

EXT COMBINATION ACTIVITIES WHITE WOOD SHELF (*Melaleuca leucadendra*) AND HONEY AS NON-SPECIFIC IMMUNOMODULATOR IN MALE WHITE RATS (*Rattus norvegicus*)

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ABSTRACT

This study aims to determine the combination of eucalyptus leaf extract (*Melaleuca leucadendra*) and honey can provide non-specific immunomodulatory activity in male white rats (*Rattus norvegicus*).

The method in this study uses a quasi-experimental approach with a pretest-posttest approach that uses a test group and a comparison (control) group. The groups are: C1 (negative control), C2 (positive control), and C3 (test group).

The results showed that the three test groups of the combination of eucalyptus leaf extract (*Melaleuca leucadendra*) and honey can increase the level of leukocytes in the blood which functions as an immunomodulator. Statistical data analysis Paired sample t-test where the significance value of P 0.05 indicates that there is activity between the combination of eucalyptus (*Melaleuca leucadendra*) and honey so that it can increase the level of leukocytes in the blood which functions as an immunomodulator.

Keywords : *Immunomodulator, Honey, Leukocytes*

INTRODUCTION

Many diseases or threats from outside will enter the body and are prevented by the human body's defense system. The body has an immune system that functions as a protector of the body from various environmental pathogens such as viruses, fungi and bacteria that can cause infections. The immune system is all the mechanisms used by the body to maintain the integrity of the body as a protection against the dangers that can be caused by various materials in the environment. Various plants in Indonesia are believed to be able to increase the immune system or the immune system

in humans, the content of compounds in them makes these plants trusted by some people. If the immune system can work properly in the body.

Based on the current facts that there is a Coronavirus Disease or commonly referred to as the Covid-19 virus which is very disturbing to the entire world community, which can cause a person's immune system to decrease. When a person's immune system weakens or declines and cannot protect the human body, pathogens from the outside environment such as the Covid-19 virus can damage the immune system.

There have been many cases of death in Indonesia and even the world caused by this Covid-19 virus[2]

One of the plants that is believed by the community to increase the body's immune system or commonly known as the immune system is the eucalyptus plant (*Melaleuca leucadendra*) and honey. The main component of eucalyptus oil is a terpenoid group. The largest component is 1,8-cineol which is a monoterpene compound. 1,8-cineol compounds act as antimicrobial, antioxidant, immune, analgesic and spasmolytic[4]

Several *in vivo* and *ex vivo* studies have been conducted on the effect of eucalyptus essential oil and eucalyptol treatment on monocytes and macrophage recruitment in response to lung inflammation and infection. The data from this study show that eucalyptus can be used as an immunomodulator which is characterized by the properties of eucalyptus oil and its active ingredient, namely eucalyptol. Besides eucalyptus (*Melaleuca leucadendra*) honey is also believed to improve the immune system or the immune system[7]

Honey is generally believed to be efficacious as a producer of energy, increase endurance, and stamina. In addition, the results of research on the content and benefits of honey for health have been widely reported, including as an antiviral and immune system booster. The virus inhibitory activity comes from the flavonoid compounds in honey. Honey also functions as an immune system booster because it can increase the production of T and B lymphocytes and antibodies. Honey can be immunomodulatory, that is, it can work by triggering macrophages to produce cytokines that are involved in killing bacteria and repairing tissue [3].

The existence of research which states that the properties of eucalyptol from eucalyptus

and flavonoid compounds from honey are also believed to increase the immune system, according to the current facts or phenomena regarding the Covid-19 virus that can attack the human immune system, thus the background for researchers to study this. , namely by looking at the combined activity of eucalyptus leaf extract (*Melaleuca leucadendra*) and honey as a non-specific immunomodulator in increasing leukocyte cells. which functions in terms of the immune system against the invasion of viruses or bacteria or the presence of other foreign objects[5]

Extracts are dry, viscous or liquid preparations made by extracting vegetable or animal *simplicia* according to a suitable method, outside the influence of direct sunlight, the dry extract must be easily ground into powder. The liquid filter used is water, ethanol and a mixture of ethanol water[6]

Extraction is a method of separating two or more components of a material which is the source of these components. The components separated by extraction can be solids from a solid-liquid system, liquids from a liquid-liquid mixed system or solids from a solid-solid system[8]

