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Research Article

Antiinflammatory Effect of The Fractions of Ethanol Extract of *Jatropa curcas* L Leaves

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ABSTRACT

Jatropha curcas leaf extract is reported to have analgesic and anti-inflammatory effects in mice induced albumin, as well as in mice induced antiarthritis Freud's Complete Adjuvant. Bioassay-guided fractionation with fractionation method is known potency active fraction of the ethanol extract of leaves Jatropha are anti-inflammatory effect. In this study, *J. curcas* leaves were maserated with ethanol, then fractionated by the n-hexane, chloroform and ethyl acetate. The fractions were tested in vivo using male Wistar rats, divided into 5 groups (n = 5), consisting of normal group (aquadest), the control group (karagenan), Na-diclofenac group with a dose of 4.5 mg / kg (per oral), and those fractions of ethanol extract of *J. curcas* leaves with a dose of 150 mg / kg, 300 mg / kg and 600 mg / kg (per oral). Oedema volume was determined every one hour during six hours. The neutrophil counts at hours six th. Data were analyzed by one-way ANOVA followed by LSD test. The results showed that the fractions of *J. curcas* leaves had anti-inflammatory activity. The ethyl acetate fraction was the best anti-inflammatory potency with inhibitory effect of 74.83 ± 3.40% at a dose of 300 mg / kg. histophatologically, Ethyl acetate fraction was also able to reduce the recruitment of neutrophils in inflamed foot tissue.

Keywords: *Jatropha curcas*, fractionation, anti-inflammatory, edema, neutrophils.

INTRODUCTION

Various types of medicinal plants have been used empirically. One of them *Jatropha curcas* is used as an herbal remedy, since it has as anti-inflammatory and antioxidant activity[8]. showed that the methanol extract of leaves of *J. curcas* has the effect of antioxidant with IC_{50} value of 90.83 ug / ml. In addition, the methanol extract of *J. curcas* also have the significant anti-inflammatory activity[4,5] (Mujumdar and Misar, 2004). Ethanol extract of the leaves of Jatropha dose of 300 mg / kg could inhibit the inflammation of 23.25% and a dose of 500 mg/kg body weight could reduce neutrophil recruitment in the legs of mice[2].

Phytochemical screening showed that the leaves of *J. curcas* contain flavonoids, phenols, glycosides, alkaloids, tannins and saponins[4]. Flavonoids, poly phenols and saponins in the potential of *J. curcas* as an antioxidant, anticancer and anti-inflammatory[7,10]. Sap and roots of *J. curcas* plant as a potential anti-inflammatory through the inhibition of the enzyme inducible Nitric Oxide Synthase (iNOS) in macrophages[6] and show the ethanol extract of leaves of J. curcas also has antiartritis activity. Anti-inflammatory and antioxidant activity in plants J. curcas indicate that this plant has the potential as an alternative therapy in conditions of acute and chronic inflammation[3].

RESEARCH METHODS

Materials and research tool

Simplesia leaves of Jatropha curcas (*J. curcas*) is obtained from the district of Sleman Kalasan Yogyakarta, chemicals such as ethanol, n-hexane, chloroform, ethyl acetate, reagent spray sitoborat and anisaldehid and GF 254 silica gel plates, chamber, glassware, micropipette.

Extraction and fractionation

Jatropha leaf powder weighed as much as 500 grams extracted with ethanol by maceration method for 3 x 24, the extract is filtered and then evaporated with an evaporator to obtain a solvent-free extract. The extract is weighed. Subsequently extract in fractionated by solid-liquid partition method using a solvent n-hexane, chloroform and ethyl acetate, then the fraction is tested antiinflammatory activity in vivo using male Wistar rats.

Test of anti-inflammatory activity

a. Testing antiinflammatory effects in vivo

Test animals were used in this study is a white male Wistar rats as much as 15 tails. Before the test all the rats acclimatized and fasted for 24 hours. The test animals were divided randomly into 5 groups as follows:

