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Antiinflammatory of *Jatropha curcas* Leaf: Role of TNF-α and IL-1

Warsinah[1], Hanif Nasiatul Baroroh[2]

Department of Pharmacy Faculty of Health Sciences, University of Jenderal Soedirman
Correspondent author: *warsinahapt[at]gmail.com*

Abstract: ***Jatropha curcas extract reported analgesic and anti-inflammation effects on albumin-induced mice and antiarthritis in Complete Freud's Adjuvant-induced mice. Phytochemical screening proves that Jatropha curcas contains flavonoid compounds, steroids, triterpenoids, alkaloids, saponins, and tannins. With a cellular approach, an invitro test can be known anti-inflammation mechanism of active compounds of Jatropha curcas leaves. In vitro tests can also be known expression of IL-1 and TNFα cytokine gene target of this research. The results showed that active isolates activity of TNF-α activity was 176, 967 µg / ml, and the IL-1 activity was 1024.077 µg / ml. The isolation was higher than the compound activity on TNF-α and IL-1. In each isolate after TLC, the isolates contained flavonoid and terpenoid compounds. The correlation between in vivo and in vitro provides clear scientific information about the pharmacodynamics of the active compounds.***

Keywords: *Jatropha curcas*, isolate, Cytokine, TNF-α dan IL-1

1. Introduction

Inflammation is an inflammatory response that needs to be serious. The medicinal herbs derived from medicinal plants are a good way of prevention because empirically used are jatropha leaves and are scientifically proven to have anti-inflammatory properties. *Jatropha curcas* has anti-inflammatory and antioxidant activity (1) . The *J. curcas* leaf methanol extract had an antioxidant effect with an IC50 value of 90.83 µg / ml. In addition, *J. curcas* methanol extract also has significant anti-inflammatory activity (2) . The dose of 300 mg/kg BW of Jatropha extract inhibits inflammation by 23.25%, and a dose of 500 mg/kg BW could reduce neutrophil recruitment in rat feet (3) . The fraction of ethyl acetate as an active fraction of ethanol extract at a dose of 150 mg/kg BW the edema volume in rat-induced rats of carrageenan (4) . Phytochemical screening (1) , and the *J. curcas* leaf, contains flavonoids, polyphenols, and saponins. Flavonoid compounds, polyphenols, and saponins in jatropha have potential as antioxidants, anticancer, and anti-inflammatory (5, 6) . The sap and roots of J. curcas plants have the potential as anti-inflammatory through inhibition of the inducible enzyme Nitric Oxide Synthase (iNOS) in macrophage cells (7) . While the ethanol extract of *J. curcas* leaf also has anti-arthritis activity (8) . The fraction of ethyl acetate containing flavonoid compounds capable of decreasing the volume of rat foot edema (7) . The anti-inflammatory and antioxidant activity shown by the J. curcas plant indicates that this plant has potential as an alternative therapy in both acute and chronic inflammatory conditions. This research on the anti-inflammatory effect in vitro the mechanism of action as cytokine inhibitor through observation of gene expression of Tumor necrosis factor (TNFα) and cytokine interleukin 1 (IL-1), the main target of this research is to get information on the mechanism of inhibition inflammation through gene expression from an isolate of active compound from jatropha leaf isolation.

2. Materials and Methods

2.1 Materials

The material used in this study is *Jatropha curcas* L. leaf collected from Kedungbanteng district of Banyumas, center of java and Anti-inflammatory mechanism test materials: IL-1 reagent kit, TNFα kit, EGF kit, VEGF Kit, 0.9% NaCl, and aqua dest.

2.2 Methods

Fractionation
The combined fraction of F3 obtained from the previous study was performed column chromatography with silica gel stationary phase GF 254 and eluent using of mixture solvent of chloroform: ethylacetate (60: 40)