The extraction method is selected based on several factors such as the nature of the raw material, adaptability to each type of extraction method and the importance of obtaining a complete or near perfect extract. The extraction process was carried out using a maceration technique with an immersion process for 24 hours or 3x24 hours. The advantage of the maceration method is that besides being easy, it also does not require heating so that it is less likely that natural materials will be damaged or decomposed. The long spelling of the maceration method and the stationary state during maceration allows many compounds to be extracted[9]

Eucalyptus (*Melaleuca leucadendra*) is one of the important plants in the essential oil industry. Eucalyptus as a tree with a height of ± 30 m. In Australian territory, eucalyptus can reach a height of over 40 m and a trunk diameter of 1.2 m. Eucalyptus trunk is gray to papery white, with a slightly silvery tree top. Meanwhile, eucalyptus leaves are green, not shiny, the edges of the leaves are flat, generally the leaves are between 5-10 cm long and 1 to 4 cm wide and the leaves are hairy. On each leaf blade there are 5 to 7 leaf bones with a length of 3 to 11 mm. The inflorescences of eucalyptus plants are grain-shaped and are found at the ends of the twigs and the axils of the leaves. Eucalyptus tree flowers are bisexual, and the petals and corolla are small. Eucalyptus fruit is in the form of capsules and is of the dehiscent type.

Eucalyptus has several chemical constituents consisting of leaves containing essential oils (by 0.5-1.5%), methyl eugenol, 1,8-cineol (by 50-65%) -terpineol, -pinene, benzaldehyde, butylaldehyde, pentanal, propionic acid, and botulin. Parameters in determining the quality of eucalyptus oil can be seen from the content of 1,8-cineol contained in eucalyptus oil. Previous studies also revealed that the mean lethal dose (LD50) of 1,8-cineol was 3849.03 mg/kg[10]

Based on (SNI) the cineol content in eucalyptus oil can be seen in table 1

Table 1. Eucalyptus oil quality standard according to SNI

Characteristics	SNI 06-3954-2006
Color	Clear To Greenish Yellow
Density (20°/20°C)	0.900 – 0.930
Refractive index (20°C)	1.450 – 1.470
Optical Rotation (20°C)	(-) 4° – 0°

Solubility in ethanol	1:1 to 1:10 clear
cineole levels	50-60 %

Source: Personal Data 2021

Various in vitro and ex vivo studies were conducted to study the effects of eucalyptus oil and eucalyptol treatment on monocytes and macrophage recruitment in response to lung inflammation and infection. The data of this study indicate an immunomodulator characterized by the properties of eucalyptus oil and its active ingredient, namely eucalyptol. Both treatments reduced the release of pro-inflammatory cytokines from monocytes and macrophages, but their phagocytic properties were not terminated. Eucalyptus (*Melaleuca leucadendra*) extract has traditionally shown antioxidant activity[12]

Honey is a natural product derived from plant flower essence and produced by bees. Honey is produced by honey bees who process nectar or flower juice. There are different types of honey, with different colors and flavors depending on the flower nectar from which it came. Honey based on the source of nectar is grouped into two, namely monofloral (one type of flower nectar is dominant) and polyfloral (a mixture of nectar of various types of flowers). Longan honey, rubber honey and randu honey are examples of monofloral honey, while forest honey is an example of polyfloral.

The flavonoid compounds are the largest group in phenolic compounds. Antioxidant activity in honey was mainly due to these two compounds because there was a strong correlation between antioxidant activity and phenolic and flavonoid compounds. In addition to fructose and glucose, other disaccharides identified include maltose, sucrose, turanose, isomaltose, laminaribiose, nigerose, kojibiose,

gentiobiose, and B-trehalose. Trisaccharides include maltotriose, erlose, melezitose, centose 3-a5, isomaltosiglucose, l-ketose, isomaltotriose, panose, isopanose, and theanderose. In addition, honey contains vitamins (B1, B2, B5, B6, and C), minerals (Ca, Na, Fe, Mg, Mn) and enzymes in the form of attaches. Honey has variability in chemical content related to its botanical and geographical origin,

