

IN SILICO SCREENING AND BIOLOGICAL EVALUATION OF THE COMPOUNDS OF  
*Justicia gendarussa* LEAVES EXTRACT AS INTERFERON GAMMA INDUCER:  
A STUDY OF ANTI HUMAN IMMUNODEFICIENCY VIRUS (HIV) DEVELOPMENT

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**Abstract**

**Background:** *Justicia gendarussa* Burm f. (Acanthaceae) has been known as traditional medicine in Indonesia. It contains flavonoids and alkaloids. This study was conducted to evaluate the effect of *J. gendarussa* on the profile of IFN- $\gamma$  on mice (*Mus musculus*). Molecular docking test was also conducted to determine the interaction of alkaloids and flavonoids on the *J. gendarussa* leaves against IFN- $\gamma$  receptor. It is expected that this research will provide scientific information on the development of *J. gendarussa* leaves as an anti-HIV drug.

**Materials and Methods:** The molecular docking test was performed by using Molegro Virtual Docker software to predict the interaction of alkaloid and flavonoid compounds of *J. gendarussa* leaves with IFN- $\gamma$  receptor. In the in vivo test, the effects of 70% ethanol extract, fractionated 70% ethanol extract, and water extract of *J. gendarussa* leaves were evaluated on the profile of IFN- $\gamma$  stimulation on mice (*Mus musculus*). The test was performed by administering the three gendarussa extracts into the nine groups of mice for 14 days.

**Results:** Based on the molecular docking test, it was found that flavonoid of *J. gendarussa* leaves have lower effects on the IFN- $\gamma$  receptor than the alkaloids. From the in vivo test on mice, it was found that the fractionated 70% ethanol extract of *J. gendarussa* leaves did not induce the level of IFN- $\gamma$ . On the other hand, both 70% ethanol and water extract of *J. gendarussa* leaves induced the production of IFN- $\gamma$ .

**Conclusion:** Fractionated 70% ethanol extract of *J. gendarussa* does not induce the production of IFN- $\gamma$ , so it can be developed as anti HIV drugs.

**Keywords:** HIV, *Justicia gendarussa* Burm f., Interferon, IFN- $\gamma$

**Introduction**

*Justicia gendarussa* Burm f. (Acanthaceae) has been known and used by many people in Papua, Indonesia, as a male contraception agent (Moeso and Agus 1985). This plant has many local names, such as besi-besi (Acehnese), gendarusa (Malay), handarusa (Sundanese), gondorusa or tetean or trus (Javanese). In the other countries, the plant is known as gandarusa, temenggong melela, urat sugi (Malaysian Malay), chin chiu (Chinese), mala bulak (Filipino), Ciang phraa mon (Thai) (Dalimartha, 2001). *J. gendarussa* spreads in tropical areas, such as : Pakistan, India, Sri Lanka, Indonesia, China, Thailand, Malaysia and Philippines. *J. gendarussa* grows wild in forests or river embankments. It is also planted as a medicinal plant or hedgerows. *J. gendarussa* grows well at 1-500 meters above the sea level. It has a shape of herbs that grow vertically to 0.8-2 meters high. Its stem is woody, branched and segmented with shiny blackish brown color. The leaves of this plant are single, short-stemmed, that lies opposite crossed. The shape of the leaves is lancet with a flat edge, a tapered end, a wedge-shaped base, and have a pinnate bone. Their color is dark green and they grow to 5-20 cm in length and 1-3.5 cm in width (Dalimartha, 2001).

Nowadays, an anti-HIV drug is being developed from *Justicia gendarussa* Burm. f. Several in vitro studies have been conducted, including the hexane, methanol and ethanol extracts of *J. gendarussa* on the inhibition of HIV virus. Based on the studies, the methanol and ethanol extracts of *J. gendarussa* could decrease the number of HIV virus (Yuliangkara, 2010). The major compound in the 70% ethanol extract of *J. gendarussa* leaves is a flavonoid apigenin glycoside called Gendarusin A (Prajogo et al., 2010b). It was reported that Gendarusin A could inhibit the growth of

