Cytotoxic Activity of Tegari (*Dianella nemorosa* Lam; Liliaceae) Leaves Methanol Extract from Papua Against Human Cells Lines *In Vitro*

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Abstract

Cancer is one of deathly diseases in the world. Many people conversely attempt to employ traditional medicine for primary health care, especially from Plants. Dianella nemorosa *Lam. has their own local name in Papua,* pra kepey *and belongs to the Liliaceae family. It is one of the anticancer plants that is empirical efficacious usage. The aim of the research was to find out cytotoxic activity of methanol extract of* D. nemorosa *leaves against selected human cell line namely hormone-dependent breast carcinoma cell line (MCF-7), human breast carcinoma cell line (T-47D), colon carcinoma cell line (WiDR) and human-ß-lymphoma cell line (Raji Cell). Leaves powder were extracted using methanol. Cytotoxic assay of the methanol extract was done using MTT [3-(4,5-dimetilthiazol-2-il)-2,5-difeniltetrazolium bromida) assay method* in vitro*. The result indicated that methanol extract of* D. nemorosa *leaves possessed cytotoxic activity in all tested cancer cell line, with the* IC_{50} *values of 1,794 µg/ml, 1,732 µg/ml, 1,719 µg/ml, and 1,049 µg/ml for MCF-7, T47D, WiDR and Raji cell line, respectively after incubation 24h. This species could be considered as potential sources of anticancer compounds. Further studies are necessary for chemical characterization of the active principles and more extensive biological evaluations.*

***Key words**:* Dianella nemorasa *Lam, cytotoxic, human cell line, MTT assay*

INTRODUCTION

Tropical rain forests, with their extremely rich vegetation, offer great possibilities for the discovery of promising new drugs. Herbal medicines, also known as botanical medicines or phytomedicines, refers to the medicinal products of plant roots, leaves, barks, seeds, berries or flowers that can be used to promote health and treat diseases. Medicinal use of plants has a long history worldwide (Li *et al.,* 2008).

Herbal medicines have a vital role in the prevention and treatment of cancer. Some herbs protect the body from cancer by enhancing detoxification functions of the body. Certain biological response modifiers derived from herbs are known to inhibit growth of cancer by modulating the activity of specific hormones and enzymes. Some herbs reduce toxic side effects of chemotherapy and radiotherapy and are often employed for cancer treatment various anticancer herbs has been identified, which execute their therapeutic effect by inhibiting cancer-activating enzymes and hormones, stimulating DNA repair mechanism, promoting production of protective enzymes, inducing

antioxidant action and enhancing immunity of the body (Sakarkar and Deshmukh, 2011).

Plants have been important sources for providing many anticancer agents with novel structures and unique mechanisms for the control and cure of cancer. Herbal medicine remedies individually or in combination have been recommended in various medical treatises for the cure of different diseases (Kintzios and Barberaki, 2004). *Dianella nemorosa* commonly known as *tegari* in Indonesia and with common name in Papua are *pra kapey* or War Plant. This plant is commonly used by the local community in Tablasupa village, Sentani, Papua. They generally consumed the leaves of this plant either in the raw form or by drinking the soup made by boiling the leaves in water (Maturbongs *et al.,* 2000; Fitowin, 2006).

According to the previous reports several active components have been isolated from some species of Dianella. Dianella containing napthoquinone, naphthalane, plumbagin, stypandrol, polifenol and saponin (Byrne *et al.,* 1987; Cheeke, 1989). Other study showed that musizin (derivative of naphthalene), benzoic acid, acetophenone, isougenitol and chromones have been isolated from the roots of *D. ensifolia* (Lojanapiwatna *et al.,* 1982; Chung *et al.,* 1998).

Several researcher have studied that naphthalene, methyl orsellinate (benzoic acid), acetophenone, isougenitol, chromones compounds possessing various anticancer activities in a wide variety of cell line (Gul *et al.,* 2002; Subramaniam *et al.,* 2008; Nakayama *et al.,* 2009). Therefore, the current study focused on examination of cytotoxic activity of methanol extract of *D. nemorosa* leaves *in vitro* against Human cancer cell line.

MATERIALS AND METHODS

Preparation of extract

D. nemorosa plant was collected from the Tablasupa village-Sentani, Papua. The plant was identified by Taxonomy Laboratory, Gadjah Mada University and Botany Laboratory-Herbarium, Indonesian Institute of Science (LIPI). The leaves are washed, dried and chopped finely using a blender. Five hundred grams of dried material were exhaustively extracted with methanol. The Methanol extract was filtered and concentrated using a rotary evaporator and then evaporated to dryness.