Honey with various types has been used for treatment by many people from various parts of the world. Honey can be used in traditional medicine to treat fever, bacterial infections and cough medicine. In addition, the results of research on the content and benefits of honey for health have been widely reported, including as an antiviral and immune booster. The virus inhibitory activity comes from the flavonoid compounds in honey with various types and concentrations for different types of honey. Honey also functions as an immune system booster because it can increase the production of T and B lymphocytes and antibodies. Consuming honey can also lead to the production of short chain fatty acids which also have immunomodulatory benefits. Various studies have stated the health benefits of honey so that in general the nutritional content of honey can increase the body's immunity. Honey helps the body actively fight bacteria and viruses. Its antioxidant activity will neutralize free radicals that are harmful to the body. In addition, it also helps stimulate tissue regeneration so that body health is maintained [15].

Rats (*Rattussp*) are harmful rodents and are pests of farmers' crops. Rats are mammals, therefore their impact on a treatment may not be much different from that of other mammals. The advantages of white rats over wild rats are that they mature faster, do not show seasonal mating, and generally

reproduce faster. As a laboratory animal, white rats are very easy to handle, can be left alone in a cage and can hear the sounds of other mice and are large enough to facilitate observation. In general, laboratory rats were lighter in weight than wild rats. Usually at four weeks the weight is 35-40 g and the average adult weight is 200-250 g, but varies depending on the breed. The use of male white rats as experimental animals can provide more stable research results because they are not influenced by the presence of the menstrual cycle and pregnancy as in female white rats. Male white rats also have a faster drug metabolism rate and a more stable biological body condition than female rats. The most common rat used in the study was the Norwegian rat which had evolved into *Rattusnorvegicus* which lived mainly in burrows. Wistar white rats have large heads and short tails, based on natural behavior, all rodent species including rats are social species that must be routinely placed in groups. Male white rats also have a faster drug metabolism rate and a more stable biological body condition than female rats. The most common rat used in the study was the Norwegian rat which had evolved into *Rattusnorvegicus* which lived mainly in burrows. Wistar white rats have large heads and short tails, based on natural behavior, all rodent species including rats are social species that must be routinely placed in groups. Male white rats also have a faster drug metabolism rate and a more stable biological body condition than female rats. The most common rat used in the study was the Norwegian rat which had evolved into *Rattusnorvegicus* which lived mainly in burrows. Wistar white rats have large heads and short tails, based on natural behavior, all rodent species including rats are social species that must be routinely placed in groups. Wistar white rats have the

characteristics of being relatively resistant to infection and have very intelligent abilities. Mice are not like mice, which are photophobic and tend to gather with each other so that their activities are not disturbed by the presence of humans. Often conduct experiments using test animals, both as samples to be studied and as comparisons. For this reason, it is necessary to know how to change the dose of humans to test animals.

Leukocytes are white blood cells that function in the body's defense system against bacterial invasion or the presence of foreign objects. White blood cells act as immune system enhancers to kill germs and diseases that are in the bloodstream. White blood cells have another name commonly called leukocytes. The increase in the total number of leukocytes can be influenced by stress factors and the inflammatory response. The number of leukocytes will increase significantly in mice if they experience inflammation and stress. The normal value of leukocytes in mice ranges from 2,000-10,000 cells/ml or leukocyte cells are said to be abnormal or less when the leukocyte cell count is less than 2,000-10,000 cells/ml. Imboost is a phytopharmaceutical herbal product that is proven to be efficacious and safe to increase immunity and prevent illness and accelerate healing. Each imboost tablet contains 250 mg of Echinacea pupurea and 10 mg of Zn picolinate. Echinacea purpurea is reported to have the ability to increase phagocytosis because of its polysaccharide content that can activate macrophages and NK cells and has been tested both preclinically and clinically as an immunostimulant [16].

RESEARCH METHODS

The research method used in this research is quasi-experimental. This study used a test group and a comparison group (control).

The control group was divided into a positive group and a negative group. The positive control group was given Imboost film-coated tablets, while the negative control group was not given any treatment. This type of research is quantitative, where the use of quantitative methods is aligned with research variables that focus on phenomena that are currently happening in the form of research results in the form of numbers that have meaning. This quantitative approach was used by researchers to conduct research on eucalyptus leaf extract (Melaleuca leucadendra) and honey to increase non-specific immunomodulatory activity in male white rats (*Rattus norvegicus*).

