

Antiproliferation Activity of Keladi Tikus (*Typhonium flagelliforme*) Leaves Ethanol Extract on MCA- B1 and MCM-B2 Tumor – Derived Cell Lines *In Vitro*

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INTRODUCTION

Tumor is a degenerative disease as the second agent cause of death in human. This disease is caused by disturbances of cell’s growth that show alteration and uncontrolled of cells proliferation. The uncontrolled of cells proliferation is also accompanied by penetration of that cells into others tissue and develop on it. Removed of tumor is usually done by operation or chemotherapy. Alternative treatment that can be used is herbal treatment. *Typhonium flagelliforme* known as “Keladi Tikus” in Indonesia; is known has a chemopreventive effect (2). Other researchers also show flavonoid glucoside of *T. flagelliforme* (1) can induce the activity of apoptosis in colon cancer cells (5). The aim of this study is to examine the *in vitro* anti-proliferation activity of *Typhonium flagelliforme* leaves ethanol extract on MCA- B1and MCM-B2 cell line.

MATERIAL AND METHODS

Extraction

Maceration method was used to extract the leave of *Typhonium flagelliforme*. Leaves powder of *Typhonium flagelliforme* were soaked in ethanol 70% as a solvent with the ratio of 1:10 for 24 hours, then the liquid extract was filtered using a filter paper. This procedure was repeated once by soaked the dregs in the same solvent. Total liquid extract was evaporated by a rotary evaporator until viscous extract was obtained.

Brine Shrimp Lethality Test (BSLT)

Eggs of *Artemia salina* were incubated in sea water with aerator for oxygen supply and the lights turned on for 48 hours. After 48 hours, the eggs hatched become a larvae.

Preparation of the leaves ethanol extract solution by dissolved it with 100% DMSO and diluted to made into 0, 10, 100, 500 and 1000 ppm concentrations. Extracts solution with different concentration were tested into 10 larvae of *Artemia salina* with three replicates for 24 hours. The dead larvae were then counted and calculated

to determined the LC50 using probit analysis (3).

Antiproliferation Assay *In Vitro*

Anti-proliferation assay of the extract on MCA-B1 and MCM-B2 cell lines was done using several extract concentrations of 0, 20, 40, 60, 80, 100, 120 ppm and doxorubicin as a positive control. Cell lines were cultivated on micro-plate 24 wells containing complete culture media (DMEM, 10% FCS, antifungi and antibiotics) with the density of 10⁴ cells/mL in three replicates. Cultivated cell line were incubated in an incubator with 37 °C, 5% CO₂.

Cell Harvesting and counting

Cell harvesting was done 3-4 days after cultivated. Harvested cells were proceed with trypan blue dye, cell suspension wer homogenized with a vortex then counting using a Neubauer Hemocytometer under the inverted microscope with 10x magnification. The total number of cells were calculated to determine the percentage of inhibiton activity by using a methode of Priosoeryanto (2009)(8).

RESULT AND DISCUSSION

Brine Shrimp Lethality Test (BSLT) showed the amounts of the death larvae using various concentration of *Typhonium flagelliforme* leaves extract (Tabel 1). The amount of deadlarvae were calculated and obtained the LC50 value (Tabel 2).

Tabel 1. Amounts of dead larvae

Sample	Replica- tion	Concentration (µg/ ml)			
		10	100	500	1000
<i>T. flagelliforme</i> Leaves Extract	1	1	7	8	10
	2	3	7	7	9
	3	7	10	9	

Tabel 2. LC50 value of *T. flagelliforme* leaves extract

Extract	Replica-tion	LC50 (µg/ ml)	Sum of LC50
<i>T. flagelliforme</i> Leaves	1	63,982	44.31
	2	39,863	
	3	29,094	

According to the results of BSLT, the leaves extract LC50 value was 44,31%. The LC50 value showed that the leaves extract was toxic substance according to Meyer *et al* (1982) (4).

Typhonium flagelliforme leaves extract could inhibit the proliferation of MCA-B1 and MCM-B2 cells. The phytochemicals components of *Typhonium flagelliforme* such as alkaloids, flavonoid, trepenoid, and steroid (7). Those components were antioxidant that potential to inhibit the proliferation of tumor cells (2) and trigger the apoptosis of the cells (4).

Inhibition activity of the leaves extract showed *dose response relationship* that the inhibition activity increased accordanced along with the increased dose. The highest dose (120 ppm) of the extract showed the highest inhibition activity (Figure 1 and 2).

Comparison between figure 1 and 2 showed the inhibition activity of the leaves extract on MCM-B2 cells was lower than MCA-B1 cells. The lower results of MCM-B2 tha MCA-B1 could be caused by different receptor among cells.

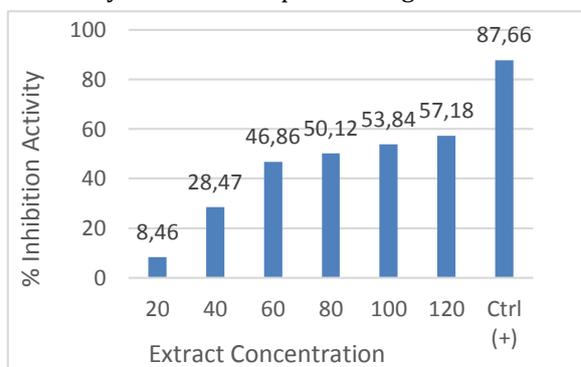


Fig. 1. Activity proliferation of *T. flagelliforme* on MCA-B1 cells

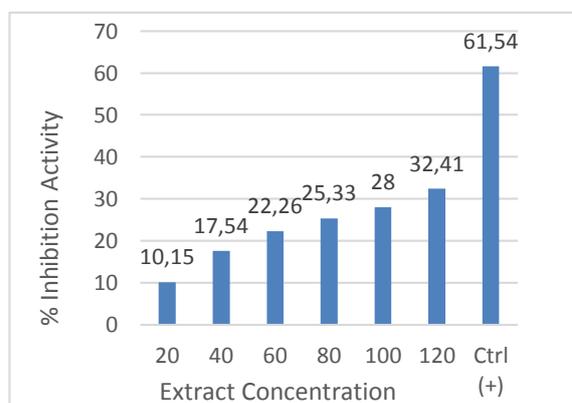


Fig. 2. Activity proliferation of *T. flagelliforme* on MCM-B2 cells

CONCLUSION

In conclusion, we showed that *Typhonium flagelliforme* leaves extract has an antiproliferative activity on MCA-B1 and MCM-B2 cells. *Typhonium flagelliforme* leaves has a potential to be develop as an antitumor drugs. Further studies on the using of several tumor cell types and combination with other substances to observe the antiproliferation, antiinvasion and antimetastatic as well as antiangiogenic are needed.

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