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Effectiveness of nutmeg flesh extract (*Myristica fragrans* H) as an immunomodulator using the carbon clearance method in mice (*Mus musculus*)

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ABSTRACT: Nutmeg (*Myristica fragrans* H) is composed of secondary metabolite compounds, among which flavonoids significantly contribute to its immunomodulatory properties. Using the carbon clearance method, this study aims to determine the efficacy of nutmeg flesh extract (NFE) as an immunomodulator. Negative control (Na-CMC 0.5%), normal control (Na-CMC 0.5%), immunostimulant positive control (Imboost® Force 0.91mg/20 g BW of mice), immunosuppressant positive control (methylprednisolone® 0.015 mg/20 g BW of mice), dose I (5 mg/20 g BW of mice), dose II (10 mg/20 g BW of mice), and dose III (20 mg/20 g BB mice) comprised the seven groups of 35 white male mice (DDY). The assessed parameters included organ index, absorbance, and the phagocytosis constant and index. The immunostimulant effect of NFE was demonstrated by the phagocytosis index values of 1.225 (indicating moderate immunostimulation), 1.512 (indicating moderate immunostimulation), and 2.202 (indicating strong immunostimulation) for NFE dose I, II, and III, respectively. The lymphoid organ index may have increased among the three NFE treatment groups, according to the results of organ index measurements. We can conclude, nutmeg flesh Extract (NFE) has an immunomodulatory effect in the immunostimulant category, as well as increased phagocytosis activity and lymphoid organ index.

KEYWORDS: Carbon clearance; immunomodulatory; immunostimulant; *Myristica fragrans* ; phagocytosis index.

INTRODUCTION

Continually proliferating and contagious infectious diseases attributed to pathogenic microorganisms, including fungi, viruses, bacteria, and parasites, are public health concerns. The primary defence mechanism against these pathogenic microorganisms is the immune system. The objective of immunomodulator therapy is to enhance the functionality and potency of the immune system [1]. Immunomodulators, such as immunostimulants, immunorestorants, or immunosuppressants, function by restoring immune system balance. It has been reported that administering immunomodulatory drugs derived from medicinal plants with immunostimulatory properties to patients causes reduced toxicity and adverse effects. Therefore, this provides an incentive for scientists to conduct additional investigations into medicinal plants and their derivative compounds, which may enhance specific immune responses [2].

Nutmeg flesh possesses promising immunomodulatory properties. The utilization of nutmeg flesh is confined to essential oil and confectioneries due to its discernible, pungent odor and delicate sweetness [3]. Nutmeg extract has been linked to numerous health benefits, including antibacterial [4], anti-inflammatory [5], and antioxidant activity [6], [7].

In order to qualify as potential immunomodulators, plants must contain specific compounds, including but not limited to limnoids, curcumin, vitamin E or tocopherol, flavonoids, catechins, and vitamin C [8]. Flavonoids[9] and Vitamin C are present in nutmeg at concentrations of 22 mg/100 g of fruit fles [10]. Epithelial barrier integrity, the initial line of defence against external pathogens, can be enhanced by vitamin C [11].

In addition to the compounds, nutmeg is known to contain Zinc (Zn) (215 mg/100 g), an essential micronutrient that is crucial for immune function, according to research done by Rosalia [12]. Zinc regulates the function and proliferation of natural killer cells (NK cells), macrophages, neutrophils, T and B lymphocytes, and cytokine production [13], contributing to cellular immunity. Using the carbon clearance

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method, this study aims to determine the efficacy of nutmeg extract (*Myristica fragrans* H) as an immunomodulator in male white mice.

