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Research Report

EFFECT OF FREE ALKALOID AND NON-FREE ALKALOID ETHANOL 70% EXTRACT OF *JUSTICIA GENDARUSSA* BURM F. LEAVES AGAINST REVERSE TRANSCRIPTASE HIV ENZYME IN VITRO AND CHEMICAL COMPOUND ANALYSIS

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ABSTRACT

HIV-AIDS is a global problem and the deadliest disease in the world. One of HIV and AIDS prevention strategy can be done with traditional medicine research program from natural resource that has anti-HIV AIDS activity. It has been found that 70% ethanol extract of Justicia gendarussa Burm.f leaves, alkaloid free and alkaloid non-free, has a strong inhibitory activity against HIV reverse transcriptase enzyme, as an effort to find a solution in the face of HIV AIDS prevalence that is still high with problem of HIV-AIDS treatment such as side effects and resistances. Justicia gendarussa had already known for having an effect anti-HIV and therefore we were looking at the mechanism of inhibition of HIV Reverse Transcriptase enzyme. Both types of extracts were tested in vitro using ELISA technique and analysed chemical content of Gendarusin A as anti-HIV using high performance liquid chromatography. ELISA test results obtained percent inhibition, respectively for 254.2, 254.2, 235.6, and 279.7 for the concentration of 5 ppm, 10 ppm, 15 ppm and 20 ppm of free alkaloid extract and 169.0, 164.0, 130.5 and 369.5 for the concentration of 5 ppm, 10 ppm, 15 ppm and 20 ppm of non-free-alkaloid extract. The results of high performance liquid chromatography obtained Gendarusin A in the free-alkaloid extract at retention time 8.402 minutes and non-free alkaloid extract at retention time 8.381. Therefore, these results concluded that the Justicia gendarussa Burm.f can be a useful resource for the isolation and development of new anti-HIV.

Key words: *Justicia gendarussa; 70% ethanol extract; free and non-free alkaloid; reverse transcriptase; anti-HIV*

ABSTRAK

HIV-AIDS merupakan permasalahan global dan penyakit yang mematikan di dunia. Salah satu strategi pencegahan HIV-AIDS dapat dilakukan dengan program penelitian pengobatan tradisional dari sumber daya alam yang memiliki aktivitas anti-HIV AIDS. Telah ditemukan bahwa ekstrak ethanol 70% daun Justicia burm.f gendarussa, bebas alkaloid dan mengandung alkaloid, memiliki aktivitas inhibitor yang kuat terhadap enzim HIV reverse transcriptase, sebagai upaya dalam rangka mencari solusi menghadapi prevalensi HIV AIDS yang masih tinggi dengan masalah pengobatan HIV-AIDS seperti efek samping dan resistansi. Justicia gendarussa telah diketahui memiliki efek anti-HIV dan perlu diketahui mekanisme penghambatan enzim HIV reverse transcriptase. Kedua jenis ekstrak diuji in vitro menggunakan teknik ELISA dan dianalisis kandungan kimia Gendarusin A sebagai anti-HIV menggunakan High Performance Liquid Chromatography (HPLC). Hasil tes ELISA diperoleh persen inhibisi, masing-masing untuk 254.2, 254.2, dan 279.7, 235.6 untuk konsentrasi 5 ppm, 10 ppm, 15 ppm, dan 20 ppm ekstrak bebas alkaloid dan 169.0 bebas, 164.0, 369.5, 130.5 untuk konsentrasi 5 ppm, 10 ppm, 15 ppm, dan 20 ppm dari ekstrak yang mengandung alkaloid. Hasil High Performance Liquid Chromatography (HPLC) menunjukkan Gendarusin A pada ekstrak bebas alkaloid pada waktu retensi 8.402 menit dan ekstrak yang

mengandung alkaloid pada waktu retensi 8.381. Hasil tersebut mengarah pada kesimpulan bahwa Justicia gendarussa burm.f dapat berpotensi bagi isolasi dan pengembangan anti-HIV baru.

