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Anti-Inflammatory Potential Of Extract Of *Nothopanax Fruticosum (L.) Miq* By Method Of Erythrocyte Membrane Stability

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Abstract
Lysosome can secrete enzyme which can induce the occurence of inflammation. Lysosome membrane is an analogue with erythrocyte membrane of human. This study is aimed to decide the potential of anti-inflammatory of extract of *Nothopanax fruticosum* (L.) Miq) seen from its ability to stabilize erythrocyte membrane. By method of erythrocyte membrane stability. This study is started by blood intake and erythrocyte suspension is made. Erythrocyte suspension is divided into 3 groups of treatment namely negative control, positive control (Natrium diclofenac), and test solution with concentration of 25, 50, 75, 100, and 125 ppm, then set aside for 30 minutes and centrifuged. Supernatant is measured by absorbance using spectrophotometer UV-Vis at wavelength 560 nm. From the result of the study it obtains that concentration of 125 ppm gives highest inhibition percentage at 80,64%.

Keyword: santi-inflammatory, *Nothopanax fruticosum* (L.) Miq), erythrocyte membrane stability

I. INTRODUCTION

Inflammation is response of normal protection to the tissue injury caused by physical trauma, dangerous chemicals, or microbiology agent. Inflammation is an effort of body to inactive or destroy organism invasion, eliminate irritant, and prepare stages for tissue improvement [1].The symptoms of inflammation process are rubor, calor, dolor, tumor, and functio laesa. Inflammation is an effort of body protection to eliminate destructive stimulation and starting the process of healing for tissue. Inflammation makes uncomfortable feeling on the individual, if it is not medicated it can cause chronic inflammation [2].

The use of anti-inflammatory drugs in a long-term can give dangerous side-effect risk. AINS (*Antiinflamasi Non Steroid* /Non Steroidal Anti-Inflammatory drugs) has side-effect such as stomach pain and AIS (*Antiinflamasi Steroid*/ Steroidal Anti-Inflammatory drugs) has side-effect such as the decrease of body immune. Nowadays there are many studies conducted to find the alternative treatment that can solve inflammation with a minimum side-effect [3].

Nothopanax fruticosum (L.) Miq) can be used as a medication, such as passing urine, rheumatic, and pain. *Nothopanax fruticosum* (L.) Miq) has chemicals namely flavonoids, steroids and triterpenoids, saponins and tannins [4]. From the reported results of studies, chemicals that has efficacy as anti-inflammatory flavonoid. It has been reported that such saponin and flavonoid give an effect to stabilize lysosome membrane either in vivo

or in vitro, while tanin and saponin have ability to bind cation, thus it can stabilize erythrocyte membrane and other biological macromolecules [5].

Some methods which can be used to test activity of anti-inflammatory of a drug *in vitro* one of them is stabilization of erythrocyte membrane. Erythrocyte of a human has been used widely as a model of study of interaction between medicine and membrane, including in term of testing an activity of a medicine such as anti-inflammatory drug screen (drug test). It is known that erythrocyte membrane of human is analogue with lysosome membrane. If a stability of erythrocyte membrane is maintained, thus stabilization of lysosome membrane will be likewise. Lysosome membrane contains around 50 degrading enzymes which consist of protease, lipase, glycosidase, nuclease, sulfatase, and phosphatase, if enzym gets out of membrane it will trigger inflammation occured. Testing anti-inflammatory potential is done in erythrocyte membrane induced by hypotonic solution. Anti-inflammatory potential of a plant will be seen if erythrocyte does not get lysis [6].

In the previous study S.Syarif (2019) "The highest anti-inflammatory activity is shown at concentration of 1000 ppm at 60,516%. This data shows that the increase of stabilization potency of extract on erythrocyte membrane is in line with increase of concentration"[7].

II. MATERIALS AND METHODS

Time and Setting of Study

This study is conducted on January 2019 until October 2019 in laboratory of Pharmacy Chemistry, Pharmacy major, Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar.