▪ MATERIALS AND METHODS

Material

The sample used was ripe nutmeg (*Myristica fragrans H*) from Karcak Village, Leuwiliang, Bogor Regency and determined at the Research Center of the National Research and Innovation Agency (BRIN), Jl. Raya Jakarta-Bogor, km.46. Cibinong, Bogor. Determination result number stated in certificate of plant identification determination results number B-710/ II.6.2/ IR.01.02/4/2023. Other ingredients used are acetic acid 1%, distilled water, iron (III) chloride nutmeg *(Myristica fragrans H*), CMC Na 0.5%, ethanol 70%, HCL P, Imboost® Force (Soho), ketamine HCl, Food for experimental animals (for 512 pellets), Methylprednisolone®, physiological NaCl, Bouchard's reagent, Dragendroff's reagent, Mayer's reagent, wood powder, V-TEC® brand carbon ink (Chinese ink), alcohol cotton, xylol. The experimental animals used were male white mice of the DDY strain.

Collection of plant material

The nutmeg fruit (*Myristica fragrans H*) was collected from Bogor, West Java, Indonesia, and determined at the Plant Conservation Research Center and Botanical Gardens (BRIN), Bogor, West Java, Indonesia.

Preparing Nutmeg Flesh Extract (NFE)

The acquired nutmeg flesh underwent a series of processes to become dried simplicia: washing, chopping, drying in an oven at 40 °C until it becomes dry simplicia, grinding, and sieving through a number 40 mesh sieve in preparation for the extraction procedure [14].

The solvent utilized in the extraction procedure was 70% ethanol, and 500 grams of simplicia powder derived from nutmeg flesh were macerated for three days at a ratio of 1:10 between simplicia powder and solvent. Occasional stirring was performed during the maceration process until filtration was performed on the third day. The filtrate obtained from the maceration was subjected to rotary evaporation until a thick extract was obtained; any remaining powder dregs were discarded. The procedure for generating a viscous extract via remaceration is identical to that of maceration [15]. Once the thick extract had been obtained, the simplicia yield was computed so that a comparison could be made between the initial and final weights; the formula for this calculation was:

$$\text{Extract yield (\%)} = \frac{weight\ of\ the\ extract\ obtained}{initial\ weight\ of\ simplicia\ powder} \text{ x } 100\%$$

Characteristics of simplicia and extracts

In addition to organoleptic evaluation, the water and ash contents of simplicia powder and nutmeg extract were assessed during characteristic testing. The organoleptic evaluation of the simplicia and extracts encompassed the following characteristics: color, odor, taste, and shape. The water and ash contents were determined using the gravimetric method specified in the Herbal Pharmacopoeia [16].

Phytochemical screening

Phytochemical screening was done on nutmeg extract to identify flavonoids, alkaloids, tannins and saponins. The method used to identify these compounds refers to Hanani (2015)[17].

Preparation of comparison solutions and test solutions

Various solutions were prepared during the course of the research: a positive control (Imboost® Force), a colloidal carbon solution, and a test solution consisting of nutmeg extract. Additionally, a CMC Na solution was prepared as a negative control.

Preparation of CMC Na negative control

Zero point five gram CMC Na is used. Hot water is added to the lumping, CMC Na is sprinkled over the hot water, left for 15 minutes, then ground until a thick and transparent mass is obtained. Add a little water, then pour into a 100 mL measuring flask, and distilled water is added to the mark. [18]

Carbon suspension preparation

A volume of 1.6 mL of carbon ink liquid was utilized. UV-sterilized carbon ink was poured into a glass beaker, followed by the addition of an adequate amount of 0.9% NaCl and thorough stirring. After adding the homogenized carbon ink to a 10 mL volumetric flask containing a concentration of 16% NaCl, a 0.9% NaCl solution was added to the same volume [2]. Animals in the experiment were injected with carbon ink at a temperature of 37 °C.

Positive control preparation

This study used Imboost® Force, an immunostimulant supplement, and methylprednisolone, an immunosuppressant drug that inhibits the immune system response, as positive controls. Imboost® Force is a supplement that increases the body's immune system and methylprednisolone is an anti-inflammatory drug that works as an immunosuppressant by suppressing the immune system response. Every single one of the ten drug tablets was finely crushed and weighed. Of the two drug powders, methylprednisolone contained 200 mg and Imboost® Force powder weighed 1508 mg for the positive control. Following this, each of the powdered tablets was pulverized once more into a mortar filled with Na-CMC. A suspension containing 0.5% is maintained in a final volume of 100 mL.

