Immunomodulator Activity Test Of Curucuminoid Extract Derived From Turmeric Rhizome (Curcuma Domestica Val.) In VCO On Rats Injected With Staphylococcus Aureus Against An Increase In The Concentration Of Leucocyte And Its Components.

Indah Pertiwi[1], Yuandani[2*], Effendy de Lux Putra[3], Denny Satria[4]

[1]Graduate School of Pharmaceutical Science Program, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
[2]Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
[3]Department of Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
[4]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
*Corresponding Author:
Email: yuandani@usu.ac.id

Abstract.

One of the medicinal Zingiberaceae plants which is regularly used by the society as a traditional remedy is turmeric rhizome. The pharmacological properties of turmeric rhizome include anti-inflammatory, anti-immuno-deficiency, anti-viral. Anti-bacterial, anti-fungal, anti-oxidant, anti-carcinogenic, and anti-infectious. This study aimed to identify the immunomodulator activity of curcuminoid extract of turmeric rhizome against the increase of leucocyte and its components, lymphocyte and neutrophil in white Galur Wistar rats that had been injected with Staphylococcus aureus. These test animals were given curcuminoid extract in VCO 100 mg/ml on a dosage of 1 ml, 0,5 ml, 0,25 ml curcuminoid powder 200 mg/kg body weight. Curcuminoid was suspended in Na CMC 0,5%, Levamisole 25 mg/kg of body weight suspended in Na CMC 0,5%, VCO 1 ml. For the negative control, merely Na CMC 0,5% was given. The extract was orally given daily for 14 days. On the 4th day, Staphylococcus aureus was injected intra-peritoneally. The rats' blood was then drawn from the heart and was observed to calculate the volume of the leucocytes, lymphocyte, and neutrophil which was conducted using flow cytometry.The findings suggested that there was a significant difference between the immunomodulatory activity of the curcuminoid extract in VCO at the doses of 1 ml, 0,5 ml, 0,25 ml on the increase in leucocyte, lymphocyte, and neutrophil compared to that of the negative control.Therefore, the findings of this study indicated that the extracts of curcuminoid in VCO could increase the number of leucocytes, lymphocytes, and neutrophils. Thus, the curcuminoid extract of turmeric rhizome in VCO potentially possessed immunomodulatory activity.

Keywords: *Curcuma domestica Val.,VCO, Immunomodulator, leucocyte,and limphocyte and neutrophil.*

I. INTRODUCTION

Bacterial infections are the second most common cause of death in the world, second only to heart diseases. Bacterial infections caused one of eight deaths in 2019. An estimated 13.7 million deaths were attributed to infections globally, in which it was investigated that 7.7 million deaths were caused by 33 pathogenic bacteria. Out of the 33 pathogenic bacteria investigated, 5 are the biggest causes of death; they are Staphylococcus aureus, Escherechia coli, Spneumoniae, Klebsiella-pneumoniae, and Pseudomonas-auruginosa [1]. One of measures taken to protect the body from infections is immunity boosting. Immunity is a system in the body which prevents or minimizes infections. This system perpetually protect our body from any intruder or foreign material that has the potential to cause various diseases in the body. A properly functioning immune system can differenciate between healthy body tissues from unwanted substances [2].Besides functioning as microorganism deterrence, the immune system can also remove dead or damaged cells for tissue repair, it can also identify and remove abnormal cells [3]. A reaction coordinated by cells, molecules, and other substances against microbes is called an immune response. [4]. White-blood cells (leucocyte) are an important part of the body's defense system in fighting infectious microorganisms, tumor cells, and harmful foreign substances. Leucocyte consists of a number of components, including basophils, eosinophils, neutrophils, lymphocytes, and monocytes. Although leucocyte is a blood cell, it mainly functions within body tissues.

Leucocyte flows only temporarily with the blood distribution throughout the body. If a body tissue experiences inflammation, leucocyte will move to the inflamed tissue by penetrating capillary walls [5].