Tools and Materials

Tools and instrument used in this study are centrifuge, micropipette (DragonLab), pH meter (Jenco), a set of maceration tools, spectrophotometer UV-Vis (*Apel®, PD 303 UV)*, analytical balances (Kern). In addition, materials used in this study are aquadest, blood,dinatrium hydrogen phosphat, natrium dihydrogen phosphat, extract of *Nothopanax fruticosum* (L.) Miq, NaCl, and Natrium Diclofenac.

Extraction of *Nothopanax fruticosum* (L.) Miq by Maceration Method

50 grams of simplicia of *Nothopanax fruticosum* (L.) Miq are put into maceration container, ethanol is added until simplicia is soaked, set aside for 3 x 24 hours by stirring several times in a place that is safe from sunlight. After 3 x 24 hours simplicia is strained and dreg is re-soaked with new solution[1]. It is done until the extraction process considered perfect. That result is strained by using filter paper. Then it is made to be concentrated until condensed extract is obtained[2].

Qualitative test of Phytochemicals of *Nothopanax fruticosum* (L.) Miq Extract

a. Flavonoid

5 grams of extractis added into 10 mL of hot water, boiledfor 5 minutesthen it is strained. 2 mL of Filtrateis added with 0,05 mg of Mg powder and 1 mL concentrated HCL, then mixed. Positive test is shown bythe colours that are created namely red, yellowor orange [8].

b. Saponin

50 mg of extract is added with 3 mL of aquadest and mixed for 1 minute, then added with 2 drops of HCl 1 N. If created foamkeeps stable ± 7 minutes, thus positive extractcontains saponin [9].

c. Tannin

50 mg of extract is added with 3 mL of waterand heated for 10 minutes, then it is cooledandstrainedthus it obtains filtrate. Filtratethat is obtainedthen addedwithiron solution (III) chloride ($FeCl_3$) 1%, ifthe bluish black color is created thus it states that it positivelycontainshydrolyzed tannin andifit is dark greenthus it states that it positivelycontainscondensed tannin [10].

Test In Vitro Potential of Anti-inflammatory

Testing anti-inflammatory potential of *Nothopanax fruticosum* (L.) Miq) extract *in vitro* including stages, such as:

a. Preparing Phosphate buffer pH 7,4 (0,15 M)

2,671 grams of dinatrium hydrogen phosphat (Na_2HPO_4. $2H_2O$) is dissolvedinto aquadestup to 100 mL (0,15 M). 2,070 grams of natrium hydrogen phosphat (NaH_2PO_4. H_2O) dissolvedin aquadestup to 100 mL (0,15 M). Then 81 mL ofsolution of Na_2HPO_4. $2H_2O$ (0,15 M) is mixed with 19 mL ofsolutionof NaH_2PO_4. H_2O (0,15 M) at room temperature. Check pH by using pH meter [11].

b. Preparing Isosaline

0,85 gram of NaCl is dissolvedinphosphate bufferpH 7,4 (0,15 M) up to 100 mLvolume at room temperature [5].

c. Preparing Hiposaline

0,25 gram of NaCl is dissolvedin phosphate bufferpH 7.4 up to 100 ml volume atroom temperature [5].

Preparing solution ofNatrium diclofenac

10 mg of Na diclofenac is dissolved in 10 Ml isosaline (1000 ppm) as stock solution. To make 100 ppm concentration from stock solution is dispensed using pipette 0,5 Ml. Then it is made to be sufficient up to 5 Ml.

Preparing solution of *Nothopanax fruticosum* (L.) Miq extract

25 mg of extract is dissolved on isosaline up to 25 Ml (1000 ppm) as stock solution. To make concentrations of 25, 50, 75, 100, and 125 ppm it is dispensed using pipette continuously from stock solution 0,125 Ml, 0,25 Ml, 0,375 Ml, 0,5 Ml and 0,625Ml. Then it is made to be sufficient up to 5 Ml.

Preparing Erythrocyte Suspension

10 Ml of blood are centrifuged with the speed of 3000 rpm for 10 minutes at temperature of 25°C. The created Supernatant is separated. Then sediment of remained blood cells is washed by isosaline solution and re-centrifuged. That process is repeated 3-4 times until isosaline is clear[3]. After that, washed blood cell is taken and suspensedwith isosaline thus it obtains erythrocyte suspension with concentration of 10% v/v. Suspension of blood cell is saved at 4°C if it has not been used [5].