Preparation of the experimental animals

For the experimental animals to become acclimated to their new environment, 35 male white mice were acclimatized for seven days. Amidst acclimatization, the mice were housed in cages with the following dimensions: 33 x 22 x 15 cm, each housing five mice. The diet consisted of Voer 512 pellets, with each animal receiving approximately 3 to 4 g per day [19] and drink indefinitely from bottles free of impurities. The experimental animals were weighed and categorized by the number of treatment groups following acclimatization. Before treatment, the experimental animals are once more weighed and the CV recalculated. When the results of the CV calculation satisfy the specified criteria, the testing process proceeds. In cases where the minimum CV requirement is 15%.

Table 1. Treatment and dosage.

Group	Treatment	Dosage
1	Normal Control	0.5% Na CMC Suspension with a volume of 0.5 mL was given
2	Negative Control (CMC Na)	0.5% Na CMC Suspension with a volume of 0.5 mL was given
3	Immunostimulant Positive Control (Imboost® Force)	Imboost® Force Suspension was given at a dose of 0.65 mg/ 20 g BW to mice with a volume of 0.5 mL
4	Immunosuppressant Positive Control (Methylprednisolone)	Methylprednisolon was given at a dose of 0.010 mg/20 g BW of mice with a volume of 0.5 mL
5	Nutmeg Flesh Extract (dose I)	NFE was given at a dose of 5 mg/ 20 g BW of mice with a volume of 0.5 mL
6	Nutmeg Flesh Extract (dose II)	NFE was given at a dose of 10 mg/20 g BW of mice with a volume of 0.5 mL
7	Nutmeg Flesh Extract (dose III)	NFE was given at a dose of 20 mg/20 g BW of mice with a volume of 0.5 mL

The arrangement of experimental animals was determined by treatment group, with five mice per group for a grand total of seven treatment groups. The dosages and treatment groups are detailed in Table 1. Both of normal and negative control was given CMC Na but the carbon ink was not used for normal control. The use of normal control is as representation of natural immune system. Otherwise negative control was used to compare with other treatment group.

NFE Immunomodulator testing with the carbon clearance method

The NFE immunomodulator test method has received ethical approval with number No. 008/KEPHP-UNPAK/06-202. Animals that have been acclimatized are given the test preparation orally for 7 days, followed by blood collection on day 8 via the mice's tails where the mice's tails were illuminated with a yellow incandescent lamp for 10 minutes. The mice's tails were given xylol to dilate the veins in the mice's tails. 50 µl of the animal's blood was taken and lysed using 4 mL of 1% acetic acid. The initial blood collection before

carbon ink was injected as a comparison (blank) was at minute 0. Then, the experimental mice were injected with 0.1 mL/20 g of mice BW carbon ink solution (black Chinese ink brand V-TEC®), which had been sterilized, incubated at 37 °C (body temperature), and administered intravenously (iv) [20]. efore injecting the mice, the tail was illuminated with a yellow incandescent lamp so that the tail veins of the mice dilated and made the injection easier. After injecting Chinese ink, 50 µl of mouse blood was taken at 15 minutes, then the blood was lysed using 4 mL of 1% acetic acid and the absorbance was measured using a UV-VIS spectrophotometer with a wavelength of 650 nm [21].

