CYTOTOXICITY STUDY OF MEZZETIAPARVIFLORA BECC. WOODBARK

UJI SITOTOKSISITAS BATANG KAYU MEZZETIAPARVIFLORA BECC.

Mufidah1*, Elly Wahyudin1, Gatot S Lawrence2, Mae Sri Hartati Wahyuningsih3, Marianti A Manggau1
1Faculty of Pharmacy, Hasanuddin University, Makassar, 90245, Indonesia
2Faculty of Medicine, Hasanuddin University, Makassar, 90245, Indonesia
3Faculty of Medicine, Universitas Gadjah Mada, Jogjakarta, 55281, Indonesia

ABSTRACT

The cytotoxic activity of acetone insoluble extract of Mezzetiaparviflora Becc. Wood bark was evaluated on HeLa cell line and it was compared with normal vero cell line to confirming the use of the plant as a traditional medicine for tumor. The experiment with normal vero cell line using MTT assay showed a percentage of cell viability of 96.8% at 1000 µg of concentration which was not increase with the increase of concentration of the extract. Whereas, the experiment on HeLa cell line showed a low cytotoxic activity with the viability percentage was found to be 87.4% at 1000 µg/ml. Therefore the extract was categorized as non-toxic and the next studies is necessary to explore the mechanism liable of the using of the plant extract as anticancer traditional.

Key words: Mezzetiaparviflora Becc., MTT, Cytotoxicity, Vero cell line, HeLa cell line

INTRODUCTION

Mezzetia comprises 4 species and indigenous in the Andaman island, peninsular Thailand, peninsular Malaysia, Sumatra, Borneo and the Moluccas. All 4 are present within Malaysia (Sosef et al., 1998; Wills, 1973). The woodbark of M. parviflora (Annonaceae) has long served as one of the most important traditional herbal medicine sources in Buton Regency, Southeast Sulawesi, Indonesia. The wood bark empirically used as antitumor, antiasthma, anti-cholesterol, anti-diabetic, etc; the damages which related to free radical activity. These activities may be contributed by its anti-oxidative compounds.

Cui (1998) isolated seven cytotoxic acylated doligomanno sides from Mezzettialeptopoda, but literature survey on M. parviflora suggest no report about the anticancer activity of M. parviflora Becc. Hence, the present study has been made to investigate the cytotoxicity properties of M. parviflora extract.

METHODOLOGY

Plant material

The woodbark of Mezzetiaparviflora Becc. Were collected from Buton forest, South east Sulawesi and identified at the Herbarium Bogoriense, Bogor. M. parviflora woodbark in powdered form were extracted with 70% ethanol
using a maceration method, and last trace soft he
solvent were evaporated in a rotary evaporator
and freeze dryer. The dry crude ethanol extract
was then subjected to partition using acetone yield
acetone extract and insoluble in acetone extract. In
soluble in acetone extract was subjected to MTT
assay.

MTT Assay
Cytotoxicity assay of woodbark extract of M. 
parviflora on Vero and HeLa cells was conducted
through the MTT assay described by Pour et al. 
(2011). Cells were diluted with medium to 1×10^6
cells/mL and aliquots (1,500-2000 cells/100μL)
were placed in individual wells in 96-well micro
plate, except for the first column wells as blank.
Cells were incubated at CO2 5%, 37.0°C overnight
to allow the cells to attach to the wells. Thereafter,
in each well, 100μL of woodbark extract was
added, which had been serially diluted 2-fold in
DMSO (final concentration 50% v/v), ranged from
7.8 to 1000μg/mL in final solution (n = 3). Column
1 and 2 were designated as cell control and
negative control (untreated), respectively. Next
micro plate were incubated at the above
conditions for 24 h and then their viability was
determined by MTT color. The MTT solution
(5mg/mL in PBS, 10μL) was added to each well
and following 5 min shaking in 150 rpm, the plates
were incubated for 4 h. SDS in HCl 0.01 N (10%)
solution was added to each well and the
absorbance of each well was read at 550nm on a
automated micro plate reader (Lab Systems
Multiskan MS). Cytotoxicity percentage was
calculated based on the following equation:

\[
\% = \frac{[\text{control} - \text{media}] - [\text{treatment} - \text{media}]}{[\text{control} - \text{media}]} 
\]

RESULT AND DISCUSSION
Vero cell line derived from African green
monkey kidney (African green monkey). Vero cell
line is commonly used as a control normal cell to
determine the level of cytotoxicity of a material
against normal cells.