1. Group I: negative controls given distilled water 5 ml / 200gBB
2. Group II: ETDJP given a dose of 100 mg / kg
3. Group III: ETDJP given a dose of 300 mg / kg

d. Group IV: ETDJP given a dose of 900 mg / kg

4. Group V: diclofenac sodium positive control dose of 4.5 mg / kg

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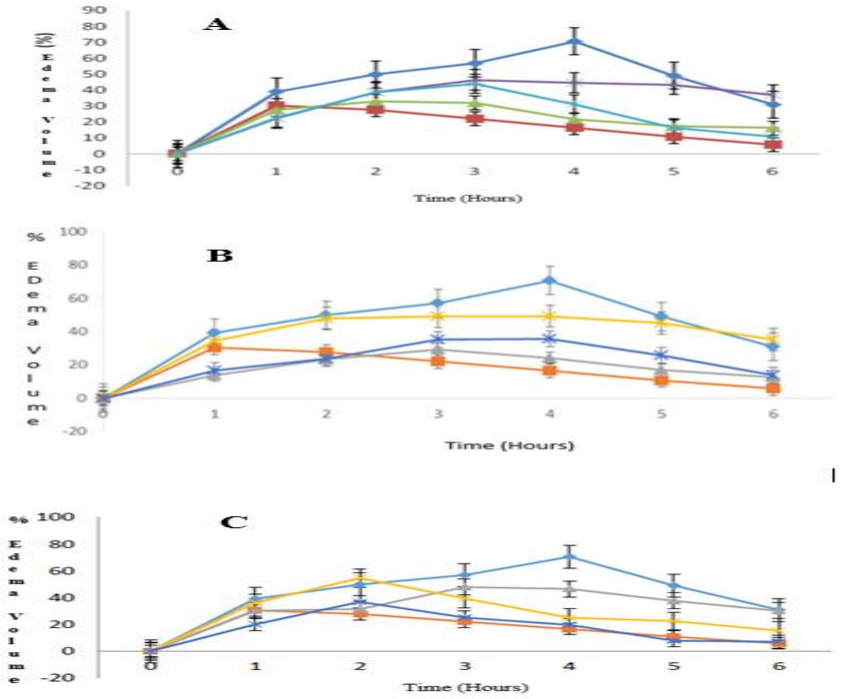

Figure 1: The volume of edema after induced carrageenan 1%. A. treatment of insoluble fraction of n-hexane, B. chloroform fraction treatment, C. Treatment of ethyl acetate fraction.

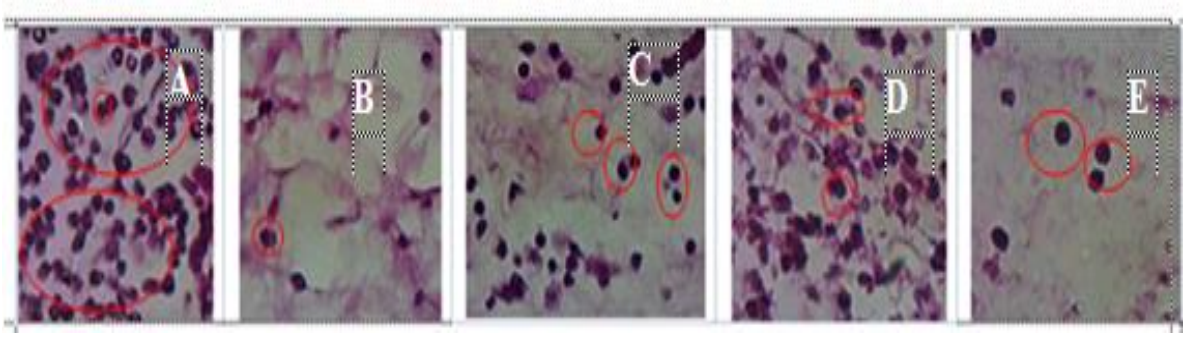

Figure 2: The shape of the neutrophils on the network-induced rat foot karagenin. A negative control, B, positive control (Diclofenac Na), C. insoluble fraction of n-hexane, D. chloroform fraction, E. Fraction ethyl acetate, the red circle is the neutrophils.

Table 1: Results of partitioning the ethanol extract with solvent n-hexane, chloroform and ethyl acetate.

fraction	colour	Weight fraction (gr)	Yield (%)
Insoluble n-hexane	Yellow	7,69	1,44
Kloroform	green	3,35	0,67
etilasetat	Green yellowness	2,98	0,52
Residue	brown	31,24	61,24

Extract dose and administration interval between the test solution and 1% carrageenan suspension adjusted to the results of preliminary experiments

Observations anti-inflammatory activity

The volume of edema was measured according to Archimedes principle using the tool pletisnometer, by way leg of mice that have been marked ankle dipped into the liquid in pletismometer and measuring the volume of liquid is spilled as the volume of the foot.

The histopathologic examination of tissue inflammation

Sampling skin

Mice from each group on anti-inflammatory activity test, taken three tails as a sample. Mice whose feet will be cut, turned off. Walking mice experienced more inflammation and then cut tissue showed symptoms of inflammation or swelling sliced crosswise. Networks put in a pot containing 10% formalin[9]. Furthermore, the network made preparations with hematoxylin-eosin staining (HE)[1].

