egg shell waste must be dried because it must be mashed for obtaining egg shell flour.

Variation Making Stage

The details of the stages of making variations are described as follows: First prepare egg shell flour and packaging milk. Then, the mass of egg shell flour and packaging milk were measured using analytical scales. Furthermore, the variation in the composition is adjusted by ratio of 1: 1, 2: 1, 1: 2, egg control and milk control (without egg shell waste additional). All variations are mixed with hot water and stirred as one.

Testing Phase

The details of the fortified milk testing stages are described as follows: fortified milk and research questionnaires were prepared in advance. Research questionnaires and fortified milk were presented to the panelists to be assessed in terms of taste, aroma and color aspects. Assessments were made for all variations of fortified milk. Research data for each variation of fortified milk were obtained and non-parameter statistical tests in this case Kruskall Wallis test and Mann Whitney U were performed.

Research Method

Organoleptic Test

Organoleptic testing is a test that uses the human senses as the main tool in measuring the acceptability of products. Organoleptic testing has an important role in determining the quality and quality of products. In this study, organoleptic testing has been carried out related to the influence of egg shell flour on the taste, aroma and color parameters of donuts. Organoleptic testing has been carried out on 40 respondents

with interview techniques and provided assessments on a likert scale ranging from (1) very less to (6) excellent[12]. All respondents are classified by those rating scales to the information according to their scores. Respondents will be asked to rate the aroma, color and taste indicator to verify the best fortified milk based on organoleptic test.

Descriptive Statistics

Descriptive statistics are a type of statistics used to describe related activities of collecting, structuring, summarizing, presenting data to make it more meaningful. Descriptive statistics are limited to providing a description or general description of the characteristics of the object under study without the intention of generalizing the sample to the population. The mean (mean) and standard deviation are part of the descriptive statistics used to report the results of research measurements[13].

Kolmogorov Smirnov Test

The Kolmogorov Smirnov test is a widely used normality test, especially after the existence of many statistical programs in circulation. The advantage of this test is that it is simple and does not cause differences in perception between one observer and another, which often occurs in normality tests using graphs. The basic concept of Kolmogorov Smirnov's normality test is to compare the data distribution (which will be tested for normality) with the standard normal distribution. The standard normal distribution is data that has been transformed into a Z-Score form and assumed to be normal. In drawing the conclusion, if the significance is below 0.05 it means that there is a significant difference, and if the significance is above 0.05 then there is no significant difference. The application to the Kolmogorov Smirnov test is that if the significance is below 0.05, it means that the data to be tested has a significant difference from the standard normal data, it means that the data is abnormal[14]. In their tests, the author used SPSS version 17 to facilitate work in terms of data processing.

Kruskall Wallis Test

The Kruskal Wallis test is a ranking-based nonparametric test whose purpose is to determine whether there are statistically significant differences between two or more groups of independent variables on dependent variables that scale numerically data (intervals/ratios) and ordinal scales. This test is identical to the OneWay Anova Test in parametric testing, so this test is an alternative to the One Way Anova test if it does not meet assumptions such as normality assumptions. Apart from being an

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alternative test, another use is as an extension of the Mann Whitney U Test test, where we know that the test can only be used on 2 groups of dependent variables. While Kruskall Wallis can be used in more than 2 groups for example 3, 4 or more. Kruskall Wallis is a non parametrical test. Because this test is a non-parametric test in which the assumption of normality can be violated, there is no need for a normality test for example the shapiro wilk or lilliefors test[15].

RESULT AND DISCUSSION

This research aims to to investigate the effect of eggshell waste in terms of aroma, color and taste in packaged milk mixtures and to investigate the optimal variation between egg shell waste and packaged milk in order to obtain the best milk formulation rich in calcium in terms of aroma, color and taste. In order to determine it, questionnaires were given to 40 respondents to judge each variation of sample. In this study, data were obtained from distributing questionnaires to 40 respondents. These respondents assessed using a Likert scale, namely (1) very bad (2) bad (3) moderate (4) good (5) very good (6) best. After distributing the data, the following research results were obtained The result can be briefly seen in table 1.

Table 1. The Result of 40 Respondents

NO	Variation	Taste	Texture	Aroma
1	Milk Control	198	190	192
2	1:1 Variation	147	155	161
3	2:1 Variation	193	178	176
4	1:2 Variation	140	153	158
5	Egg Shell Flour	93	87	85

Information:
40 – 73: Very Bad
74 – 107: Bad
108 – 141: Enough
142 – 175: Good
176 – 209: Very Good
210 – 240: Best

In the table above, it can be seen that the highest value of all parameters, namely 580 (very good), is milk control followed by 2: 1 variation with a value of 547 (very good). If examined further, the addition of eggshell powder did not differ significantly in the variations of 1: 2, 2: 1 and 1: 1, but variations in egg control were quite different. This will be proven in non-parametric statistical tests. The addition of eggshell powder can disguise the smell of milk for respondents who do

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not really like the aroma of milk. Therefore, the addition of eggshell powder can be an alternative solution for packaged milk so that it can still get a high calcium content with a mild milky aroma see in table 2.

Table 2. Chi Square table for Kruskall Wallis Test

Chi-Square Statistics	103.598
Probability	0.000

The table above informs that testing the differences in giving egg shell flour to the taste, color and aroma of fortified milk produces a Chi-square test statistic of 103.598 with a probability of 0.000. It can be seen that the probability < alpha (5%), so that H0 is rejected. Therefore, it can be stated that there is at least one pair of treatments giving egg shell flour to the taste, color and aroma of fortified milk which is significantly different.

To determine the effect of giving egg shell flour on the taste, color and aroma of fortified milk which was significantly different, it was carried out using the Mann Whitney test with the criteria that if one pair of treatments produced a probability ≤ level of significance (alpha = 5%), it could be stated that there was a difference in the effect of giving egg shell flour to the taste, color and aroma of fortified milk which was significantly different see **Table 3.**

Table 3. All Parameters Aspect for All Variations to Milk Control

Parameter	Variation			
	Egg control	**Variation 2:1**	**Variation 1:2**	**Variation 1:1**
Mann- Whitney U	822.5	110.0	466.50	544.50
Wilcoxon	1812.5	1100.0	1456.50	1534.50
Z	-1.382	-7.37	-4.55	-4.01
Asymp.Sig	.167	.000	.00	.00

The results of the above analysis indicate that the average value of taste, texture and aroma assessment in fortified milk, in the control group milk is the highest and significantly different from the average assessment of taste, color and aroma in egg controls. However, it was not significantly different from the taste, color and aroma ratings of

the milk variations 1:2 , 2:1 and 1:1. With no significant difference, the addition of egg shell flour can be used as an alternative solution to still get the high calcium content from packaged milk with a mild milky aroma.

On the other hand, due to usage of waste, it does not affect much cost production for processing egg shell waste into egg shell flour. Processing egg shell waste only requires drying process by using sun shine which is not adding any cost of production but only its duration. However, using additional egg shell waste increase the profits because it can tune down the milky aroma compared to normal donuts. Therefore, by applying egg shell waste in packaging milk will rise the profit and will not affect much the cost of production.

CONCLUSION

The addition of eggshell powder can reduce the aroma of milk which causes the aroma diminished. The most optimal variation in this study is the milk control variation of 580 points (very good) followed by 2 : 1 variation (powdered milk : egg shell powder) with a value of 547 points (very good). There was no significant difference between control variations, 1:2, 2:1, 1:1 but there were significant differences between control eggs. This research requires more iterations are needed in this study to produce better research results. In addition, this research is needed regarding the calcium content in fortified milk through clinical testing laboratories to obtain detailed content results.

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Relationship Between Hemoglobin Levels (Hb) and Nutritional Status with Academic Achievements of STIK KESOSI TLM Students in 2022

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Abstract

Students who excellence academically are always associated with good health status. One indicator of health status that is commonly used is hemoglobin (Hb) and nutritional status. Hb levels can be an indicator of anemia and nutritional status can be a measure of a person's brain development ability. The purpose of this study was to determine the relationship between hemoglobin (Hb) levels and nutritional status with the academic achievement of TLM STIK KESOSI students in 2022. The research design was cross-sectional, and sampling was carried out in October - November 2022. Sampling using a non-random technique, with a total sample of as many as 36 students. Data collection was carried out by measuring Hb levels, body weight, and height. The data obtained were analyzed by univariate and bivariate. The results showed that 47.2% of students were in the category of normal nutritional status, and 91.7% were not anemia. The relationship between Hb levels and student nutritional status on student academic achievement showed no significant relationship (P> 0.05). In conclusion, normal or not Hb levels and nutritional status did not affect student learning achievement. However, efforts are still needed to maintain the stability of Hb levels and nutritional status in students. So that the impact is not only to increase academic achievement but also to maintain the quality of long-term health.

Keywords: *TLM Students, hemoglobin, nutritional status.*

Abstrak

Mahasiswa yang berprestasi secara akademik selalu dikaitkan dengan status kesehatan yang juga baik. Salah satu indikator status kesehatan yang umum digunakan adalah hemoglobin (Hb) dan status gizi. Kadar Hb dapat menjadi indikator kondisi anemia dan status gizi menjadi ukuran kemampuan perkembangan otak seseorang. Tujuan penelitian ini untuk mengetahui hubungan kadar hemoglobin (Hb) dan status gizi dengan prestasi akademik mahasiswa TLM STIK KESOSI tahun 2022. Desain penelitian ini cross sectional, pengambilan sampel dilakukan pada bulan Oktober – November 2022. Pengambilan sampel dengan teknik non random, dengan jumlah sampel sebanyak 36 mahasiswa. Pengumpulan data dilakukan dengan mengukur kadar Hb, berat badan dan tinggi badan. Data yang diperoleh dianalisa secara univariat dan bivariat. Hasil penelitian menunjukkan 47,2% mahasiswa berada dalam kategori status gizi normal, dan 91,7% berada dalam kondisi tidak anemia. Hubungan kadar Hb dan status gizi mahasiswa terhadap prestasi akademik mahasiswa menunjukkan tidak ada hubungan yang signifikan (P>0,05). Kesimpulannya normal atau tidak nya kadar Hb dan status gizi ternyata tidak mempengaruhi prestasi belajar mahasiswa. Namun tetap diperlukan upaya untuk tetap menjaga kestabilan kadar Hb dan status gizi pada mahasiswa. Agar dampaknya bukan hanya kepada peningkatan prestasi akademik namun juga untuk menjaga kualitas kesehatan jangka Panjang

Kata kunci*: Mahasiswa TLM, kadar hemoglobin, status gizi*

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INTRODUCTION

Hemoglobin level is generally used as an indicator in determining anemia status in a person. The normal value of Hb levels based on WHO standards are 12 gr/dL. Anemia can be experienced by individuals in every age group, but children and women of childbearing age are a vulnerable group [1,2]. This problem is often referred to by most lay people as anemia. The impacts arising from this condition include endurance and inhibition of growth and development, and if in a pregnant condition it will certainly endanger the condition of the mother and fetus. Anemia itself is not a disease but a sign of a disease process rather than a disease in itself. Anemia conditions are directly correlated with nutritional status or intake of iron consumed.

