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THE EFFECT OF *GENDARUSSIN* A ISOLATES OF *JUSTICIA GENDARUSSA BURM.F*. LEAF IN REVERSE TRANSCRIPTASE INHIBITION OF HIV TYPE I IN VITRO

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ABSTRACT

Screening has been done to a few extracts from the leaves Justicia gendarussa Burm.f to see the growth rate of the virus from the blood plasma of HIV patients at Dr Soetomo Hospital. It is known that J. gendarussa leaf extract inhibits HIV type 1 reverse transcriptase. In addition, its main content is gendarussin A, besides gendarussin B, JGF1, JGF2 and JGF3, which have just identified. At the beginning, extraction and fractionation were performed with 3 models that highlight the absolute methanol, 70% methanol and 70% ethanol with the release of alkaloids. Furthermore, samples of each fraction were incubated in plasma of HIV patients with a titer of 3.6 10^6 copies for 1 h in concentrations of 1.64 ppm, 4.1 ppm, 8.2 ppm, 16.4 ppm and 41.0 ppm. After incubation, examination was performed by using Nucli sens a machine, which is a combination of PCR and Elisa, thus avoiding direct contact with the highly pathogenic virus. The result showed that the activity sequence from the most potential to the weak, among others, was 1.64 ppm > 4.1 ppm > 8.2 ppm > 16.4 ppm > 41.0 ppm, each with barriers value of 0.62 10^6, 1.4 10^6, 1.6 10^6, 2.4 10^6 and 5.2 10^6 cells/ml. In conclusion, highest anti-HIV activity comes from the concentration of gendarussin A isolate at 1.64 ppm. Furthermore, after linear regression of y = -3.063 x + 81.37 was done, the IC_{50} of 10.24 ppm was obtained.

Keywords:, *Justicia gendarussa, gendarussin A,reverse transcriptase, inhibition, anti HIV*

ABSTRAK

Penelitian telah dilakukan pada beberapa ekstrak daun Justicia gendarussa Burm.f untuk melihat angka pertumbuhan virus plasma darah pasien HIV di Rumah Sakit Dr. Soetomo. Telah diketahui bahwa ekstrak daun J. gendarussa menghambat HIV tipe 1 reverse trancipitase. Selain itu, kandungan utama dari J. gendarussa adalah gendarussin A disamping gendarussin B, JGF1, JGF2, dan JGF3 yang telah diidentifikasi. Pada awalnya, ekstraksi dan fraksinasi ditunjukkan oleh 3 model yang ditandai oleh absolute methanol, 70% methanol dan 70% ethanol dengan melepaskan alkoid. Selanjutnya, sebagian dari masing-masing sampel diinkubasi ke dalam plasma HIV pasien HIV dengan titer 3.6 10^6 sebanyak masing-masing 1h dengan konsentrasi 1.64 ppm, 4.1 ppm, 8.2 ppm, 16.4 ppm dan 41.0 ppm. Setelah inkubasi, pengujian ditunjukkan menggunakan mesin Nucli sens yang dikombinasikan dengan PCR dan Elisa untuk menghindari kontak langsung dengan virus yang memiliki resiko pathogen tinggi. Hasil menunjukkan bahwa rangkaian aktivitas tersebut dari yang paling berpotensi tinggi ke yang paling berpotensi rendah diantaranya adalah 1.64 ppm > 4.1 ppm > 8.2 ppm > 16.4 ppm > 41.0 ppm dengan masing-masing nilai penghalang 0.62 10^6, 1.4 10^6, 1.6 10^6, 2.4 10^6 and 5.2 10^6 cells/ml. Kesimpulannya. Aktivitas anti-HIV tertinggi diperoleh dari konsentrasi gendarussin A yang dipisahkan pada 1.64 ppm. Selanjutnya, setelah regresi linier y = -3.063 x + 81.37 selesai, diperoleh IC_{50} of 10.24 ppm.