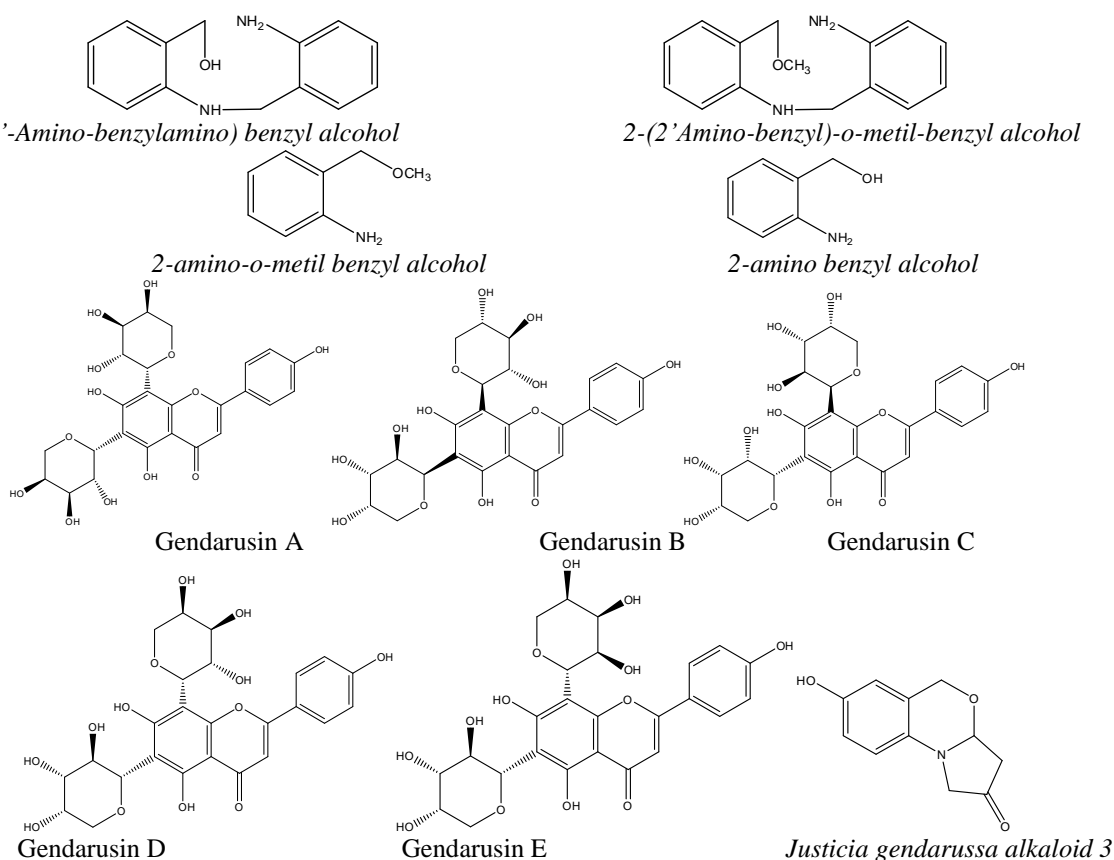
virus in the blood plasma of patients with HIV (Prajogo *et al.*, 2010a). In addition, several class of flavonoids, such as apigenin, kaempferol, luteolin, mirisetin, quersetin, hesperitin, naringenin, catechin hydrate, apikatekin, galokatekin, epigallokatekin, amentoflavon, daidzein, genistein and skutellarein, showed the activity of anti HIV *in vitro* by the mechanism of inhibition of reverse transcriptase and protease enzymes (Yeon-Ju *et al.*, 2009).

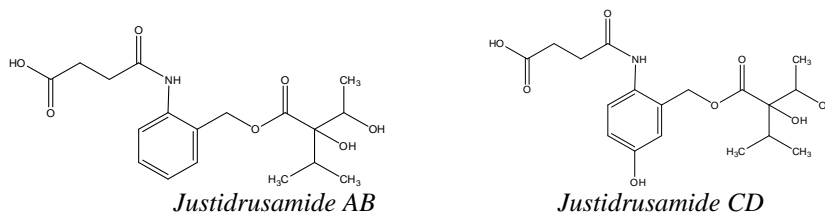
Another research regarding *J. gendarussa* was also done to examine the activity of water and ethanol extracts of *J. gendarussa* as anti-HIV agents. The results of the study indicated that, at the same concentration of 200 µg/mL, the inhibitory activity of the water extract against reverse transcriptase of HIV-1 was greater than the ethanol extract (Woradulayapinij *et al.*, 2005). Furthermore, a study with a 70% ethanol extract and a fractionated 70% ethanol extract of *J. gendarussa* leaves showed that the extracts had the activity to inhibit HIV-1 reverse transcriptase activity with IC<sub>50</sub> of respectively 220.98 ppm and 393.02 ppm (Riza, 2014).