College students in general are a community group of adolescents aged 10-24 years and are not married [3]. Based on the results of the population census in 2020, Indonesia is dominated by Generation Z and Millennial Generation. Proportion Gen. Z as many as 74.93 million people (27.94% of the total population) and as many as 69.38 million people Millennial Generation (25.87% of the total population). This generation has the potential to be actors in development to determine the future of Indonesia, that thing can be an opportunity as well as a challenge to Indonesia both in the present and in the future front [4].

One of the problems that often arise during adolescence is anemia. Symptoms of anemia that are often experienced include weakness, lethargy, dizziness, dizzy eyes, and a pale face. The diverse activities of adolescents in their daily life require a lot of nutritional intakes, especially iron [5]. Because iron is an important component in the manufacture of hemoglobin and is a micronutrient to help brain development [6]. If this is not sufficient, it can cause various effects such as decreased endurance. If the immune system decreases, the disease will easily attack, and the impact on learning activities and achievement will decrease as a result of decreased concentration [5].

Nutritional problems in adolescents can be caused by several factors, namely bad eating habits. This could come from bad eating habits in the family since childhood. In addition, adolescents have a wrong understanding of nutrition, such as limiting food to maintain a slim body and liking certain foods, such as eating only junk food [7]. A person's nutritional status is closely related to cognitive intelligence [8]. The learning process in a person will be disrupted if the incoming nutritional intake is not balanced with learning activities. In other words, nutritional intake in a person affects the learning process.

Several studies have explained the link between anemia and the achievement status of female students.

There is a significant relationship between anemia status and learning achievement which is measured using the academic achievement index [9]. Nutritional status and anemia status among midwifery academy students in the Kendal Regency government have a significant relationship [7]. Nutritional status has a positive relationship with student achievement in midwifery FK UNS [10]. Other studies explain that iron consumption is not related to the incidence of anemia in students of SMP Negeri 27 Padang [11]. Based on some of these varied research results, researchers are interested in conducting an assessment to see the relationship between anemia and nutritional status with academic achievement in STIK KESOSI Medical Laboratory Technology students in 2022.

METHODOLOGY

This research is analytically observational with a cross-sectional approach. The sample was determined based on a non-random technique (consecutive sampling), with inclusion criteria: (1). Registered as an active student in the TLM study program, (2). Not currently taking iron supplements or blood-boosting tablets, (3). Maximum age of 22 years, (4). Not menstruating during the examination and (5). In good health or not currently suffering from an infectious disease (Diarrhea, TB, Malaria). Respondents who met the inclusion criteria then filled out a questionnaire and were explained informed consent. The questionnaires distributed contained open-ended questions regarding the characteristics of the respondents (age, GPA, Hb levels, nutritional status). The samples collected based on the inclusion criteria totaled 36 students. Student nutritional status is measured using Body Mass Index (BMI). The tools used to measure BMI are weight scales and a microtoise / height meter. Anemia status was measured by determining the students' hemoglobin (Hb) level using a Hb meter. Academic achievement/grade point (GPA) is determined by looking at the Student Study Result Card in the academic information system. Data analysis used a statistical program (SPSS ver 22) which included univariate and bivariate analysis. Univariate analysis was carried out to look at the characteristics of the students' weight, hemoglobin level, height, age, and GPA. Bivariate analysis was conducted to see the relationship between anemia and nutritional status with student academic achievement using the Pearson correlation test.

RESULT AND DISCUSSION

Based on the inclusion criteria, the number of student respondents collected in this study total 36 samples. Most of the students were in normal nutritional status (47.2%), and only a small number were obese (8.3%) (Table 1). Meanwhile, based on learning achievement, as measured by the grade

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point average (GPA) it was found that 72.2% were in the GPA range of 3.51 – 4.00 (Table 2). The measured student hemoglobin levels showed that most of the respondents (91.7%) were in good Hb condition or not anemic (>12 g/dL) (Table 3).

Table 1. Respondent distribution based on nutritional status

Nutritional status	n	F (%)
Normal	17	47,2
Underweight	6	16,7
Overweight	10	27,8
Obesity	3	8,3
Total	36	100

Table 2. Respondent distribution based on academic achievement

GPA	n	F (%)
2,76 – 3, 50	10	27,8
3,51 – 4,00	26	72,2
Total	36	100

GPA = achievement index

Table 3. Respondent distribution based on Hb levels

Hb levels (g/dL)	n	F (%)
<12 (anemia)	3	8,3
>12 (non-anemia)	33	91,7
Total	36	100

Hb = hemoglobin

The results of the examination of Hemoglobin (Hb) levels in TLM STIK KESOSI students showed that most of the students were in very good Hb condition (> 12 g/dL). This is an indication that TLM STIK KESOSI students in this case are generally free of anemia. Concerning learning achievement, it is known whether Hb levels are normal or do not affect student learning achievement. Low Hb levels or anemia are also said to not affect academic achievement in Al-Hikmah Jepara Islamic AKBID students [12]. Although the results of other studies argue that Hb levels in the body will affect cognitive function and the development of motor skills [13]. Poor cognitive function is usually characterized by poor memory, difficulty concentrating, fatigue, and decreased problem-solving abilities. Low Hb levels correlate with low student academic achievement [14,15]. However, student achievement is not only determined by Hb levels as an indicator of anemia status but as well as student nutritional status.

The relationship between Hb levels and nutritional status with student academic achievement was analyzed using the person correlation

test. It was found that there was no relationship between Hb levels and student academic achievement (P> 0.05) (Table 4). Likewise, the relationship between nutritional status and student academic achievement showed no significant relationship (P > 0.05) (Table 5).

Table 4. Correlation test results for Hb levels and student academic achievement

r	P	correlation
-0,213	0,21	negative

Table 5. Correlation tests results of levels of nutritional status and student academic achievement

r	P	correlation
0,118	0,492	positive

Many factors can be behind the high and low academic achievement of a student. One of the determining factors is the motivation to learn and the learning experience. The higher the motivation and learning experience, the higher the student will get good learning achievement [16]. In addition, the competence of educators also provides opportunities for students to obtain good learning outcomes [17].

Nutritional status in this study was also found to be unrelated to student academic achievement. The same results were obtained for UNNES engineering faculty students in 2019, which stated that physical activity and nutritional status were not significantly related [18]. Likewise, the results of Nurzia's research (2018) stated that there was no significant effect between nutritional status on student achievement in semester III of the Prima Jambi Nursing Academy in 2016 [19].

Although no association was found between Hb levels and nutritional status with student academic achievement in this study, efforts are still needed to maintain the stability of Hb levels and nutritional status in students. So that the impact is not only on increasing academic achievement but also on maintaining the quality of long-term health

CONCLUSION

The results of the Pearson correlation test showed that there was no relationship between hemoglobin (Hb) levels and the nutritional status of TLM STIK KESOSI students in 2022 on academic achievement (P > 0.05).

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Analysis of Examination Results of Erythrocyte Index in Pulmonary TB Patients Labuang Baji General Hospital, Makassar City

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Abstract

The high rate of transmission of tuberculosis (TB) in Makassar City is very concerning. Data from the Makassar Health Office (Dinkes) in 2019 there were 5,412 TB cases. The data then decreased during the pandemic to 3,260 cases in 2020. And 3,911 cases in 2021 with a recovery rate of 83% in 2019 and 85% in 2020. The purpose of this study was to determine the value of the erythrocyte index in patients with pulmonary tuberculosis at the Hospital of Labuang Baji, Makassar City. The type of research used is laboratory observation with descriptive method. The population in this study were all patients with pulmonary tuberculosis as many as 11 people with pulmonary tuberculosis. The results obtained are known for the MCV value, 8 tuberculosis patients have a normal MCV value, 2 patients have a low MCV value and one has a high MCV value, for the MCH value, 6 tuberculosis patients have a normal MCH value, 4 patients have a high MCH value. low and one patient had a high MCH value, for the MCHC value 4 people with tuberculosis had a normal MCHC value and 7 patients had a low MCHC value.

Keywords: *Tuberculosis, Erythrocyte, Transmission of TB*

Abstrak

Tingginya angka penularan tuberkulosis (TB) di Kota Makassar sangat memprihatinkan. Data Dinas Kesehatan (Dinkes) Makassar tahun 2019 terdapat 5.412 kasus TB. Data tersebut kemudian menurun pada masa pandemi menjadi 3.260 kasus pada tahun 2020. Dan 3.911 kasus pada tahun 2021 dengan angka kesembuhan 83% pada tahun 2019 dan 85% pada tahun 2020. Tujuan dari penelitian ini adalah untuk mengetahui nilai indeks eritrosit pada pasien dengan tuberkulosis paru di RSUD Labuang Baji Kota Makassar. Jenis penelitian yang digunakan adalah observasi laboratorium dengan metode deskriptif. Populasi dalam penelitian ini adalah seluruh penderita tuberkulosis paru sebanyak 11 orang penderita tuberkulosis paru. Hasil yang didapatkan diketahui nilai MCV, 8 pasien tuberkulosis memiliki nilai MCV normal, 2 pasien memiliki nilai MCV rendah dan 1 pasien memiliki nilai MCV tinggi, untuk nilai MCH, 6 pasien tuberkulosis memiliki nilai MCH normal, 4 pasien memiliki nilai MCH yang tinggi. rendah dan satu pasien memiliki nilai KIA tinggi, untuk nilai MCHC 4 orang penderita tuberkulosis memiliki nilai MCHC normal dan 7 pasien memiliki nilai MCHC rendah.

Kata kunci: *Tuberculosis, Eritrosit, Transmisi TB*

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INTRODUCTION

Tuberculosis can affect all series of hematopoiesis, especially in erythrocytes, when infected erythrocytes a reaction will occur where the life span of erythrocytes is about 10-20 days shorter while in normal conditions the life span of erythrocytes is 120 days. In this condition, it affects the poor production of erythrocytes so that they are damaged and can affect lower hemoglobin concentrations and experience anemia. Laboratory tests are needed in diagnosing tuberculosis (TB), one of the laboratory tests in the field of hematology that can be done is the examination of the erythrocyte index. This examination is carried out to determine the classification of various types of anemia. Erythrocytes are produced by red bone marrow. In a day Produced about 3.5 million cells/kg body weight. These red blood cells survive and function for 90-120 days, then are destroyed by macrophages in the spleen and liver[1,2,3].

Tuberculosis is still a health problem for the people of Indonesia, especially for active smokers. There are many risk factors that can cause tuberculosis in active smokers including age at starting smoking, number of cigarettes consumed per day, and how long smoking has been done. The purpose of this study was to determine the results of the identification of mycobacterium tuberculosis in active smokers with the Ziehl–Neelsen staining acid resistance test method. This type of research is a descriptive analytic study that aims to determine the results of the identification of Mycobacterium tuberculosis in active smokers. The Ziehl–Neelsen staining acid resistance test was conducted at the Tourism Hospital Laboratory of the University of East Indonesia Makassar on September 22 to October 6, 2022. The population in the study this is 10 people. The sample was determined using a total sampling technique so that 10 samples were obtained. The research variables were active smokers. The data obtained are presented in the form of tables and narratives. The conclusion after laboratory testing using the Ziehl–Neelsen staining method was found that 1 out of 10 samples of active smokers was identified as Mycobacterium Tuberculosis[4].