Kata kunci: *Justicia gendarussa, gendarussin A, reverse transcriptase, inhibisi, Anti-HIV*

INTRODUCTION

The transmission of HIV-AIDS in Indonesia continues to widen, particularly in the group of young and productive individuals. Data from the Ministry of Health showed that up to June 2008 there were approximately 6782 individuals aged 20-29 years who suffered from AIDS. The number of people with AIDS in this age group is the highest compared to other groups, the second highest were those with age 30-39 years, comprising only 3539 people. The young generation is as if being chased by this deadly disease, and, unfortunately, the spread of this disease is like an iceberg phenomenon. The problem that comes to the surface is actually just a piece of the reality in the field. Integrated Biological and Behavior Survey (Survey Terpadu Biologi dan Perilaku, STBP) regarding HIV prevalence in Indonesia in 2007 showed that about 43-56% percent of drugs (narcotics, psychotropic substances and additives) users or injection drug users in four cities, Medan, Jakarta, Bandung and Surabaya has been infected with HIV.[1]

HIV-AIDS becomes the fifth leading cause of death in population aged 25-44 years in the United States. At global level, 25 million people have died in vain since the epidemics of this infectious disease and 40.3 million people worldwide are currently living with HIV-AIDS. HIV causes AIDS, the virus attacks immune system and stay in the body that can spur the onset of infection and cancer. Generally, bacteria, yeast, and viruses seem not to become a serious illness when the immune system in healthy condition. This may be different and will be fatal in people who suffer from AIDS. HIV is found in saliva, tears, nervous tissue and spinal fluid, blood, semen (seminal fluid in ejaculate), vaginal fluid and breast milk. But only through blood, semen, vaginal secretions and breast milk generally these infections can be transmitted.[2]

AIDS begins with HIV infection. HIV-infected person may be no symptoms for 10 years or more, but remain infected and can transmit infection to others. Meanwhile, if the infection is not detected or without treatment, immune system gradually weakens, and AIDS develops. Generally, symptoms may present as flu with fever, rash, sure throat, sweats, chills, swollen lymph nodes, weakness and weight loss. HIV infection is associated with decreased CD4 cells, a type of immune cells called "T cell" or "helper cell". Indications of a viral infection is when the number of "CD4 cells" below 350 cells/ml, and, specifically in HIV infection, if CD4 cell count is below 50 cells/ml. Furthermore, for monitoring HIV patients the number of CD4 cells, known as HIV-RNA, is used.[3]

Traditional medicine contributes much to the discovery of new compounds that has anti-HIV activity. There are the plants that have proteins that can inhibit HIV reverse transcription in vitro.[4] Some isolated single chain ribosom inactivating protein (SCRIP) showed the power of the antiviral action of DNA and RNA viruses. For example, MAP30 and TAP 29 are SCRIP protein isolated from *Momordica charantia* seeds and tubers of *Trichosanthes kirilowii*. Both materials can inhibit the replication of HIV-1 infected cells and also the activity of inhibitors of HIV-1 virus associated with reverse transcriptase.[5]Water extracts and 80% ethanol extracts of the plants *Andrographs paniculata, Justicia gendarussa, Vitex trifolia* and *Tinospora crispa* have the activity of inhibitors of HIV-1 reverse transcriptase.[6] Natural materials, particularly the class of polyphenol, have anti-HIV activity, whose action can work, for example, by inhibiting HIV cycle: (1). Virus adsorption, (2) Virus-cell fusion (3) Reverse transcription, (4) Integration, (5) Proteolytic cleavage, (6) Glycosylation and (7) Assembly/release.[7]

Justicia gendarussa is a plant often used by Indonesian people as medicine, either as a drug for internal and external use. As a male contraceptive, research has been carried out on its biological and pharmacological activity, even reaching preclinical and clinical trials phase I and II.[8,9,10] From phytochemical studies, the major component of *gendarussin* A has been identified, in addition to minor components of other flavonoids. There is also alkaloid content Bhagya. Furthermore, the anti-HIV screening showed that 70% EtOH extracts *Justicia gendarussa* in 100 ppm can reduce HIV growth inhibition (viral load) to 1.47 10^5 copies/ml after incubation of 60 minutes. It is even more potential if *gendarussin* A isolates are used.[10]Due to the high potential of *gendarussin* A isolates and the needs of reconfirmation with different concentrations, it was necessary to carry out reisolation, which is quite difficult, even it has already standardized method. This effort is aimed to gain the stabil gendarusi A level in extracts. Due to humanity consideration, this research is very important, since until now there is no potential anti-HIV drugs originating from Indonesia that have been subjected to clinical trials. Therefore, this study examined the ability of the isolates of *gendarussin* A, a polyphenol compound from *Justicia gendarussa* leaves, as the activity inhibitor of HIV-1 reverse transcriptase.

MATERIAL AND METHODS

Justicia gendarussa leaf powder were obtained from Pacet Mojokerto, Methanol pa, Methanol pro HPLC, *gendarussin* A isolate, JGF1 isolate, JGF2 isolate, JGF2 isolate, and Silica Gel F_{254}.

