IN VITRO ANTI-PROLIFERATION ACTIVITY OF *Impatiens balsamina* PLANT EXTRACTS ON TWO HUMAN TUMOR-DERIVED CELL LINES

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ABSTRACT

An anti-proliferation activity study of *Impatiens balsamina* plant extracts on two cell lines derived from human tumor was performed. The plant was extracted using ethanol and chloroform solutions. Brine Shrimp Lethality Test (BSLT) for determination of lethal concentration-50 (LC_{50}) of the extracts was used. The growth inhibition activity of the extracts was assayed by using a Trypan Blue dye exclusion method and the cells were counted using a haemacytometer. The plant extracts were significantly inhibited the proliferation of leukemia (K-562) and myeloma (137)-derived cell lines in vitro ($P<0.05$).

The LC_{50} for *Impatiens balsamina* ethanol extract was 11.1734 ppm, and the dose tested for this extract were 4, 8, 12, 16 and 20 ppm. The highest anti-proliferation effect of *Impatiens balsamina* ethanol extract on each cell lines were 60.80% for leukemia cell, while for myeloma cell was 64.29%, this activity was occurred on the dose of 20 ppm. For the chloroform extract, the LC_{50} level was achieved at 4.6623 ppm. The tested doses were 2, 4. 6, 8 and 10 ppm and the maximum activity on both cell lines were occurred on the dose of 10 ppm, they were 38.63% for leukemia cell and 85.71% for myeloma cell.

The anti-proliferation activity of *Impatiens balsamina* plant extracts indicated that this extracts contained substance(s) that have ability on the inhibition of the growth of some human derived-tumor cell *in vitro*. From the above result, in conclusion we suggest that *Impatiens balsamina* have a possibility and could be used as a source of anti-tumor substance(s). Isolation and identification of the bioactive compounds of this plant extracts is in progress.

Key words: Anti-proliferation, in vitro, tumor cells, *Impatiens balsamina*

INTRODUCTION

In the end of 20-th century, tumor diseases become a seriously disorders affected human and animals all over the world especially in the big city. This condition due to the increasing of air, water, light and land pollutions, behavior changes and many other factors including genetic abnormalities. In Indonesia, about 190,000 new cases of tumors were reported every year and the mortality rate is around 20% (Tjahjono, 1999). Household survey of the Department of Health the Republic of Indonesia in 1995 showed that the incidence of cancer in Java and Bali islands about 100 cases per 100,000 peoples. In the United States, the number of death peoples due to cancer diseases was 538,000; this number account about 23% of all death cases (Contran *et al,* 1994).

Natural metabolites especially from plants are widely use for medical purposes. In some Asian countries, the use of plants for traditional medicine in the treatment of some disorders in human and animal is a common practice. Indonesia is a "mega-biodiversity", rich of plants and animals. For plants, not less than 30,000 species were encountered while 7,000 out of them known or have a potential as a medicinal plants; this number accounted as a 90% of Asia's medicinal plants (Indonesia Drugs and Food Agency, 2002). The Agency also accounted that 45 important drugs in the USA are originated from tropical medicinal and aromatic plants; in fact, 14 plants species are coming from Indonesia. This figure showed that Indonesia's medicinal plants have a potential for the source of medical substances in the future.

The aim of this study is to clarify the potential anti-proliferation effect of chloroform and ethanol extracts from one of Indonesia medicinal plant *Impatients balsamina* on tumor-derived cell lines in order to find the anti-tumor drugs for medical purposes both in human and animal medicine.

MATERIAL and METHODS

Extraction of the Plants

The plant use was identified and determined by the Research Center for Biology, Indonesian Institute of Science/LIPI-Bogor. The extracts of *Impatients balsamina* were prepared using chloroform and ethanol solutions following the method of Anonimous (1985).

Briefly, 50 grams of seed powder were macerated in 400ml of ethanol or chloroform and kept for 5 days in a tightly isolated bottle, and were then filtered. The wastes were dissolved in to 100 ml ethanol or chloroform, mixed gently and filtered until the total volume of 100 ml. The extracts were kept for 2 days in the room temperature and kept out from the light. The extracts were evaporated to get the desired concentrated filtrates and were kept on refrigerator until use. Working concentrations of each extracts were made by dilution the extracts until the desired concentration was achieved.

Brine Shrimp Lethality Test

Ten larvae of *Artemia salina* on 18 vials each were used (5 concentrations of extracts and one control with 3 replicates). The tested dose for each plants extracts were : 0, 4, 8, 16, 32 and 64 ppm. After 24 hours of extracts treatment, the dead *Artemia salina* was counted (Meyer *et al,* 1982; Colegate *et al*, 1993). The data were processed statistically using Probit Test.

Anti-proliferation Activity Assay

The cell lines (K-562, leukemia and 137, myeloma) 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 (Prijosoerjanto *et al*, 1995; Prijosoerjanto *et al,* 2001). 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 : 2, 4, 6, 8 and 10 ppm and for Ethanol extract : 4, 8, 12, 16 and 20

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 plant extracts were 11.1734 ppm for ethanol extract and the dose tested for this extract were 4, 8, 12, 16 and 20 ppm; For the chloroform extract, the LC_{50} was achieved at 4.6623 ppm and the tested doses were 2, 4, 6, 8 and 10 ppm.