From the 2010 WHO Global Report, obtained data on pulmonary TB in Indonesia, the total of all pulmonary TB cases in 2009 was 294,731 cases, of which 169,213 were cases of new smear-positive pulmonary TB, 108,616 cases of smear-negative pulmonary TB, 11,215 extra pulmonary TB cases, 3,709 pulmonary TB cases[5] Relapse and 1,978 cases of re-treatment outside of relapse cases. Pulmonary TB

is not a new disease in Indonesia, but until now it is still a major health problem. It is estimated that the number of pulmonary TB cases in Indonesia accounts for around 5.8 percent of the total number of pulmonary TB in the world. The prevalence of tuberculosis in Indonesia is 281 cases per 100,000 population with a treatment success rate of 90.3%. This number decreased compared to 2010 of 289 per 100,000 population[6]. On March 3 2014, the Stop TB Partnership Forum for the Southeast Asia, West Pacific and East Mediterranean Regions was held in Jakarta. The forum involved 100 participants from 13 countries consisting of national TB program managers, national stop TB partnerships, and related NGOs[6 7].

Previous studies have shown a relationship between pulmonary TB and the erythrocyte index, that anemia of chronic disease is more common in tuberculosis patients compared to iron deficiency anemia, anemia of chronic disease occurs due to suppression of erythropoiesis by inflammatory mediators. Severe tuberculosis infection with anemia status will disappear with successful adherence to treatment such as Anti-Tuberculosis Drugs (OAT) such as iron (Fe), hydroxycobalamin (Vitamin B12) and folic acid, erythropoietin, and consuming foodstuffs that contain lots of protein. So for that erythrocyte indices such as Mean Corpuscular Volume (MCV) are used to determine cell size, Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) are used to determine the size, shape and color of erythrocytes and the value of hemoglobin can support laboratory diagnosis in classifying anemia or as a support in distinguishing various types of anemia[8].

Red blood cells (erythrocytes) are the most abundant cells in comparison with other blood cells which have an erythrocyte count of approximately 5 million/mm2 and function to transport oxygen gas (O2) into all cells and tissues of the body to enable metabolic activities in them. Normal erythrocytes are bioncaf or disc-shaped with a diameter of about 8 microns[9]. Red blood cells do not have a nucleus but have a central pallor. The mature erythrocyte is a biconcave disk about 7 microns in diameter. Erythrocytes are cells with an incomplete structure. This cell only consists of a cytoplasmic membrane without a cell nucleus. Red blood cells in the body are useful for transporting oxygen needed in the lungs to be circulated throughout the body and transporting carbon dioxide to be removed from the body[10].

Review of the mean cospuscular values or erythrocyte index provides information about the average size of erythrocytes and the amount of hemoglobin per erythrocyte. a. Mean Corpuscular Volume (MCV) MCV is also called the Average Erythrocyte

Volume (VER) is an average of an erythrocyte called a femtoliter. b. Mean Corpuscular Hemoglobin MCH is also called the Average Erythrocyte Hemoglobin (HER) is the amount of hemoglobin per erythrocyte called picogram. c. Mean corpuscular hemoglobin concentration (MCHC) MCHC is also called the Average Erythrocyte Hemoglobin Concentration (KHER) is the hemoglobin level obtained per erythrocyte, expressed as a percentage.

METHODOLOGY

The type of research used is laboratory observation with descriptive method, The population in this study were all patients with pulmonary tuberculosis in Labuang Baji Hospital, Makassar City with a total sample of 11 people. The sampling technique used was saturated sampling, where all populations were used as samples. This research took place in August 2022, with the object of research being the erythrocyte index in patients with pulmonary TB.

The research procedures were:
(1) Sampling in the sampling room which consisted of a 3cc syringe, tourniquet, EDTA vacutainer tube, the ingredients included EDTA blood, alcohol cotton and plaster.
(2) working procedure by preparing the tools and materials to be used and then asking patient to stretch out his hand, then palpate the patient's arm, then put a tourniquet on the patient's upper arm + 7cm from the elbow fold then disinfect the skin around the place where the blood was taken (the median cubital vein area) with alcohol cotton and let it dry, then puncture the vein with the needle position 30° from skin, if blood is seen flowing into the syringe, immediately release the tourniquet and pull the pin slowly until the blood is obtained as needed. after that carefully remove the needle and insert it into the EDTA vacutainer tube which has been given an ID for each patient, after that close the injection site with dry cotton and plaster. then homogenize the blood sample until smooth to avoid hemolysis, or blood clots after that throw away the needle that has been used into the safety box.
(3) Examination of the Erythrocyte Index The method used is the Hematology Analyzer method with tools such as the material analyzer, including EDTA blood through pre-analysis, preparation of venous blood samples with EDTA anticoagulant, then the analytical prepares the tools and materials to be used. Then the words "please wait" will appear on the display screen, it will automatically carry out the operation. Pressing the analysis button and making sure it's on the whole blood method (the writing is in the lower middle position). Pressing

the button (patient demographics). Enter the patient ID then press the "OK" button. The blood sample to be used must first be homogenized. Insert the sample into the probe until it touches the bottom of the tube. Pressing the probe button then the sample will be processed. Then after the sample has been sucked in and the examination results will appear on the screen, record the results according to the patient's ID, in post-analytic Interpretation of Results MCV = 80-96 fl MCH = 27-33 pg/cell MCHC = 33-35 g/dl.

RESULT AND DISCUSSION

Tuberculosis is an infectious disease caused by the bacterium Mycobacterium tuberculosis which results in hematological abnormalities, especially in the erythrocyte index so that it can affect the process of erythropoiesis, namely shortening the life span of erythrocytes in the bone marrow before they go to body tissues. A limit for the size and content of hemoglobin is expressed by the red cell index. The erythrocyte index consists of the contents/volume and size of the MCV (mean corpuscular volume) in femtoliter (fL), MCH (mean corpuscular hemoglobin) in picogram (pg), MCHC (mean corpuscular hemoglobin concentration or average erythrocyte hemoglobin level) in units of grams/deciliter (g/dL). Erythrocyte index or Mean Corpuscular Value is an average value that can provide information about the average erythrocyte and the amount of hemoglobin per erythrocyte. Examination of the erythrocyte index is used as a screening test to diagnose anemia and determine anemia based on its morphology.

Based on the results of a study of 11 tuberculosis patients at the Labuang Baji Hospital, Makassar City on 15-25 August 2022, it was found that 8 of them had normal MCV values and 2 people had low MCV while 1 person had high, for normal MCH values, namely 6 people, the low 4 people and the high 1 person, then the normal MCHC value is 4 people, the low is 7 people, as shown in the table 1 below.

The erythrocyte index in patients with pulmonary tuberculosis is usually low. This is due to the occurrence of anemia (iron deficiency) in patients. This situation is characterized by decreased transferrin saturation and decreased bone marrow ferritin or hemosiderin levels. From the results of the study it was known that some TB sufferers had a normal erythrocyte index, some had a low erythrocyte index and some had a high erythrocyte index. For patients who have a normal erythrocyte index, this could be influenced by the consumption of anti-tuberculosis drugs that have been carried out for 5-6

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months or because there are no haematological changes in patients that can be caused by the daily habits of pulmonary TB patients.

Tabel 1. Examination Results of Erythrocyte Index in Patients with Pulmonary Tuberculosis

No	Sample Code	Sex	Age	MCV (fL)	MCH (pg)	MCHC (g/dL)
1	1	L	39	79.2	24.1	30.4
2	2	P	22	86	27.8	32.4
3	3	P	39	85	27.3	32.1
4	4	L	53	87.1	27.8	31
5	5	L	29	85.2	29	34
6	6	L	28	85.4	28.3	33.2
7	7	P	26	72.7	21.1	29.1
8	8	L	81	87.7	31.1	35.4
9	9	P	58	80.2	23.4	29.2
10	10	L	65	108.4	36.6	33.8
11	11	L	51	83.1	28.4	34.2

(Source: Primary data, 2022)
Normal value of Erythrocyte Index
MCV = 80.0 – 96.1 (fL)
MCH = 27.5 – 33.2 (pg)
MCHC = 33.4 – 35.5 (g/dL)

Patients who have an abnormal (low) erythrocyte index due to reduced iron. This is caused by a low intake of total iron in food or decreased bioavailability of consumed iron. This can occur due to reduced appetite, insomnia in tuberculosis sufferers. For those who have a high erythrocyte index, it is usually caused by interference from other diseases such as infection, sample lysis and because the hemoglobin concentration is more than normal. The results of the above research are in line with research that has been conducted by, Jonuarti, R[11], which found that out of 20 pulmonary TB sufferers it was found that the MCV value was normal, namely 11 sufferers and 9 patients were abnormal, while the normal MCH values were 12 patients and those who were not normal were 8 patients, and for normal MCHC values were 14 patients and those who were not normal were 6 patients. For the abnormal results above, there is an indication of anemia. From the research conducted by Arma Yunis (2020) at the Kendari City Hospital, it was also found that out of 12 patients with pulmonary tuberculosis, it was found that 8 patients had normal MCV values and 4

patients who were abnormal, while 4 patients had normal MCH values and those who were not normal 8 patients, and for normal MCHC values there were 10 sufferers and 2 patients who were abnormal. Of the 12 patients with pulmonary tuberculosis who were examined for the erythrocyte index, the normal values were more than abnormal.

CONCLUSION

Based on the results of the research on the description of the results of examining the erythrocyte index in patients with pulmonary tuberculosis at the Labuang Baji Hospital, Makassar City, it was found that out of 11 people with pulmonary tuberculosis, the results of examination of normal MCV values were 8 patients, 2 patients who were low and 1 patient high, the results of the examination normal MCH values were 6 patients, 4 patients who were not normal and 1 patient who had high, MCHC examination results were normal in 4 patients and those who were low were 7 patients.

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Effectiveness of Resam Fern Leaves Extract (*Gleichenia linearis*) as Bioinsecticide on Mortality of Black Ant (*Dolichoderus thoracicus*)

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Abstract

Insecticides are type of pesticide used to eradicate or control pests. Generally, there are two types of insecticides, namely natural and synthetic insecticides. Natural insecticides are scarce due to their inability to eradicate pests such as black ants. Meanwhile, the use of synthetic insecticides is very practical and fast but can cause pollution that is harmful to humans, animals and other living things. Thus, natural insecticides are needed that can eradicate black ants effectively and efficiently and do not pollute the environment or poison other organisms. The research method used is combination of experimental methods, observation, and literature studies. In determining the best insecticide variation for mortality, a black ant mortality rate was measured using timer for 50 black ants. After the data was obtained, it was analyzed descriptively to determine the value of the mortality rate for each experimental sample. Based on the data obtained, the best variation of natural insecticides was the control variation of fern leaf extract with 3.646 seconds per ant. This is because fern leaf extract in insecticides contains tannin compounds which have several benefits as active pesticide ingredients. Thus, fern insecticides are effective in exterminating black ants.