Activity Testing of Extract on Stabilization of Erythrocyte Membrane [10].

To decide activity of extract on stabilization of erythrocyte membrane, solution of used are mentioned in the following:

a. Preparing of extract

Test solution is madeby adding 1 Ml phosphate bufferPh 7,4 (0,15 M) + 0,5 Ml 34uspense erythrocyte + 1 Ml samplesolution (25, 50, 75, 100, and 125 ppm) + 2 Ml hiposaline.

b. Preparing of blanko

Solution of blanko is made by adding phosphate bufferPh 7,4 (0,15 M) + 0,5 Ml isosaline + 1 Ml of extract solution/ solution of Natrium diclofenacbased on concentrationfor each + 2 Ml hiposaline

c. Preparing of positive control

Solution of positive34uspens is made by adding 1 Ml of phosphate bufferPh 7,4 (0,15 M) + 0,5 Ml 34uspense erythrocyte + 1 Ml of solution of Na diclofenac + 2 Ml hiposaline.

d. Preparing of negative control

Solution of34uspens is made by adding 1 Ml of phosphate bufferPh 7,4 (0,15 M) + 0,5 Ml 34uspense erythrocyte +1 Ml of isosaline (as a alternative of sample solution) +2 Ml of hiposaline.

Every solution above then is incubated at 37°C for 30 minutes and centrifuged with speed of 3000 rpm for 20 minutes. Obtained supernatant fluid is taken and the content of hemoglobin is calculated by using spectrophotometer UV-Vis on wavelength of 560 nm.

III. RESULTS AND DISCUSSION

RESULTS

Table 1. Result of extraction and percentage of rendament of ethanol extract of *Nothopanax fruticosum* (L.) Miq

Sample	Weight of fresh sample (g)	Weight ofethanol extract (g)	Rendament ofethanol extract (%)
Nothopanax fruticosum (L.) Miq	50	8,32630	16,6526

Table 2. Result of qualitative test of ethanol extract of *Nothopanax fruticosum* (L.) Miq

Sample	Testing	Reactant	Colour	Result
Nothopanax fruticosum (L.) Miq	Flavonoid	Magnesium powder +concentrated HCL	Red	+
	Saponin	HCL 1N	Foam	+
	Tannin	$FeCl_3$	dark green is created	+

Table 3. Result of measurement of absorbance from test solution, positive control and negative control.

Concentration (ppm)	Absorbance
Extract25 ppm	0.044
Extract50 ppm	0.037
Extract 75 ppm	0.031
Extract 100 ppm	0.022
Extract 125 ppm	0.012
Natrium diclofenac 100 ppm	0.021
Negative control	0.069