TNF-α/ IL-1 activity
TNF-α / IL-1 inspection procedure with indirect ELISA technique (Sandwich) with HS Quanticine kit reagent. The microplate with a murine monoclonal antibody specific to TNF-α at the well, then a 50 µL diluent HD1-11 assay was added to each well, and 200 µL standard (TNF-α / IL-1 standard) or sample the. The existing TNF-α / IL-1 will be bound by the antibody-coated in the well. The well is covered with an adhesive cover and incubated for 14-20 hours at 2-8 ° C. The well was four times, and a 200 µL conjugate solution containing polyclonal antibody labeled specific enzyme for TNF-α / IL-1, then covered with an adhesive cover, incubated for 3 hours at room temperature. Subsequently, washed four more times, 50 µL substrate solution was added to each well; covered with a new adhesive cover, then incubated for 60 minutes at room temperature. The well will not wash, but coupled with 50 µL of the amplifier solution; covered with a new adhesive cover, then incubated for 30 minutes at room temperature. The addition of this amplifier solution begins the appearance of the color change. It is on a 50 µL stop solution (2N sulfuric acid) in each well. Then read on a microplate reader with 490 nm waveform (in 30 minutes), Reads of length

waves 650 nm or 690 nm. The addition of this stop solution does not affect the color of the well.

3. Results

The fractionation with n-hexane, chloroform, and ethyl acetate by liquid-liquid partition methods. The solvent selection and each solvent having a different polarity that the compound produced also has different polarity so that when its activity on each solvent based on polarity. The fractionation resulted in 4 fractions of the n-hexane fraction, the chloroform fraction, the ethylacetate fraction, and the residual fraction (Table 1) with different colors and yields. The yield of residue is higher and red-brown. The ethyl acetate is a smaller yield and is yellow.

Table 1: Partition results of ethanol extract with n-hexane, chloroform, and ethyl acetate solvent

Fraction	colour	weight (g)	% Yields
n-heksan	yellow	7, 69	1, 44
Kloroform	green	3, 35	0, 67
etilasetat	Yellowish green	1, 98	0, 42
Residu	brown	31, 24	61, 24

Fractionation on its polarity. Withdrawal of non-polar compounds such as fat by n-hexane solvent. Repeatedly all non-polar compounds are interested in n-hexane, which is by the color of the solvent. The more polar with chloroform and ethyl acetate Table 2), Each test for its antiinflammatory activity to determine the active fraction. The fractionation with column chromatography yielded four isolates. Each isolates with TNF-α and IL-1 tests. Observations based on TNF-α and IL-1 activity of the isolates were obtained from the most active combined fractions to obtain pure isolates. The most active fraction was the Fg3 fraction with a TNF-α value of 244 µ / ml purified by separated by column chromatography method and obtained four isolates then tested TNF-α and IL-1 activity in each isolate. The isolates, namely isolate I have y of 176, 967 µ / ml, isolate II of 353.655 µ / ml, isolate III of 314, 799 µ / ml, and isolate IV of 197.019 µ / ml. The TNF-α is the most active.

Table 2: TNF-α observation of isolate of Jatropha leaves

Control (-)		Control (+)				treatment			
Std	TNF-α	Sampel	TNF-α	TNF-α	Mean	sampel	TNF-α	TNF-α	Mean
1	40	SNI	207.115	76.639	141.877	I1	161.921	192.052	176.967
2	80	SN2	57.852	62.438	60.145	I2	348.033	359.277	353.655
3	160	SN3	214.078	85.307	149.693	I3	357.885	271.713	314.799
4	320	SN4	61.487	74.731	68.109	I4	233.380	160.658	197.019
5	640	SN5	194.772	241.699	218.236				

Information: SN= standard, I= Isolate

Table 3: IL-1 Observation of isolates of Jatropha leaves

Control (-)		Control (+)				treatment			
Std	IL-1	Sampel	IL-1	IL-1	Mean	sampel	IL-1	IL-1	mean
1	300	SN1	1111.008	816.990	963.999	I1	1120.835	927.318	1024.077
2	600	SN2	917.837	917.837	917.837	I2	2312.950	2191.053	2252.002
3	1200	SN3	669.776	1090.467	880.122	I3	1842.001	2645.810	2243.906
4	2400	SN4	1030.016	867.687	948.852	I4	2141.417	1028.911	1585.164
5	4800	SN5	1071.003	1294.282	1182.643				

