Growth inhibition effect of plants extract (*Mussaenda pubescens* and *Curcuma zedoaria*) on tumor cell lines *in vitro*

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ABSTRACT

A growth inhibition effect study of *Mussaenda pubescens* and *Curcuma zedoaria* extracts was conducted using two tumor derived cell lines in order to evaluate their activities on cell proliferation. The plants were extracted using ethanol and chloroform solutions. Lethal concentration-50 (LC_{50}) of the extracts were determined using Brine Shrimp Lethality Test. The growth inhibition activity of the extracts was performed using a Trypan Blue staining methods and the cells were counted with a hemacytometer. The extract of both plants were significantly inhibited the proliferation of myeloma and carcinoma derived cell lines in vitro ($P<0.05$).

The LC_{50} for chloroform extract of *Curcuma zedoaria* was 21 ppm, and the tested dose for this extract were 10, 20 and 30 ppm, while for *Mussaenda pubescens* the LC_{50} was achieve at the 1,749 ppm and the tested dose were 1600, 1800 and 2000 ppm. The highest growth inhibition effect of *Curcuma zedoaria* chloroform extract on each cell lines were 86.57% for myeloma cell, and 78.43% for carcinoma cell this activity was occurred on the dose of 30 ppm. On the *Mussaenda pubescens* chloroform extract, the highest activity for both cell lines were achived at the concentration of 2000 ppm, they were 83.90% for myeloma cell and 65,80% for carcinoma cell.

For the ethanol extract of *Curcuma zedoaria*, the LC_{50} level was 69 ppm. The tested doses were 30, 60 and 90 ppm and the highest activity on both cell lines were occurred on the dose of 90 ppm, they were 83.7% for myeloma cell and 80.8% for carcinoma cell. On the ethanol extract of *Mussaenda pubescens*, LC_{50} was achieved at 32.45 ppm, while the highest growth inhibition activity was exhibited at the concentration of 50 ppm with the percentage of 86.80 for myeloma cell and 87.66 for the carcinoma cells.

From this study we concluded that *Curcuma zedoaria* and *Mussaenda pubescens* contained substances that can inhibit the growth of some tumor cell *in vitro*, therefore we suggest that both plants have a possibility and could be used as a source of anti-tumor substances. Isolation and identification of the bioactive compounds of both plants are in progress.

Key words: Growth inhibition, in vitro, tumor cells, *Curcuma zedoaria*, *Mussaenda pubescens*

INTRODUCTION

A tumors or neoplasm can be defined as a disturbance of growth characterized by excessive, abnormal and uncontrolled proliferation of transformed or altered tissue at one or more primary points within the host, and frequently at one or more metastatic sites. In the course of spontaneous development of tumor in human and animals, groups of neoplastic cells may be present for years before a tumor. Even after the neoplastic growth becomes detectable, it may remain at relatively stable size and degree of invasiveness for prolonged periods of time before its full malignant potential is manifest.

Organics substances isolated from plants are known as metabolite substances. These natural metabolites are widely use in the medical, pharmacy, agro-chemistry and chemical industries (Harborne, 1996). In some Asian countries, metabolites derived from several plants are use for the alternative treatment or traditional medicine for some disorders in human and animals.

Indonesia is spread out in the tropical area are rich of medicinal plants. Indonesia Drug and Food Control Agency indicated that medicinal herbs produced in Indonesia by 326 manufacturers are use not less than 180 medicinal and aromatic plants. The total of raw materials consumption annually reach about 6.223 tons. The Agency was also counted for 45 important drugs in the USA are originated from tropical medicinal and aromatic plants, in fact, 14 plants species are coming from Indonesia. The big number of medicinal and aromatic plants species grow in Indonesia is an indicator that the land and climate conditions of Indonesia have a potential change for the cultivation development of medicinal and aromatic plants.

The aim of the present study is to elaborate the anti-proliferation activity of two extract plants on the tumor cell line in vitro, in order to find the potential anti-tumor drugs for medical purposes.