The design of this study was experimental with a pretest-posttest approach with a control group where before being given observation and treatment, both groups were randomized (have the same opportunity). The randomization method used by the researcher is Single Blind, which means that one of the research subjects or the researcher does not know to which group the subject is allocated. Research design is the methods used to collect research data so that research results can be proven. The research design used by the researcher is a laboratory experiment. This research was conducted with the preparation of experimental white rats which were acclimatized for 7 days before being given treatment. During acclimatization the rats were fed fresh fruit and drinking water. Eucalyptus oil is commonly used as a topical remedy. The dosage forms of drugs circulating in Europe according to the European Medicines Agency include oral preparations containing 0.3 and 0.6 mL of essential oil equivalent to 100 and 200 mg of 1,8-cineol, with a frequency of administration of 2 to 5 times

a day and an oral dose. the daily maximum is 600 mg. The dose of honey in test animals was determined based on the results of the conversion from humans to mice which was equivalent to giving 1 full tablespoon (15 ml) and 2 full tablespoons (30 ml) in adults weighing 70 kg. Giving honey to white rats with doses of 0.25 ml and 0.5 ml is equivalent to human doses of 1 and 2 tablespoons[17] 3 and 0.6 mL of essential oil are equivalent to 100 and 200 mg of 1,8-cineol, with a frequency of administration of 2 to 5 times daily and a maximum daily oral dose of 600 mg. The dose of honey in test animals was determined based on the results of the conversion from humans to mice which was equivalent to giving 1 full tablespoon (15 ml) and 2 full tablespoons (30 ml) in adults weighing 70 kg. Giving honey to white rats with doses of 0.25 ml and 0.5 ml is equivalent to human doses of 1 and 2 tablespoons[17] 3 and 0.6 mL of essential oil are equivalent to 100 and 200 mg of 1,8-cineol, with a frequency of administration of 2 to 5 times daily and a maximum daily oral dose of 600 mg. The dose of honey in test animals was determined based on the results of the conversion from humans to mice which was equivalent to giving 1 full tablespoon (15 ml) and 2 full tablespoons (30 ml) in adults weighing 70 kg. Giving honey to white rats with doses of 0.25 ml and 0.5 ml is equivalent to human doses of 1 and 2 tablespoons[17] The dose of honey in test animals was determined based on the results of the conversion from humans to mice which was equivalent to giving 1 full tablespoon (15 ml) and 2 full tablespoons (30 ml) in adults weighing 70 kg. Giving honey to white rats with doses of 0.25 ml and 0.5 ml is equivalent to human doses of 1 and 2 tablespoons[17] The dose of honey in test animals was determined based on the results of the conversion from humans to

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On the basis of previous studies, it was stated that the effective dose for the use of honey was 6-8 tablespoons consumed 2 times a day if converted, it was equivalent to 90 ml, 105 ml and 120 ml [18].

The research subject is a source of information that is excavated to reveal the facts in the field. Determination of the subject in this study is used to obtain the required information clearly and in depth. The researcher determined the research subject based on the problem to be studied about how the activity of the combination of eucalyptus extract and honey as a non-specific immunomodulator in male white rats of the wistar strain. Thus, the research subjects taken by the researchers were male white rats of the wistar strain which will be used as experimental animals in this study with a weight of 200-250 grams as many as 25 tails. The process of adapting the male white rat wistar strain is called acclimatization. Preparation of experimental animals begins with acclimatization. Acclimatization is the process of adjusting experimental animals to changes in the environmental climate. It is feared that regional and climatic differences will cause physiological differences in this case the Wistar male white rat. This adjustment process takes place in a fairly varied time depending on how far the conditions differ between the new environment that will be faced, it can last from several days to several weeks. Therefore, acclimatization is needed to observe the behavior and adaptability of mice to their new environment. Mice that are not well adapted to their environment, have different

behavior from the others, will not be used as research subjects. Some conditions that are generally adjusted are ambient temperature, acidity (pH), and oxygen levels [19].