Information: SN= standard, I= Isolate

4. Discussion

The fraction of Fg3 is higher in anti-inflammation activity. The active fraction was purified with the column chromatography method and obtained in four isolates. The isolates were identified with TLC and sprayed with chemical reagents. Four Isolate, its I1 contains terpenoid, I2 is a flavonoid, I3 is also flavonoid, and I4 is a flavonoid compound. The four isolates then TNF-α tests. Tumor necrosis factor alpha (TNF-α) is a major cytokine in acute inflammatory responses to Gram-negative bacteria and other microbes. The Infections can trigger the production of TNF in large quantities to systemic reactions. The TNF-a on a historical basis and to differentiate it from TNF-β or lymphotoxins. The TNF-α are mononuclear phagocytes and activated T cells, antigen, NK cells, and mast cells. The Lipopolysaccharides are a potent stimulus against macrophages to secrete TNF. The IFN-γ has produced T-cells, NK, and stimulates macrophages by increasing TNF synthesis (9) . TNF-is prothrombotic, stimulates adhesion molecules, and induces endothelium. The regulation of macrophages, the immune response with growth factors, and other cytokines. The functions of hematopoietic, T cells, B cells, and the activity of neutrophils and macrophages. TNF-α also has additional beneficial functions in the immune response to bacteria, viruses, fungi, and parasitic invasion (9, 10) . All inflammatory processes in the activation of tissue macrophages and infiltration of blood monocytes. This activation causes many changes in cells. The cytokines cause multiple effects in the host. These effects include 1) fever induction; 2) acute hepatic phase response with leucocytosis and production of acute phase proteins such as C-Reactive Protein (CRP); and 3) differentiation or activation of T cells, B cells, and macrophages (11) . Interleukin-1 is also known as leukocyte activating factor, B cell activating factor, mononuclear cell factor, endogenous mediator leukocyte, hemopoietic-1, and some other names. Interleukin-1 is the name for several IL-1α, IL-1α, and IL-1R cytokine polypeptides, which an important in immune system regulation and inflammatory response. IL-1α and IL-

1ß have genetic bundles IL1A and IL1B on the 2nd chromosome of the same series of 2q14 and pleiotropic cytokine of monocyte and macrophage secretions in the form of prohormone, in response when the cell is injured. The COX-2 induction of these cytokines in the central nervous system is the cause of hypersensitivity. In observation of interleukin-1 activity (IL-1), Similar to TNF-α observation, the purer the compound higher activity by the interleukin level. It is lymphokines, killer T cells, B cells, and others of the immune system. Interleukin is a group of cytokines (secreted hormones) by white blood cells (leukocytes). The immune activity and the lack of a number with complete or infectious immune deficiency. The purification isolates against IL-1. The four isolates, namely I1 of 1024.077 µ / ml, I2 of 2252.002 µ / ml, I3 of 2243.906 µ / ml, and I4 of 1585.164 µ / ml. The most active IL-1 activity measurements occurred in I1 by I4, then I3 and I2. The isolates with the lowest activity were I2 and I3, and the highest in isolate 1 (table 3). Although IL 1 and TNF are biochemically different and bound to cell membrane receptors, each represents a few biological properties and has many of the same activities. The overlapping effects of IL 1 and TNF to the same induction of the spectrum of cytokines and receptors. The IL-1, TNF, IL2, and IL-6 receptors, contribute to immune enhancing, inflammatory, and radioprotective. The IL 1 and TNF are often also released cooperatively by cells and have cooperative effects. For example, IL 1 with TNF has a radioprotective effect and in vitro terminal differentiation effect on tumor cells. These data suggest a common and different post-receptor signal transduction pathway for IL 1 and TNF. IL 1 and TNF can each induce several cell types to produce IL 6, which seems to act as other "broad spectrum" cytokines. In addition, IL 1 and TNF induce the production of NAP-1 by human monocytes and fibroblasts. New cytokines are purified, sequenced, cloned, expressed, and synthesized, which can explain some of the acute inflammatory effects of this cytokine. The inflammatory activity of IL1 and TNF, Although in vitro, IL-1 and TNF-α of inflammation exhibit almost the same amount and have similarities, this effect is also shown in vivo studies.

5. Conclusion

The fractionation was n-hexane with yellow color and 1.44% yield, green of chloroform fraction with 0.57% yield, yellowish green in the ethylacetate fraction with 0.62% yield, and brown of residue with 61.4% yield. The TNF-α in active isolates was 176, 967 µg / ml, and IL-1 activity was 1024.077 µg / ml2. The purer the compound activity of TNF-α and IL-1.

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Conflicts of Interest

All authors declare that they have no conflict of interest.

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