Anti-proliferation Activity

Anti-proliferation activity of the *Impatiens balsamina* extracts were detected in all cancer-derived cell lines. The degree of this activity was varied in each cell lines. The highest anti-proliferation activity of *Impatiens balsamina* ethanol extract on each cell lines were 60.80% for leukemia cell and 64.29% for myeloma cell. This activity was occurred on the dose of 20 ppm (Figure 1). In the chloroform extract, the maximum activity on both cell lines were occurred on the dose of 10 ppm, they were 38.63% for leukemia cell and 85.71% for myeloma cell (Figure 2).

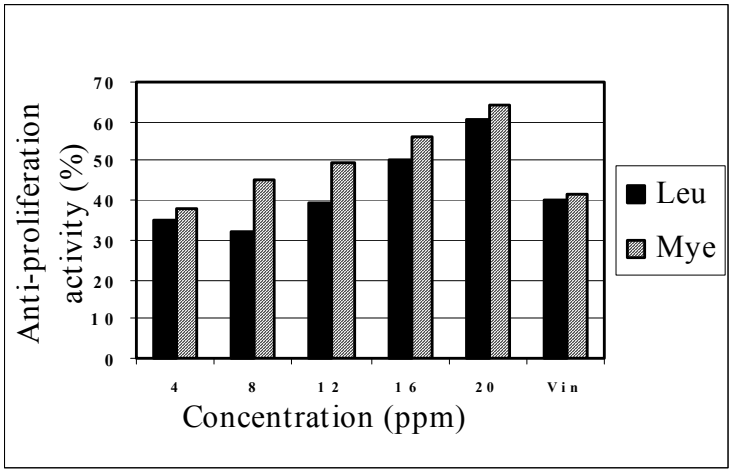

Figure 1. Anti-proliferation activity of *Impatients balsamina* ethanol extract on leukemia and myeloma cell lines. Vin = Vinblastine; Leu = Leukemia cell; Mye = Myeloma cell

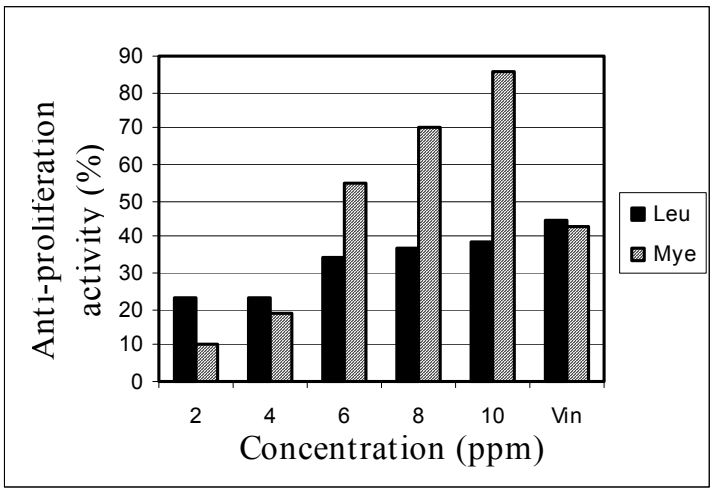

Figure 2. Anti-proliferation activity of *Impatients balsamina* chloroform extract on leukemia and myeloma cell lines. Vin = Vinblastine; Leu = Leukemia cell; Mye = Myeloma cell

The anti-proliferation activity detected of two extracts from *Impatients balsamina* on the leukemia and myeloma-derived cell lines seem to have had a similar pattern, although there were differences in the dose and the inhibition degree. The difference activity of both extracts on the tested cell-lines indicated that this phenomenon was seem due to the different dose level, it means that activity was increase parallel with the increasing of the extract concentration. Besides the dose level, the anti-proliferation activity was also different among these two cell types. In the ethanol extract, the anti-proliferation activity is seem to be effective on the both of cell lines, but in the chloroform extract the inhibition effect is more effective on the myeloma cell compared to the leukemia cell.

Wijayakusuma *et al,* (1992) and Dalimartha (1999) indicated that *Impatients balsamina* seed contain chemical substances such as parinarat acid, balsaminasterol, α-spinasterol and β-ergosterol, while in the flower antosianin, sianidin, pelargodinin, dephinidin, kaemperol and quersetin was found. Sianidin was also found in the seed.

Traditionally, *Impatients balsamina* was used in the menstruation period (emenagog), give birth smoothness (parturifasient), treatment of upper tract intestinal cancer, dermatitis, abscess, anti-rheumatic and anti-fungal.

Result of the present study indicated that ethanol and chloroform extracts of *Impatients balsamina* have an anti-proliferation activity on the human derived leukemia and myeloma cell lines. We suggest that this plant extracts could be develop and use as an anticancer substances and further study for isolation and identification of the bioactive compound from this plant extracts including toxicity and safety should be conducted before field application in human and animal medicine.

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