This research was conducted with several different doses of treatment. The dosage distribution and treatment are as follows:

1. Positive control group = White mice given imboost
2. Negative control group = White mice not given treatment
3. Test group = White rats given a dose of eucalyptus extract + honey, namely 120 mg/kg (24mg/200gr) (24 ml/200gr) + 500ml (2.16 ml)

The population in this study were eucalyptus and honey plants in Harapan Village, Wonosari District, Boalemo Regency, Gorontalo Province. The sample in this study was collected by purposive sampling, namely the technique of collecting samples or plants without comparing them with the same plants from other areas. This eucalyptus plant (*Melaleuca leucadendra*) grows natively in the Maluku Islands, especially on Ambon Island, Buru Island and Seram Island. However, it is known that the largest eucalyptus oil production in Indonesia comes from Java Island, namely from eucalyptus stands in the PerumPerhutani area on Java Island, and the Production Forest Management Unit (KPH) Yogyakarta.

The tools used in this research are: vortex mixer, beaker, jar, bistur, microscope, dispo, measuring cup, hemocytometer, surgical board. The materials used in this study were: 400 ml eucalyptus leaf extract, 10.8 ml honey, Wistar strain male white rats, cotton, tissue, aluminum foil, filter paper, 96% ethanol, imboost, aquades, Turk's solution, ether, Acid sulfate (H₂SO₄).

The research procedures at the maceration stage are:

- a. Eucalyptus leaf sample preparation (washing, chopping, and drying)
- b. Weighing 500 g of eucalyptus leaf simplicia
- c. Prepare the jar (maerator)
- d. Clean the macerator with aquades
- e. Measuring 5 Liters of 96% ethanol solvent
- f. Put all the simplicia eucalyptus leaves into a dry and clean maserator
- g. Enter 96% ethanol solvent into the macerator
- h. Stirring is done every day for 3 days within 10 minutes
- i. Strain the maceration pulp from the maserate with a clean filter cloth and use a funnel
- j. Strain little by little until completely filtered
- k. The result of maceration is ready to be evaporated to form a thick extract

The research procedures at the phytochemical screening stage are:

- a. 1 ml of eucalyptus extract added 5 drops of sulfuric acid reagent (H₂SO₄)
- b. If a blackish green color is formed, it indicates the presence of terpenoids

The immunomodulator test research procedures are:

1. Blood sampling of rats belonging to the negative control group
2. The blood that has been taken is put into the EDTA tube
3. *White blood cellcount* (Wbc) or counting the total number of leukocytes carried out in the laboratory using a Hemocytometer
4. After Treatment (Positive Control Group and Test Group)
5. Blood collection for rats after treatment (day 7)
6. The blood that has been taken is put into the EDTA tube

7. White blood cell count Wbc or counting the total number of leukocytes is done in the laboratory

Data is a unit of information recorded by media that can be distinguished from other data, can be analyzed and relevant to a particular program. Data collection is a systematic and standard procedure to obtain the required data. To collect research data, the author uses the method of observation (observation), where observation is a data collection technique carried out by observing and recording systematically, logically, objectively and rationally about various phenomena. *White blood cell count* (Wbc) or known as counting the number of leukocytes was carried out by taking blood from experimental animals in the negative control group (day 1) and the positive control group and the test group (day 7) giving treatment, in the technique of taking blood through the heart. The blood taken will be put into the EDTA tube before the WBC count is performed. Data collection techniques were carried out such as taking blood on male white rats that had been given treatment, aiming to count leukocytes to be carried out in the laboratory [20].

The data obtained from the immunomodulator test were analyzed using the SPSS version 21 statistical test data processing program using the one way ANOVA method. Data were obtained from each test group, both from the negative control group, the positive control group and the EKP + honey test group, the statistical test used in this observation was the T test (Paired sample T-test) aimed at seeing the occurrence of the effect in terms of probability activities. < 0.05 . This test is carried out on two paired samples (Paired), paired samples are defined as a sample with the same subject but experiencing two different treatments by looking at the final

probability results produced. If probability > 0.05 then H_0 is accepted but if probability < 0.05 then H_0 is not accepted. In knowing whether the information has been distributed normally and uniformly.

