Journal of Experimental and Clinical Pharmacy (JECP)
Doi: 10.52365/jecp.v1i1.202
http://jurnal.poltekkesgorontalo.ac.id/index.php/JECP/
2021, 1(1), 47-55

Research Article

Uji Aktivitas Antiinflamasi Ekstrak Metanol Daun Tembelekan (*Lanatana camara* Linn.) Secara In Vitro Menggunakan Metode Stabilitasi Membran Sel Darah Merah

Sukmawati A Damiti[1], Ysrafil Ysrafil[2*], Zaenal Abidin[3], Rahmawati Rahmawati[3], Vyani Kamba[2], Hartati Hartati[2], Pratiwi Ishak[2], Ghaitsa Zahira Sopha Yusuf[2]

[1]*Department of obstetrics, Health Polytechnic of Palangkaraya, Centre Kalimantan*
[2]*Program Studi Farmasi Poltekkes Kemenkes Gorontalo, Indonesia*
[3]*Faculty of Pharmacy, Muslim Indonesia of University, Makassar, South Sulawesi*

INFO ARTIKEL

Dikirim : Feb 2021

Revisi: Feb 2021

Diterima: Feb 2021

***Corresponding Author:**
Ysrafil Ysrafil,
Program Studi Farmasi
Poltekkes Kemenkes
Gorontalo, Indonesia.
Telp/Mail:
ysrafil0155@gmail.com

ABSTRAK

Pengobatan berbagai penyakit saat ini perlahan kembali menerapkan konsep back to nature yakni menggunakan produk herbal untuk menyembuhkan penyakit tidak terkecuali inflamasi. Tembelekan adalah tanaman yang telah lama digunakan seara empiris untuk mengobati penyakit radang. Namun, bukti kemanfaatannya secara ilmiah untuk mengobati inflamasi masih kurang. Penelitian ini bertujuan untuk mengetahui aktivitas antiinflamasi ekstrak metanol daun tembelekan dengan menggunakan metode stabilisasi membran sel arah merah. Kontrol positif yang digunakan adalah natrium diklofenak pada konsentrasi 400 ppm, 600 ppm, 800 ppm dan 1200 ppm sedangkan pada larutan ekstrak digunakan konsentrasi 250 ppm, 500 ppm, 1000 ppm dan 2000 ppm. Pengukuran aktivitas antiinflamasi dihitung berdasarkan persen stabilitasnya. Semakin tinggi nilai % stabiitas, maka dianggap semakin baik efek inflamasinya. Hasil pengujian menunjukan bahwa pada ekstrak dengan konsentrasi 2000 ppm mempunyai daya stabilitas tertinggi yakni sebesar 62,171%. Dari hasil dapat dilihat bahwa semakin tinggi konsentrasi ekstrak, maka daya inflamasinya juga semakin baik.

Kata kunci: Antiinflamasi, Daun tembelekan, Ekstrak metanol, Stabilisasi membran sel darah merah

PENDAHULUAN

Inflammation is local protective response caused by injury or tissue damage, with the aim to destroy, reduce, or contain both the

eISSN 2775-1368

injuring agent and the injured tissue (Dorland 2002). This causes various physiological reactions in the body such as activation of enzymes, release of mediators, diapedesis or movement of white blood cells through capillaries to inflammation areas, cell migration, tissue damage and repair. (Kumar et al. 2012).

Clinical symptoms of inflammation include rubor (redness), heat (heat), dolor (pain), tumor (swelling) and dysfunction and tissue damage due to the movement or migration of leukocytes from the bloodstream to the site of injury or infection. (Katzung 2012; Jutila et al. 1989; Springer, 1994). Although inflammation is the body's protective responses to eliminate adverse stimuli and initiate the healing process for the tissues, if inflammation (inflammation) is not treated, it will generate other diseases such as vasomotor rhinitis, rheumatoid arthritis and atherosclerosis (Ilakkiya et al. 2013).

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used anti-inflammatory drugs to treat inflammation. Based on the ARAMIS database 2000 NSAIDs consume 30 million daily. Furthermore, according to the Intercontinental Medical Statistics (IMS) Health in 2000 in the United States, 111.4 million NSAIDs were prescribed in yearly. (IMS Health 2000). However, these drugs often cause unwanted reactions such as indigestion, heart and kidney disorders. Recently, research and development of medicinal plants both at home and abroad are growing rapidly, especially in pharmacological properties, one of which is as an anti-inflammatory, such as tembelekan leaf (Oyedapo 2010). The Tembelekan (*Lantana camara* Linn.) has been used empirically as fever, swelling therapy, and is commonly used when meet wound. (Suwertayasa, Bodhy, & Edy 2013).