Lymphocyte is a derivative of leucocyte which has an essential role in fighting infectious diseases. Lymphocytes originated in the pluripotent stem cell which differentiates through lymphocyte lane in the liver, bone marrow, and timus to form a number of major classes [6]. Lymphocytes consit of T-cells (TH, TC, TR), B-cells, and NK cells. The T and B cells will then function in immune-specific/adaptive and the NK-cell functions in the non-specific/hereditary immunity (Hadi, M.I. et al, 2020) [7]. Neutrophil functions as a body's defense line against foreign substances, especially against bacteria, which are phagocytic and capable of entering the infected tissue. Neutrophil circulates in the blood for 10 hours and has a life-time of 1 to 4 days in an extravasculary tissue [8].An immune abnormality can be corrected with synthetic drugs indicated as immunomodulators, both as immunostimulants or immunosupressants. However, the use of synthetic drugs is toxic to the kidney and liver, and also can induce hypertension, gastrointestinal problems, and other adverse side effects [9]. Therefore, the use of plants as immunomodulating agents begin to gain rising interest because they are regarded to be safer [10]. An immunomodulator is a compound which can restore immune balance.

The mechanisms of action of immunomodulators are to correct the abnormality in immune function (immuno-restoration), enhance the function of the immune system (immuno-stimulant), and suppress the immune response (immuno-suppressant). Immuno-modulator is mainly used in immuno-deficiency, chronic infections, and infection cases [11]. There have been many studies examining the immunomodulatory activities of Zingiberaceae plants, both in-vitro, in-vivo, and in clinical trials. Immunomodulator plants include curcuma longa linn, curcuma kwangsiensis, zingeber officinale roscoe, etc [12]. One of the medicinal plants that are most frequently used by society in traditional medicine is turmeric rhizome. The pharmacological properties of turmeric rhizome include anti-inflammatory, anti immuno-deficiency, antiviral, anti-bacterial, anti-fungal, anti-oxidant, anti-carcinogenic, and anti-infectious. [13]. Turmeric rhizome contains yellow chemical substance called curcuminoid. Curcuminoid can function as an antioxidant which can prevent cell damage caused by free radicals. In addition, curcuminoid can also function as an anti-inflammatory agent [14].Nowadays, turmeric extraction technology has been developed. This technology no longer uses chemical solvents, it instead uses plant-based solvents, one of which is VCO. The use of VCO as a solvent serves to lower costs and increase profit of the fact that VCO is edible if the preparation process is a food product [15]. A better method to extract curcumin is required; and one of the methods is known as Green Extraction, that is a Microwave-Assisted Extraction (MAE), which can minimize the use of solvents in the extraction process.

[16] Green extraction is a design and extraction method which reduces or eliminates harmful substances, and is environment-friendly [17]. The MAE method is expected to be able to extract curcumin compounds in turmeric perfectly when compared to maceration and percolation [18]. Many studies have been conducted using VCO as solvent because most of the solvent does not need to be removed [19]. The increasing use of VCO in society is in line with the increasing trend of "back to nature" treatment. According to Santosa et al, (2020) Virgin Coconut Oil (VCO), a processed product from coconut. It is a transparent and tasteless liquid with a distinctive odor of coconut. VCO contains a high concentration of medium saturated fat and short chain [20]. The benefits of VCO include boosting human's immune against diseases and accelerating recovery from an illness. [21] According to Emilia et al (2021), VCO has also gained popularity around the world owing to its chemical-free process. A further use of VCO is as an additive [22]. VCO is a non-polar solution making it suitable for dissolving natural coloring agents. It possesses a dielectric constant value of 2.82 x 10-18 [23]. Based on the above exposition, the researcher is interested in finding out the immunomodulator activities using the curcumin extract of turmeric rhizome in VCO, by observing an increase in the leucocyte, lymphocyte, and neutrophils. In this study, the use of VCO as a green solvent combined with the Microwave Assisted Extraction (MAE) method is expected to produce extraction conditions which are efficient, easy, quick and environmentally friendly. The MAE method has a number of advantages, including a short extraction process, less heat gradient, minimal solvent use, and high yield of target solution [24]..