Some equipments such as Percolator, HPLC Shimadzu LC10AD, coloumn Waters Novapack C18, EasyQ HIV-1 v2.0 Worksheet Nuclisens Magnetic Extraction, Rotavapor BUCHI R-114, Rotavapor BUCHI R-153.

Sample Extraction and Isolation

Justicia gendarussa extract were obtained by maceration of 1.5 kg of powdered *gendarussa* leaves which was soaked with 3 L. methanol for 24 hours with stirring. Extraction was repeated 2 times with the same method and solvent volume. *Gendarussin* A isolate was obtained by several times preparative HPLC running with a 200 µl per injection.

Furthermore, the collections of isolates were subjected to lyophilization with freeze dryer until constant weight was obtained.

Identification of isolate preparation

Amorphous-shaped *gendarussin* A isolate was identified and compared with standard *gendarussin* A isolates through HPLC analysis, ^{1}H-^{13}C NMR, chemical reactions and physical characterization that could be done. Inhibitions to HIV-1 replication were tested in several concentrations from 5, 10, 20, 40, 80, 100 ppm (each in triplicate).

Determination of IC_{50} of *gendarussin* A isolate

Obtained *gendarussin* A isolates was diluted in methanol with various concentrations, which could be done by making more than six points around 10 ppm, and analyzed by probit which would reveal the regression equation, so that the chart pattern would also be obtained between the concentration and the viral load (HIV), and from the extrapolation we would have the IC_{50}.

HIV Monitoring Procedure

EasyQ HIV-1 v2.0 Worksheet Nuclisens Magnetic Extraction was used and it has some steps such as lysis, binding,washing and dilution. In Lysis step, lysis buffer tube was centrifugated for 10 seconds at 1500 g, and then 0.1 ml or 0.5 ml or 1.0 ml sample were added into it. The mixed solution was vortexed and was incubated for 10 minutes at room temperature. Then premix solution was prepared by entering 550 CAL diluent into grain-shaped CAL and 550 ul salica solution was added and was vortexed maximum 20 minutes, premix solution must be added to the sample. In binding step, Premix solution was vortexed and 10 µl premix solution was added. The solution then was vortexed and was incubated for 10 minutes at room temperature