Histopathological observations

Table 2: Area Under the Curve (AUC) in the treatment of fractions of ethanol extract of *J. Curcas* leaves.

The treatment	AUC values (% / h) ± SD				
	Negative Control	Positive Control	insoluble fraction of n-hexane	Chloroform fraction	Ethyl Acetate fraction
without treatment	280.97±0.93				
diclofenac Na		110.35±35.94			
dose of 150 mg			139.32±7.43	119,66± 12,51	208,92 ± 14,39
dose of 300 mg			214.41±4.83	243,39 ± 25,05	185,30 ± 23,94
dose of 600 mg			157.47±4.42	144,25 ± 13,09	112,69 ± 15,00

Table 3: Number of recruitment of neutrophils in the mice given leg-insoluble fraction of n-hexane, chloroform fraction, ethyl acetate fraction.

Group	Neutrophils Total				
	Control (-)	Control (+) diclofenac Na	Insoluble fraction of n-hexane	chloroform fraction	Ethyl acetate fraction
	66	40			
1			16		19
2			20		14
3			17		13

Subsequent staining results read under a microscope with a magnification 10x40 and counted the number of neutrophils as parameters that affect inflammation[9]. Observations of the number of polymorphonuclear (neutrophils) using a Nikon microscope N-100 and shoot with video pictures in 5 visual field with an area of each field of view is 37 909 μm2.

Data analysis

The identification of compounds in fraction was analyzed by TLC profile diskriftif kualtatif and data AUC0-5 between edema volume against time, the value of the percentage of antiinflammatory, and histopathological Observation data, namely the number of polymorphonuclear (neutrophils) then Kolmogorof-Smirnov test. If the data are normally distributed then followed by one-way ANOVA with 95% confidence level.

RESULTS AND DISCUSSION

Extraction and fractionation

Simplesia were extracted with ethanol, produced Jatropha leaf extract. The yield of ethanol extract is 10.66%. Then fractionated to produce 4 fractions, namely fraction of insoluble n-hexane, chloroform fraction, ethyl acetate fraction and residual fraction (Table 1) with a different color and yield. The highest yield obtained in the residue with a red-brown color and the yield of the smallest in ethyl acetate in yellow.

Antiinflammatory study

Observation of edema volume

The ethanol extract of *J curcas* leaves given first released from phorbolester by way of the ethanol extract was fractionated using a solvent n-hexane to take phobol thereof as toxic to the test animals. Then the insoluble fraction obtained n-hexane, followed by fractionation with chloroform, ethyl acetate fraction and residual fraction. The antiinflamatory effect of the fractions were determined with the paw edeme methode. The rats were induced by karagenin 1%, and administered orally (po) with a dose of 150mg, 300 mg and 600 mg, and then observed a decrease in the volume of edema having obtained the percentage increase in the value of edema volume, further AUC 0-6 value calculation of each group against time.

In observation AUC showed the same pattern as the volume of edema, ie the greater the dose fractions are given the smaller the value of AUC means. The greater was the dose fractions more effective as anti-inflammatory drugs, it was proven by the AUC on the control negative (without treatment) with values high, while on treatment Diclofenac Sodium give the smallest AUC values because diclofenac sodium has been recommended as an anti-inflammatory drug and then performed observations of the number of neutrophils

In observation of the number of neutrophils, as the data of other anti-inflammatory (figure 1), showed that the insoluble fraction of n-hexane, chloroform and ethyl acetate fraction showed a decrease of neutrophils. On the positive control neutrophil number greater than the insoluble fraction of n-hexane and ethyl acetate, whereas the negative control gives the number of neutrophils, the most. In granting ethyl acetate fraction gives the number of neutrophils, the smallest and the greater the dose given to indicate the number of neutrophils, the less, it may be said that the ethyl acetate fraction was the fraction of the most effective anti-inflammatory. In observation of neutrophils also showed the same pattern with the observation volume edema and AUC were dose dependent have a pattern ie the higher the dose the greater the reduction of recruitment neutropils or in other words the higher the concentration the greater the antiinflammatoryeffects. The next observation is pathological picture of the number of recruitment, the results presented observations of control, administration of diclofenac Na and insoluble fraction of n-hexane, chloroform, ethyl acetate (figure 2).

CONCLUSIONS

The ethyl acetate fraction has the greatest anti-inflammatory activity compared with the chloroform fraction and insoluble fraction of n-hexane. The ethyl acetate fraction Jatropha leaves (*J. curcas*) has antiinflammatory activity of power of 74.83 ± 3.40% at a dose of 300 mg / kg.

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