Alkaloids, lignans, flavonoids and terpenoids (iridods, diterpenoids and triterpenoids) are frequently found in Acanthaceae. Flavonoids apigenin and vitexin have been isolated from ethanol extract of *J. gendarussa* (Correa and Alcantara, 2011). *J. gendarussa* leaves also contains of potassium, justisin, steroids or triterpenoids, and tannins. It is also containing essential oils, calcium oxalate, and quite toxic alkaloids (Dalimartha, 2001). Some alkaloids has been isolated from the leaves, i.e.: 2-amino benzyl alcohol, 2-amino-*o*-methyl benzyl alcohol, 2-(2'-amino-benzylamino)benzyl alcohol, 2-(2'amino benzyl)-*o*-methyl-benzyl alcohol (Chakravarty *et al.*, 1982).

Previously, it were reported that at least 12 flavonoids could be detected from *n*-butanol extract of *J. gendarussa* leaves. The main flavonoid in the extract was characterized to be Gendarusin A. Although as a minor constituent, Gendarusin B, Gendarusin C, Gendarusin D, and Gendarusin E was also found in the extract (Prajogo *et al.*, 2010b).

In order to develop a phytopharmaceutical preparation of anti-HIV that meets the requirements of safety, quality and efficacy, WHO has set some steps that should be taken to determine whether a traditional medicine is potential as an anti-HIV. The steps include are: 1) The provision of the extracts that are going to be analyzed and which is followed by the use of an *in vitro* test method to detect the activity of the compounds in the plants and to determine if there is a potential toxicity; 2) Research should be terminated if it is known that the toxicity of the plant is greater than the anti-HIV activity; 3) An extract should be purified and a review on the content of the extract should be done; 4). An extract that shows an interferon-inducer activity should be examined separately; and 5) Antiviral activity and toxicity of an extract in several cell systems should be confirmed. If the toxicity of the extract is no greater than the anti-HIV activity, the research can be continued (WHO, 1989).





**Figure 1:** Structures of flavonoids that were characterized from a n-butanol extract of *J. gendarussa* leaves

Interferon (IFN) is a cytokine produced in response to viral and microbial infections. The main characteristic of interferon is its ability to inhibit the growth of virus by inducing an antiviral state in the cells. Interferons in human are proteins that consist of 165-208 amino acid residues and are mostly modified by the process of glycosylation by post translation. Interferons are divided based on their amino acid sequence and their receptors. Type I IFN consists of IFN- $\alpha$  and IFN- $\beta$ . Both of these interferons can be induced by a viral infection in any kinds of cells. The only member of the type II IFN is IFN- $\gamma$ . This type of interferon is not related to IFN I and it involves IFN- $\gamma$  heterodimeric receptor (IFNGR). IFN II is produced mainly by the activation of T and NK cells (Fensterl, 2009).

Therapeutic approaches that involve immunomodulatory compounds can improve the effectiveness of anti-HIV therapy. Ideally, the drug can stimulate the immune response that increases HIV-infected cell destruction. The reduction of the number of infections can lower the dose of ARVs that are considered toxic. However, it is hypothesized that the activation of the immune system can increase the number of the infected host cells that increases the HIV proliferation (Clerici *et al.*, 2000). Interferon is determined by the effectiveness of the antivirus. The virus can be eliminated from the infected cells, but on the other hand, the uninfected cells can develop antiviral state after the interferon exposure (Fensterl, 2009).