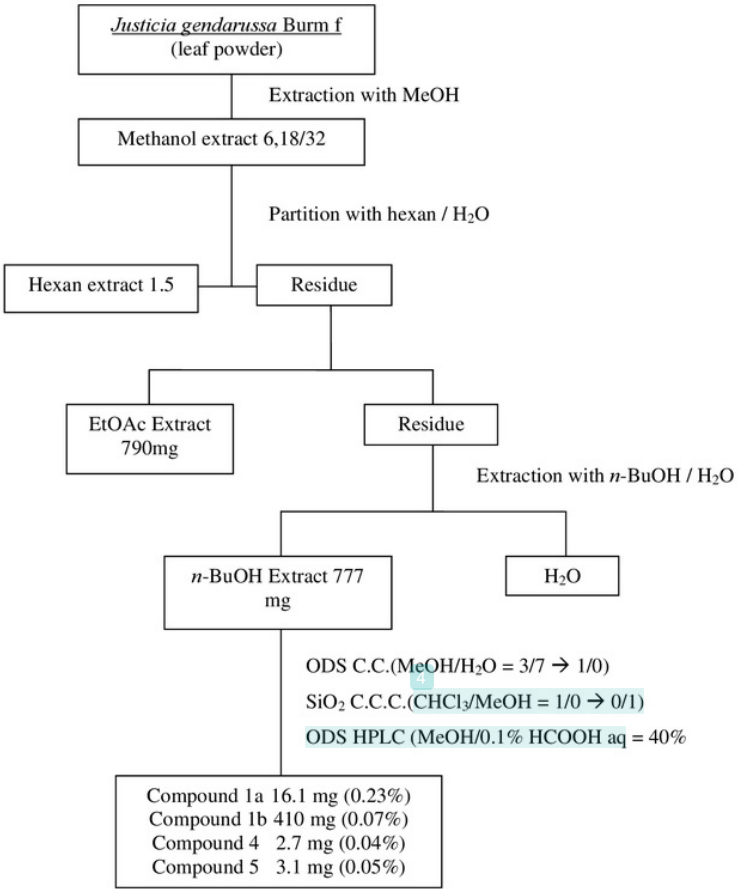

Figure 1. Scheme of flavonoids isolation

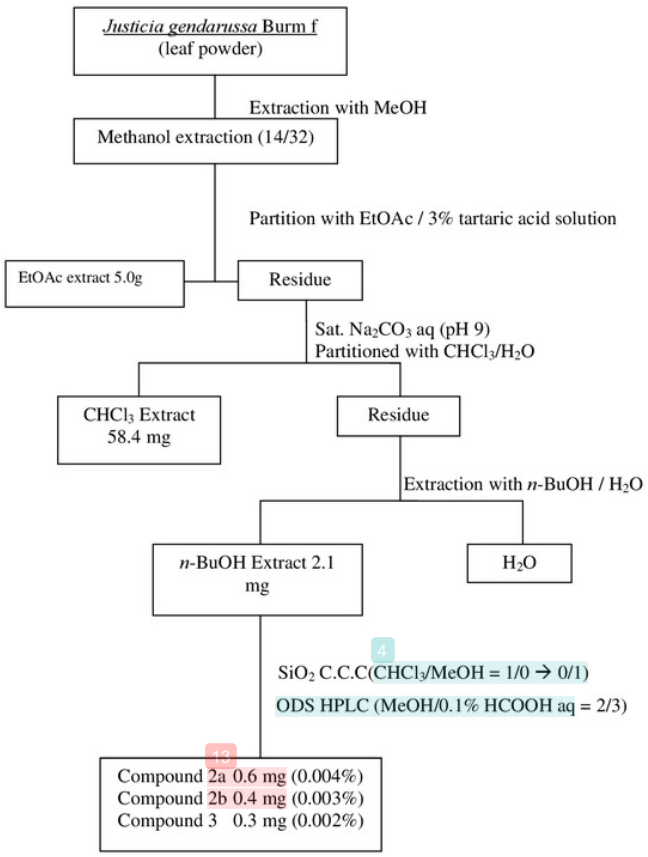

Figure 2. Alkaloid isolation scheme

without homogenization (without mixing). In washing step, the lysis buffer tube was vortexed for 2 minutes at 1500g and then was discarded the supernatant.400 µl wash buffer 1 (transparent) was added, homogenized and transfered this solution into 1.5 ml tubes and was washed for 30 seconds on the menu STEP 1 on the NucliSens mini MAG (magnetic On). After that the supernatant was discarded. 400 µl wash buffer 1 (transparent) (magnet off) was added step 4 and 5 was repeated. 500 µl wash Buffer 2 (red●) (Magnet off) was added and was repeated step 4 and 5. After that step 7 was repeated.500 µl wash Buffer 3 (yellow●) (Magnet off) was added and was washed for 15 seconds on the menu STEP 1 at nuclisens min MAG (magnet on). All supernatant/liquid (magnet on) was discarded. In dilution step, 25 µl elution buffer (yellow●) was added and was incubated for 5 min, 60°C in thermoshaker (speed 1400 rpm). After that tube was placed in magnetic rack with open tube, and 15 µl extracted samples was moved to 8 tube strips in the amplification area or store at a certain temperature.

RESULTS AND DISCUSSION

In some extraction isolation models, the type of solvent, methanol and ethanol, and alkaloid-free or not extract were influenced the quantity of fractions which were obtained. The largest amount is in the polar fraction from the methanol and ethanol in various concentrations. Whereas, in non-polar solvents, like *n*-hexane and chloroform, it was found in small amounts. It is known that the main content in the polar fraction is *gendarussin* A, which is a type of flavonoid glycosides.[11] The process of isolation in butanol fraction revealed isolates JGF1 (2 mg), JGF2 (4.2 g) and JGF3 (1.3 mg), while from chloroform fraction we obtained JGA1 alkaloids (0.96 mg). From the physicochemical analysis, it was found that JGF1, JGF2 and JGF3 are derivatives of apigenin as that in *gendarussin* A and has the same molecular weight of 534.14. They are different only in ribose, xylose and arabinose sugar structures. Then, in JGA1 alkaloid is also observed. It is the derivative of benzyl