Calculating the phagocytosis constant and index

The phagocytosis constant and phagocytosis index values were calculated using the absorbance data values obtained from the mice's blood measurements using a UV-visible spectrophotometer subsequent to the carbon clearance method involving the injection of a carbon ink solution. The formula for determining the phagocytosis constant and phagocytosis index, as stated by Aldi et al. (2016) [21].

$$(K) = \frac{Log\ A\ (n) - Log\ A\ (n-1)}{t\ (n-1) - t(n)}$$

Note :

K = Phagocytosis Constant

A(n) = Absorbance at time n

A(n-1) = Absorbance at time n-1

T = Time (15)

N = n^{th} observation (1)

The calculation of the phagocytosis index price is performed using the following formula:

$$(IF) = \frac{Phagocytosis\ constant\ of\ Mouse\ x}{Mean\ phagocytosis\ constant\ of\ controls}$$

Note:

IF = Phagocytosis Index

Mouse X = required Mouse

Lymphoid organ index testing

The mice were re-weighed on the ninth day following treatment with the carbon clearance method. Subsequently, they were intraperitoneally (IP) injected with ketamine HCL at a dose of 44 mg/kgBW and a wait was applied until their consciousness diminished and they lapsed into unconsciousness. An operation was performed in order to remove and quantify each organ, specifically the thymus gland, liver, and spleen. The procedure is executed utilizing specialized instruments: the liver, situated in the abdominal cavity and identified by its red-brown coloration; the spleen, situated on the left side of the abdominal cavity, beneath the diaphragm, curved in front of the stomach; and finally, the thymus gland, designated sequential organ harvesting [1], positioned in front of the heart at the rear of the chest cavity and characterized by its flat, triangular configuration with its base facing the neck. Following a comparison of the weighed lymphoid organs to the negative control group, the lymphoid organs' relative weight percentage was calculated.

▪ RESULTS

Determination results

As per the results of the determination, the species utilized in this investigation was *Myristica fragrans Houtt*., which is a member of the Myristicaceae family. The fine, dark brown powder of nutmeg simplicia generated in this investigation possesses a distinct nutmeg aroma and a chewy texture. The measured yield was 14.48%, surpassing the 12.87% yield achieved in prior research by Dareda et al. (2020) [23]. This signifies the process's efficacy and potentially suggests the existence of a greater concentration of the compound. The extract obtained is viscous, blackish-brown in hue, and emits a discernible scent reminiscent of nutmeg. The yield achieved was 12.45%, surpassing the yield of 10.36% reported in a study conducted by Makanaung et al. (2021) [24]. Variations in results may arise from many factors, including but not limited to the solvent

employed and the extraction method utilized, disparities in planting location, and environmental conditions, including temperature, soil, and rainfall.

Water content and ash content of simplicia powder and nutmeg flesh extract

In this study, the water and ash contents of simplicia powder and thick extract of nutmeg flesh were ascertained via the gravimetric method. To enhance the precision of the findings, both analyses were replicated twice (duplo). The obtained ash and water contents are detailed in Table 2.

Table 2. Results of test parameters for water content and ash content.

Test parameter		Result (%)	Requirement*	Explanation
Water Content	Simplicia Powder	6.65%±0.003	≤10%	Qualified
	Condensed Extract	7.51%±0.006	≤16%	Qualified
Ash Content	Simplicia Powder	2.7%±0.003	≤4.1%	Qualified
	Condensed Extract	1.41%±0.006	≤1.7%	Qualified

As shown in Table 2, all test samples complied with the water and ash content specifications of Edition II of the Indonesian Herbal Pharmacopoeia [16]. The thin extract of simplicia (6.65%±0.006) and powder (6.65%±0.003) contain minimal water content, thereby reducing the likelihood of microorganism growth that may compromise quality. Additionally, the low ash content signifies an inorganic contaminant level that conforms to established standards. The findings of this research suggest that the samples utilized were of satisfactory quality and adhered to the established criteria for quality.

Results of simplicia and nutmeg extract phytochemical screening test

Phytochemical screening aims to qualitatively determine the concentration of secondary metabolite compounds in simplicia and extracts. The results of phytochemical tests are presented in Table 3.

Table 3. Phytochemical screening results of simplicia and nutmeg flesh extract.