Keywords: *black ants, fern leaves, insecticides*

Abstrak

Insektisida adalah pestisida yang mengandung senyawa kimia yang digunakan untuk membasmi atau mengendalikan hama. Secara garis besar, terdapat dua jenis insektisida yaitu insektisida alami dan sintetis. Insektisida alami cukup sulit ditemukan dikarenakan ketidakmampuan insektisida dalam membasmi hama seperti semut hitam. Sedangkan, penggunaan insektisida sintetis memang sangat praktis dan cepat tetapi dapat menimbulkan polusi yang membahayakan manusia, hewan dan makhluk hidup lainnya. Dengan demikian, dibutuhkan insektisida alami yang dapat membasmi semut hitam secara efektif dan efisien serta tidak mencemari lingkungan maupun meracuni organisme lainnya. Metode penelitian yang digunakan adalah kombinasi metode eksperimen, observasi, dan studi literatur. Dalam menentukan variasi insektisida terbaik terhadap mortalitas tersebut, dilakukan pengukuran kecepatan mortalitas semut hitam dengan menggunakan timer kepada 50 semut hitam. Kemudian data dianalisis menggunakan statistik deskriptif untuk mengetahui efektifitas mortalitas untuk setiap sampel percobaan. Berdasarkan data yang diperoleh, variasi terbaik insektisida variasi kontrol ekstrak daun fern leaves dengan kecepatan 3,646 detik per semut. Hal ini dikarenakan ekstrak daun fern leaves dalam insektisida mengandung senyawa tanin yang mempunyai beberapa manfaat sebagai bahan aktif pestisida. Dengan demikian, insektisida fern leaves efektif dalam membasmi semut hitam secara efektif.

Kata kunci: *insektisida, fern leaves, semut hitam*

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INTRODUCTION

Ants are eusocial insects which are also known as the third largest household pest after mosquitoes and cockroaches. Ants have various types of species, ranging from beneficial to detrimental. Beneficial ants such as the type of skeleton ant which is used as a predator of fruit plant pests, can automatically reduce the use of chemical pesticides in eradicating plant pests. Whereas harmful ants are those that interfere with the lives of other living things because they are classified as omnivore animals or eat everything, both plants and animals[1]. One of the harmful ants is black ants on food with stinging bites and odors. However, to eradicate pests in the form of insects themselves, people tend to use chemical or synthetic insecticides that contain harmful substances, to control pests such as cockroaches, mosquitoes, flies, termites, ants, rats because they are considered more practical, effective and efficient. Ideally, these synthetic insecticides are used to kill only the target organisms. But in fact, insecticides that contain a lot of chemicals are not selective enough and instead poison other organisms that play a role in the environment[2].

Insecticides are usually packaged in various formulations including liquid, mosquito coil, aerosol, mat, vaporizer, insect repellent lime and burning paper with various active ingredients and concentrations used. The use of synthetic insecticides is indeed very practical and fast but can cause pollution that is harmful to humans, animals and other living things. This pollution can spread to the environment so that it is inhaled by the upper respiratory organs and has an impact on the lower respiratory organs[3]. Meanwhile, the insecticide that is often used to control ants is magic lime which contains the chemical substance deltamethrin of 0.6%. Deltamethrin is a broad-spectrum insecticide that acts as a contact poison and stomach poison[4].

Meanwhile, on the other hand, there are many plants that can be used as natural or vegetable insecticides without harmful chemicals, one of which is ferns. The resam fern plant (*Gleichenia linearis*) is a type of fern plant that is easy to find in Indonesia because it attaches a lot to oil palm trees and roadside cliffs, so it is called a weed[5]. Weeds are plants that often grow where they are not wanted because they cause disturbance to the plant. Within a plant commodity there is always competition in food absorption, especially competition between cultivated plants and weeds fighting over nutrients[6]. So, instead of being allowed to become weeds on other plants, fern is better extracted

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to be used as a vegetable insecticide. *Gleichenia linearis* itself contains compounds that affect insect metabolism, such as antifeedants, which are compounds that inhibit eating. Ferns also contain many allelochemical compounds in the form of flavonoids, triterpenoids, saponins, tannins, alkaloids and steroids which are theoretically harmless to humans, but sufficient to fulfill the role as a vegetable insecticide. So far, ferns have been widely used as traditional medicines, vegetables, ornamental plants, building poles, etc., but no one has optimized ferns as natural insecticides to control black ant pests that are effective and environmentally friendly[7].

The use of fern fern extract has previously been the object of research but not for controlling black ant pests. In his research conducted a toxicity test of a vegetable insecticide from fern fern leaves on the larvae of *Plutella xyostella L*, which is the main pest of cabbage plants[8]. The sample used in this study was 250 larvae of *Plutella xyostella L* instar 3 with 4 treatments, namely the concentration of *G. Linearis (Burm.f.)* S.W Clarke leaf extract. 5%, 10%, 15%, 20% and control (0%) with 5 repetitions. Data were analyzed using the Kruscal Walis and the Probit test. It was concluded that *Gleichenia linearis* leaf extract could be used as a pest controller for *Plutella xyostella L larvae* because it was proven to be toxic and the lowest LC50 value was the best, namely 0.127% -1.546% at 48 hours.

The study conducted to determine the concentration of *G.linearis* leaf extract on the germination and growth of *Cynodon dactylon* weed seedlings[9]. This study used a completely randomized design with 5 replications consisting of control, 0.1; 0.2 and 0.3 gr/ml (germination) compared to control, 0.1 and 0.2 gr/ml (grow). The results showed that the effective concentration that could inhibit the germination of *C. dactylonis* at a concentration of 0.3 g/ml and inhibit the growth of other plants was a concentration of 0.2 g/ml.

In the same year, a study was also conducted to determine the concentration of *G.linearis* leaf extract on the germination and growth of *Mimosa pudica L*. This study also used a Completely Randomized Design (CRD) with 5 replications consisting of control, 0 ,1; 0.3; 0.5 and 0.7 g / ml[10]. Germination and growth treatments were started from control, 0.1; 0.3; 0.5 and 0.7 g / ml. The results showed that the concentration that inhibited the germination of *M. pudica* weeds was 0.5 g/ml and the growth concentration was 0.5 g/ml. As explained above, it can be concluded that no research has been found that

optimizes Fern leaves leaf extract as an insecticide to control black ant pests. So that this research can be called a new idea regarding the optimization of Fern leaves leaf extract as an insecticide tested based on the mortality of black ants.

Therefore, an experiment will be carried out entitled " Effectiveness of Resam Fern Leaves Extract (*Gleichenia Linearis*) as Bioinsecticide on Mortality of Black Ant (*Dolichoderus thoracicus*). This study will put forward a new idea related to optimizing ferns as bioinsecticide to control black ants. This fern insecticide is environmentally friendly because it is made from natural ingredients without a mixture of chemicals that are harmful and pollutes the environment. As an alternative solution to reduce the use of synthetic insecticides, the number of negative impacts produced by synthetic insecticides is calculated. In this fern extract insecticide, the composition will be combined with alcohol, to obtain the best variation that is most effective in controlling black ant pests.

METHODOLOGY

Research Type

This type of research is a type of quantitative experimental research using a combination of experimental methods, direct observation and literature study. The research began with a literature study on ferns and black ant and the manufacture of vegetable insecticides using the internet and continued with field observations to obtain ferns and black ants. To determine the success of this study, an experimental method was used to obtain information regarding the effect and effectiveness of the Fern leaves Plant Insecticide on the mortality of black ants. With this combination of methods, maximum results will be obtained both quantitatively and qualitatively.

Tools and Materials

The tools used in this study are described as follows, knife, cutting boards, big bowl, frying pan, spatula, gas stove, tea filter, bottle, analytical balance, spoon. The materials used in this study are described as follows, resam fern leaves, alcohol, water.

Research Variable

Independent Variable

Independent variables or independent variables are variables that influence or cause changes or the emergence of the dependent variable[11]. The independent variables in this study were fern and alcohol with varied compositions. Variations are made as much as:

1. Alcohol control (Without Fern leaves leaf extract)
2. Control of Fern leaves leaf extract (without alcohol)
3. Alcohol: Fern leaves leaf extract (1: 1)
4. Alcohol: Fern leaves leaf extract (1: 2)
5. Alcohol: Fern leaves leaf extract (2: 1)

Dependent Variables

The dependent variable or dependent variable is the variable that is affected or becomes the result, because of the independent variables [11]. The dependent variable in this study is insecticides which are seen based on mortality of black ants (*D. Thoracicus)* and duration of insecticidal effect.

Research Steps

This research was conducted in three stages, with the first stage being the preparatory stage, namely the extraction of Fern leaves leaves. The second stage, is the stage of making insecticides. And finally, the third stage, is the testing stage in the form of interviews related to insecticides. The following is a description of the stages of the assessment.

Preparation phase

The details of the extraction stage are described as follows; the middle part of the fern leaves is cut so that each sorus is separated (into small pieces) then roasted. Next, The leaves of fern leaves have been roasted and soaked for 3 days. Finally, the fern water is strained until there are no leaves left.

Insecticide Manufacturing Stage

The details of the insecticide manufacturing stage are described as follows, fern leaves *(G. linearis*) leaf extract and alcohol are prepared. Next, the mass of fern leaves extract and alcohol was measured using an analytical balance. Then the variation of the composition is adjusted by comparing the composition between fern leaves leaf extract and alcohol. Variations in the composition were made with a ratio of 1:1, 1:2, 2:1, alcohol control (without added Fern leaves leaf extract), and fern leaves leaf extract control (without added alcohol)

Testing Stage

The details of the insecticide testing stages are described as follows; insecticides and research questionnaires are prepared in advance. Research questionnaires and insecticides were presented to the panelists to be assessed in terms of black ant mortality and duration of insecticide effects. Next, assessment is carried out for all

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insecticide variations. Finally, Research data for each insecticide variation were obtained by using statistical tests were carried out.

Data Collection Stage

In this study, data was collected through 2 stages. The first stage of data was collected through experimental activities, carried out by applying variations of alcohol and fern leaf extract. The second stage, the data or information that supports this research is analyzed through literature study and studied more deeply to obtain the required information.

Data Analysis Method

Descriptive Statistics

Descriptive statistics are a type of statistics used to describe related activities of collecting, structuring, summarizing, presenting data to make it more meaningful. Descriptive statistics are limited to providing a description or general description of the characteristics of the object under study without the intention of generalizing the sample to the population. The mean (mean) and standard deviation are part of the descriptive statistics used to report the results of research measurements[12].