Preparation of selected cancer cell line

Selected Human cell line: hormone-dependent breast carcinoma cell line (MCF-7), human breast carcinoma cell line (T-47D), colon carcinoma cell line (WiDR) and human-ß-lymphoma cell line (Raji Cell) was obtained from Laboratory stock of LPPT Unit III, UGM. MCF-7, T47D and Raji cancer cell line was grown on RPMI 1640 medium, for WiDR cell line used DMEM Medium (Sigma) medium containing 5% v/v Fetal Bovine Serum (FBS) (Sigma) and 1% v/v Fungsison (Sigma) in the presence of 1% w/v of Penicillin-streptomycin (Sigma) under 5% CO_2 at 37^oC.

In vitro assay for cytotoxic activity

One hundred microliter the cell suspension 2.10^4 cell mL^{-1} was plated into 96 well microplate (Nunc, Germany). Different concentration of the sample were added with two time serial dilution (2000, 1000, 500, 250, 125, 62.50, 31.25 and 15.625µg/ml. Plate was incubated at 37^oC, with 5% CO_2 for 24h in incubator. After 24h of incubation, MTT (5mg/ml FBS) was added to each well and incubation was continued further 4h and the media removed. After that 100µl of 10% sodium dodecylsulfate (SDS) was added to stop MTT reaction and incubation at 37^OC, with 5% CO_2 for 15h. The absorbance was read at wavelength of 540 nm using ELISA reader. The percentage of cellular viability was calculated with appropriate control taken into account. The concentration which inhibited 50% of cellular growth (IC_{50}) was determined.

RESULT AND DISCUSSION

Cytotoxic test used to find out potential anticancer properties from methanol extract of *D. nemorosa* leaves againts breast carcinoma cell line (MCF-7), human breast carcinoma cell line (T-47D), colon carcinoma cell line (WiDR) and human-ß-lymphoma cell line (Raji Cell). The cytotoxic activity of methanol extract of *D. nemorosa* leaves was measured using 3-(4,5-dimethylthiazol-2yl)-2,5 diphenyl tetrazolium bromide assay (MTT assay).

The assay was based on the capacity of mitochondrial dehydrogenase to convert the yellow water-soluble substrate MTT into dark blue formazan product which was insoluble in water. The amount of formazan product is directly proportional to the viable cell number in a variety of cell type (Berridge *et al.,* 1996). This augmentation of enzyme activity leads to the increase in the amount of formazan dye formed, which directly correlates to the number of metabolically active cells in the culture.

Table 1. IC_{50} values after treated with extract methanol of *D. nemorosa* leaves after incubated 24h.

No.	Cell line	IC50 (µg/ml)
1.	MCF-7	1,794 µg/ml
2.	T47D	1,732 µg/ml
3.	WiDR	1,719 µg/ml
4.	Raji cell	1,049 µg/ml

Cell mortality data could be used to determine the IC_{50}. The result demonstrated methanol extract of *D. nemorosa* had cytotoxic activity with IC_{50} values of 1,794 µg/ml, 1,732 µg/ml, 1,719 µg/ml, and 1,049 µg/ml for MCF-7, T47D, WiDR and Raji cell line respectively after incubated 24h. The result showed that methanol extract exhibited cytotoxic activity againts all cell line was tested. The IC_{50} values are summarized in Table 1.

As shown in Figure 1, 2, 3 and 4. In concentration of 2000µg/ml average percentage of cell death almost 100% compared to untreated cell. This was indicated by decreased of the formazan dye formed, compared to the control which have high intensity of dark blue purple colour. Microscope analysing showed all cell line have morphological differences between control and extract.

Herbal medicine refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes and any parts of the plant may be contains different active compounds (Barrett *et al.,* 1999). Many studies have reported that active compounds have been isolated from the roots some of *Dianella* like *D. revoluta, D. ensifolia* and *D. longifolia* (Lojanapiwatna *et al.,* 1982; Colegate *et al.,* 1986).

In this study focus on the anticancer activity of methanol extracts from leaves of this plant. The leaves were chosen in this study because it was reported that the leaves were commonly consumed by the local ethnic groups in Tablasupa, Sentani-Papua, and no data are available about cytotoxic activity of *D. nemorosa* leaves and this is may be the first study report cytotoxicity of methanol extract of *D. nemorosa* leaves against on different human cell line. It is possible that the bioactive compounds with anticancer activity may be concentrated in other parts of plants as the plant's natural chemical defence ecology. Some studies have reported that extract from different parts of plant showed different effect on cell lines. The stem and leaves exhibited weaker cytotoxic activity against murine P388 leukaemia when compared with the roots and tubers of *Typhonium flagelliforme* (Chee *et al.,* 2001).

Other study reported the leaf and seed of *Annona squamosa* showed exhibited higher potency cytotoxic activity than the stem bark (Shirintorn *et al.,* 2004).