Compound content	Reagent	Parameter	Simplicia powder	Extract
Alkaloids	Reagent mayer	Yellowish white precipitate	+	+
	Reagent dragendorff	Orange to red precipitate	+	+
Flavonoids	Ethanol 95% + Mg Powder + HCL P	Red to orange color	+	+
Tannin	Ethanol 80% + FeCl3	Blue-green to blackish color	+	+
Saponins	Hot distilled water	Foam is formed	+	+

Note:
+ : The test results showed that the extract contained the compounds tested

Table 3 presents the outcomes of the phytochemical screening test, which indicates the presence of secondary metabolites, including flavonoids, alkaloids, saponins, and tannins in the fruit flesh powder and extract. The findings acquired are consistent with the research carried out by Sirait and Enriyani (2021) [25]. Each employs an identical solvent, which is 70% ethanol. The solvent's influence on the compounds that can be attracted throughout the extraction process is significant. 70% ethanol can attract polar and nonpolar compounds with immunomodulatory properties, including alkaloids, saponins, tannins, steroids, and flavonoids.

Immunomodulatory effects of nutmeg flesh extract

The immunomodulatory effect of NFE was assessed using the carbon clearance method. This method is one of the standard procedures in immunomodulatory testing fot non-specific immune responses, particularly utilised to observe the reticuloendothelial system (RES) activity in removing colloidal carbon suspensions from the bloodstream [27]. Carbon is a marker in this test due to its advantages, such as small and stable particles that do not cause blockages in blood vessels and lungs. Injecting a colloidal of carbon ink as a foreign substance into the reticuloendothelial system in vivo aims to quantify the phagocytosis rate of phagocytic cells,

including monocytes and macrophages and other phagocytic cells, are engaged in this procedure to remove carbon ink particles from the blood.

The phagocytic activity was determined by counting the clearance rate of carbon introduced as a foreign substance in the blood. This was achieved by measuring the absorbance of blood samples treated with 1% acetic acid at specific time intervals. The absorbance measurements were conducted at a wavelength of 675 nm using a UV-Vis spectrophotometer, and the result is shown in Table 4.

Table 4. Average value of absorbance in each group.

Treatment Group	Average value of absorbance in the nth minute		Difference
	0	15	
Negative Control (Na-CMC 0.5%)	0.542±0.028	0.646±0.041	+19.18% ^{b}
Normal Control (Na-CMC 0.5%)	0.587±0.068	0.599±0.025	+2.04% ^{a}
Imboost® Force	0.479±0.062	0.378±0.046	-21.08%^{a}
Methylprednisolone®	0.539±0.046	0.676±0.031	+25.41% ^{b}
Dose I (NFE)	0.595±0.104	0.589±0.082	-1.01% ^{a}
Dose II (NFE)	0.602±0.483	0.515±0.024	-14.45% ^{a}
Dose III (NFE)	0.473±0.057	0.381±0.027	-19.45% ^{ab}

Note:
NFE: Nutmeg Flesh Extract
(+) : increase in absorbance percentage
(-) : decrease in absorbance percentage

The decrease in absorbance values indicates increased phagocytic activity, especially by leukocytes, including monocytes, neutrophils, eosinophils, and macrophages. This suggests that the faster the phagocytic cells eliminate carbon from the bloodstream, the more significant the decrease in absorbance values observed. This study showed that all doses of NFE and Imboost® influenced phagocytic activity, as evidenced by decreased absorbance values significantly different than the negative control ($P<0.05$). In contrast, the normal, methylprednisolone®, and negative controls demonstrated increased absorbance, indicating diminished phagocytic function.

The phagocytosis activity, an essential part of innate immunity, is crucial in eliminating infections by engulfing and destroying pathogens [21]. The obtained absorbance value was subsequently utilized to calculate the constant and index phagocytosis value. The phagocytic constant is one of the parameters that indicate the rate of phagocytosis in immunomodulatory testing using the carbon clearance method. The greater the value of the phagocytic constant, the greater the rate of carbon clearance [29]. NFE doses II and III, and Imboost® Force, significantly increase the phagocytosis constant and index compared to the negative control ($P<0.05$), whereas NFE dose I did not show a significant difference.

