CYTOTOXIC ACTIVITY AGAINST P-388 MURINE LEUKEMIA CELL FROM *Lygodium microphyllum* HERB

Hadi Kuncoro[1,2], Laode Rijai[2], Euis Julaeha[1] & Unang Supratman[1*].
[1]Departement Of Chemistry, Faculty Of Mathematics and Science, Padjajaran University, Jatinangor
[2]Pharmaceutical Research & Development Laboratory of FARMAKA TROPIS Pharmacy Faculty, Mulawarman Univesity, Samarinda, East Kalimantan, Indonesia
* Corresponding author email: E-mail : u_supratman@unpad.ac.id

ABSTRACT

Lygodium microphyllum is an invasive climbing fern weed that has become aggressive in humid habitats in tropical regions. We studied its cytotoxic activity of methanol extract, n-hexane, Ethyl acetate, and n-butanol fraction. Various extract tested for cytotoxic activity on murine P388 leukemia cells using the MTT assay method. The results of the work indicated that IC50 values of methanol extract >100 µg/mL, n-hexane fraction 62,186 µg/mL, Ethyl acetate fraction 50,166 µg/mL , and n-butanol fraction >100 µg/mL on the growth of the P-388 murine leukemia cells.

Keywords: Cytotoxic, *Lygodium microphlyllum*, MTT assay, P-388 murine leukemia cell

INTRODUCTION

The majority of anticancer agents are derived from natural sources, including plants, marine organisms and micro-organism (Cragg, 2005). Plants are the source of some of the most frequently used cytostatic drugs examples paclitaxel, vinblastine and vincristine, topotecan and irinotecan, the camptothecin derivatives, and etoposide. Today, crude extracts from traditional medicinal plants are systematically subjected to an assessment of their anticancer activity. If found to be effective, these extracts are then analysed, and the active compounds responsible for their effects are identified and tested. A large number of compounds isolated from both terrestrial and marine phytosynthetic organisms are currently in preclinical development [Cragg, 2005].

Identification of cytotoxic compounds led the development of anticancer therapeutics for several decades. Advances in cancer treatment, however, continued to be limited by the identification of unique biochemical aspects of malignancies that could be exploited to selectively target tumor cells. Conventional anticancer drug discovery and development have focused on the cytotoxic agents. The drug discovery paradigms selected agents that had significant cytostatic or cytotoxic activity on tumor cell lines and caused tumor regression in murine tumor allografts or xenografts. The anticancer agents were discovered mainly by serendipity or inhibiting metabolic pathways crucial to cell division. Their exact mechanisms of action were often a subject of retrospective investigation [Narang, 2009].

Besides flowering plants (angiosperms), which represent the vast majority of extant vascular plant species, many seedless vascular plants, namely ferns and lycopods, are now also the focus of anticancer research. Most people think that there are limited uses for ferns. However, these plants have given many health benefits to humans since ancient times. Not surprisingly, herbal medicines of Chinese, Indian, and Native American peoples include ferns. These cultures have used them for food, tea, and drugs. In the present day, the functional activities of ferns and fern allies for human health have been studied using several advanced scientific technologies. Compared to flowering plants, ferns and fern allies have limited use to human health in modern times

Lygodium microphyllum (Cav) R. Br. (Genus Lygodium), an invasive weed that is widespread throughout wetland and mesic habitats in south Florida, and degrades critical ecosystem services and habitats of rare and endangered species [Pemberton, 1998]. Its rapid spread was by the establishment of large monospecific stands. It can overgrow trees to reach a height of more than 30 m [Langeland, 2001; Lott, 2003]. It is one of the worst non-indigenous invasive plant species and has

expanded its range in southern Florida, USA [Lott, 2003 ; Volin, 2004], hence, listed as Category I noxious weed by the Florida Exotic Pest Plant Council, with the ability to change ecosystem function, community structures and alter the native plant communities [Hutchinson, 2013]. The selfing and outcrossing which occur, in L. microphyllum may give the ability to adapt to local conditions and then invade distant habitats [Lott, 2003]. Field investigations in China showed that it has become more invasive in Xiqiao Mountain, Guangdong Province, China and damaged the nativeforests [Ou, 2008; Peng, 2009].

Hexane extract from Lygodium flexuosum (L.) Sw. (Lygodiaceae), a species ranging from Southeast Asia to northern Australasia, also inhibited the viability of hepatoma cells, where it further induced apoptosis, inhibited TNF-α and activated NF-κB [Wills, 2009].

Wang et al. (2014) determined allelopathic and antibacterial activity from essential oil of L. microphyllum. The chemical constituents in essential oils were analyzed by GC-MS and identified fifteen component which main component were α-monoolein (28,3%), ethylene glycol oleate (25,7%), and undecyne (15,5%).

This research was designed to discover the ability of cytotoxic activity from the plant extract against murine leukemia P-388 cell.

MATERIAL AND METHODS

Collection of Plant Material

Fresh herb of L.microphyllum were collected from Lempake village, Samarinda, East Borneo, Indonesia in April 2014. They were identified by the staff of the Dendrology Laboratory, Faculty of Forestry, Mulawarman University, Indonesia.