Results of cell viability assay on HeLa and
normal vero cell line (Table I). Cytotoxicity of the
extract was decreased in line with the increasing
of concentrations, even at a concentration of
1000µg indicated a very low inhibition percentage
i.e. 3.2% and 12.8% for vero and HeLa cell line,
respectively. So that the inhibition percentage of
both of veroan HeLa cell vs log concentration
curve obtained in the form of a parabolic curve.
The chart below shows the changes in cytotoxicity
against Vero and HeLa cells line incubated with
various dilutions of the extract for 24 hours.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>HeLa Cell Viability (%)</th>
<th>Vero Cell Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>7.81 ug</td>
<td>97.5</td>
<td>90.04</td>
</tr>
<tr>
<td>15.62 ug</td>
<td>97.5</td>
<td>88.2</td>
</tr>
<tr>
<td>31.25 ug</td>
<td>90.4</td>
<td>88.2</td>
</tr>
<tr>
<td>125 ug</td>
<td>92</td>
<td>82.15</td>
</tr>
<tr>
<td>250 ug</td>
<td>85.1</td>
<td>83.96</td>
</tr>
<tr>
<td>500 ug</td>
<td>83.1</td>
<td>86.42</td>
</tr>
<tr>
<td>1000 ug</td>
<td>87.2</td>
<td>96.8</td>
</tr>
</tbody>
</table>

Parabolic curve of Lantana camara leaf
eextract concentration vs. cytotoxicity against vero
cells line also reported by Pour et al (2011) which
suggest the presence of pro- and anti-toxic phytochemicals in the extract that influence the
cytoprotective or cytotoxic effects of each other in
a concentration-dependent manner.

The insoluble in acetone extract of M.Parviflora
showed a low anti proliverative effect
on  HeLa  cell line which indicated by its inhibition
which was not linearly increase in line with the
increasing of the concentration. This profile result
indicated that the using of extract as traditional
anticancer was not caused by its anti-proliferative
activity.

The major phytochemical contained in
insoluble in acetone extract of M. parviflora
was condensed tannin, i.e26.46±0.315μg/mg of extract
(calculated as quebracho tannin), which was
justified its antioxidant activity toward DPPH
(IC50 21.79μg/mL) and NO (229.09μg/ml)
(Murdifin et al., 2012). Therefore, the traditional
anticancer using of M. parviflora extract was
possessed by its chemopreventive properties.
Further more, comprehensive studies comprising
chemopreventive mechanism are essential to
characterize the M.parviflora extracts as natural
anticancer.

Yang et al. (2001) and Middleton et al.
(2000) suggested mechanisms of anticancer
effects of polyphenols include antioxidant, anti-
inflammatory, antiproliferative, as well as their
effects on sub cellular signaling path ways,
induction of cell-cycle arrest and apoptosis. But,
Yang et al., 2002 in Jeong and Kong, 2004 have
demonstrated that the anticancer effect of
the natural products, including polyphenolic were
possessed by their chemopreventive effects. Effect
on Nuclear factor-kappa B (NF-KB), Activator
protein-1 (AP-1), and mitogen-activatedprotein
kinase (MAPK) underlying the cancer
chemopreventive properties (Jeong and Kong,
2004).
CONCLUSIONS

The insoluble in acetone extract of *Mezzetiaparviflora* Becc. Woodbark was categorized as non-toxic and the using of the plant extract as anticancer traditional not caused by its anti-proliferative effect.

ACKNOWLEDGEMENT

We are grateful to Prof. Mae Sri Hartati Wahyuningsih for this collaborative work and providing all facilities to carry out the work.

REFERENCES