MATERIAL and METHODS

Extraction of the Plants

Two plants (*Mussaenda pubescens* and *Curcuma zedoaria)* were used in this study. The extracts of these plants were prepared using ethanol or ether solution according to the method of Anonimous, (1985). Briefly, 50 grams each of *Mussaenda pubescens* and *Curcuma zedoaria* roots powder were macerated using 500 ml of chloroform or ether and kept for 5 days, and were then filtered. The wastes were dissolved into a sufficient amount of chloroform and were filtered until the total volume of extracts was 100 ml. The extracts were evaporated to get the desired concentrated filtrates and were kept until use. Working concentrations of each extracts were made by dilution the extracts until the tested concentration was achieved.

Brine Shrimp Lethality Test

Ten larvae of *Artemia salina* on 12 vials each were used (3 concentrations of extracts and one control with 3 replicates). The tested dose for each plants extracts were : 0, 10, 100 and 1000 ppm. After 24 hours of extracts treatment, the dead *Artemia salina* was counted (Meyer *et al,* 1982). The data were processed statistically using Probit Test.

Anti-proliferation Activity Assay

The cell lines were cultured with the density of 10^3 cell/ml on the 24-well dish using a growth medium comprises from DMEM and 10% FCS (Priosoeryanto *et al*, 1995). The tested dose of each plants extracts was determined after the LC_{50} of each extracts were

recognized. The dose for each extract are as follows : chloroform extract of *Mussaenda pubescens* : 1600, 1800 and 2000 ppm and for *Curcuma zedoaria* : 10, 20 and 30 ppm; for Ethanol extract of *Mussaenda pubescens* : 10, 30 and 50 ppm and for *Curcuma zedoaria* : 30, 60 and 90 ppm). The extracts were added to the culture dish (3 holes for each dose). For the control positive, anti-tumor commercially drugs Vinblastine was used. After the confluence of cell growth was achieved on the control negative dishes, the cells were harvested and the average of the total number of cells on each dishes were counted using a hemacytometer with Trypan Blue dye. The data were then statistically analyzed to determine the anti-proliferation activity level.

RESULT and DISCUSSION

Brine Shrimp Lethality Test

The LC_{50} for each plants extracts were 21 ppm for chloroform extract of *Curcuma zedoaria,* 1,749 ppm for chloroform extract of *Mussaenda pubescens,* ethanol extract of *Curcuma zedoaria* was 69 ppm and 32.45 ppm for ethanol extract of *Mussaenda pubescens.*

Anti-proliferation Activity

The anti-proliferation activity was detected in all extracts. The degree of this activity on all extracts was varied. The highest anti-proliferation activity of *Curcuma zedoaria* chloroform extract on each cell lines were 86.57% for myeloma cell, and 78.43% for carcinoma cell, this activity was occurred on the dose of 30 ppm (Figure 1). For the ethanol extract of *Curcuma zedoaria,* the highest anti-proliferation activity on both cell lines were occurred on the dose of 90 ppm, they were 83.7% for myeloma cell and 80.8% for carcinoma cell (Figure 2).

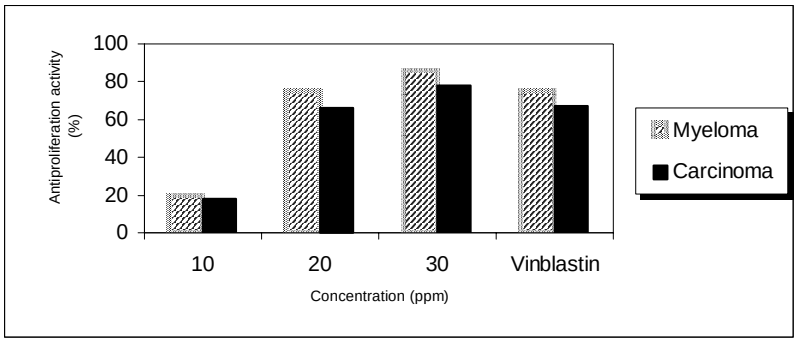

Figure 1. Anti-proliferation activity of *Curcuma zedoaria* chloroform extract on myeloma and carcinoma cell lines

