VOL. 6. NO. 1 JANUARY–APRIL 2016

CYTOTOXICITY OF JUSTICIA GENDARUSSA BURM. F. LEAF EXTRACTS ON MOLT-4 CELL

Prihartini Widiyantia,b,1, Bambang Prajogoc, Ni Putu Ermi Hikmawantic

a Faculty of Science and Technology, Universitas Airlangga, Surabaya, East Java, Indonesia
b Institute of Tropical Disease (ITD), Universitas Airlangga, Surabaya, East Java, Indonesia
c Department of Pharmacognosy, Faculty of Pharmacy, Universitas Airlangga, Surabaya, East Java, Indonesia

Corresponding author: drwidiyanti@yahoo.com

ABSTRACT

Justicia gendarussa Burm. (Acanthaceae) is known for its activity as a male contraceptive and anti-HIV properties. The present study was designed to evaluate extracts of J. gendarussa for cytotoxicity activity against MOLT-4 cells. The cytotoxic activity of the fractionated-extract and 70% ethanol extracts of J. gendarussa leaves on MOLT-4 cells were evaluated using a WST-1 assay. The treatment cells, control cells without treatment and control media were also tested in duplicate. The absorbance was measured at a wavelength of 450 nm using a microplate absorbance reader (Bio-Rad). The average absorbance measures formazan produced by viable cells that metabolize the WST-1 reagent. Then the data was analyzed with regression analysis Microsoft Excel 2007 program to determine the concentration with 50% cell viability (50% Cytotoxicity Concentration, CC50). The CC50 values of the fractionated-extract and 70% ethanol extract of J. gendarussa leaves were 94 μg/ml and 78 μg/ml, respectively. The cytotoxicity of fractionated-extract and 70% ethanol extract of J. gendarussa leaves were not significantly different (p > 0.05). It can be concluded that the fractionated-extract and 70% ethanol extract of J. gendarussa leaves are not toxic to MOLT-4 cells.

Key words: cytotoxicity; Justicia gendarussa Burm.f; MOLT-4 cell; WST-1 assay, anti HIV

INTRODUCTION

Justicia gendarussa Burm.f. (Acanthaceae) leaves are often used in traditional medicine to treat fever, headache, rheumatism, myalgia, respiratory disorders, and back pain.1 J. gendarussa is also used in Papua as a male contraceptive. A pre-clinical study of an alkaloid-free 70% ethanol extract of J. gendarussa leaf extract has confirmed male contraceptive activity.2 The 70% ethanol leaf extract (with alkaloids and without alkaloids) from J.
**Materials and Methods**

**Materials**

**Plants**

*Justicia gendarussa* Burm. f. leaves used in this study were obtained from a cultivated crop in Trawas, Mojokerto, East Java-Indonesia. The medicinal plants were identified by Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya.

**Methods**

**Preparation of sample**

*J. gendarussa* leaves powder was divided into 2 fractions, a leaf powder with releasing alkaloids and a leaf powder with non-releasing alkaloids. Both powders were extracted using 70% ethanol during 324 hours in macerator and the filtrate obtained evaporated using a rotary evaporator (Buchi). The two extracts were dried at 50 °C until fractionated with 70% ethanol extract (alkaloid-free; 2.4% w/w) and 70% ethanol extract (10.8% w/w).

A stock solution was made by dissolving 100 mg of each extract in 1000 μl DMSO and then diluted using RPMI-1640 medium with 10% FBS to obtain various concentrations (7.8; 15.6; 31.3; 62.5; 125.0; 250.0; 500.0; and 1000.0 μg/ml) for each trial extract. The concentration of DMSO used was less than 1% which does not affect viability.

**Detection of Flavonoid in J. gendarussa Leaf Extract**

The content of gendarusin A, the major flavonoid in *J. Gendarussa* leaves, was analysed by a Waters HPLC (Agilent 1100, reverse phase NovaPack® column C-18 3.9150 mm using a water:methanol (30:70) eluent with a flow rate of 1 ml/min, and a UV detector at 254 nm wavelength).

**Cytotoxicity Assay**

Cytotoxicity of the extracts on MOLT-4 cells was measured using a colorimetric method with WST-1 reagent (Roche). Briefly, 50 μl MOLT-4 cells (110^5 cells/well) were plated in each well on a 96-well microplate. 50 μl of extract at various concentrations were also added to each well, and incubated for 72 hr at 37 °C in a 5% CO₂ incubator. The treatment cells, control cells without treatment and control media were also tested in duplicate. Total volume in each well was 100 μl. After 72 hr of incubation, 10 μl WST-1 reagents was added into each well and incubated
for an additional 2 hrs at 37 °C in a 5% CO₂ incubator. The absorbance was measured at a wavelength of 450 nm using a microplate absorbance reader (Bio-Rad). The average absorbance measures formazan produced by viable cells that metabolize the WST-1 reagent. The percentage cell viability was determined by the equation below:

\[
\text{Cell viability } (\%) = \frac{\text{treatment absorbance} - \text{control media absorbance}}{\text{control cells absorbance} - \text{control media absorbance}} \times 100
\]

### Statistical Analysis

The collected data was analyzed with regression analysis Microsoft Excel 2007 program to determine the concentration with 50% cell viability (50% Cytotoxicity Concentration, CC\textsubscript{50}). The comparison of cytotoxicity activities of both extracts were tested using a paired t-test analysis (Microsoft Excel 2007 program). The difference was considered to be significant if the probability was \( p < 0.05 \).