Wagner's theory categorizes substances with a phagocytosis index below 1 as weak, between 1 and 1.5 as moderate, and above 1.5 as strong immunostimulants. Accordingly, NFE doses I, II, and III demonstrated varying degrees of immunostimulation as we can see on the table 5, NFI doses I and II had immunostimulant index between 1 and 1.5 (1.225 and 1.512), indicating moderate immunostimulation and NFE doses III and Imboost® Force (immunostimulant positive control) had immunostimulant index above 1.5 (2.202 and 2.225), indicating strong immunostimulation. Conversely, Methylprednisolone® demonstrated weak immunostimulation, with a phagocytosis index below 1 (0.888), aligning with its role as an immunosuppressant. Methylprednisolone® is a positive immunosuppressant. Dosage-dependent enhancement of the immunostimulatory effect of NFE is observed.

DISCUSSION

As we could see in the table 4, the enhancement of dosage induced the immunostimulant by NFE becomes stronger.

Table 5. Average data results of constant and phagocytosis index.

Treatment group	Phagocytosis constant	Phagocytosis index	Classification of immunomodulating effects
Negative Control (-)	0.0127±0.0012^{ab}	0.998±0.150 ^{ab}	-
Normal Control	0.0152±0.0021 ^{b}	1.165±0.094 ^{b}	-
Positive Control (Imboost® Force)	0.0283±0.0036 ^{d}	2.225±0.281^{d}	Strong immunostimulant
Positive Control (Methylprednisolone®)	0.0113±0.0013 ^{a}	0.888±0.104 ^{a}	Immunosuppressive
NFE Dose I (5 mg/20 g BW)	0.0164±0.0041 ^{b}	1.225±0.322 ^{b}	Moderate immunostimulant
NFE Dose II (10 mg/20 g BW)	0.0192±0.0013 ^{c}	1.512±0.103 ^{c}	Moderate immunostimulant
NFE Dose III (20 mg/20 mg BW	0.0271±0.0021 ^{d}	2.202±0.163 ^{d}	Strong immunostimulant

Note:
The addition of superscript letters (a, b, c) in the numbers in the column shows a significantly different effect on each lymphoid organ (Sig. > p 0.05)

The lymphoid organ index is a non-specific immune response test method that evaluates the function of lymphoid organs, such as the thymus gland and spleen, which play a vital role in the immune system. The thymus gland is a primary lymphoid organ that functions as a site for B-cell and T-cell maturation [30]. Based on this explanation, the results that comes from percentage of lymphoid organ index refer to illustrate of non specific immune response. We could use titer antibody calculation to determine the specific immune response. The spleen is a secondary lymphoid organ containing B and T lymphocytes, dendritic cells, and macrophages that act as APCs (Antigen Presenting Cells). The liver plays a crucial role in the immune system, contains various active immunological cells, and plays a role in nonspecific phagocytosis, which is a key component in preventing the invasion of pathogenic organisms from the intestines [31].

As shown in Table 6, the spleen and liver index in the NFE doses II and III were increased and significantly different than the negative control ($P<0.05$). The thymus index in all doses of NFE was increased than the negative control, but NFE III was significantly different ($P<0.05$). Generally, NFE doses II and III have no significantly different spleen and liver index ($P>0.05$) with Positive Control (Imboost® Force). Positive Control (Methylprednisolone®) has a lower spleen, liver, and thymus index than all groups, including negative control.

Table 6. Results of % lymphoid organ index.