In this study, the effect of 12 compounds in *J. gendarussa* leaves on the stimulation of IFN- $\gamma$  was tested. The molecular docking test was performed by using Molegro Virtual Docker software to predict the interaction of alkaloid and flavonoid compounds of *J. gendarussa* leaves with IFN- $\gamma$  receptor. Then the *in vivo* test on mice (*Mus musculus*) was also conducted to find out the effects of 70% ethanol extract, fractionated 70% ethanol extract, and water extract of *J. gendarussa* leaves on the profile of IFN- $\gamma$  stimulation.

## Materials and Methods

### *In silico* study

The molecular docking test is performed to determine the interaction of the alkaloid and flavonoid compounds of *J. gendarussa* leaves with IFN- $\gamma$  receptor. The structure of the alkaloid and flavonoid compound is determined by ChemBioOffice Ultra version 12.0 software. The structure of the IFN- $\gamma$  receptor is obtained from Protein Data Bank (<http://www.pdb.org/pdb/home/home.do>) with 2R3Z code. Molecular docking analysis is performed by using Molegro Virtual Docker (MVD) version 5.0 software. From molecular docking test, it obtains the re-rank score. Then the re-rank score is used as activity prediction.

### *In vivo* study

#### Plant Materials and Extraction Procedure

Leaves of *J. gendarussa* were obtained from a cultivated crop in Pacet, Mojokerto, East Java province, Indonesia. This plant was identified by the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University under the voucher number 18/H3.1.5/DT/2012. The dried powder of *J. gendarussa* leaves was divided into two groups: acidified leaves powder to release alkaloids and non-acidified leaves powder. Both powders were extracted using 70% ethanol for 3x24 hours in a macerator device, and then the resulting filtrate were concentrated using a rotary evaporator. The extract is dried at 50°C a temperature of to obtain a 70% ethanol extract (17.4% w/w) and fractionated-70% ethanol extract (6.4% w/w) of *J. gendarussa* leaves. Water extract of leaves of *J. gendarussa* is made by blending fresh *J. gendarussa* leaves in cold water. Then the resulted filtrate was collected and was dried using the freeze-dry method to obtain water extracts (1.8% w/w) of *J. gendarussa* leaves.

#### Laboratory Animals

The animals used were 6-7 weeks of age BALB/c mice, weighed 25-30 grams, and were not in a state of estrus. The mice had been adapted in the laboratory for 7 days to optimize their body condition to the new environment. The animal adaptation was done due to the ethical clearance of the laboratory animal treatment. Animals should be free from hunger and thirst, discomfort, pain, injury and disease, fear and stress in the long-term. They also should be free to express their natural behavior, have enough space to move, and get the appropriate facilities (Ridwan, 2013).

The animals were fed and watered regularly every morning and evening. The health was monitored to ensure that no animal got injured due to fighting among the animals in a single cage. The animals were euthanized on the 15<sup>th</sup>

day after they received treatment. The animals were made unconscious by giving them anesthetic using ether. Subsequently, intra cardiac blood samples were drawn by doing surgery to take the blood from their heart. The blood samples were sucked up using 1 ml syringe and collected in lithium heparin tube and then centrifuged at 20000 rpm for 15 minutes to obtain blood plasma. The samples obtained were stored at -80 °C for being tested in the following day (Clerici *et al.*, 2000).

## Results

### *In Silico* Study

The parameter values of physico-chemical properties of the compounds of *J. gendarussa* Burm f. leaves were determined using the program ChemBio Office Ultra 11.0. The results of the analysis can be seen in the Table 1.

**Table 1:** The parameter value of physico-chemical properties of the compounds of *J. gendarussa* Burm f. leaves

Compound	BM	LogP	ClogP	MR	CMR
2-Amino benzyl alcohol	228,13	1,89	0,774	71,05	7,0179
2-Amino- <i>o</i> -metil benzyl alcohol	137,18	1,02	0,663	42,12	4,138
2-(2'-Amino-benzylamino) benzyl alcohol	123,07	0,66	-0,173	37,37	3,6742
2-(2'Amino-benzyl)- <i>o</i> -metil-benzyl alcohol	242,14	2,25	1,61	75,81	7,4817
Gendarusin A	534,47	-2,27	-1,18271	129,92	12,6415
Gendarusin B	534,14	-2,27	-1,18271	129,92	12,6415
Gendarusin C	534,14	-2,27	-1,18271	129,92	12,6415
Gendarusin D	534,47	-2,27	-1,18271	129,92	12,6415
Gendarusin E	534,47	-2,27	-1,18271	129,92	12,6415
<i>Justicia gendarussa</i> alkaloid 3	205,07	0,65	0,128649	54,63	5,3634
<i>Justidrusamide</i> AB	369,14	-0,62	-1,146	88,17	9,0317
<i>Justidrusamide</i> CD	383,16	-0,36	-0,6712	93,6	9,4955

Hydrogen receptor interferon (2R3Z) binding interaction with Amixin and the *J. gendarussa* Burm f. compounds can be seen in Table 2.

