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5 messages

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Clement Adewunmi <athmsi2012@gmail.com>
To: Restry Sinansari <r.sinansari@gmail.com>  
Tue, Jan 30, 2018 at 4:41 PM

Dear Authors,

Please check your paper for errors. Please do not send back the whole paper but only portions that need to be corrected in MS word, stating the page, paragraph and lines where required in the paper. But if you could not identify any error in your script, kindly give your consent to publishing the article as it is.

Best regards

--

COJA House,  
7, Road 1, Otnnmaiye Square, Ajaibamidele,  
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Restry Sinansari <r.sinansari@gmail.com>
To: Agustinus Widodo <aureliasesy1@gmail.com>  
Thu, Feb 8, 2018 at 11:50 AM

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Restry Sinansari <r.sinansari@gmail.com>
To: Clement Adewunmi <athmsi2012@gmail.com>  
Cc: "Dr.Widiyanti,dr.g,M.Kes Wibowo" <drwidiyanti@yahoo.com>, Bambang Wardowo <prajogo_eu@yahoo.com>, gseid Seminar <gseid2016@gmail.com>  
Thu, Feb 8, 2018 at 2:25 PM

Dear Mr. Clement,  
Herewith I send you my proofread revision of my jurnal  
Thank you for you kind review

Best regads,  
Restry  
[Quoted text hidden]

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**v12sajid-sinansarietal rev 20180208.doc**

342K
Clement Adewunmi <athmsi2012@gmail.com>  
To: Restry Sinansari <r.sinansari@gmail.com>  

Dear Author,

Your manuscript have been redacted and its hereby attached for your perusal. Kindly let us know if the changes made are adequate and if we could proceed with publishing the script.

Warm regards

[Quoted text hidden]

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Restry Sinansari <r.sinansari@gmail.com>  
To: Clement Adewunmi <athmsi2012@gmail.com>  

Dear Mr. Clement,
I already read the attached manuscript.
You may proceed it with publishing the script
Just have one correction for table 2. The table are still have the vertical line
Thank you

Best regards,
Restry

Sent from my iPad

[Quoted text hidden]

<v12sajid-sinansarietal.pdf>
Submission for African Journal of Infectious Disease (AJID)

**gseid Seminar** <gseid2016@gmail.com>  Thu, Mar 2, 2017 at 12:54 PM

To: abdurachman abdurachman <rachman1166@yahoo.com>, Agustin Widodo <widodoagustinus@yahoo.com>, Aryati <dr_aryati@yahoo.com>, Betty pk <betty_pksurabaya@yahoo.co.id>, cck_cecilia <cck_ceciliah@yahoo.com>, Densy Violina <densy.viliona1980@gmail.com>, Desi Indriarini <desi.indriarini@gmail.com>, farindira vesti@gmail.com, Fauna Herawati <fauna@staff.ubaya.ac.id>, gondo mastutik <gondomastutik@gmail.com>, heni puspitasari <heni.puspitasari486@gmail.com>, Kurnia Dwi <kurnia.dwi.z@gmail.com>, Ninadifa Muflikah <ninadifa@gmail.com>, prihartini widiyanti <pwidiyanti@fst.unair.ac.id>, Resty Sinansari <r.sinansari@gmail.com>, Retno Budianti <retnobudianti@yahoo.com>, Ricardo Adrian Nugraha <ricardo.unair@gmail.com>, Dharin Serebrina Arfiputri <dharinserebrina@yahoo.com>

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Dear all GSEID participants,

Thank you for your patience during the journal reviewing process of the International Seminar GSEID 2016.

By writing this email, we would like to announce that the submission of your journal could be registered from now in **African Journal of Infectious Diseases (AJID)**.

**Kindly submit your manuscript on its website:** [http://ajid.edmgr.com/](http://ajid.edmgr.com/).

Your manuscript must be followed the AJID guidelines on its website.

Herewith we attach the following steps of registering your manuscript.

The manuscript that has been submitted, must pay $20. Importantly, the payment should be 3 days after the submission. Furthermore, whether your journal is accepted, you are required to pay $300.

The due date for submission is **March 16, 2017**. Kindly submit your manuscript before the date.

We are thankful for your cooperation and consideration.