RESULT AND DISCUSSION

This study aims to determine the effect of the insecticide Fern leaves leaf extract on black ant mortality as well as to determine the most appropriate and effective variation of Fern leaves leaf extract insecticide in black ant mortality. In determining the best insecticide variation for mortality, the speed of ant mortality was measured using a timer for 50 respondents (ants). The data collection process was taken by spraying resam fern insecticide on the respondent with a distance of 3 cm from spraying, then measuring the time needed to kill the ant. After spraying the insecticide liquid, the ants become weak and eventually die. From there the time data was obtained, then we could know the value of the mortality rate produced by each respondent. The recorded mortality rate data is shown in table 4.1 of the measurement results. In this research, we measured the mortality time of black ants (*D, thoracicus*) from the effects of 5 variations of insecticides which were carried out in 10 trials. The mortality time will be the data to obtain the value of the mortality rate of an ant per second which will be used as a test parameter. The measurement result data is shown by the following data table 1.

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Table 1. Results of Measuring the Time of Death of Black Ants from 5 Variations of Insecticides Carried Out 10 Times

Variation	1	2	3	4	5	6	7	8	9	10
1	4.6	5.1	5.8	4.8	3.5	3.8	3.6	4.9	5.6	7.5
2	5.2	5.4	8.1	5	4.4	4.2	4.4	5.3	6.1	7.5
3	3.2	3.9	4.0	3.9	4.3	3.6	4.6	5.6	3.2	4.2
Control Fern Leaves Extract	4.1	2.8	3.3	3.6	3.5	4.1	3.2	4.2	4.2	3.5
Control Alcohol	3.1	4.8	4.2	3.5	4.8	5.1	3.3	4.9	4.1	4.1

It has been explained previously that the time data that has been obtained from the black ant mortality will be processed into data on the speed of the black ant mortality itself. Mortality rate data is presented in graphical form as follows:

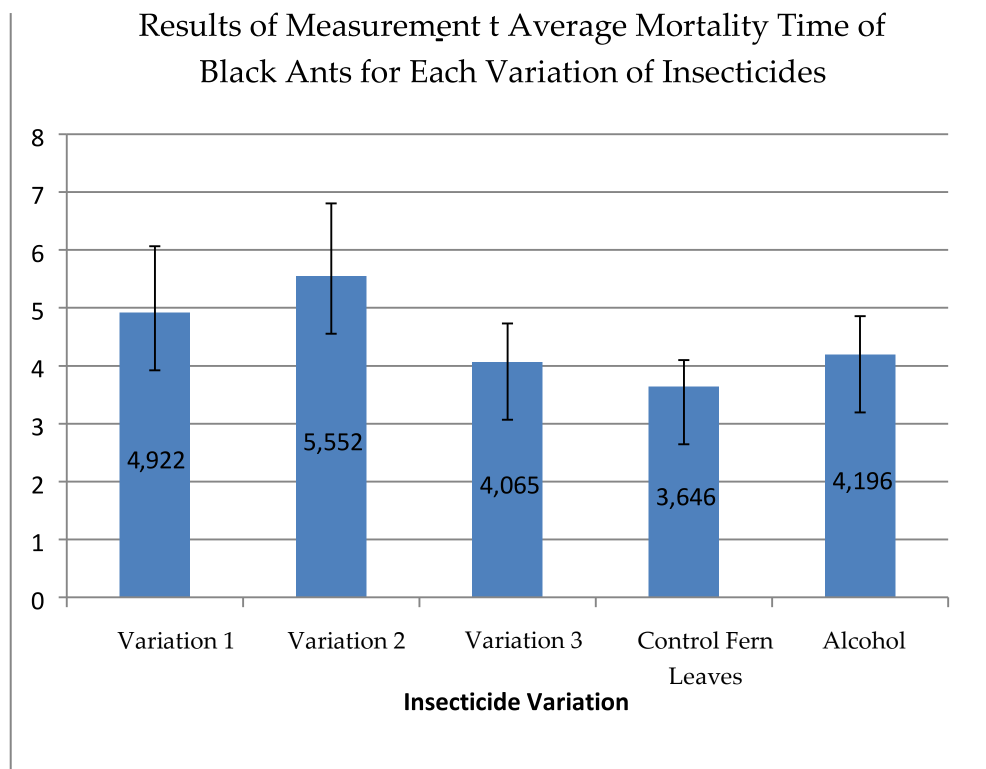

Figure 1. Results of Measurement t Average Mortality Time of Black Ants for Each Variation of Insecticides

The average t value of the time indicates the speed of effect of the insecticide. This speed will affect the effectiveness of each variation of

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the insecticide itself in killing black ant pests. In addition to obtaining data on the mortality rate of each respondent for each variation, the most effective variation in killing ants was also obtained. Based on comparison table 1 it can also be concluded that the best variation is the variation that has the fastest mortality rate, namely the control variation of fern extract with an average value of 3.646 seconds per ant. This is because Fern leaves leaf extract in insecticides contains allelochemical compounds, one of which is tannin. Tannins are active compounds of secondary metabolites that have several functions, including astringents, anti-diarrhea, anti-bacterial and antioxidants.

CONCLUSION

Based on the analysis and processing of the data, the best variation of natural insecticides was the control variation of fern leaf extract with 3.646 seconds per ant. This is because fern leaf extract in insecticides contains tannin compounds which have several benefits as active pesticide ingredients. Thus, fern insecticides are effective in exterminating black ants.

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Improved Quality of Spermatozoa Mice (*Mus musculus*) Model of Diabetes Mellitus Type 1 After Being Given Cinnamon Bark Methanol Extract (*Cinnamomum burmanii* Bl.)

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Abstract

Diabetes mellitus has to do with male fertility, a hormone disorder that affects spermatogenesis. This study aims to determine the effect of cinnamon bark methanol extract (Cinnamomum burmani) on the quality of diabetes mellitus mice spermatozoa. This study used a complete randomized design with 4 treatments and 3 repeats. 22 mice were divided into 4 groups. Mice have injected with streptozotocin dose of 0.1 mL and observed blood sugar levels were, if sugar levels increased, they are given cinnamon bark methanol extract at a dose of 250 mg/kg BB and 500 mg/kg BB, and blood sugar levels are measured. After that, the mice are dissected for observation of spermatozoa's morphology, viability, and motility after administration of the extract. The results showed that cinnamon bark methanol extract at a dose of 250 mg/kg BB and 500 mg/kg BB can reduce the number of abnormal spermatozoa and increase the viability and motility of diabetes mellitus mice spermatozoa. This indicates that cinnamon bark extract as an antioxidant has a positive effect in maintaining structure and development, as well as the function of spermatogenesis cells so that in the presence of these active substances, the number of seed cells that experience developmental failure, degeneration, death due to free radicals can be suppressed or reduced.

***Keywords**: Cinnamon, Morphology, Motility, Viability of Spermatozoa*

Abstrak

Diabetes mellitus mempunyai hubungannya dengan fertilitas pria adalah gangguan pada hormon yang mempengaruhi spermatogenesis. Penelitian ini bertujuan untuk mengetahui efek ekstrak methanol kulit batang kayu manis (Cinnamomum burmani) terhadap kualitas spermatozoa mencit diabetes mellitus. Penelitian ini menggunakan Rancangan acak lengkap dengan 4 perlakuan dan 3 kali ulangan. Mencit sebanyak 22 ekor dibagi menjadi 4 kelompok. Mencit diinjeksi streptozotocin dosis 0,1 ml dan diamati kadar gula darah, apabila kadar gula sudah mengalami peningkatan maka diberi ekstrak methanol kulit batang kayu manis dengan dosis 250 mg/kg BB dan 500 mg/kg BB dan dilakukan pengukuran kadar gula darah. Setelah itu mencit dibedah untuk pengamatan morfologi, viabilitas dan motilitas spermatozoa setelah pemberian ekstrak. Hasil penelitian menunjukkan bahwa ekstrak methanol kulit batang kayu manis dengan dosis 250 mg/kg BB dan 500 mg/kg BB dapat menurunkan jumlah spermatozoa abnormal dan meningkatkan viabilitas dan motilitas spermatozoa mencit diabetes mellitus. Hal ini mengindikasikan bahwa ekstrak kulit batang kayu manis sebagai antioksidan berpengaruh positif dalam memelihara struktur dan perkembangan, serta fungsi sel-sel spermatogenesis, sehingga dengan adanya zat aktif tersebut maka jumlah sel-sel benih yang mengalami kegagalan perkembangan, degenerasi, kematian akibat radikal bebas dapat ditekan atau dikurangi.

***Kata Kunci**: Kayu Manis, Morfologi, Motilitas, Viabilitas Spermatozoa*

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INTRODUCTION

Diabetes mellitus is a chronic disease and will persist for life.[1] Ironically, the disease exerts a systemic influence that leads to the occurrence of functional disorders of the body in general. One of the impacts caused by this disease is in the form of impaired male reproductive function related to a decrease in sperm quality which is one of the causes of infertility in men.[2]

Diabetes mellitus has to do with male fertility, a hormone disorder that affects spermatogenesis.[3] In addition, there are abnormalities in the process of spermatogenesis itself followed by the presence of ejaculation disorders. In diabetics, there is a significant decrease in testosterone levels accompanied by a decrease in LH and FSH levels.[4]

Spermatogenesis is a fundamental process in the male reproductive system that involves a series of high-level genetic and epigenetic events within germ cells that play an important role in converting spermatogonia into spermatozoa ((Irdalisa, 2021) by being controlled by gonadotropin hormones, namely follicle-stimulating hormone (FSH) and luteinizing hormone (LH).[5]

The quality parameters of spermatozoa that are important to determine the fertility of a man include viability, number, morphology, and motility of spermatozoa. The motility and speed of motion of spermatozoa are closely related to the fertilization process. If the spermatozoa swim or move very slowly then the total number of spermatozoa that fertilize the ovum is too small. To approach the ovum, spermatozoa must swim quickly and move like a spiral, which is what is referred to as the capacitating motility pattern.[6]

The use of herbal medicine in treating diabetes mellitus has been used for generations because, in addition to the relatively small side effects, the price is also more economical. This traditional treatment places more emphasis on subjective complaints. Traditional medicine is one of the alternatives in medicine because its side effects are considered smaller and cheaper than modern medicine.[7]

Cinnamon (*Cinnamomum burmani*) is a spice in the form of bark that is commonly used by Indonesians in their daily lives. Apart from being an enhancer of the taste of cooking and baking, the cinnamon plant is known to have various properties. Cinnamon contains chemical compounds in the form of Metylhydroxy Chalcone Polymer and Tyrosine Phosphatase IB (PTP1B), phenols, terpenoids, tannins, and saponins which are sources of antioxidants.[8] The compounds Metylhydroxy Chalcone Polymer and Tyrosine Phosphatase IB (PTP1B) can serve as insulin substitutes by protecting the beta pancreas cells of mice.

The purpose of this research was to prove that cinnamon bark methanol extract (*Cinnamomum burmani*) can

improve the quality of the spermatozoa mice model of diabetes mellitus type 1.

METHODOLOGY

Research Type

This research is a laboratory experimental research.

Tools and Materials

The tools used in this study were mice cages, digital Ohaus Scales, Erlenmeyer, sonde tools, experimental animal surgical tools consisting of scalpels, tweezers, scissors, needles, candle tables, object glass, Petri dishes, microscopes, haemocytometers, hand counters, and digital cameras.