**Table 2:** Hydrogen receptor interferon (2R3Z) binding interaction with 2,7-Bis(2- diethylaminoethoxy)fluoren-9-one (Amixin) and the *J. gendarussa* Burm f. compounds

Compound	Amino Acid						
	Ile 24	Lys 46	Arg 22	Arg 20	Arg 52	Lys 47	Pro 18
+	+	+	-	-	-	-	-
A	-	-	+	-	-	-	-
B	-	-	-	-	-	-	-
C	-	-	+	-	-	-	-
D	-	-	++	+	-	-	-
E	-	-	++	-	+	-	-
F	-	-	++	-	+	-	-
G	-	-	-	++	-	+	-
H	-	-	++	-	+	-	-
I	+	+	+	-	+	-	-
J	-	-	-	-	-	+	-
K	-	-	+	-	-	-	+
L	-	-	+	-	-	-	+

Steric receptor interferon (2R3Z) binding interaction with 2,7-Bis(2-diethylaminoethoxy) fluoren-9-one (Amixin) and the *J. gendarussa* Burm f. compounds are presented in table 3.

**Table 3:** Steric receptor interferon (2R3Z) binding interaction with 2,7-Bis(2- diethylaminoethoxy) fluoren-9-one (Amixin) and the *J. gendarussa* Burm f. compounds

Compound	Amino Acid												
	Lys 46	Ile 24	Arg 52	Val 19	Ala 23	Arg 22	Ala 23	Pro 18	Arg 20	Lys 47	Met 45	Gly 17	Val 19
+	+	+	+	+	+	-	-	-	-	-	-	-	-
A	-	-	-	-	-	+	++	-	-	-	-	-	-
B	-	-	-	+	-	+	+	+	-	-	-	-	-
C	-	-	-	-	-	+	+	-	-	-	-	-	-
D	-	-	-	+	-	+	+	-	-	-	-	-	-
E	-	-	+	+	+	+	-	+	+	-	-	-	-
F	-	-	+	+	-	+	-	+	+	-	-	-	-
G	+	-	+	-	-	+	+	-	-	+	+	-	-
H	+	-	+	+	+	+	-	+	+	-	+	-	-
I	+	+	+	+	+	+	-	+	+	-	+	-	-
J	+	-	-	+	+	-	-	-	-	+	-	-	-
K	-	+	-	+	-	+	-	+	-	-	+	-	-
L	-	+	-	-	-	+	-	+	+	-	+	+	+

Explanation:

A : 2-(2'-Amino-benzylamino) benzyl alcohol  
 B : 2-Amino-o-metil benzyl alcohol  
 C : 2-Amino benzyl alcohol  
 D : 2-(2' amino-benzyl)-o-metil-benzyl alcohol  
 E : Gendarusin A  
 F : Gendarusin B

G : Gendarusin C  
 H : Gendarusin D  
 I : Gendarusin E  
 J : *Justicia gendarussa* alkaloid 3  
 K : *Justidrusamide AB*  
 L : *Justidrusamide CD*