II. METHODS

This study was an experimental study with a control group, conducted in the laboratory using curcuminoid extract from turmeric rhizome (curcuma domestica Val) in VCO, and a male white Galur Wistar rat. This study measured immunomodulator activities of curcuminoid extract of turmeric rhizome (curcuma domestica Val) in VCO against the increasing number of leucocyte and its components, namely lymphocyte and neutrophil, in white Galur Wistar rat which had been induced by Staphylocuccua aureus. As well as evaluating the concentration of fatty acid in pure VCO and VCO in the curcuminoid extract using gas chromatographic (GC) method.

Extraction Procedure

The initial step was the extraction of turmeric rhizome powder using VCO solvent in the Microwave Assisted Extraction method (MAE). In microwave extraction, 6 grams of symplisia powder was weighed and VCO was added to a volume of 60mL. The solution is stirred until homogenous. Then the mixture was placed into a 270-watt microwave for 30 minutes. The mixture was left to cool and then filtered through filter paper [25].

Immunomodulator Activity Test

The initial step was the extraction of turmeric rhizome powder using VCO solvent in the Microwave Assisted Extraction method (MAE). Next, the fatty acid composition was evaluated using gas chromatography (GC). Afterward, an experiment was conducted on the test animal, by counting the amount of leucocytes, lymphocytes, and neutrophils in a white Galur Wistar rat which had been induced by Staphylococcus aureus. In microwave extraction, 6 grams of symplisia powder was weighed and VCO was added to a volume of 60mL. The solution is stirred until homogenous. Then the mixture was placed into a 270-watt microwave for 30 minutes. The mixture was left to cool and then filtered through filter paper [25].Ethical approval for the test animal should be obtained from the ethic commission. This study had been granted ethical approval from the Ethic Committee for Animal Study, Faculty of Mathematics and Natural Sciences, Universitas Sumatra Utara.

The approval letter number was 0228/KEPH-FMIPA/2023. The sample was derived from a test animal with sample size calculated using the Faraday formula.The test animals in this study were male white Galur Wistar with an average body weight of 160 grams which were divided into 7 groups, each consisting of 5 rats.Curcuminoid extract from turmeric rhizome (Curcuma domestica Val) in 100 mg/mL VCO was given orally to the rats for 14 days at the doses of 1mL, 0.5mL, and 0,25 mL. The control group was given curcuminoid powder 250 mg/kg bodyweight suspended in Na CMC 0.5%, Levamisol 25 mg/kg bodyweight suspensioned in Na CMC 0.5%, and 1 mL of VCO. The negative control group was given only 0.5% Na CMC. Staphylococcus aureus was injected on day 4 intra-peritonially. Then the rat's blood was drawn from the heart and then stored in sterile vacuntee tube containing 0.1% EDTA. The EDTA 0,1 % would function as anticoagulant for the blood. Then the blood plasma was isolated and the leucocyte along with its components were evaluated using flow cytometry method.

III. RESULT AND DISCUSSION

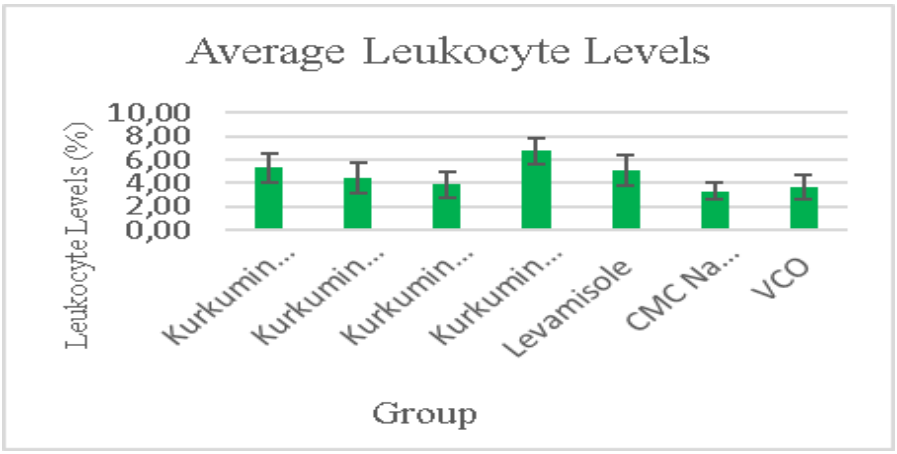
Evaluation was conducted to determine the fatty acid composition of pure VCO and the VCO used as solvent in curcuminoid extract using Gas chromatography (GC). This was performed to see the difference in the test results of fatty acid composition of pure VCO and in VCO as solvent in curcuminoid extract. The evaluation results can be seen in Tabel 1.