Previous studies have demonstrated that tembelekan extract contains alkaloids, flavonoids, alkaloids, steroids, tannins, saponins, terpenoids, phitosterols and anthocyanins (proanthocyanidin) which are known as effective componen in treating inflammation. (Parwanto, Senjaya, & Edy, 2013). Quercetin is flavonoid derivative compounds contained in the leaves of the tembelekan and had known to had anti-inflammatory effect (Anand David, Arulmoli, & Parasuraman, 2016; Kempuraj et al., 2005). This study aims to determine the anti-inflammatory activity of the methanol extract of the tembelekan leaves using the red blood cell membrane stabilization method.

MATERIAL DAN METODE

Material

The type of research is Laboratory Experimental Method with an experimental design used is Posttest Only with Nonequivalent Control Group Design by testing the anti-inflammatory activity of the methanol extract of tembelekan leaf (Lanatana camara Linn.) on normal human red blood cells.

Metode

Extraction

The tembelekan leaves was weighed as much as 30.014 grams and further macerated with 250 mL of 70% methanol for 2 days. During maceration stir occasionally. This procedure was then repeated 2 times (remaceration) until the filtrate was clear. Furthermore, each filtrate is filtered using filter paper and concentrated until a thick extract is obtained.

Preparation of Red Blood Cell Suspension

As 10 mL of blood was centrifuged at 3000 rpm for 10 minutes at 25^{o}C. The supernatant formed was separated using sterile pipette. The sediment of blood cells was further washed with isosalin solution and recentrifuged. The process was repeated 3-4 times until the isosalin was clear. After that, blood cells that have been washed and suspended with isosalin were taken to obtain red blood cell suspension with a concentration of 10% v/v.

Anti-Inflammatory Activity Testing

We used the following solutions to determination of extract effect on erythrocyte membrane stabilization,:

Preparation of test solutions

The test solution (4 mL) consist of 1 mL of phosphate buffer pH 7, plus 0.5 mL of red blood cell suspension, 0.5 mL of extract solution, and 2 mL of hyposalin.

Positive control solution

Positive control solution (4 mL) consists of 1 mL of phosphate buffer pH 7, 0.5 mL of red blood cell suspension, 0.5 mL of diclophenac sodium, and 2 mL of hyposaline..

Preparation of control solution

The control of test solution (4 mL) consists of 1 mL of phosphate buffer pH 7, 0.5 mL of isosalin as substitututions for red blood cell suspension, 0.5 mL of extract and/or diclofenac sodium, and 2 mL of hyposalin..

Preparation of negative control solutions

The negative control solution consist of 1 mL of phosphate buffer pH 7, 0.5 mL of red blood cell suspension, 0.5 mL of ethanol as substitute for the sample solution, and 2 mL of hyposalin..

Each of solutions were incubated at 37^{0}C for 30 minutes and centrifuged at 5000 rpm for 10 minutes. The obtained supernatant was taken and the hemoglobin content was measured using a UV-Vis spectrophotometer at a wavelength of 540 nm..

Analysis persentage of rendamen was carried out to assessed the effectiveness of using the solvent and the extraction method. Analysis of % rendamen of the extract can be calculated by the formula (Saputra 2015, p. 24):

$$\% \text{ Rendamen} = \frac{the\ total\ extract\ weight}{weight\ of\ total\ simplicia\ powder} \text{ x } 100\%$$

Analysis of the anti-inflammatory effect of the methanol extract of the leaves of tembelekan (Lantana camara Linn.) was carried out by looking at the percentage of stabilization which can calculated by the formula (Oyedapo et al 2010, h. 48) :

$$\% \text{ Stability} = 100 - \frac{(Abs\ test\ solution - Abs\ control\ test\ solution)}{(Abs\ negative\ control\ solution)} \text{ x } 100\ \%$$

HASIL

The results of tembelekan leaf extract extraction using 70% methanol can be seen in table 1.

Table 1. Rendamen persentage of Methanol extrac of Tembelekan Leaf

	Sample wight	Solvent	Extract	Persentage of Rendamen	mean of Rendamen
1.	30,014 gram	250 mL	8,856 gram	29,506 %	27,9365 %
2.	30,014 gram	250 mL	7,914 gram	26,367 %	

The high yield is due to the universal nature of methanol lead it can dissolve and attract secondary metabolites, both polar and nonpolar, in the tembelekan leaves. The results of positive control testing, with

concentrations of 400, 600, 800 and 1200 ppm can be seen in the following table:

Table 2. Percentage of red blood cell membrane stability of diclofenac sodium (ND) at concentrations of 400, 600, 800 and 1200 ppm.