Extraction of Plant Material

Dried Powder Herb of L. microphyllum (3,5 kg) was extracted with methanol exhaustively at room temperature for 3 days. After removal of the solvent under vacuum condition, the viscous concentrate of Methanol extract (526,7 g) was first suspended in H2O and then partitioned with n-hexane, Ethyl Acetate, and n-butanol successively.

Cytotoxic assay using P-388 murine leukemia cells

The P-388 cells were seeded into 96-well plates at an initial cell density of approximately 3 _ 104 cells cm_3. After 24 h of incubation for cell attachment and growth, varying concentrations of samples were added. The compounds added were first dissolved in DMSO at the required concentration. Subsequent six desirable concentrations were prepared using PBS (phosphoric buffer solution, pH = 7.30– 7.65). Control wells received only DMSO. The assay was terminated after a 48 h incubation period by adding MTT reagent [3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; also named as thiazol blue] and the incubation was continued for another 4 h, in which the MTT-stop solution containing SDS (sodium dodecyl sulphate) was added and another 24 h incubation was conducted. Optical density was read by using a micro plate reader at 550 nm. IC50 values were taken from the plotted graph of percentage live cells compared to control (%), receiving only PBS and DMSO, versus the tested concentration of compounds (mg/mL). The IC50 value is the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate and averaged.

RESULT AND DISCUSSION

Preparation and extraction

Fresh Herb of L. microphyllum were grounded and successively extracted with methanol at room temperature. The methanolic extract from the dried Herb of L. microphyllum was concentrated and extracted successively with n-hexane, ethyl acetate, and n-butanol. n-hexane and ethyl acetate extracts exhibited a cytotoxic activity against P-388 murine leukemia cells with IC50 values 62.186 and 50.166 µg/mL, respectively.

Figure 1. *Lygodium microphyllum*

Result of cytotoxic activity against P-388 murine leukemia cells.

The cytotoxic effect of the four extract and fraction against the P-388 Murine Leukemia cell were conducted according to the method described in previous paper [Alley, 1988]. The cytotoxicity activities of extract and fraction are shown in table 1.

Table 1. *Cytotoxic activity of extract L. microphyllum against P-388 murine leukemia cells (IC50).*

Sample	IC_{50} (µg/mL)
Methanol extract	>100
n-hexane fraction	62.186
Ethyl acetate fraction	50.166
n-butanol fraction	>100

The assay results for the extracts summarised in table 1 were separated into three categories: score 1—weak (IC50 ≥ 50 µg ml-1), score 2—moderately active (10 µg ml-1 < IC50 < 50 µg ml-1) and score 3—active (IC50 ≤ 10 µg ml-1). An IC50 value of less or equal to 10 µg ml-1 was used as the cut-off point to select the potential fractions for further bioassay guided isolation. IC50 values of methanol extract >100 µg/mL, n-hexane fraction 62,186 µg/mL, Ethyl acetate fraction 50,166 µg/mL , and n-butanol fraction >100 µg/mL on the growth of the P-388 murine leukemia cells. Ethyl Acetate and n-hexane fraction exhibited higher cytotoxic effect than methanol extract and n-butanol fraction. This significant difference in IC50 values of Ethyl Acetate fraction has prompted us to carry out on-going work to determine the active ingredients that may have potential to be developed as valuable lead compound(s). This speculation, however, needs further studies for confirmation and therefore, Ethyl Acetate fraction was subjected to bioassay guided isolation. According to the United States National Cancer Institute plant screening program, a plant extract is generally considered to have active cytotoxic effect if t IC_{50} value, following incubation between 48 to 72 h, is 20 µg/mL or less.

The crude Methanol extract) and n-butanol fraction was found to have no cytotoxic effect on P-388 Murine Leukemia cells tested (IC50 > 100 µg/mL). However, ethyl acetate and n-hexane fraction extracts shown better cytotoxic activity than crude Methanol extract and n-butanol fraction, as shown in Table 1. The n-hexane extract having IC50 values in the range of 62.186 µg/mL and ethyl acetat 50.166 µg/mL can be classified as possessing mild cytotoxic activity against the selected human cell lines.

The drugs used to combat cancer belong to one of two broad categories. The first is cytotoxic (cell killing) drugs and the second is cytostatic (cell stabilising drugs). Both categories lead to a reduction in the size of the tumour because cancer cells (for various reasons) have such a high mortality rate that simply preventing them from dividing will lead to a reduction in the population.

Cytotoxic drugs work by interfering with DNA replication. Because cancer cells are rapidly dividing they are rapidly synthesizing new DNA - and if this is damaged the cell will die. There are three main groups of molecules that can be used to interfere with DNA replication : antimetabolites, alkylating groups and DNA- binding agents.

These observation are indicative of potentials of the extract in the cytotoxic activity against P-388 meurine leukemia. cancer cell lines. Hexane extract from Lygodium flexuosum (L.) Sw. (Lygodiaceae), genus lygodium a species ranging from Southeast Asia to northern Australasia, also inhibited viability of hepatoma cells, where it further induced apoptosis, inhibited TNF-α and activated NF-κB [Wills, 2009].

CONCLUSION

The results of cytotoxic activity against murine leukemia P-388 cells indicated that IC_{50} values of methanol extract >100 µg/mL, n-hexane fraction 62,186 µg/mL, Ethyl acetate fraction 50,166 µg/mL , and n-butanol fraction >100 µg/mL on the growth of the P-388 murine leukemia cells.

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