Sincerely yours,

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GSEID Committee
"Global Strategy to Combat Emerging Infectious Diseases " in Boderless Era
Faculty of Medicine - Institute of Tropical Disease, Universitas Airlangga
Jl. Mayjen Prof. Dr. Moestopo 47 - Kampus C UNAIR Mulyorejo, Surabaya, East Java, Indonesia
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3 attachments

- [Steps Submitting manuscript on AJID.pdf](attachment:Steps Submitting manuscript on AJID.pdf) 457K
- [Instruction for Author.pdf](attachment:Instruction for Author.pdf) 30K
- [Sample Article - AJID.pdf](attachment:Sample Article - AJID.pdf) 228K
Submission Confirmation for AJID-D-17-0

African Journal of Infectious Diseases <em@editorialmanager.com> to me

Ref.: Ms. No. AJID-D-17-00035R1
IN SILICO SCREENING AND BIOLOGICAL EVALUATION OF THE CO INDUCER (A STUDY OF ANTI HIV)

Dear Ms Sinansari,

African Journal of Infectious Diseases has received your revised submis

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Kind regards,

Restry Sinansari <r.sinansari@gmail.com> to wati_lana
Submission Confirmation for IN SILICO SCREENING AND BIOLOGICAL EVALUATION OF THE COMPOUNDS OF Justicia gendarussa L.

African Journal of Infectious Diseases <em@editorialmanager.com> to me

Dear Ms Sinansari,

Your submission entitled "IN SILICO SCREENING AND BIOLOGICAL E INTERFERON GAMMA INDUCER (A STUDY OF ANTI HIV)" has been

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African Journal of Infectious Diseases <em@editoralmanager.com> to me

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IN SILICO SCREENING AND BIOLOGICAL EVALUATION OF THE CO INDUCER (A STUDY OF ANTI HIV)
African Journal of Infectious Diseases

Dear Ms Sinansari,

Reviewers have now commented on your paper. You will see that they, required, I would be pleased to reconsider my decision.

For your guidance, reviewers’ comments are appended below.

If you decide to revise the work, please submit a list of changes or a reb

Your revision is due by Aug 17, 2017.

To submit a revision, go to <http://ajid.edmgr.com/> and log in as an autho

Yours sincerely
Abstract

Background: Justicia gendarussa Burm f. (Achanthaceae) has been known as traditional medicine in Indonesia. It contains of flavonoids and alkaloids. This study was conducted to evaluate the effect of J. gendarussa on the profile of IFN-γ 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-γ 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-γ 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-γ 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-γ 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-γ. On the other hand, both 70% ethanol and water extract of J. gendarussa leaves induced the production of IFN-γ.

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

Keywords: HIV, Justicia gendarussa Burm f., Interferon, IFN-γ

Introduction

Justicia gendarussa Burm f. (Achanthaceae) 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), gondorus or tetean or trus (Javanese). In the other countries, the plant is known as gendarusa, 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 140
viruses in the blood plasma of patients with HIV (Prajogo et al., 2010a). In addition, several classes of flavonoids, such as apigenin, kaempferol, luteolin, mirisetin, quersetin, hesperitin, naringenin, catechin hydrate, apikatekin, galokatekin, epigallocatekin, 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 IC₅₀ of respectively 220.98 ppm and 393.02 ppm (Riza, 2014).

Alkaloids, lignans, flavonoids and terpenoids (iridoids, 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 contain potassium, justisin, steroids or triterpenoids, and tannins. It is also containing essential oils, calcium oxalate, and quite toxic alkaloids (Dalimartha, 2001). Some alkaloids have been isolated from the leaves, i.e.: 2-amino benzyl alcohol, 2-amino-0-methyl benzyl alcohol, 2-(2′-amino-benzylamino) benzyl alcohol, 2-(2′amino benzyl)-o-methyl-benzyl alcohol (Chakravarty et al., 1982).

Previously, it was 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 were 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).