Table 1. Comparison of fatty-acid composition test in pure VCO and VCO used in curcuminoid extract.

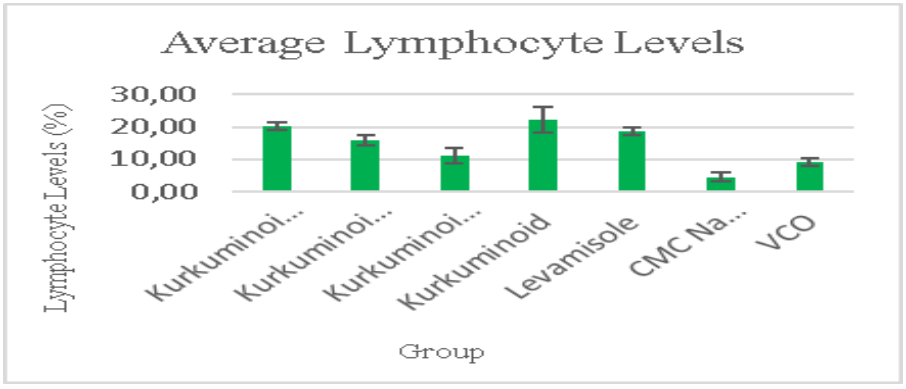
Parameter	Unit	VCO Test result	Test result in VCO + curcuminoid
Fatty acid composition			
Kaprilat Acid (C8:0)	%	9,0	9,2
Kaprat Acid (C10:0)	%	6,3	6,3
Laurat Acid (C12:0)	%	49,1	49,1
Miristat Acid (C14:0)	%	17,5	17,4
Palmitat Acid (C16:0)	%	8,5	8,4

Stearat Acid (C18:0)	%	2,8	2,8
Oleat Acid (C18:1)	%	5,6	5,6
Linoleat Acid (C18:2)	%	1,2	1,2
Linolenat Acid (C18:3)	%	0,1	0,1

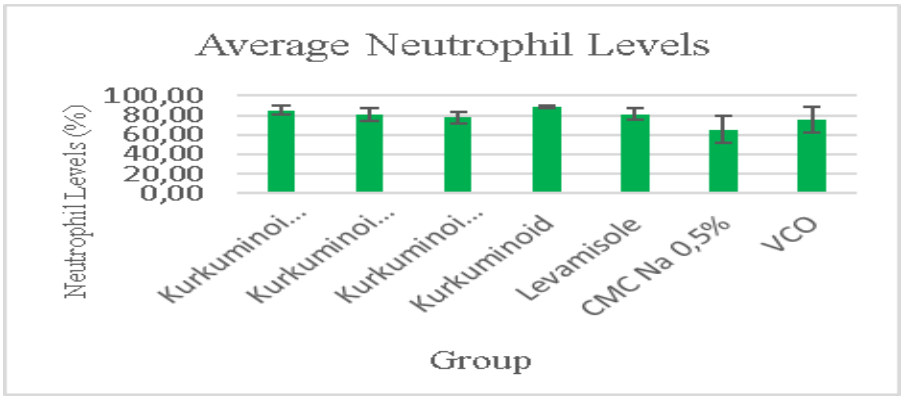
The result of routine tests on the hematologic parameter of the male white rats which had been given curcuminoid extract in VCO 100 mg/mL with dosages of 1mL, 0,5 mL, and 0,25 mL of curcuminoid powder, 200 mg/kg bodyweight, suspended in Na CMC 0,5%, Levamisol 25 mg/kg bodyweight suspended in Na CMC 0,5%, VCO 1 mL under a test on leucocyte, neutrophil, and lymphocyte can be seen in Picture 1.