Treatment group	Lymphoid organ index (%)		Thymus gland
	Spleen	Liver	
Negative Control (-)	0.904 ± 0.225^{b}	6.412 ± 0.470^{ab}	0.392 ± 0.04^{b}
Normal Control	0.997 ± 0.137^{b}	6.829 ± 0.434^{b}	0.415 ± 0.032^{b}
Positive Control (Imboost® Force)	1.135 ± 0.104^{cd}	7.255 ± 0.150^{cd}	0.512 ± 0.026^{c}
PositiveControl (Methylprednisolone®)	0.783 ± 0.142^{a}	5.907 ± 0.492^{a}	0.344 ± 0.048^{a}
NFE Dose I (5 mg/20 g BW)	0.859 ± 0.088^{b}	5.966 ± 0.583^{a}	0.401 ± 0.048^{b}
NFE Dose II (10 mg/20 g BW)	1.043 ± 0.081^{c}	7.091 ± 0.188^{cd}	0.434 ± 0.035^{b}
NFE Dose III (20 mg/20 mg BW)	1.249 ± 0.128^{d}	7.593 ± 0.223^{d}	0.501 ± 0.016^{c}

Note:
The addition of superscript letters (a, b, c) in the numbers in the column shows a significantly different effect on each lymphoid organ (Sig. > p 0.05)

The increase in lymphoid organ indices suggests NFE's role as an immunostimulant. Increase the lymphoid organ by stimulating the proliferation of immune cells, thereby facilitating the activation and fortification of the body's immune response—a critical component in safeguarding against pathogens and infections [32].

This result shows that NFE had immunostimulant properties, its ability to increase the phagocytosis index and lymphoid organ index responsible for nonspecific and humoral immune responses. The effectivity of NFE as an immunostimulant might be accredited to the presence of flavonoids, alkaloids, saponins, and tannins, which contribute to its immunostimulating properties. Flavonoids are recognized for their antioxidant and natural immunostimulant properties [33],[34]. To counteract free radicals, this compound can enhance the phagocytosis of macrophage cells within the body. Furthermore, by stimulating phagocytosis, flavonoids can increase the quantity of B or T lymphocytes and lymphokines produced by T lymphocytes, thereby enhancing phagocytic activity. The activation of these macrophages is induced by interferon-gamma (IFN-gamma), which is stimulated by Th1 cells. To eliminate pathogenic microorganisms, activated macrophages generate nitric oxide and other cytotoxic substances, which stimulate T-Toxicity (TC) cells responsible for removing infected cells. Subsequently, B cells secrete antibodies that eliminate extracellular substances [21].

One of the flavonoids from nutmeg is myristicin. In vivo studies show that this compound has an immunostimulating effect characterized by increased immune biomarkers such as lysozyme in fish mucus [35]. Apart from flavonoids, alkaloids also trigger the release of the cytokine IL-12 by T cells [36]. Where IL-12 helps stimulate interferon-gamma production by NK cells. IL-12 plays a role in activating macrophage cells [30]. Saponins also play an essential role in influencing macrophage phagocytosis, which can improve the immune system by producing cytokines, including interferon and interleukin [36]. Furthermore, tannin compounds can have physiological activities, including increasing cell phagocytic activity, such as stimulating phagocytic cells, and anti-tumour and anti-infection [37].

In addition to its secondary metabolite content, nutmeg flesh is rich in Vitamin C and Zinc, both of which are crucial for cellular functions in the innate and adaptive immune systems [11],[12],[13]. The Vitamin C content in fresh nutmeg pulp ranges from 17.59 to 21.47 mg per 100 grams of fruit flesh. Research by Sipaheuleut (2013) has indicated that the Vitamin C level in nutmeg flesh stands at 22 mg per 100 grams. This vitamin plays a significant role in bolstering the integrity of the epithelial barrier, serving as the primary defense against external pathogens [11]. Furthermore, nutmeg flesh contains 215 mg of Zinc per 100 grams [12]. Zinc's contribution to cellular immunity encompasses the regulation of function and proliferation of neutrophils, natural killer cells (NK cells), macrophages, T and B lymphocytes, and the production of cytokines, highlighting its essential role in the immune response [29],[38].

▪ CONCLUSION

Nutmeg flesh extract has an immunomodulatory effect, with the effect increasing as the dose increases. Dose III (20 mg/20 g BW) is the best dose in the strong immunostimulant classification category.

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