No.	Concentration (ppm)	Absorbance		% Stability
		Control ND	ND	
1.	Negative control	0,00	0,645	0
2.	400	0,00	0,391	39,38
3.	600	0,002	0,306	52,868
4.	800	0,00	0,244	62,171
5,	1200	0,00	0,132	79,535

ND : Natrium Diclofenac

Furthermore, results of the membrane stabilization power of Tembelekan leaf methanol extract against the induction of hypotonic solution at concentrations of 250, 500, 1000 and 2000 ppm can be seen in table 3.

Table 3. Percentage of red blood cell membrane stability from methanol extract of tembelekanleaf (EMDT) at concentrations of 250, 500, 1000 and 2000 ppm.

	Concentration (ppm)	Absorbance		% Stability
		Control EMDT	EMDT	
1.	Negative control	0,00	0,645	0
2.	250	0,001	0,550	14,884
3.	500	0,007	0,455	30,543
4.	1000	0,045	0,420	41,861
5,	2000	0,133	0,376	62,326

The results show that concentration of 250 ppm the methanol extract of tembelekan leaves could inhibit hemolysis of red blood cell membrane by 14.884%, while concentration of 1000 ppm obtained stabilizing power of 41.861%. The highest anti-inflammatory activity was shown at concentration of 2000 ppm, namely 62.326%. From these data, it was shown that the increase of extract concentration generates increasing of ability in stabilization of the red blood cell membrane.

PEMBAHASAN

In this study, we assessed anti-inflammatory effect of methanol extract of tembelekan leaves using the red blood cell membrane stabilization method. Dry powder of tembelekan simplicia was extracted using the maceration method. The method was cold extraction method suitable for extracting secondary metabolites. In the process, the solvent used to soak the simplicia will break down the cell walls and membranes of the simplicia due to the pressure difference between inside and outside the cell. Damage to the cell walls and membranes causes secondary metabolites in the cytoplasm to dissolve in the solvent used.

The solvent used was 70% methanol that considered effective in attracting secondary metabolites. Methanol was universal solvent that can dissolve both polar and nonpolar analytes. Methanol can attract secondary metabolites such as alkaloids, steroids, saponins, and flavonoids from plants (Thompson, 1985). Methanol was proven effective in the extraction of tembelekan leaves in this study. It could seen from the average percentage of rendamen extract which was 27.9365%. Previous research revealed that 70% methanol was chosen as solvent because according to research conducted by Zuhair et al, this solvent is effective in attracting phenolic compounds such as flavonoids which can act as anti-inflammatory.

Furthermore, the anti-inflammatory activity of the extract was traced using the red blood cell membrane stabilization method. The red blood cell membrane stabilization method was one of the test methods to determine in vitro anti-inflammatory activity. The use of this method was based on the similarity (analogue) of the red blood cell membrane with the lysosomal membrane, so that when the red blood cell membrane was stabilized, the same will happen to the lysosomal membrane. Lysosomal membrane stabilization was important to treat inflammatory response, because it prevents the release of lysosomal content that can cause and exacerbate inflammation.

In this study, the anti-inflammatory activity of sample showed by its ability to prevent hemolysis of erythrocytes induced by hypotonic solutions. Hemolysis in this test occurs due to an osmosis event in red blood cells where the hypotonic solution (low concentration) will move into red blood cells which have a higher concentration. The displacement that occurs continuously will result in the rupture of the red blood cell membrane.

The prevention of hemolysis showed by decreasing of absorbance in the test solution mixture. The smaller the absorption detected in the test solution mixture means that the red blood cell membrane is more stable

and does not undergo lysis. However, anti-inflammatory activity is not only seen through the absorbance value, should continued with the calculation of lysis inhibition using the percent stability formula..

The results of this study showed that at concentration of 250 ppm, the methanol extract of leaves of tembelekan lead inhibition hemolysis on the red blood cell membrane as much of 14.884%, while the concentration of 1000 ppm obtained a stabilizing power of 41.861%. The highest anti-inflammatory activity was shown at a concentration of 2000 ppm, namely 62.326%. From these data, it was shown that the increase in the stabilization power of the extract against the red blood cell membrane was in line with the increase in the concentration of the extract.

Compounds with membrane stabilizing properties were known for their ability to interfere with the initial phase of the inflammatory reaction, where prevention will trigger the release of phospholipase A2 which will form inflammatory mediators (Aitadafoun et al., 1996). The mechanism of extract stabilization in this study was to prevent oxidative stress caused by the hypotonic solution. Oxidative stress will affect the stability of the red blood cell membrane which was analogous to the lysosome membrane (Kumar 2011). Allegedly, the secondary metabolites contained in the extract can stabilize the red blood cell membrane. Secondary metabolites that were thought to have an important role in stabilizing red blood cell membranes are saponins, flavonoids and tannins..

