

## CYTOTOXICITY STUDY OF *MEZZETIAPARVIFLORABECC. WOODBARK*

### UJI SITOTOKSISITAS BATANG KAYU *MEZZETIAPARVIFLORABECC.*

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#### 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

#### ABSTRAK

Aktivitas sitotoksik ekstrak larut aseton batang kayu *Mezzetiaparviflora* Becc. dievaluasi pada sel HeLa dan dibandingkan dengan sel normal vero untuk memastikan penggunaan sebagai tanaman obat tradisional untuk pengobatan tumor. Percobaan dengan sel normal vero menggunakan uji MTT menunjukkan persentase viabilitas sel sebanyak 96,8% pada konsentrasi 1000 µg dan tidak meningkat dengan meningkatnya konsentrasi ekstrak. Sedangkan pada percobaan dengan sel HeLa menunjukkan aktivitas sitotoksik yang rendah dengan persentase viabilitas sebesar 87,4% pada konsentrasi 1000 µg/ml. Oleh karena itu ekstrak ini dikategorikan sebagai tidak beracun dan diperlukan studi selanjutnya untuk mengeksplorasi mekanisme yang bertanggung jawab terhadap efek antikanker dari tanaman tersebut.

Kata kunci: *Mezzetiaparviflora* Becc, MTT, sitotoksitas, sel Vero, sel HeLa.

#### 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 (Sosefet *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 acylatedoligorhamno 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

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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  $1 \times 10^5$  cells/mL and aliquots (1,500-2000 cells/100 $\mu$ L) were placed in individual wells in 96-well micro plate, except for the first column wells as blank. Cells were incubated at CO<sub>2</sub> 5%, 37.0°C overnight to allow the cells to attach to the wells. Thereafter, in each well, 100 $\mu$ L of woodbark extract was added, which had been serially diluted 2-fold in DMSO (final concentration 50% v/v), ranged from 7.8 to 1000 $\mu$ g/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, 10 $\mu$ L) 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 1000 $\mu$ g 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 I. Determination of cytotoxicity by MTT assay

Concentration	HeLa Cell Viability (%)	Vero Cell Viability (%)
control	100	100
7.81 ug	97.5	90.04
15.62 ug	97.5	88.2
31.25 ug	90.4	88.2
125 ug	92	82.15
250 ug	85.1	83.96
500 ug	83.1	86.42
1000 ug	87.2	96.8

Parabolic curve of *Lantana camara* leaf extract 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 proliferative 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.e.  $26.46 \pm 0.315 \mu\text{g}/\text{mg}$  of extract (calculated as quebracho tannin), which was justified its antioxidant activity toward DPPH (IC<sub>50</sub> 21.79 $\mu$ g/mL) and NO (229.09 $\mu$ g/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 been 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-activated protein kinase (MAPK) underlying the cancer chemopreventive properties (Jeong and Kong, 2004).

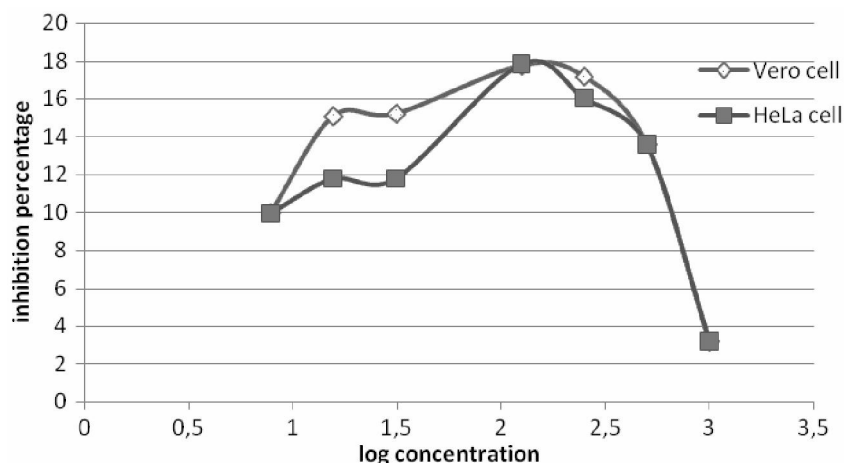


Figure 1. Correlation between log concentration of *M. parviflora* wood bark insoluble in acetone extract versus percentage of cell inhibition

## 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.

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