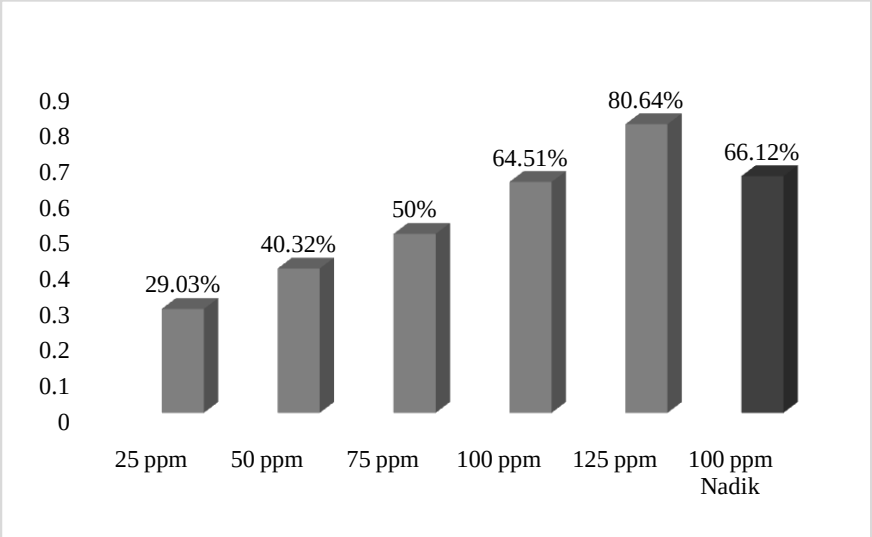

Figure 1. Histogram of Stability Erythrocyte Membrane

IV. DISCUSSION

In this study, testing anti-inflammatory potential on extract of *Nothopanax fruticosum* (L.) Miq with method of stability of erythrocyte membrane. The aim of this study is to determine anti-inflammatory potential on extract of *Nothopanax fruticosum* (L.) Miq viewed from its ability to stabilize erythrocyte membrane.

Extract of *Nothopanax fruticosum* (L.) Miq is obtained through extraction of maceration because it is the easiest and simplest extraction method; it is because it does not need heating thus compund contained on the plant is not damaged [12].Result of extraction of *Nothopanax fruticosum* (L.) Miq can be seen in Table 1.

Result of rendement is needed to know the number extract that is obtained during extraction from a sample. Moreover, there is a relation between data of rendement result and the number of content of active compound from a sample thus if number of rendement is much higher it can be concluded that content of active compound is also much higher [13].

Furthermore, qualitative test is done from extract of *Nothopanax fruticosum* (L.) Miq. The obtained result can be seen in table 2.From qualitative test that is done it is known that extract of *Nothopanax fruticosum* (L.) Miq positively contains flavonoid, saponin, and tannin.

The study is started by managing code of ethics then continued with blood intake. Erythrocyte that is used in this study is erythrocyte which is obtained from healthy volunteer and taken directly by paramedic. Then erythrocyte disentrifuge with speed of 3000 rpm for 10 minutes and it obtains supernatant and sediment. Sediment that will be used is washed by isosaline solution and suspension of erythrocyte 10% is made. Erythrocyte that is used is new erythrocyte because if suspension of erythrocyte is saved thus isosaline will stimulate lysis happened that will influence data or results of study.

Suspension of erythrocyte is divided into 3 groups of treatment namely negative control, positive control, and test solution with concentrations of 25, 50, 75, 100, and 125 ppm. After getting a treatment then it is incubated for 30 minutes at 37^0C and centrifuged with the speed of 3000 rpm for 20 minutes. Moreover, absorbance measurement is done by spectrophotometer UV-Visible at wavelength 560 nm. Result of absorbance testing can be seen in the table 3.

Based on table 3, it can be seen that extract with concentration at 125 ppm gives lowest absorbance rate. The lower the absorbance rate is, the greater avoidance of erythrocyte lysis is. Furthermore, from the absorbance data, inhibition percentage is calculated, result of calculation is presented in histogram in Figure 1.

Based on Figure 1, it is seen that concentration of 125 ppm shows % highest inhibition even greater than positive control. From that data it shows that increase of stability of erythrocyte membrane is in line with increase of concentration of extract.

The ability of inhibition of extract of *Nothopanax fruticosum* (L.) Miq is assumed because the chemicals that have ability to stabilize erythrocyte membrane. It is reported that certain saponin and flavonoid gives effect in stabilizing lysosome membrane either in vivo or in vitro, while tannin and saponin have ability to bind cation, thus it can stabilize erythrocyte membrane and other biological macromolecules [5].

Stabilization of erythrocyte membrane has been used as a method to know the activity of anti-inflammatory *in vitro*. It is because component of erythrocyte is similar to lysosome membrane. Lysosome membrane contains around 50 degrading enzymes which consist of protease, lipase, glycosidase, nuclease, sulfatase, and phosphatase, if enzym gets out of membrane it will stimulate inflammation occured. Because lysosome membrane is similar to erythrocyte membrane thus extract that have ability to stabilize erythrocyte also has ability to stabilize lysosome [14].

V. CONCLUSION

Extract of *Nothopanax fruticosum* (L.) Miq has potential as anti-inflammatory; seen from its ability to stabilize erythrocyte membrane with concentration 125 ppm it gives highest inhibition percentage at 80,64 %

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