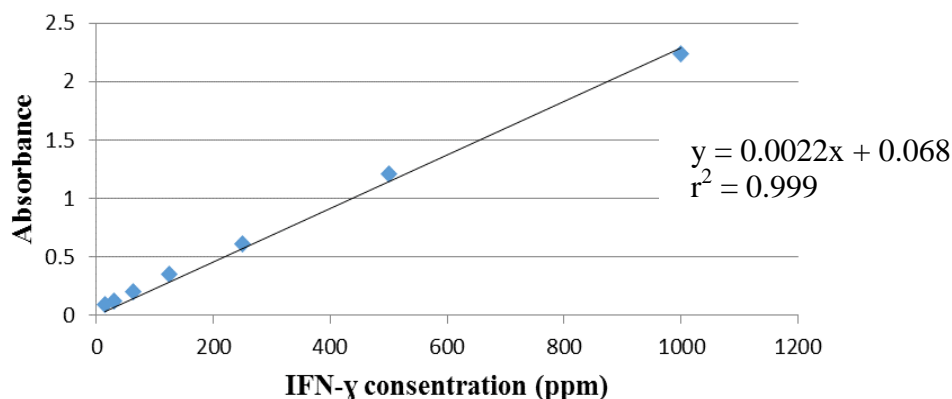
The docking scores of the 12 compounds of *J. gendarussa* Burm f. leaves and 2,7-Bis (2-diethylaminoethoxy) fluoren-9-one (Amixin) in the interaction with interferon receptor (2R3Z) are presented in table 4.

**Table 4:** The docking scores of the 12 compounds of *J. gendarussa* Burm f. leaves and 2,7-Bis(2-diethylaminoethoxy)fluoren-9-one (Amixin) in the interaction with interferon receptor (2R3Z)

Compound	MolDock Score	Rerank Score	Hbond
Amixin	-106,709	-64,8847	-2,61402
2-Amino benzyl alcohol	-75,0046	-58,3096	0
2-Amino-o-metil benzyl alcohol	-72,4387	-46,7266	-2,2733
2-(2'-Amino-benzylamino) benzyl alcohol	-48,4629	-25,0705	-1,92141
2-(2' Amino-benzyl)-o-metil-benzyl alcohol	-43,8892	-23,7146	-5,02994
Gendarusin A	-65,1477	10,8063	-8,57626
Gendarusin B	-63,7849	46,7675	-6,21807
Gendarusin C	-51,8104	-46,0295	-8,31234
Gendarusin D	-62,8825	-27,2349	-6,95245
Gendarusin E	-72,3911	-3,01849	-8,17422
<i>Justicia gendarussa</i> alkaloid 3	-57,2643	-36,5153	-2,27018
<i>Justidrusamide AB</i>	-89,187	-66,4454	-3,30514
<i>Justidrusamide CD</i>	-106,709	-64,7522	-4,4665

### In Vivo Study

Before measuring the amount of IFN- $\gamma$  in the serum of mice which were treated with the sample, it was necessary to determine the standard curve of IFN- $\gamma$  with various concentrations of 15.6; 31.3; 62.5; 125; 250; 500 and 1000 pg/ml against the absorbance of the solution. The Absorbance of FN- $\gamma$  at various concentrations measured at 450 nm with microplate absorbance ELISA reader are presented in figure 2.



**Figure 2:** Graphic of linearity of the IFN- $\gamma$  standard concentration against absorbance measured at 450 nm using a microplate absorbance ELISA reader

The effects of the various doses of *J. gendarussa* leaves extracts on the profile of IFN- $\gamma$  on mice are presented in table 5.

**Table 5:** The effects of various doses of *J. gendarussa* leaves extracts compared to the negative control and the positive control measured at 450 nm using a microplate absorbance ELISA reader

Sample	Dosage of extract	Absorbance Average $\pm$ SD	IFN- $\gamma$ (ppm)
CMC Na 2% (Negative control)	CMC Na 2%	0.061 $\pm$ 0.002	15.151
Stimuno tablet <sup>®</sup> (Positive control)	6.5 mg/kgBW	0.064 $\pm$ 0.005	190.455
Ethanol extract 70% I	0.571 g/kgBW	0.059 $\pm$ 0.002	66.818
Ethanol extract 70% II	1.114 g/kgBW	0.058 $\pm$ 0.002	115.758
Ethanol extract 70% III	2.228 g/kgBW	0.059 $\pm$ 0.002	330.455
Fractionated 70% ethanol extract I	0.571 g/kgBW	0.055 $\pm$ 0.005	42.424
Fractionated 70% ethanol extract II	1.114 g/kgBW	0.061 $\pm$ 0.006	41.970
Fractionated 70% ethanol extract III	2.228 g/kgBW	0.064 $\pm$ 0.003	40.152
Water extract I	0.489 g/kgBW	0.061 $\pm$ 0.002	72.879
Water extract II	0.977 g/kgBW	0.057 $\pm$ 0.004	149.394
Water extract III	1.954 g/kgBW	0.063 $\pm$ 0.006	339.546