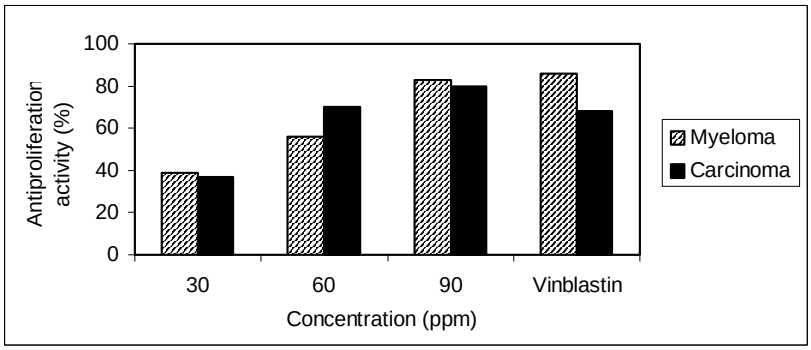

Figure 2. Anti-proliferation activity of *Curcuma zedoaria* ethanol extract on myeloma and carcinoma cell lines.

On the *Mussaenda pubescens* chloroform extract, the highest activity for both cell lines were achived at the concentration of 2000 ppm, they were 83.90% for myeloma cell and 65,80% for carcinoma cell (Figure 3). For the ethanol extract of *Mussaenda pubescens,* the highest anti-proliferation activity was achieved at the concentration of 50 ppm with the percentage of 86.80 for myeloma cell and 87.66 for the carcinoma cells (Figure 4).

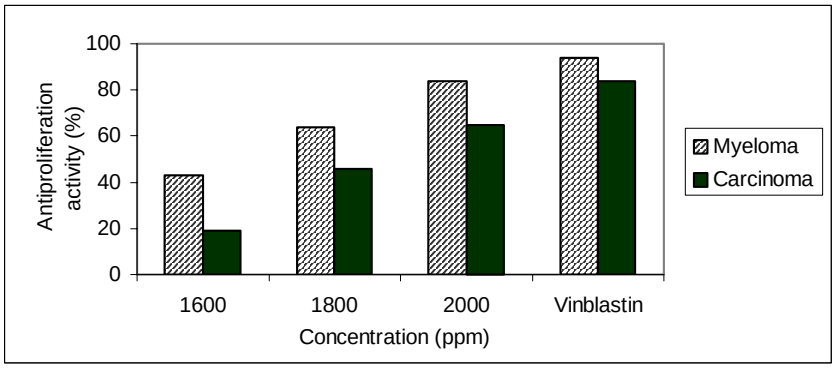

Figure 3. Anti-proliferation activity of *Mussaenda pubescens* chloroform extract on myeloma and carcinoma cell lines

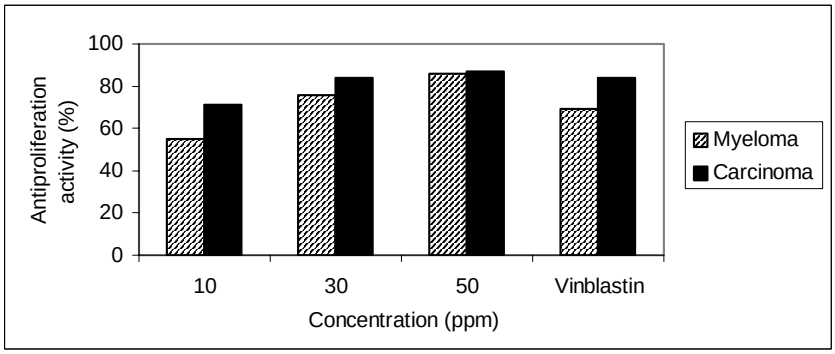

Figure 3. Anti-proliferation activity of *Mussaenda pubescens* ethanol extract on myeloma and carcinoma cell lines.

The anti-proliferation activity pattern of both extracts of *Curcuma zedoaria* on myeloma and carcinoma cell lines was generally similar. The different was only in the degree and effective dose activities, with ethanol extract need more high concentration compared to chloroform extract. Anti-proliferation activities of both extracts seem to be a dose dependent (increasing dose was also increasing in anti-proliferation activity). The different activity of this extracts on two types of tumor cells indicated that this activity is depended on cell type. The anti-proliferation activity of *Curcuma zedoaria* shown that this plant contains some substances that can inhibit the proliferation of tumor cells in vitro.