### RESULTS AND DISCUSSION

The leaf extracts of \( J. \) gendarussa tested had low toxicity to MOLT-4 cells with decreased MOLT-4 cell viability with increasing extract concentrations (Table 1). Based on regression analysis, the CC\textsubscript{50} of the 70% ethanol extract was 78 \( \mu \)g/ml, while fractionated-70% ethanol extract was 94 \( \mu \)g/m.

Table 1. Cytotoxicity test of 70% ethanol extract and fractionated-70% ethanol extract to MOLT-4 cells incubated for 72 hr

<table>
<thead>
<tr>
<th>Extract Concentration (( \mu )g/ml)</th>
<th>Cells Viabilities (%) ± SD</th>
<th>70% Ethanol Extract</th>
<th>Fractionated-70% Ethanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.8</td>
<td>101.4 ± 2.9</td>
<td>100.4 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>15.6</td>
<td>91.3 ± 4.9</td>
<td>89.8 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>31.3</td>
<td>72.2 ± 8.8</td>
<td>81.1 ± 12.1</td>
<td></td>
</tr>
<tr>
<td>62.5</td>
<td>51.2 ± 1.3</td>
<td>67.7 ± 6.1</td>
<td></td>
</tr>
<tr>
<td>125.0</td>
<td>35.1 ± 6.1</td>
<td>37.4 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>250.0</td>
<td>15.1 ± 6.1</td>
<td>22.5 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>500.0</td>
<td>8.9 ± 5.6</td>
<td>7.3 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>1000.0</td>
<td>3.2 ± 11.8</td>
<td>4.7 ± 0.9</td>
<td></td>
</tr>
</tbody>
</table>

A comparison of extract cytotoxicity activity was performed using a paired t-test analysis based on the percentage of cell viability for each treatment. Based on the t-test, \( t = -1.786, \) \( t \text{ table } (0.05) = ± 2.365, \) the significance value is 0.117 or 11.7% which is larger than 0.05 or 5%. Based on t-test results, there was no significant difference for cytotoxicity activity on MOLT-4 cells between the fractionated-70% ethanol extract and the 70% ethanol extract of \( J. \) gendarussa leaves (\( p > 0.05 \)).

HPLC chromatograms show gendarusin A content in the fractionated-70% ethanol extract (Fig. 1b) and the 70% ethanol extract (Fig. 1c) as a major flavonoid component of \( J. \) gendarussa leaves. Fractionated-70% ethanol extract and 70% ethanol extract of \( J. \) gendarussa leaves contain 0.53% and 0.95% of gendarusin A, respectively.

Cytotoxicity activity were evaluated to identify the relative toxicity of fractionated-70% ethanol extract and 70% ethanol extracts from \( J. \) Gendarussa leaves to MOLT-4 human T-lymphocytes line cells using a WST-1 test. The test is based on the reduction of tetrazolium sodium salt (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzendisulfonate) (water-soluble tetrazolium salt, WST-1) by a succinate-tetrazolium reductase system of the mitochondria respiratory chain. This enzyme system is only active in viable cells. The WST-1 reduction process produces soluble formazan with a bright colour. Absorbance measurements of formazan are directly related to cell viability.

The cytotoxicity activity of the extract was measured as the concentration of extract that reduces cell viability or cell growth by 50% (CC\textsubscript{50}). The cytotoxicity levels were based on previous studies where CC\textsubscript{50} values less than 20 \( \mu \)g/ml were considered as cytotoxic, 21–40 \( \mu \)g/ml as low cytotoxicity, and over 41 \( \mu \)g/ml as not cytotoxic.
Using this criteria, fractionated-70% ethanol extract (CC\textsubscript{50} 93 μg/ml) and 70% ethanol extract (CC\textsubscript{50} 78 μg/ml) of \textit{J. gendarussa} leaves are considered non-cytotoxic to MOLT-4 cells.

The cytotoxicity activity of fractionated-70% ethanol extract and 70% ethanol extract of \textit{J. gendarussa} leaves at various concentrations on MOLT-4 cell viability are found in Fig 2. The reduction of cell viability is reflected by the inhibition of cell growth related to the suppression of cell proliferation activities so that the total number of dividing or living cells are decreased. Various cell signalling activities involved in protein expression of the programmed cells death such as \textit{bid}, \textit{bax}, and \textit{bcl}-2 are likely to be activated when cell lines are exposed to active compounds contained in the extract\textsuperscript{25} causing an increase in programmed cell death (apoptosis).\textsuperscript{26} Testing by using tetrazolium only measures the formation of formazan (which is related to mitochondrial living cell activities) but it is not able to determine the cause of cell death.\textsuperscript{27}

There was no statistical difference between the two extracts despite having twice the amount of gendarusin A in the extract which indicates that gendarusin A probably does not significantly contribute to the cytotoxicity of the extract. The alkaloids present in the 70% ethanol extract probably contributed to the cytotoxicity but also did not result in a significant difference between the alkaloid free and the alkaloid containing extracts.

\section*{CONCLUSIONS}

Fractionated-70% ethanol extract and 70% ethanol extract of \textit{J. gendarussa} leaves are relatively non-cytotoxic to MOLT-4 cells with no significant difference of cytotoxicity between the fractionated-70% ethanol extract and 70% ethanol extract (p > 0.05).

\section*{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, Surabaya for Bio-safety Level-3 facilities.

\section*{REFERENCES}