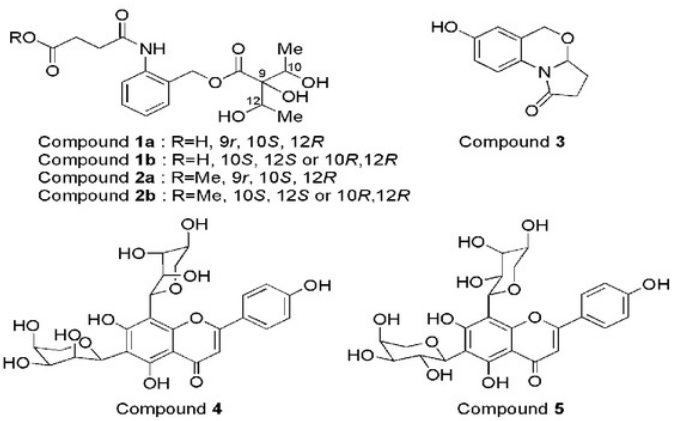

Figure 3. Alkaloid and flavonoid compounds in *Justicia gendarussa* leaves

amino alcohol bound to carboxylic acid on both sides of the core. Of the various isolates, such as *gendarussin* A, JGF1, JGF2 and JGF3 with various concentrations, anti-HIV test was done using Nucli Sens Machine (Table 1). From the results of incubation of isolates tested against human plasma HIV for 60 minutes in vitro, it was shown that the *gendarussin* A isolate provided the strongest activity (3.6 10^6) at 793 ppm compared to other isolates. In various concentrations, it was observable that anti-HIV activity is determined by the viral load. Determination of anti-HIV activity can be seen with inverse proportion between viral load and CD4 count. This indicated that the effect is positive when viral load is lower than the negative control (patients' titer) or CD4 count increases compared to a negative control. If *gendarussin* A isolate provides good effect compared to other isolates, it means that those isolates contain apegenin glycoside compounds with xylose and arabinose sugar. In tested sample 70% ethanol fraction contained 1.4% *gendarussin* A, as determined by HPLC method. In previous clinical trials, it was found that bioavailability test in plasma or blood serum detected *gendarussin* A metabolite, which also appeared in ejaculate and urine. [12] Thus, this in vitro test can then be used as a model of direct interaction with the virus and it has been known previously that the virus growth inhibition is due to the inhibition of transcriptase enzyme function of HIV type 1, whose function is to replicate itself. The certainty of viral death can be tested through the identification of proteins, since the virus is highly pathogenic.

In the HIV viral load measurement results, 200µl sample after 60 minutes incubation is showing the result sample Gendarussin A with concentration 793ppm, JGF_1, JGF_2, JGF_3 show the viral load value 3.1×10^6; 6.4×10^6; 3.1×10^6 and 8.1×10^6 subsequently. This effects of flavanoid compounds in HIV sample could be seen in the Figure 4.

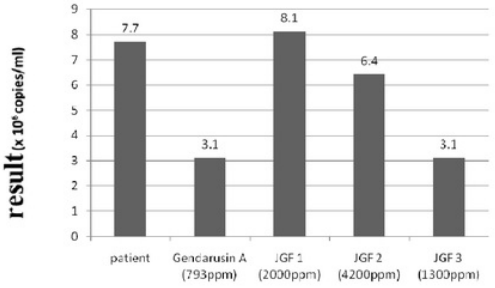

Figure 4. Diagram of the effects of flavonoid compounds in the plasma of HIV in vitro

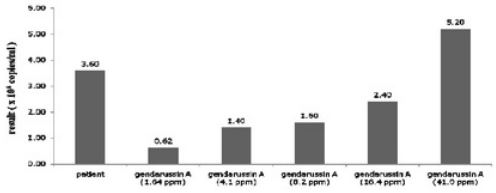

Figure 5. The Effect of Gendarussin A on Human Plasma in Patients with HIV-1

The concentration of Gendarussin A showed the tendency to be increased as high as the viral load result. The data show in Figure 5.

The isolates of *gendarussin* A, JGF1, JGF2 and JGF 3 of the leaf *J. gendarussa* at concentrations 793, 2000, 4200, and 1300 ppm produces viral load 3.1×10^6 ; 8.1×10^6 ; 6.4×10^6 and 3.1×10^6 copies/ml. The Inhibition Concentration 50% (IC_{50}) of *gendarussin* A is 235.3 ppm.

In conclusion, this fact is referred to the protency and strength of *gendarussin* A and other active compound of Jucticia gendarussa leaf in inhibition of HIV replication.

CONCLUSSION

In conclusion, this fact is referred to the protency and strength of gendarussin A and other active compound of Jucticia gendarussa leaf in inhibition of HIV replication.

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