While the ingredients used are cinnamon bark, methanol, equates, STZ (streptozotocin), 22 male mice, aluminum foil, 70% alcohol, filter paper, cotton, tissue, formalin, feed mice, and PAM water, Giemsa solution, 70% alcohol, NaCl 0.9, equates, and rice husks.

Implementation of Research

Trial Animal Setup

Before the study begins, a place for keeping experimental animals is first prepared, namely cages (plastic tubs), chaff, and places for eating, drinking, and feeding mice. After that, acclimatization is carried out in the laboratory for 1 week.

Mice are divided into 4 groups, namely group I: the negative control group (not diabetes) consisting of mice tails, group II: the positive control group i.e. mice injected with streptozotocin but not given cinnamon bark methanol extract, group III: mice group injected with streptozotocin and given cinnamon bark methanol extract dose 250 mg/kg BB and Group IV: the group of mice injected with streptozotocin and given cinnamon bark methanol extract at a dose of 500 mg/kg BB. Each group consists of 5 mice.

Injection Solution Preparation

Before injection, STZ was dissolved in citrate buffer with pH 4.5 (5.25 g of citrate buffer solution (Na_2HNO_4) weighed and 22.5 mL of aqueous), homogenized then inserted 39.8 mg STZ was so that the final concentration of streptozotocin solution was obtained was 22.5 mg/mL STZ. STZ solution is injected intraperitoneally as much as 0.1 mL per head. Mice with glucose levels exceeding 200 mg/dl are considered diabetes.[9]

Test Material Preparation

The bark of the cinnamon stick is taken and dry air at room temperature. After drying, it is mashed with a blender and the smooth powder is weighed. The manufacture of cinnamon bark methanol extract is carried out using the maceration method using ethanol solvent. After obtaining the liquid extract, the extract is then evaporated using a rotavapor at a temperature of 40°C until a concentrated extract of methanol is obtained from the bark of the cinnamon stick.

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Testing Procedure

Before the treatment, cinnamon bark was given to blood glucose levels to ensure that 12 mice had diabetes. Measurement of blood glucose levels is carried out by taking mice blood through the tail which is first cleaned with alcohol. Then the blood is dripped on the glucometer strip and put in the glucometer to read the glucose level.

The test procedure is carried out by testing 4 groups each consisting of 3 mice. The extract was given for 2 weeks and in the last week of testing, blood sugar levels were measured using glucose.

Spermatozo Quality Observations

Observations on the quality of spermatozoa mice include morphology, viability, and motility carried out when administering STZ and after administration of cinnamon bark methanol extract.

Data Analysis

The observational data were analyzed with Analysis of Variance (ANOVA) with the SPSS 16.00 program. The difference between treatments using the BNT test with a confidence level of 0.05%.

RESULTS AND DISCUSSION

Abnormal Spermatozoa Count

The results of observations of spermatozoa morphology in diabetic Mellitus mice and given cinnamon bark extract (*Cinnamomi burmanii*) where what is calculated is the abnormal morphology of spermatozoa can be seen in Table 1.

Table 1. The average number of abnormal spermatozoa of diabetes mellitus mice after administration of cinnamon bark methanol extract.

No.	Treatment	Average Viability of spermatozoa (%) ± SD
1	Negative control	2,33 ± 1,00[a]
2	Positive control	80,33 ± 3,00[b]
3	Dosage 250 mg/kg BB	26,33 ± 2,52[c]
4	Dosage 500 mg/kg BB	16,67 ± 1,52[d]

Description: Superscripts with the same letter do not differ markedly (P > 0.05).

Based on Table 1, shows that in the negative control group the average number of abnormal spermatozoa was 2.33. The average number of abnormal spermatozoa in the positive control group was 80.33. In the group of diabetic mice treated with cinnamon bark methanol extract before dosing 250 mg/kg BB, the average number of spermatozoa was 26.33. Meanwhile, in the diabetic mice group treated with cinnamon bark methanol extract at a dose of 500 mg/kg BB, the average number of abnormal spermatozoa was 16.67.

The decrease in normal spermatozoa during STZ injection is caused by an increase in primary

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abnormalities due to toxins present in streptozotocin. STZ makes the production of superoxide and Nitrite Oxide (NO) in mitochondria increase, further activating protein kinase C (PKC) and the formation of advanced glycosylated end-products (AGEs) which will both interfere with cell function β.[10]

Reactive Oxygen Species (ROS) will damage the integrity of DNA in the spermatozoa nucleus so that it will induce cell apoptosis.[11] Apoptosis of such cells causes changes in the morphology of spermatozoa, especially at the time of spermatogenesis. The results showed that the administration of streptozotocin caused the occurrence of abnormal spermatozoa, namely on the head, neck, and tail. Abnormalities in the head, neck, and tail in this study are thought to have occurred at the time of the process of spermatogenesis.[12,13]

Spermatogenesis can occur through several stages of division. In the early stages, the spermatogonia will change to primary spermatocytes, then to secondary spermatocytes, and spermatids. Before a spermatid becomes spermatozoa there is a phase that the spermatid passes through called the spermiogenesis phase. This phase consists of the Golgi, cap, acrosome, and maturation phases aimed at forming a normal morphology of spermatozoa consisting of the head, neck, and tail. This disorder can be caused by hormonal consequences, free radicals, and foodstuffs.[14]

According to Prastowo,[15] abnormal abnormalities of the head and tail are characterized by a condition where spermatozoa only have the head, tail, and neck. Abnormalities in this state are thought to occur primarily or secondary, abnormalities occur primarily due to disturbances during the process of spermatogenesis, while secondary abnormalities occur due to damage to spermatozoa during their passage inside the epididymis. The head and tail of spermatozoa are connected by a cell membrane allowing separation during cell movement and cytoplasmic displacement.[16] In spermatozoa that have abnormalities in the posterior part of the head, sometimes a perfect membrane is not formed so that contact with the tail basal is less strong. This is due to damage to the spermatozoa membrane by ROS. In addition, according to Zulfa,[17] lipid peroxidation of unsaturated fatty acids on the head and neck of spermatozoa causes changes in the morphology of spermatozoa.

The administration of cinnamon bark methanol extract in the research showed a decrease in the number of abnormal spermatozoa. The decrease in the average of these abnormal spermatozoa is thought to be due to the content contained in the bark of cinnamon sticks such as flavonoids. The function of most flavonoids in our body is as an antioxidant.[18] Flavonoids are natural antioxidants that can act as

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hydroxyl radical reducers (*OH), superoxides (O2*-), and peroxyl radicals (ROO*).[19] It also contains 313 ppm of tocopherol which dampens 96% of free radicals at a concentration of 12 ppm. Tannins are astringent, bitter-tasting plant polyphenols that can bind and precipitate proteins.

Viability of Spermatozoa

The results of observations on the percentage viability of spermatozoa mice after administration of cinnamon bark methanol extract can be seen in Table 2.

Table 2. The average percentage of viability of spermatozoa mice diabetes mellitus after administration of cinnamon bark methanol extract.

No.	Treatment	Average Viability of spermatozoa (%) (%) ± SD
1	Negative control	68,00 ± 1,00[a]
2	Positive control	22,00 ± 3,00[b]
3	Dosage 250 mg/kg BB	53,67 ± 2,52[c]
4	Dosage 500 mg/kg BB	60.33 ± 1,52[d]

In Table 2, it can be seen that the average viability percentage of spermatozoa mice is 68.00%. In the positive control group, the average percentage of spermatozoa viability was 22.00%. The average percentage of spermatozoa viability in the diabetic mice group treated with cinnamon bark methanol extract dose 250 mg/kg BB was 53.67%. Meanwhile, in the group of diabetic mice treated with cinnamon bark methanol extract at a dose of 500 mg/kg BB, the average percentage of viability of spermatozoa mice was 60.33%.

STZ injection in the study led to a decrease in the percentage of viability (viability of spermatozoa). This can be seen in the average viability percentage of spermatozoa mice in the negative control group was higher when compared to the average percentage of mice viability in the positive control group, the dose group was 250 mg/kg BB and 500 mg/kg BB before being given cinnamon bark methanol extract, where the percentage of viability was lower.

With the low percentage of viability, it is suspected that fertilization will not occur because spermatozoa die before fertilizing the egg. Spermatozoa on their way to vas deferens are not all able to maintain their lives so some die. Decreased viability of spermatozoa can occur due to the presence of obstacles in the epididymis as a place of maturation of spermatozoa that occur due to reduced testosterone hormone. Testosterone is necessary for the viability of spermatozoa in the epididymis.[20] The disruption of hormonal work due to streptozotocin causes the life force of

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spermatozoa to decrease so that many spermatozoa die.[21]

Free radicals contained in STZ will affect Sertoli cells which act as determinants of spermatozoa cell reproduction and a source of nutrition for spermatozoa cells.[22] Free radicals will cause the occurrence of lipid peroxidation of cell membranes and damage the organization of cells. This cell membrane is very important for receptor function and enzyme function so lipid peroxidation results in a complete loss of cellular function.[23]

Giving cinnamon bark methanol extract can increase the percentage of viability of spermatozoa. This can be seen by increasing the percentage of viability of spermatozoa mice given cinnamon bark methanol extract at doses of 250 mg/kg BB and 500 mg/kg BB, while in the positive control group the percentage of spermatozoa viability decreased. The increasing percentage of spermatozoa viability after being given cinnamon extract is caused because cinnamon bark has natural antioxidants such as flavonoids, saponins, and tannins that can provide spontaneous and rapid stimulation power to the hypothalamus. The way antioxidants enter the body is through blood circulation to the hypothalamus and the rest of the body. The hypothalamus will produce the hormone GnRH (Gonadotropin Releasing Hormone) which will then stimulate the anterior pituitary to produce the hormones LH and FSH.[24]

Spermatozoa motility

The application of cinnamon bark methanol extract in this study can improve the motility of mice spermatozoa. The average number of spermatozoa mice during the study can be seen in Table 3.

Table 3. Average motility of spermatozoa mice diabetes mellitus after administration of cinnamon bark methanol extract

No.	Treatment	Average Motility of spermatozoa (%) (%) ± SD
1	Negative control	68,33 ± 1,16[a]
2	Positive control	18,67 ± 2,52[b]
3	Dosage 250 mg/kg BB	53,00 ± 2,00[c]
4	Dosage 500 mg/kg BB	60.33 ± 1,53[d]

In Table 3, it can be seen that the average number of spermatozoa motility in the negative control group was 68.33%. In the positive control group, the average motility of spermatozoa mice was 18.67%. In the group of diabetic mice treated with cinnamon bark methanol extract at a dose of 250 mg/kg BB, the average amount of motility of spermatozoa mice was 53.00%. Meanwhile, in the group of diabetic mice treated with cinnamon bark methanol extract at a dose of 500 mg/kg BB, the

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average motility of spermatozoa mice was 60.33%.