Hutapea (1993) explain that *Curcuma zedoaria* contain volatile oils, saponin, flavonoids and polifenol. Heyne (1987) indicated that *Curcuma zedoaria* usually use as an oral medicinal herbs for women after getting birth in order to promote the restoration of uterus. Some researchers reported that this plant has an anti-tumor activity (Soedibyo. 1998). The mechanisms of this extracts inhibit the proliferation of tumor cells is still non-understood clearly.

The similar anti-proliferation activity on tumor cells as *Curcuma zedoaria* was also encountered in the *Mussaenda pubescens* extracts. The chloroform extract of is less active compared to the ethanol extract of *Mussaenda pubescens* this was indicated by the higher concentration of its $LC_{50.}$ The chloroform extract seem to be more effective in the

inhibition of myeloma cell proliferation compared to the carcinoma cells. This phenomenon indicated that the activity of *Mussaenda pubescens* ethanol extract seem are cell and dose dependent. On the contrary, the ethanol extract is slightly similar on their activity into both tumor cell lines.

Mussaenda pubescens contains some substances such as arjunolic acid, beta-sitosterol, stigmasterol and triterpenoid saponin named mussaendosides G and K (Wijayakusumah et al, 1992; Zhao *et al*, 1996; Dalimartha, 1999; Anonimous, 2001a,b). Traditionally, *Mussaenda pubescens* is could be use as anthelminthic in child, back pain and tumors (Wijayakusumah et al, 1996; Dalimartha, 1999), and also for fever, heat schok, anti-inflamation and diare (Wijayakusumah et al, 1992).

Result of the present study showed that the chloroform and ethanol extract of *Mussaenda pubescens* and *Curcuma zedoaria* have an anti-proliferation activity on some tumors-derived cell lines. We suggest that these extracts plants could be developed and use as an alternative treatment for combat the tumors disorders. Further study on the isolation and identification of the bioactive compound of these two plants as well as toxicity and safety should be conducted before field application.

REFERENCES

1. Anonimous. 1985. Special extract preparation and production. Directorate General Food and Drugs Control, Department of Health the Republic of Indonesia.
2. Anonimous. 2001a. Traditional Chinese Medicine. tcm: Buddha's Lamp. http://www. Online.daylight.com.26551/tcm/bio/B157.html.
3. Anonimous. 2001b. Traditional Chinese Medicine. tcm: family Rubiaceae. http://www. Online.daylight.com.26551/tcm/fam/Rubiaceae.html.
4. Dalimartha,S. Traditional medicinal herbs for cancer treatment. 1999. Penebar Swadaya. Jakarta. *(in Indonesian language).*
5. Heyne, K. 1987. Indonesian medicinal plants. Vol I. *(in Indonesian language).*
6. Hutapea, JR. 1993. Indonesian medicinal plants inventory. 2nd. Ed. Research and Development Division, Department of Health the Republic of Indonesia. *(in Indonesian language).*
7. Meyer, BN., NR. Ferrigni, JE. Putnam, LB. Jacobson, DE. Nichols and JL. McLaughlin. 1982. Brine shrimp : A convenient general bioassay for active plant constituents. *Planta Medica* **45**:31-34.
8. Priosoeryanto, BP., S. Tateyama, R. Yamaguchi and K. Uchida. 1995. Antiproliferation and colony forming inhibition activities of recombinant feline interferon on various cells in vitro. *Canadian Journal of Veterinary Research* **59**:67-69.
9. Soedibyo, M. 1998. Nature the source of health, benefit and use. Balai Pustaka. Jakarta. *(in Indonesian language).*
10. Wijayakusuma, HMH., SA. Wirian, T. Yaputra, S. Dalimartha, and B. Wibowo. 1992. Medicinal plants in Indonesia. Vol. II. Pustaka Kartini . Jakarta. *(in Indonesian language).*
11. Zhao, W.,R. Xu, G. Qin, T. Vaisar and MS. Lee. 1996. Saponin from *Musaenda pubescens*. Phytochemistry **42**(4):1131-1134.