Kata kunci: *Justicia gendarussa; ekstrak ehanol 70%; bebas dan tidak bebas alkaloid; anti-HIV*

INTRODUCTION

HIV-AIDS is a global problem and the deadliest disease in the world. According to WHO global report, the number of AIDS deaths in the world in 2009 reached 1.8 million people[1]. Whereas based on the Health Ministry data, although the total HIV and AIDS cases nationwide declined from 2011 as many as 21.031 and 4162 into 9.883 and 2.224 in 2012, but it is still relatively high.[2] Seen from a treatment, medical efforts of the HIV-AIDS treatment service still face some problem. For example, the antiretroviral utilization which is the dose and side effect are very limited. Toxicity and side effects affecting patient obedience to antiretroviral. Furthermore, antiretroviral utilization this time are resistant that cause the failure of therapy.[3]

One of HIV and AIDS prevention strategy can be done with traditional medicine research program from natural resource that has anti-HIV AIDS activity. Traditional medicine research is directed to find a scientific evidence on it.[4] Natural resources still have an important role as an initial material invention of new drugs.[5] Based on a published report in 2007, there were 974 molecular compound that 63% of them come from natural or semisynthetic derivatives from natural materials.[6]

The reverse transcriptase enzyme has an important role in the life cycle of HIV because reverse transcription is an early phase of viral replication in the cell host. All the proteins and enzymes that play an important role in the new virus formation are not carried by the virus but using enzymes and proteins in host cells.[7] Furthermore, the reverse transcriptase enzyme together with an integrated enzyme is derived from a virus that enters the host cell in fusion phase.[8] Therefore, the drugs development that act on the reverse transcriptase enzyme will inhibit the next cycle process directly, starting from reverse transcription of the virus RNA into DNA, the integration of DNA virus on host cell DNA, and core replication to the virus proteins formation. Inhibition on phase after transcription is still possible to set an infection in the host cell because the virus DNA can settle along with the host cell DNA. Therefore, inhibition of the enzyme reverse transcriptase can reduce a HIV infection.[7]

Currently, it is being developed an anti-HIV drugs derived from natural medicine, namely *Justicia gendarussa* Burm.f. Research has been done on them to test the effect of hexane, methanol and ethanol of *Justicia gendarussa* Burm.f drug against HIV virus in vitro methanol and ethanol extracts obtained 70% alkaloid-free give a decrease in the amount of virus results.[9]

The part of *Justicia gendarussa* Burm.f herb showed inhibitory activity analog reverse transcriptase enzyme substrates in vitro.[10] Isolate of the pure compound flavonoid apigenin showed inhibition of HIV reverse transcriptase enzyme activity as a substrate analog and HIV protease enzyme in vitro.[11] The main content of 70% ethanol extract *Justicia gendarussa* Burm.f is apigenin. A few compounds either major or minor component can give a synergistic effect as an anti-HIV through the same or different mechanism.[12] Therefore, this study aimed to test the inhibitory activity of the Reverse Transcriptase HIV enzyme using 70% ethanol extract free and non-free alkaloid.

MATERIALS AND METHODS

Materials

*Justiciagendarussa*Burm.f leaf obtained from cultivated plants in Trawas, Mojokerto, East Java. Roche RT Activity Kit obtained from PT. Roche, Germany. Materials to extract, alkaloid test and chemical content are 70% ethanol, dichloromethane, methanol, hexane, distilled water, citric acid, filter paper, dragendorf reagent, Silica gel GF 254.Extraction tool set such as macerator extraction, Memmert oven, evaporator buchi, Julabo USR 3 ultrasonic, iMark Microplate Absorbance Reader, HPLC tool set: Agilent1100, reversephase column C18 Nova-pak® sized 3,9 × 150 mm.

Research Place

Research had been done in two laboratories, Laboratory Pharmacognosy Faculty of Pharmacy, Airlangga University and Institute of Tropical Disease laboratory, Airlangga University.

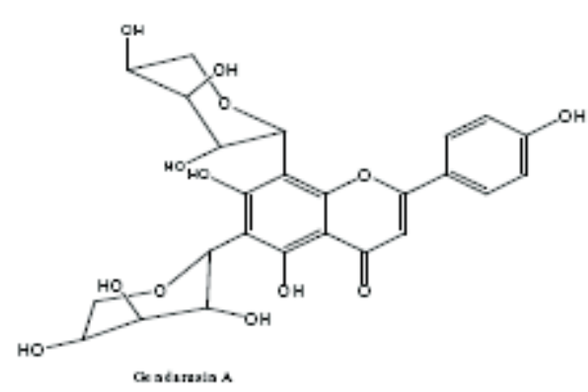


Figure 1. Gendarusin A Structure. (Prajogo BEW, 2010)

Methods

Simplicia of *Justicia gendarussa* Burm.f leaves were divided into two sample groups. First group acidified to release the alkaloids and the second group did not to acidified. Both two sample groups were macerated with 70% ethanol and then concentrated. To ensure of free alkaloid, the first group tested the alkaloid free using Thin Layer Chromatography with stationary phase Silica Gel GF 254 and the mobile phase dichloromethane: methanol, 9:1 with the spray Dragendorf reagent. Both of samples tested its activity in an enzyme activity inhibition Reverse Transcriptase HIV using Elisa and determined the type of inhibitor. Both samples were also analysed to determine levels of chemical content Gendarusin A based on previous research that isolates apigenin has Reverse transcriptase HIV inhibitory activity. The conditions that used in HPLC was methanol eluent: water (30:70), flow 1 ml/min, stop time 25 minutes, a wavelength of 254 nm.