STZ injection in this study also led to a decrease in the motility of spermatozoa mice. The number of spermatozoa motility in the normal control group was greater when compared to the number of spermatozoa motility in the mice group given streptozotocin. STZ will cause inhibition of the energy supply for the process of spermatogenesis. Energy for the motility of spermatozoa is supplied in the form of adenosine triphosphate synthesized by mitochondria on the caudal body. So if there is damage to the mitochondrial membrane, it will be able to interfere with the motility of the spermatozoa. Streptozotocin will cause weight loss of the epididymis because it can stimulate the formation of estrogen in the body which will increase estrogen levels.[25,]

The epididymis is a long circular channel leading into the vase deferens that enlarges de the ampule of the vas deferens.[26] An epididymis is a place of maturation and storage of spermatozoa The epididymis is used to transport spermatozoa coming from the testes to be taken to the vas deferens. The proximal part of the epididymis duct is a place for the absorption of fluid secreted by the testicles and then the maturation of spermatozoa, while the distal part works as a place to store spermatozoa.[27]

Spermatozoa that leave the testicles are not yet fully capable of movement. As it passes through the epididymis, the sperm maturates and gets motility and capacity for fertilization. Sperm motility is enhanced by relaxants that may be produced by the prostate. Sperm maturation in epididymis includes morphological, histochemical, biochemical physiology, biophysical, and metabolic changes.[28]

Giving cinnamon bark methanol extract at a dose of 250 mg/kg BB and 500 mg/kg BB can improve the quality of mice spermatozoa. In the dose group of 250 mg/kg BB, the average amount of motility of spermatozoa mice before the administration of the extract was 43.33 and after the administration of cinnamon bark extract increased by 53.00. Meanwhile, in the dose group of 500 mg/kg BB, the average amount of motility of spermatozoa mice before administration of the extract was 42.67 and increased after administration of cinnamon bark methanol extract by 60.33.

Cinnamon bark extract contains antioxidant compounds such as flavonoids, eugenol, tannins, saponins, and methyl hydroxy chalcone polymer (MHCP). Flavonoids as antioxidants (AH) give hydrogen atoms quickly to free radicals (R*, ROO*) or convert them to a more stable form, while antioxidant radical derivatives (A*) have a more stable state than free radicals. Flavonoids can stimulate cells β the pancreas to produce and secrete insulin.[29] Elevated insulin levels can trigger IGF–1 synthesis in the liver that increases the effects of LH and FSH on Leydig cells and Sertoli cells. Such increases can trigger an increase in

testosterone and ABP levels used in the process of spermatogenesis. The process of spermatogenesis that is not inhibited will produce spermatozoa in a higher quantity.[30,31]

Flavonoid compounds and saponins also inhibit the metabolism of Low-Density lipoprotein (LDL) in secondary atherosclerosis lesions through inhibition of LDL oxidation in the endothelial and stop the proliferation of smooth muscle cells (SMCs) stimulated by oxidized LDL.[32] In addition, it reduces the toxicity of oxidized LDL to endothelial cells, smooth muscle cells, and macrophages. Thus inhibiting the thickening of the lining of the vascular wall, which consists of fat, foam cells, muscle cells, and matrices. Then the narrowing of blood vessels will be reduced so that blood flow as a nutrient distributor in the reproductive organs is not hampered and the quality of sperm is not disturbed (sperm count and motility). Blockage of blood vessels (arterial and venous incompetence) results in the flow of nutrients for spermatogenetic cells that develop in the seminiferous tubules will be disrupted, which further affects spermatogenesis and the quality of the sperm produced.[33,34]

CONCLUSION

From the results and discussion, it can be concluded that giving cinnamon bark methanol extract can affect the improvement of the quality of spermatozoa mice model of diabetes mellitus type 1. Cinnamon bark methanol extract dose of 500 mg/kg BB is more effective in lowering the number of abnormal spermatozoa and increasing the percentage of viability and motility of spermatozoa mice (*Mus musculus*) with Diabetes Mellitus.

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Evaluation of the Antihyperuricemia Activity of Bajakah Tampala Stem Ethanol Extract (*Spatholobus littoralis* Hassk.) in Male White Mice (*Mus musculus*)

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Abstract

Hyperuricemia is elevated blood uric acid. Blood uric acid can harm health. Allopurinol can lower uric acid, but it has harmful side effects. The community has treated various diseases with Bajakah stems. This study examined Bajakah Tampala stem ethanol extract secondary metabolites and antihyperuricemia activity. This research identified the bajakah tampala stem plant (Spatholobus littoralis Hassk.), manufactured simplicia, characterized simplicia, phytochemically screened, prepared the ethanol extract, and tested its antihyperuricemia activity against male white mice (Mus musculus). The simplicia and Bajakah tampala stem ethanol extract contained flavonoids, tannins, steroids/triterpenoids, alkaloids, and saponins. At 400 mg/kgBB (3.88 mg/dl), the bajakah tampala stem ethanol extract reduces uric acid at the 6th hour, similar to allopurinol at 10 mg/kgBB (3.46 mg/dl).

Keywords: *Bajakah tampala stem,Spatholobus littoralis, Hyperuricemia, Allopurinol*

Abstrak

Hiperurisemia adalah peningkatan asam urat darah. Asam urat darah dapat membahayakan kesehatan. Allopurinol dapat menurunkan asam urat, namun memiliki efek samping yang berbahaya. Masyarakat telah mengobati berbagai penyakit dengan batang Bajakah. Penelitian ini menguji metabolit sekunder ekstrak etanol batang Bajakah Tampala dan aktivitas antihiperurisemia. Penelitian ini mengidentifikasi tanaman batang bajakah tampala (Spatholobus littoralis Hassk.), pembuatan simplisia, karakterisasi simplisia, skrining fitokimia, preparat ekstrak etanol, dan uji aktivitas antihiperurisemia terhadap mencit putih jantan (Mus musculus). Simplisia dan ekstrak etanol batang bajakah tampala mengandung flavonoid, tanin, steroid/triterpenoid, alkaloid, dan saponin. Pada 400 mg/kgBB (3,88 mg/dl), ekstrak etanol batang bajakah tampala menurunkan asam urat pada jam ke-6, mirip dengan allopurinol pada 10 mg/kgBB (3,46 mg/dl).

Kata Kunci: *Batang bajakah tampala, Spatholobus littoralis, Hiperurisemia, Allopurinol*

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INTRODUCTION

The prevalence of hyperuricemia has increased worldwide, both in developed and developing countries. Based on data from the Global Burden of Diseases (GBD), it shows that the prevalence of hyperuricemia in Indonesia is 18%. Hyperuricemia data were also obtained from Tomohon City and Denpasar, where the prevalence

reached 25% and 18.2%, respectively. Whereas in Bandungan (Central Java), the figures were 24.3% for men and 11.7% for women, with a total prevalence of both sexes of 17.6%[1].

Hyperuricemia is a condition where uric acid levels in the blood are greater than the normal value, which in men is said to be hyperuricemia if the uric acid levels are above 7 mg/dl and in women above 6 mg/dl. Mice are said to have hyperuricemia when their uric acid levels are 1.7–4.0 mg/dl[2].

Medications used to lower blood uric acid levels can be used by either reducing uric acid production or increasing uric acid excretion by the kidneys. Allopurinol is an example of a drug that works to inhibit the formation of uric acid through inhibition of the activity of the enzyme xanthine oxidase, and probenisid is an example of a uricosuric drug that can increase uric acid excretion by inhibiting its reabsorption in the kidney tubules[3].

One of the plants that has the potential to be used as a traditional anti-hyperuricemia medicine is bajakah tampala (Spatholobus littoralis Hassk.). Compounds that have potential as antihyperuricemia agents are flavonoids, tannins, and saponins. Flavonoid group compounds work by inhibiting xanthine oxidase so as to reduce excessive uric acid production; tannin compounds are known to bind free radicals during the conversion of purines to uric acid; and saponin compounds work by reducing the activity of the xanthine oxidase enzyme in serum[4]. Aside from being antihyperuricemia, flavonoids also function as anti-inflammatories by inhibiting cyclooxygenase and lipooxygenase enzymes so that they can reduce pain resulting from hyperuricemia[5].

According to previous research, the ethanol extract of the Bajakah tampala stem has secondary metabolite compounds like phenolics, flavonoids, tannins, and saponins and helps male rats heal wounds[6]. The Bajakah Tampala stem ethanol extract is antibacterial[6] and antioxidant[7]. The ethanol extract of Bajakah tampala stem was tested for antihyperuricemia (*Spatholobus littoralis* Hassk).

METHODOLOGY

Identification of the bajakah tampala stem plant (*Spatholobus littoralis* Hassk.), collection of the bajakah tampala stem plant, production of simplicia, characterization of simplicia, phytochemical screening, preparation of the ethanol extract of the bajakah tampala stem, and antihyperuricemia activity test of the

ethanol extract of the bajakah tampala stem (*Spatholobus littoralis* Hass (Mus musculus).

Sample Processing

The samples used were tampala pirate stems taken from Central Kalimantan. The stems of the Bajakah Tampala that have been collected are washed thoroughly with running water, then drained and spread on parchment paper. The ingredients are then chopped, weighed, and dried in a drying cupboard at 40°–50°C until dry; when crushed, they are brittle. The dried simplicia was then powdered using a blender[8]. The simplicia powder is stored in plastic to prevent moisture and other impurities before being extracted.

Preparation of Bajakah Tampala (Spatholobus littoralis Hassk.) Stem Ethanol Extract

The ethanol extract of the stem of Bajakah tampala (*Spatholobus littoralis* Hassk.) was made by percolation using 96% ethanol solvent by means of 500 g of simplicia powder moistened with 250 ml of solvent, closed, and left for 3 hours, then put into a percolator. Pour enough of the 96% ethanol solvent solution until all the simplicia is submerged and there is a layer of solvent above it[9]. The mouth of the percolator tube is covered with aluminum foil and left for 24 hours, then the faucet is opened and the extract droplets are allowed to flow at a rate of 1 ml per minute, and the percolate is collected. Add the filter solution repeatedly so that there is always a layer on top, so that you get 4 liters (80 parts). The percolation was stopped when the last 500 g of percolate had been evaporated without leaving any residue, and then sufficient distiller solution was added to obtain 5 liters (100 parts)[10]. The extract was evaporated using a rotary evaporator until a thick extract was obtained[11].

Preparation of 0.5% Na Carboxymethyl Cellulose (Na CMC) Suspension

As much as 0.5 g of CMC Na was put into a mortar containing 10 ml of hot distilled water and allowed to stand for 15 minutes until a transparent mass was obtained, then crushed until homogeneous. Then it was diluted with distilled water and put into a 100-ml volumetric flask. The volume is turned all the way up[12].

Preparation of Bajakah Tampala Stem Ethanol Extract Suspension (Spatholobus littoralis Hassk.)

The dose of the ethanol extract of the Bajakah tampala stem was determined based on the orientation of the dose in experimental animals. The doses of the ethanol extract of

Bajakah stems given to experimental animals were 200, 300, and 400 mg/kgBB. The ethanol extract of the bajakah stem (EEBBT) was weighed at 200, 300, and 400 mg, respectively, and put into a mortar containing a small amount of 0.5% Na CMC suspension, homogenized, and then added to 10 ml of the 0.5% Na CMC suspension.