RESEARCH RESULT

The research was conducted at the Laboratory of BinaMandiri University, Gorontalo. BinaMandiri University Gorontalo is one of the campuses located in Gorontalo Province. Initially, the campus was still under the name STIKES BinaMandiriGorontalo which has 4 (four) study programs, namely S1 Pharmacy, S1 Hospital Administration, S1 Nutrition, D3 Health Analyst and in 2019 changed its name to BinaMandiri University Gorontalo. UBMG Laboratory is a place or room used for academic services, especially practicum services and research for student final assignments and lecturer research as well as other activities related to the improvement and development of the Tridharma of Higher Education. The laboratory is led by a Head of Laboratory which consists of several teaching staff/lecturers as members of the laboratory and several analysts/technicians. UBMG Laboratory consist of several rooms used by students to conduct research including pharmacy students. The rooms include a pharmaceutical technology laboratory, a phytochemical laboratory, a chemistry laboratory and a microbiology laboratory. UBMG Laboratory have adequate practicum equipment for practitioners or student.

Wet simplicia used as much as 1 kg which then became dry simplicia as much as 500 gr. The extraction process of eucalyptus leaves (*Melaleuca leucadendra*) was carried out for 3 x 24 hours by soaking dried simplicia leaves of eucalyptus (*Melaleuca leucadendra*) using 5 liters of

96% ethanol as solvent. Extraction results in the form of a liquid extract in the amount of 400 ml.

Table 2 shows the number of doses given to each group of mice from each sample. In the negative group, the mice were not treated at all and only as a benchmark for testing the positive group and the test group. The positive group of white rats was given a solution containing 4.7 mg of boosted tablets dissolved in 5 ml of water, while the test group was given EKP + honey as much as 24 ml/200gr + 2.16 ml.

Table 2 The number of doses given to each group of mice from each sample

Subject	Sample	Dose
Group (-)	Not given action	-
group (+)	Boost solution	4.7 mg/5ml water
Test Group EKP + honey	Eucalyptus extract + honey	24ml/200gr + 2.16ml

Source: Personal Data 2021

The result of maceration of 500 g of simplicia powder of eucalyptus leaves (melaeucaledendra) with 96% ethanol solvent obtained 400 ml of liquid extract which was then evaporated using a method evaporator and obtained an extract yield of 80%.

Table 3.Eucalyptus Leaf Extract Yield Results

Simplicity	Simple weight	Amount of extract (ml)	marinade
Eucalyptus Leaves (Melaeucaledendra)	500 gr	400 ml	80%

Source: Personal Data 2021

The examination carried out on eucalyptus leaf extract (Melaeucaledendra) was an examination of the class of flavonoid

compounds, saponins, terpenoids, and alkaloids. The results of phytochemical screening of simplicia powder ethanol extract of eucalyptus leaves (Melaeucaledendra) can be seen in table 4.

Table 4The result of the screening of the White Wood Shelf(Melaeucaledendra)

No	Compound	Reactor	Results	Note:
1.	Terpenoids	Sulfuric Acid (H2SO4)	Blackish green color is formed	+ (Positive)
2.	Saponins	Hot Water + HCL	Foam Formed	+ (Positive)
3.	Alkaloids	Dragendroff	No Orange Deposits Formed	- (Negative)
4.	Flavonoids	Concentrated HCL + Mg	Formed Orange Color	+ (Positive)

Source: Personal Data 2021

The immunomodulator test aims to compare the number of values between the positive control group given the boost, and the test group given the eucalyptus extract + honey which is associated with the immune system, namely leukocytes.

Table 5.The results of the immunomodulatory test of each test group on male white rats (Rattusnorvegicus)

Sampl e	Test	Normal value of leukocytes
Group (-)	group (+)	test group EKP + honey

C1	5.325 cells/ml	7,025 cells/ml	7,750 cells/ml	2,000- 10,000c ell/ml
C2	5.175 cells/ml	7.150 cells/ml	8050 cells/ ml	2,000- 10,000c ell/ml
C3	5,500 cells/ml	7,400 cells/ml	7.550 cells/ ml	2,000- 10,000c ell/ml
C4	5.125 cells/ml	6,975 cells/ml	7,975 cells/ ml	2,000- 10,000c ell/ml
C5	5,650 cells/ml	7,450 cells/ml	8175 cells/ ml	2,000- 10,000c ell/ml

Source: Personal Data 2021

DISCUSSION

Research on the activity of a combination of eucalyptus extract (*Melaleucadendron*) and honey as a non-specific immunomodulator in male white rats (*Rattus norvegicus*) was conducted at the Laboratory of BinaMandiri University, Gorontalo. The sample used was eucalyptus leaves (*Melaleucadendron*) weighing 1 kg, samples of eucalyptus leaves were washed and dried. In this study, a plant determination key was used with the aim of identifying eucalyptus leaves (*Melaleucadendron*) so that there were no errors in the plants used in the study.