![Chemical structures of Gendarusin A, B, C, D, E and Justicia gendarussa alkaloid 3](image-url)
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-α and IFN-β. 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-γ. This type of interferon is not related to IFN I and it involves IFN-γ 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-γ 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-γ 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-γ 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-γ receptor. The structure of the alkaloid and flavonoid compound is determined by ChemBioOffice Ultra version 12.0 software. The structure of the IFN-γ 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 15th
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>
<thead>
<tr>
<th>Compound</th>
<th>BM</th>
<th>LogP</th>
<th>ClogP</th>
<th>MR</th>
<th>CMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Amino benzyl alcohol</td>
<td>228,13</td>
<td>1,89</td>
<td>0,774</td>
<td>71,05</td>
<td>7,0179</td>
</tr>
<tr>
<td>2-Amino-o-metil benzyl alcohol</td>
<td>137,18</td>
<td>1,02</td>
<td>0,663</td>
<td>42,12</td>
<td>4,138</td>
</tr>
<tr>
<td>2-(2'-Amino-benzylamino) benzyl alcohol</td>
<td>123,07</td>
<td>0,66</td>
<td>-0,173</td>
<td>37,37</td>
<td>3,6742</td>
</tr>
<tr>
<td>2-(2’Amino-benzyl)-o-metil-benzyl alcohol</td>
<td>242,14</td>
<td>2,25</td>
<td>1,61</td>
<td>75,81</td>
<td>7,4817</td>
</tr>
<tr>
<td>Gendarusin A</td>
<td>534,47</td>
<td>-2,27</td>
<td>-1,18271</td>
<td>129,92</td>
<td>12,6415</td>
</tr>
<tr>
<td>Gendarusin B</td>
<td>534,14</td>
<td>-2,27</td>
<td>-1,18271</td>
<td>129,92</td>
<td>12,6415</td>
</tr>
<tr>
<td>Gendarusin C</td>
<td>534,14</td>
<td>-2,27</td>
<td>-1,18271</td>
<td>129,92</td>
<td>12,6415</td>
</tr>
<tr>
<td>Gendarusin D</td>
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<td>-2,27</td>
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<tr>
<td>Gendarusin E</td>
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<td>-2,27</td>
<td>-1,18271</td>
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<td>12,6415</td>
</tr>
<tr>
<td><em>Justicia gendarussa</em> alkaloid 3</td>
<td>205,07</td>
<td>0,65</td>
<td>0,128649</td>
<td>54,63</td>
<td>5,3634</td>
</tr>
<tr>
<td><em>Justidrusamide AB</em></td>
<td>369,14</td>
<td>-0,62</td>
<td>-1,146</td>
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<td>9,0317</td>
</tr>
<tr>
<td><em>Justidrusamide CD</em></td>
<td>383,16</td>
<td>-0,36</td>
<td>-0,6712</td>
<td>93,6</td>
<td>9,4955</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ile 24</th>
<th>Lys 46</th>
<th>Arg 22</th>
<th>Arg 20</th>
<th>Arg 52</th>
<th>Lys 47</th>
<th>Pro 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
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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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Lys</th>
<th>Ile</th>
<th>Arg</th>
<th>Val</th>
<th>Ala</th>
<th>Pro</th>
<th>Arg</th>
<th>Lys</th>
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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)

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

In Vivo Study

Before measuring the amount of IFN-γ in the serum of mice which were treated with the sample, it was necessary to determine the standard curve of IFN-γ 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-γ at various concentrations measured at 450 nm with microplate absorbance ELISA reader are presented in figure 2.
The effects of the various doses of J. gendarussa leaves extracts on the profile of IFN-γ 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

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

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 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-γ 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 Justidrisamide A, Justidisamide B, Justidisamide C, and Justidisamide 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.

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The results of the study conform to the comparison results of the IFN-γ values of the nine treatment groups with the positive control using Tukey and Bonferroni test. The result 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-γ. On the other hand, fractionated 70% ethanol extract of J. gendarussa leaves has no effect on IFN-γ 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-γ.

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-γ, while both 70% ethanol extract and water extract of J. gendarussa leaves have the effect of IFN-γ 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.

Acknowledgements

The authors would like to thank the Collaborative Research Center for Emerging and Reemerging Infectious Disease (CRC-ERID), Institute of Tropical Disease (ITD), Airlangga University for supporting Biosafety Level-3 facility, and Prof. Dr. Siswandono, Apt., MS. from Faculty of Pharmacy, Airlangga University who has a license of the Molegro software.

References


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