Preparation of 1% Allopurinol Suspension

Allopurinol 100 mg, as much as 1 tablet, was crushed into a mortar, then 0.5% Na CMC suspension was added little by little while grinding until homogeneous, and the volume was made up to 100 ml with 0.5% Na CMC.

Preparation of Potassium Oxonate Solution

The preparation of a potassium oxonate solution is carried out in the following way: 250 mg of potassium oxonate are weighed, then put into a 10-ml volumetric flask, and 0.9% NaCl solution is added to the mixture[12].

Uric Acid Lowering Effect Testing

This study used healthy male white mice (Mus musculus) weighing 20–30 g. The study was conducted with five treatment groups. The total number of animals used from each treatment group was determined based on the Federer formula[13].

The study began with mice being fasted for 18 hours (not given food but given water). Then each mouse was weighed and marked. Then the fasting uric acid level was measured by dripping blood from the tail vein of the mice onto the test strip and waiting a few seconds until the blood was evenly distributed in the reaction zone automatically. Within 20 seconds, the level of uric acid in the blood of the mice will appear on the screen of the tool. The mice were then given an intraperitoneal (i.p.) injection of 0.2 mL potassium oxonate. Mice were randomly divided into 5 groups, and each group's uric acid levels were measured after one hour of injections of potassium oxonate. After measuring uric acid levels, each rat was treated as follows:

1. Group 1: As a negative control, give CMC Na 0.5% orally.
2. Group 2: Allopurinol 1% was given orally as a positive control.
3. Group 3: Given EEBBT at 200 mg/kgBB, orally
4. Group 4: Given EEBBT at 300 mg/kgBB, orally
5. Group 5: Given EEBBT 400 mg/kgBB, orally

Blood uric acid levels were measured again one hour after the test material was administered, then again after two hours, four hours, six

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hours, and until the mice's uric acid levels returned to normal[14].

Data analysis

Data analysis from this study was carried out by calculating the average decrease in uric acid levels for each group using the normality and homogeneity method, one-way ANOVA (analysis of variance), followed by the Tukey test to see significant differences between treatment groups. This statistical analysis uses the SPSS (Statistical Product and Service Solution) program.

RESULT AND DISCUSSION

Plant Identification

In a study involving plants, the initial step is determination. This plant identification aims to ascertain and confirm the correct identity of the plants used and to prevent sampling errors. Plant identification performed at the Medanense Herbarium (MEDA) of the Biology Research Center, Faculty of Mathematics and Natural Sciences, University of North Sumatra revealed that the plant used in this study was the stem of the tampala bajakah (*Spatholobus littoralis* Hassk.). Local name: (Bajakah Tampala) In Figure 1, the tampa steel rod can be seen.

Figure 1. Tampala Bajakah Stem

Simplicia Characterization of Bajakah Tampala (Spatholobus littoralis Hassk.) Stems

The results of the characterization of the simplicia powder from the Bajakah Tampala stem can be seen in Table 1.

Table 1. Powder Characterization Results of Bajakah Tampala Stem Simplicia

No	Parameter	Check up result (%)	MMI terms (%)
1.	Water content	7.3%	< 10%
2.	Water soluble essence content	13.3%	> 9%
3.	Ethanol soluble essence content	16%	> 7%
4.	Total ash content	3.17%	< 10%
5.	Acid insoluble ash content	0.5%	< 1%

According to Materia Medica Indonesia, the results of the characterization tests for water content, water-soluble essence

content, ethanol-soluble extract content, total ash content, and acid-insoluble ash content are satisfactory.

Phytochemical Screening

A phytochemical screening was conducted to determine the class of secondary metabolites present in the stem of the Bajakah Tampala plant. Table 2 displays the screening results for simplicia powder and the ethanol extract of the Bajakah Tampala stem.

Table 2. Results of Phytochemical Screening of Bajakah Tampala Stems

No	Secondary Metabolites	Powder Results	Extract Results
1.	Alkaloids	+	+
2.	Flavonoids	+	+
3.	Saponins	+	+
4.	Tanins	+	+
5.	Steroids/Triterpenoids	+	+

Information
(+) indicates that secondary metabolites are present.
(-) Negative: Does not contain secondary metabolite

The ethanol extract of the Bajakah tampala stem contains secondary metabolites of alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids. The presence of flavonoids contained in this plant is very supportive as an antihyperuricemia drug. Flavonoids act as xanthine oxidase inhibitors because flavonoids are reducing agents, so they can inhibit the action of the xanthine oxidase enzyme to convert xanthine compounds into uric acid.

Measurement of Uric Acid Levels in the Ethanol Extract of Bajakah Tampala Stems

This study used experimental animals, namely mice, because mice have a digestive system absorption process and a metabolic system for testing drugs that are relatively similar to the human digestive system. The selection of male mice as test animals is because male mice have greater hormonal stability compared to female mice, because female mice experience estrus cycles during pregnancy and lactation, which will affect the psychological condition of the test animals. Male mice do not have the hormone estrogen, even though there is a very small amount. The hormone estrogen is useful for increasing uric acid excretion through urine. Increased levels of uric acid in each group can be observed after the

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induction process using potassium oxanate. Then the decrease in uric acid levels was observed for six (six) hours after being given treatment in the form of 0.5% Na CMC, allopurinol, EEBBT 200 mg/kgBB, EEBBT 300 mg/kgBB, and EEBBT 400 mg/kgBB. The decrease in uric acid levels can be seen in Figure 2.

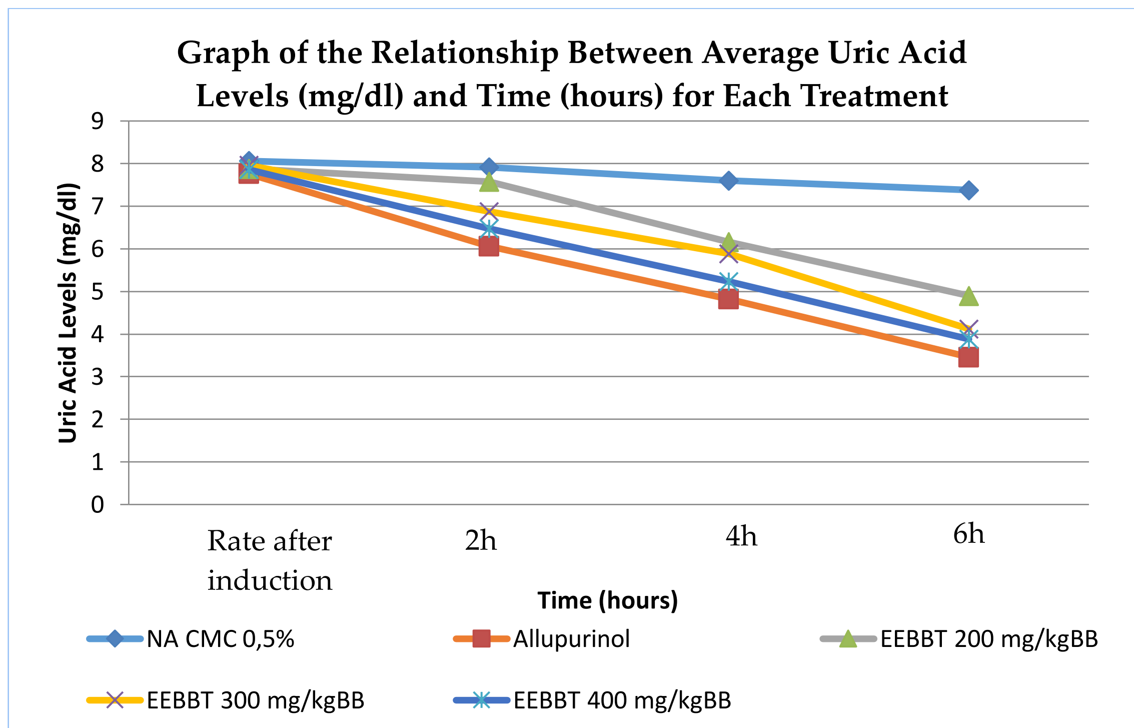

Figure 2. Graph of the Relationship Between Average Uric Acid Levels (mg/dl) and Time (Hours) for Each Treatment

Based on Figure 2 above, the EEBBT uric acid level test of 200 mg/kgBB decreased uric acid levels at the 4th hour and continued to decrease uric acid levels until the 6th hour. where EEBBT at 300 mg/kgBB and 400 mg/kgBB decreased uric acid levels at the 2nd hour. At the sixth hour, EEBBT of 400 mg/kgBB was nearly as effective as allopurinol uric acid reduction. Based on the graph above, to find out the differences in uric acid levels in mice obtained from various different treatments, whether significant or not, it is necessary to proceed with the one-way ANOVA test and the Tukey test.

In the one-way ANOVA test, if the significant value shows (0.05), it means that there is a significant difference in ability, and if the value shows (0.05), it means that there is no significant difference in ability[15].

In this study, measurements of decreased uric acid levels in mice had a significant value of 0.05, indicating a significant difference. The Tukey test was continued to see if there were differences in the data on the decrease in uric acid levels in the test animals from before treatment to after treatment between the 5 different treatment groups. Whereas data from normal levels and levels after induction show no significant change, allopurinol is already significant at 2 hours EEBBT at 400 mg/kgBB. At 4 hours EEBBT, 400 and 300 mg/kgBB were significant with allopurinol. while the EEBBT dose of 200 mg/kg BB was not significant until 6 hours.

The effectiveness of reducing uric acid from the Bajakah Tampala stem is strongly supported by the presence of secondary metabolites contained therein. The flavonoid group is one of the secondary metabolite compounds found in the stem of Bajakah tampala as a result of phytochemical screening[16]. Flavonoids are one of the compounds that are most likely to act as xanthine oxidase inhibitors because flavonoids are reducing agents, so they can inhibit the action of the xanthine oxidase enzyme to convert xanthine compounds into uric acid[17].

Overall, it can be concluded that the ethanol extract of the Bajakah Tampala stem has effectiveness for reducing uric acid in male white mice induced with potassium oxonate; the best dose is 400 mg/kgBB, which is not significantly different from allopurinol 10 mg/kgBB in the 6th hour after administration of the test substance.

CONCLUSION

The ethanol extract of the Bajakah tampala stem contains secondary metabolites of alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids. The ethanol extract of bajakah tampala stems has an antihyperuricemia effect in mice at the three doses used: 200, 300, and 400 mg/kgBB. EEBBT 300 and 400 mg/kgBB have comparable effectiveness with allopurinol 10 mg/kgBB; the statistical test results for EEBBT 300 and 400 mg/kgBB are not significantly different ($p > 0.05$) with allopurinol 10 mg/kgBB at 4 hours and 6 hours. The 200 mg/kgBB dose was significantly different from the 10 mg/kgBB allopurinol dose (p 0.05). The most effective dose of the ethanol extract of Bajakah Tampala as an antihyperuricemic is 400 mg/kgBB.

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