Eucalyptus (*Melaleucadendron*) is a plant that grows in the lowlands up to 400 meters above sea level. Eucalyptus can grow in bad soil, is heat resistant, and can sprout again after a fire. Eucalyptus is used in traditional medicine as a respiratory medicine for the treatment of the nasal passages [22]

Eucalyptus extract (*Melaleucadendron*) which contains cineol which is a class of terpenoid compounds (monoterpenoids). Cineol is the main component of the essential oil in eucalyptus, which is 85%. Based on the 2006 SNI design drawings, the

minimum content of 1,8-cineol in eucalyptus oil is 50%. Referring to previous studies, simplicia as much as 5-7 kg or 5,000-7,000 g contains 60% cineol, from the highest amount of simplicia, which is 7,000 g and 60% cineol compound (60% / 7,000g) is converted into gram form to 4,200g/7,000g, which was then adjusted to the results of the extract in this study, which was 400 ml of the 500 g of simplicia so that the final result was 300g/400 ml of extract, meaning that there were about 300g of cineol contained in 400 ml of eucalyptus leaf extract (*Melaleucadendron*).

The use of honey as one of the samples combined with eucalyptus extract which also contains flavonoid compounds has viral inhibitory activity and strengthens the immune system because honey can increase T and B lymphocytes (white blood cells) and antibodies. attack body cells that have been exposed to viruses while B lymphocytes work by producing antibodies that function to attack bacteria, viruses and toxins [21]

Before the extraction process is carried out, first the eucalyptus leaves that have been collected are then dried by airing. Drying simplicia by means of air is a method used to dry soft plant parts such as flowers and leaves and plants that contain volatile compounds. Extraction is the initial stage carried out in this study to obtain extracts from the leaves of the Eucalyptus (*Melaleucadendron*) plant. The extraction process was carried out using the maceration method with 96% ethanol solvent which was carried out for 3x24 hours. Ethanol is used as a solvent because ethanol is a universal solvent that can attract most of the compounds contained in plants [23].

In previous studies the use of the maceration extraction method in the separation of essential oils due to the

advantages of this method is easy and does not require heating so that natural ingredients are less likely to be damaged or decomposed. The choice of solvent based on its solubility and polarity facilitates the separation of natural substances in the sample. The operation of the maceration method for a long time and at rest during maceration allows many compounds to be extracted [24].

Maceration extraction method was also chosen to prevent the destruction of compounds that are not resistant to heating. In the maceration extraction process, the simplicia eucalyptus leaves were previously dried and then soaked using 96% ethanol solvent which produced 400 ml of liquid extract. Ethanol solvent was chosen because ethanol has a polarity close to water according to its use. Ethanol solvent is safer and less toxic, because the extract obtained will be tested on rats, besides that ethanol can inhibit the growth of microorganisms compared to using water as a solvent.

After the extraction process is complete, the next step is phytochemical skinning. Phytochemical screening was carried out with the aim of knowing the compounds contained in the Eucalyptus (*Melaleuca leucadendra*) plant. Before carrying out phytochemical screening, first calculate the yield of eucalyptus extract (*Melaleuca leucadendra*). The marinade has units of percent (%) and the results obtained from the calculation of the extract yield are 80%, from the weight of the wet simplicia produced is 1 kg then dried to 500g, the drying process is carried out first with the aim of stopping the enzymatic process and preventing the emergence of microbes that can damage chemical content. The amount of liquid extract produced from the maceration process is 400 ml,

Based on the results of the phytochemical screening carried out, there are several

compounds contained in the eucalyptus plant (*Melaleuca leucadendra*), namely phytochemical screening using sulfuric acid reagent (H_2SO_4) positive to produce terpenoid compounds which are characterized by the formation of a blackish green color, hot water reagent + positive HCl produces saponin compounds which are characterized by the formation of foam, negative Dragendroff reagent produces alkaloid compounds because there is no orange precipitate formed, and the last is phytochemical screening of flavonoid compounds using concentrated HCl + Mg reagent, producing positive results marked by the formation of an orange color.