(a)



(b)



(c)

Fig 1. Immunomodulatory Activities of Curcuminoid extract derived from turmeric rhizome (curcuma domestica val.) in VCO on white Galur Wistar rats injected with Staphylococcus aureus regarding the increase in leucocyte (a), Limphocyte, (b) and neutrophil (c).

IV. DISCUSSION

Based on the results obtained in Picture-1, it can be seen that the percentage of test result of the fatty acid composition of the pure VCO or VCO as solvent in curcuminoid extract does not show any difference. According to the findings of a study by Romadhoni, S.N. (2022), the addition of turmeric simplicial does not affect the percentage of fatty acid within VCO. From the identification using GC-MS instruments, 7 peaks were found, including 6 saturated fatty acids, namely: methyl caproate, methyl capricate, methyl caprylate, methyl laurate, methyl myristate, and methyl palmitate, and 1 unsaturated fatty acid, that is methyl oleate [26].It can be seen in the picture that during a routine hematological examination after administration of curcuminoid extract, there is a significant increase in leucocyte, lymphocyte, and neutrophil compared to the negative control. At a dose 1mL curcuminoid extract, there was an increase as follows: leucocyte 5.29 ± 1.25 103/mL. Limhocyte 84.87 ± 4.62% and neutrophil 20.18 ± 1.24%. At a dose of 0.5 mL of curcuminoid extract, the following increases occurred: Leucocyte 4.44 ± 1.34103/mL, limphocyte 80.72 ± 6.47% and neutrophil at 5.94 ± 1.62%. At a dose of 0.25 curcuminoid extract the increases are as follows: Leucocyte 3.88 ± 1.07103/mL, limphocyte, 77.65 ± 6.36% dan neutrophil 1.23 ± 2.32%.An increase in the number of leukocytes is a response mechanism of the body against antigen. Leucocyte possesses various functions which are closely related with the expulsion of foreign particles (including pathogenic microorganisms) [27].

Leucocyte comes in 6 types and functions in the immune system. Neutrophil, eosinophil, basophil, and monocyte are non-specific immune system, whereas lymphocyte cells are classified as specific immune system. Neutrophil cells function in the initial defence of non-specific immunity against bacterial infections. Lymphocyte's role is to develop anti-body and circulates in the blood or in the cellular immune system [28].Lymphocyte is an abundant part of the white blood cells. Lymphocyte consists of both naive and active T-Cells and B-Cells. When dendritic cells detect an antigen (antigen presenting cell), the naive T and B cells found in bone marrow will enter secondary lymphoid organs, such as lymph and spleen, and are then activated by the antigens to become effector cells and memory cells. The active cells then migrate to the peripheral tissues which are the area of the infections. In addition, there are null cells in about 20% of peripheral lymphocyte. Null cells are lymphocyte without the T and B cells' characteristic and differentiation or superficial clusters of antibodies, but instead, it has a role of cells destruction carried out by antibody [29].Neutrophil serves as the first line in the defense against microorganisms, tissue trauma, and many factors causing inflammations. [30]. Neutrophyl will increase because the body already has a defence mechanism, so when a bacterial infection occurs, neutrophyl will be produced by the spleen to be dispatched to the site of infection. [31]. This situation was also reported by Mones (2008) who argued that the increased in neutrophil concentration was caused by a pathogenic infection [32].

IV. CONCLUSION

This study suggests that curcuminoid extract derived from turmeric rhizome (Curcuma domestica Val.) in VCO can increase the number of leucocyte cells and their components, thus a conclusion can be drawn that curcuminoid extract in VCO potentially possesses activity as an immunomodulator.

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