## Discussion

Based on the in-silico study on the flavonoids in *J. gendarussa* leaves, such as: Gendarusin A, Gendarusin B, Gendarusin C, Gendarusin D, and Gendarusin E showed that the five flavonoids have specific amino acids bond that similar with the positive control. The alkaloids compounds, such as: 2-(2'-amino-benzylamino)benzyl alcohol, 2-amino-o-methyl benzyl alcohol, 2-amino benzyl alcohol, 2-(2' amino-benzyl)-o-methyl- benzylalkohol, Justidrusamide A, Justidrusamide B, Justidrusamide C, and Justidrusamide D are also have the specific amino acids bond that similar with the positive control.

The reranked score value was also used to predict the IFN- $\gamma$  inducer activities. It represents the bond energy. The bond energy is the energy required to bind the ligand to its receptor. The lower the binding energy is, the more stable and easier the binding of ligand-receptor. The more stable ligand receptor binding, the greater the activity (Thomson and Christensen, 2006). The rerank scores the *J. gendarussa* compounds that presented at table 4 showed that the flavonoids, i.e: Gendarusin A, Gendarusin B, Gendarusin C, Gendarusin D and Gendarusin E, has higher rerank score than the alkaloids, i.e: Alkaloid Justridisamide A, Justridisamide B, Justridisamide C, and Justridisamide D. From the table it is showed that the alkaloids group has similar rerank score with the positive control.

The in vivo test on mice data were analyzed using SPSS 23 and Tukey and Bonferroni test. From the analysis, it is known that the 70% ethanol extract of *J. gendarussa* leaves group at a dose of 2.23 g/kgBW, 1.11 g/kgBW and the water extract of *J. gendarussa* leaves groups at a dose of 1,95 g/kgBW, 0.98 g/kgBW had significant differences with the negative control. On the other hand, the fractionated 70% ethanol extract of *J. gendarussa* leaves groups at a dose of 2.23 g/kgBW, 1.11 g/kgBW and 0.57 g/kgBW, the 70% ethanol extract of *J. gendarussa* leaves group at dose of 0.57 g/kgBW and the water extract of *J. gendarussa* leaves groups at a dose of 0.49 g/kgBW showed no significant differences with the negative control.

The results of the study conform to the comparison results of the IFN- $\gamma$  values of the nine treatment groups with the positive control using Tukey and Bonferroni test. The results demonstrated that there were no significant differences of the 70% ethanol extract of *J. gendarussa* leaves groups at a dose of 2.23 g/kgBW; 1.11 g/kgBW and the water extract of *J. gendarussa* leaves groups at a dose of 1.95 g/kgBW; 0.98 g/kgBW. However, the results also indicated significant differences of fractionated 70% ethanol extract of *J. gendarussa* leaves at a dose of 2.23 g/kgBW, 1.11 g/kgBW, and 0.57 g/kgBW; 70% ethanol extract of *J. gendarussa* leaves with at a dose of 0.57 g/kgBW; and a water extract at a dose of 0.49 g/kgBW.

From the in vivo study, it can be concluded that 70% ethanol extract and water extract of *J. gendarussa* increase the levels of IFN- $\gamma$ . On the other hand, fractionated 70% ethanol extract of *J. gendarussa* leaves has no effect on IFN- $\gamma$  mice. This in vivo study results conform with the in-silico test. The fractionated 70% ethanol extract of *J. gendarussa* leaves has been freed from alkaloids through the process of acidification. As in the silico study result, it is showed that the alkaloids may give a great influence on the induction of IFN- $\gamma$ .

Based on the WHO standards that mention the requirements of the phytopharmaceutical preparation of anti-HIV drugs should not have the interferon-inducer activities, it can be concluded that the fractionated 70% ethanol extract of *J. gendarussa* leaves can be developed into the anti HIV drugs.