Steps to calculate the total number of leukocytes by accommodating as much as 1 ml of rat blood samples into a container in the form of an EDTA tube first containing an anticoagulant to prevent blood clots. Measurement of leukocytes is carried out because leukocytes are a component of the non-specific immune system which is the main line to fight if there are foreign objects or microorganisms that enter the body [25]. Based on the results of Wbc or leukocyte examination of male white rats (*Rattus norvegicus*) in the negative control group, the positive control group and the EKP + honey test group with the number of rats in each group as many as 5 mice, getting different results. In the negative control group, which was not treated at all, the WBC results showed results ranging from 5,000 cells/ml, in the positive control group, which was induced using an imboost tablet that had been made a solution by means of an imboost tablet which had been weighed as much as 4.7 g, then crushed. dissolved in 5 ml of water and showed WBC results in the range of 6000-7000 cells/ml. The increase in the number of leukocytes in the positive control compared to the negative control was due to the

administration of booster tablets containing *Echinacea purpurea* 250 mg which can be efficacious as an immunomodulator. *Echinacea purpurea* contains alkylamides (mostly isobutylamide), esters of caffeic acid (ecinacoside, cycoric acid, cynarin), polyacetylene essential oil, polysaccharides, non-toxic pyrolizidine alkaloids and flavonoids. The mechanism of action of *Echinacea* is by stimulating macrophages to produce IL-1 cytokines, a small amount of IL-2 and IL-6, IL-10, IL-12, IFN- γ and TNF- thus helping to overcome the infection[28]

Meanwhile, in the test group that was induced by EKP + honey as much as 500 ml which was then converted into a rat dose of 2.16 ml/200g mice, and EKP as much as 24 ml/200g mice showed WBC results ranging from 6,000-8,000 cells/ ml. The increase in the number of leukocytes was caused by the administration of eucalyptus extract which contains cineol compounds that can increase the immune system. Cineol compounds contained in eucalyptus have an effect and response to the recruitment of white blood cell groups such as monocytes and macrophages [29].

Giving honey can also stimulate non-specific immune responses, especially increasing the number of leukocytes to the maximum because the content of flavonoid compounds works by donating hydrogen ions so that they can neutralize the toxic effects of free radicals. Honey can also be an immunomodulator that works by triggering macrophages to produce cytokines that are involved in tissue repair [26].

Several studies have stated that flavonoids are able to increase the activity of IL-2 (Interleukin-2) and T-cell lymphocyte proliferation which causes Th1 cells (T helper 1) to be activated. Activated Th1 cells will affect MAF (Macrophage

Activation Factor), which are molecules that cause macrophage activation [27].

So, the results of these three test groups showed an increase in results between the negative control group that was not treated at all then with the positive control group and the EKP + honey test group due to the administration or treatment of each group.

From the test results obtained from each group, the paired sample T-test was tested. However, initially a pre-test was conducted on the untreated negative control group, which will show whether the information is normally distributed or not. Then a post-test test was carried out after being given treatment in the positive control group and the EKP + honey test group. In the Paired sample T-test statistical test, starting with the normality test, the resulting data was declared to have been normally distributed in the negative control group and the EKP test group, the significance value was $0.000 < 0.05$, and in the positive control group and the EKP test group the significance value was $0.009 < 0.5$, then the results of the test showed that the EKP test group had significant activity on immunomodulatory activity. In this case, H_0 is rejected and H_1 is accepted, where a combination dose of eucalyptus extract (*Melaleuca leucadendra*) and honey can increase the level of leukocytes in the blood which functions as an immunomodulator.

CONCLUSION

In accordance with the results obtained, it can be concluded that eucalyptus extract (*Melaleuca leucadendra*) combined with honey can increase the level of leukocytes in the blood which functions as an immunomodulator with a dose concentration of eucalyptus extract (*Melaleuca leucadendra*) and honey, namely 24 ml/200g rat + 2.16 ml/200g rat.

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