Flavonoid compounds play important role in protecting erythrocyte membranes from hypotonic solutions. The effect of the hypotonic solution was related to the amount of fluid that enters the erythrocyte membrane, resulting rupture of the erythrocyte membrane which is called hemolysis. Flavonoid compounds contained in the extract will interact with the induced hypotonic solution generated inhibition membrane destroying. The number of secondary metabolites present in the extract reacts in the same amount as the hypotonic solution added to the suspension so that it does not damage the erythrocyte cell membrane. It was said that the membrane stabilization activity was influenced by the high content of polyphenols such as tannins, steroids and flavonoids which function as free radical scavengers and stabilize the erythrocyte membrane from the induction of hypotonic solutions (Sankari et al., 2009).

KESIMPULAN

Based on the results obtained, we conclude that methanol extract of tembelekan leaves has anti-inflammatory effect that shown in its ability to stabilize the red blood cell membrane against the induction of hypotonic solutions. The percentage of stability of methanolic extract of tembelekan

leaves at concentration of 250 ppm (14,884%), 500 ppm (30,543%), 1000 ppm (41,861%) and 2000 ppm (62,326%) which indicates that higher the concentration used, the better too its anti-inflammatory activity.

UCAPAN TERIMAKASIH

Tidak ada deklarasi

DAFTAR PUSTAKA

Anand David, A. V., Arulmoli, R., & Parasuraman, S. (2016). Overviews of Biological Importance of Quercetin: A Bioactive Flavonoid. *Pharmacogn Rev, 10*(20), 84-89. doi:10.4103/0973-7847.194044

Dorland, N 2002, *Kamus Kedokteran Dorland*, edk 29, EGC, Jakarta

Ilakkiya, R, Neelvizhi, K, Tamil, SS, Bharathidasan, R, & Rekha, D 2013, 'A comparative study of anti-inflammatory activities of certain herbal leaf extracts', *International Journal of Pharmacy and Integrated Life Sciences*, vol. 1, no. 2, pp. 67-77.

Jutila, MA, Berg, EL, Kishimoto, TK, Bargatze, RF, Bishop, DK, Orosz, CG, Wu, NW, Butcher, EC 1989, 'Inflammation-induced endothelial cell adhesion to lymphocytes, neutrophils, and monocytes. Role of homing receptors and other adhesion molecules', *Transplantation*, vol. 48, no. 5, pp. 727-731.

Kempuraj, D., Madhappan, B., Christodoulou, S., Boucher, W., Cao, J., Papadopoulou, N., Theoharides, T. C. (2005). Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. *Br J Pharmacol, 145*(7), 934-944. doi:10.1038/sj.bjp.0706246

Kumar, V, Zulfiqar, AB, Kumar, D, Khan, NA, & Chashoo, IA 2012, 'Evaluation of anti-inflammatory potential of leaf extracts of Skimmia anquetilia, *Asian Pacific Journal of Topical Biomedicine'* , vol. 2, no. 8, pp. 627-630.

Katzung, BG, Masters, BS, Trevor, AJ, 2012, *Basic & Clinical Pharmacology*, 8th edn, The McGraw-Hill Companies, United States.

Oyedapo, OO, Akinpelu, BA, Akinwunmi, KF, Adeyinka, MO, & Sipeolu, FO, 2010, 'Red blood cell membrane stabilizing potentials ofextracts of Lantana camara and its fractions, *International Journal of Plant Physiology and Biochemistry*', vol. 2, no. 4, pp. 46-51.

Parwanto, M. E., Senjaya, H., & Edy, H. J. (2013). Formulasi Salep Antibakteri Ekstrak Etanol Daun Tembelekan (lantana camara L). *Pharmacon, 2*(3).

Springer, TA 1994, Traffic Signals For Lymphocyte Recirculation and Leukocyte Emigration: The Multistep Paradigm. *Cell*, vol. 76, no. 2, pp. 301-314.

Suwertayasa, IM, Bodhy, W, & Edy, HJ 2013, 'Uji Efek Antipiretik Ekstrak Ethanol Daun Tembelekan (*Lantana Camara* L.) pada Tikus Jantan Galur Wistar', *Pharmacon* , vol. 2, no. 3, pp. 45-49.

Thompson, E. B. 1985. Drug Bioscreening. America: Graceway Publishing Company, Inc. pp. 40, 118.