## Conclusion

From the study, it can be concluded that fractionated 70% ethanol extract of *J. gendarussa* does not induce the production of IFN- $\gamma$ , while both 70% ethanol extract and water extract of *J. gendarussa* leaves have the effect of IFN- $\gamma$  inducer at a dose of 1.11 g/kgBW and 2.23 g/kgBW for 70% ethanol extract and 0.98 g/kgBW and 1.95 g/kgBW for water extract of *J. gendarussa* leaves. Based on the result, it is also can be concluded that the fractionated 70% ethanol extract of *J. gendarussa* can be developed into the anti HIV drugs.

**Conflict of Interest:** The authors declare that there are no conflicts of interest.

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## References

1. Chakravarty, AK., Dastiar, PPG., and Pakrashi, SC. (1982). Simple Aromatic Amines from *Justicia gendarussa* 13C NMR Spectra of the Bases and Their Analogues. *Tetrahedron*. Elsevier, 18 (12):1797-1802.
2. Clerici, M., Seminari, E., Suter, F., Castelli, F., Pan, A., Biasin, M., Colombo, F., Trabattoni, D., Maggiolo, F., Carosi, G. and Maserati, R.. (2000). Different Immunologic Profiles Characterize HIV Infection in Highly Active Antiretroviral Therapy-Treated and Antiretroviral-Naïve Patients with Undetectable Viraemia. *AIDS* 2000, 14(2):109-116
3. Corrêa, GM and Alcântara, AF. de C. (2011). Chemical Constituents and Biological Activities of Species of *Justicia*. *Brazilian Journal of Pharmacognosy* 22 (1): 220-238
4. Dalimartha S. (2001). Atlas Tumbuhan Obat Indonesia. Jilid 1. Jakarta: Trubus Agriwidya.
5. Fensterl, V. And Sen, GC. (2009). Interferons and Viral Infections. *International Union of Biochemistry and Molecular Biology*. 35(1):14-20.
6. Prajogo, B.E.W., Nasronudin, Widiyanti, P., Aksono, EB. (2010a). Pengaruh Isolat Gendarusin A daun *Justicia gendarussa* Burm. f pada Penghambatan Reverse Transcriptase tipe 1 HIV In Vitro. Laporan Akhir Hibah Penelitian Strategis Nasional 2010, Surabaya: Universitas Airlangga.
7. Prajogo, B.E.W., Nasronudin., Cholies, N., Neni. (2010b). Development of *Justicia gendarussa* Burm. f as anti-HIV. Joint Symposium Emerging & Re-emerging Infections Diseases Update III From Research to Clinical Practice with Stem Cell II Symposium. Graha BIK Iptekdok – Fakultas Kedokteran UNAIR.
8. Riza H., 2014. Pengaruh Ekstrak Etanol 70% Daun *Justicia gendarussa* Burm.F Terhadap Aktivitas Enzim Reverse Transcriptase Hiv In Vitro. Tesis. Fakultas Farmasi, Universitas Airlangga, Surabaya.
9. Woradulayapinij W, Soonthornchareonnon N, and Wiwat C, (2005). *In vitro* HIV Type 1 Reverse transcriptase Inhibitory Activity of Thai Medicinal Plants and *Canna indica* L. *rizhomes, Journal of Ethnopharmacology* 101, p. 84-89.
10. World Health Organisation (1989). *In vitro* screening of traditional medicines for anti-HIV activity: Memorandum from a WHO meeting. *Bulletin of the World Health Organization*, vol. 87, No. 6, p. 613-618.
11. World Health Organization, (2013). Global Report: UNAIDS Report on the Global AIDS Epidemic 2013. WHO Library Cataloguing-in-Publication Data.

12. Yeon-Ju K, Hyun-Jeong O, Hyo-Min A, Ho-Jung Kang, Jung-Hyun K, and Young-Hwan K, 2009. Flavonoids as Potential Inhibitors of Retroviral Enzymes, *Journal of the Korean Society for Applied Biological Chemistry* 52, p.321-326.
13. Yuliangkara B, 2010. Pengaruh Ekstrak Heksan, Metanol, dan Etanol Tanaman Obat *Justicia gendarussa* Burm.f terhadap Virus HIV In vitro, Skripsi, Fakultas Farmasi, Universitas Airlangga, Surabaya.