RESULTS AND DISCUSSION

In this study, it had been tested to know the inhibitory activity of *Justicia gendarussa* Burm.f 70% ethanol extract against Reverse Transcriptase HIV enzyme and acidification differences treatment to know the effect of alkaloid on the inhibition of the enzyme Reverse Transcriptase HIV. Extraction was done by ethanol 70% maceration because the previous studies showed the extract can decrease the amount of HIV virus in vitro culture.

The first group was tested to ensure that it was free from alkaloid as shown in Figure 2. The stain A that was free from alkaloid extract was not showed red-orange stain like stain B and C.

Inhibition testing of Reverse Transcriptase HIV enzyme was obtained by using the formula (1).

$$\%\text{Inhibition} = \left[\frac{A_0 - A_1}{A_0}\right] \times 100\% \quad (1)$$

Explanation:
A_0: blank absorbance
A_1: sample absorbance

Table 1 shows the both extracts had inhibitory activity of the Reverse Transcriptase HIV enzyme that was indicated by the percent inhibition.

Table 1. Inhibition percentage of 70% ethanol extract alkaloid-free and non-free of *Justicia gendarussa* Burm.f leaves to the activity of the Reverse Transcriptase HIV enzyme

Sample	Concentration	% Inhibition
A	20 ppm	279,7
	15 ppm	235,6
	10 ppm	254,2
	5 ppm	254,2
B	20 ppm	369,5
	15 ppm	130,5
	10 ppm	164,0
	5 ppm	169,0
K+	100 µg/ml	83
K-	-	-

Explanation:
A : Non-free alkaloid extract
B : Free alkaloid extract
K^+ : Positive control (Doksorubisin 100 µg/ml)
K^- : negative control (tested solution without enzyme and sample)

For the chemical substitute analysis of Gendarusin A obtained at retention time 8.402 minutes for the alkaloid-free extract and retention time 8, 381 minutes in Fig 3 compared with standard Gendarusin A 9.6 ppm with a retention time of 8.590 minutes as shown in Fig 2.

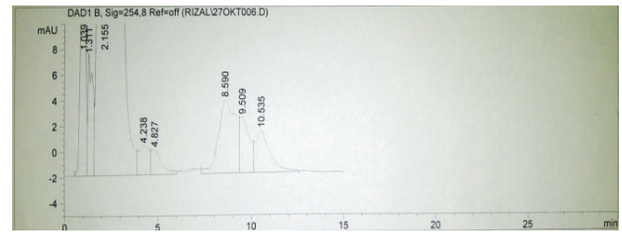
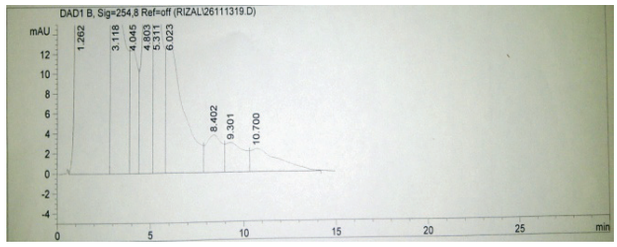
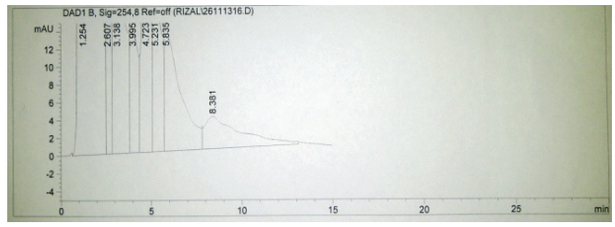


Figure 2. Gendarusin A 9,6 ppm standard chromatogram



a



b

Figure 3. Ethanol 70% Extract of *Justicia gendarussa* Burm.f, Leaves Chromatogram (a) alkaloid-free and (b) non alkaloid-free.

CONCLUSIONS

From these results, it can be concluded that the 70% ethanol extract of *Justicia gendarussa* Burm.f leaves 60th alkaloid- free and non-alkaloid-free have inhibitory activity of the Reverse Transcriptase HIV enzyme.

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