Many medicinal plants contains large amounts of chemical compounds having broad spectrum of pharmacological activities like anticancer, antitumor and antioxidant activities. Experimental pharmacology researches in animal models

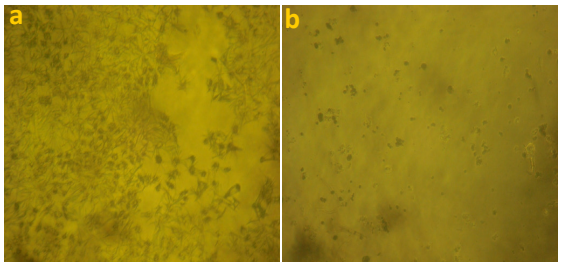

Figure 1. Morphology MCF7 cell line after treated by MTT. (a). control MCF7 in RPMI medium, (b). treated cell with concentration 2000 µg/ml methanol extract of *D. nemorosa* after incubation 24h .

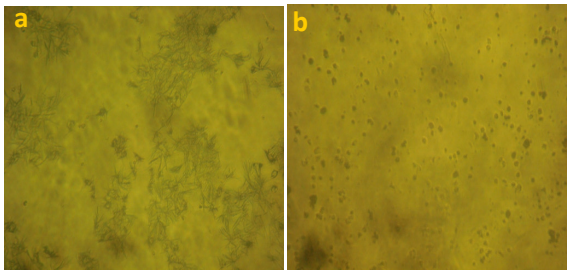

Figure 2. Morphology T47D cell line after treated by MTT. (a). Control T47D in RPMI medium, (b). treated cell with concentration 2000 µg/ml methanol extract of *D. nemorosa* after incubation 24h .

or on cell lines have confirmed that the extracts and/or isolated purified compounds derived from Liliaceae family possess many beneficially biological effects, including cytotoxic activity (Kintzios and Barberaki, 2004)

In our preliminary study using paper chromatography also showed that the methanol extract of *D. nemorosa* contained phenolic, alkaloid, tannin and terpenoid compounds. These compounds found in plant sources, with a great variety of different effect on each cell line. The cytotoxicity activity of methanol extract of *D. nemorosa* against human cell lines, may be due to the presence of these compounds and responsible for cytotoxic activity. Many compounds that interact only with a very specific receptor in spesific cell line (Rajesh and Sahil, 2011)

Several studies reported many extract from herbal plant different cytotoxicity activity or selective on the differents cell line. This selectivity could

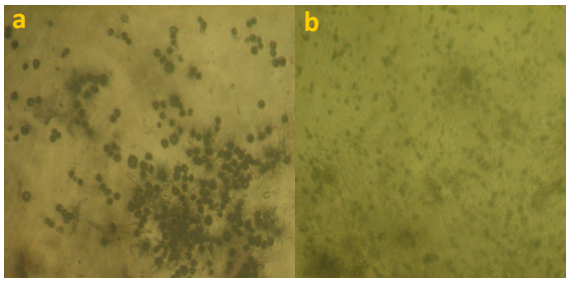

Figure 3. Morphology WiDR cell line after treated by MTT. (a). control cell WiDR in DMEM medium, (b). treated cell with concentration 2000 µg/ml methanol extract of *D. nemorosa* after incubation 24h.

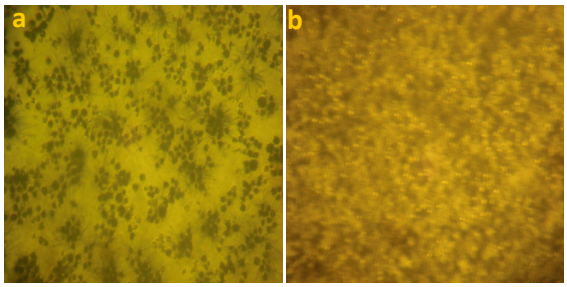

Figure 4. Morphology Raji cell line after treated by MTT. (a). control cell Raji in RPMI medium, (b). treated cell with concentration 2000 µg/ml methanol extract of *D. nemorosa* after incubation 24h .

be due to the sensitivity of the cell line to the active compounds in the extract or to tissue specific respon (Kirana *et al.,* 2003). Some studies have reported that the water extract of *Dioscorea birmanica* was selectively toxic against cell line, some activity against COR-L23 (Large cell lung carcinoma) but it showed no cytotoxic activity against MCF-7 (Human breast cancer) and LS-174T (Human colon adenocarcinoma), and other result have showed that etanolic extract of *Siphonodon celastrineus* leaves have cytotoxic activity against breast cancer but had low activity against lung cancer and no activity against colon cancer was studied but some extract had no specific cytotoxic activiy (Arunporn *et al.,* 2004).

The result showed that methanol extract of *D. nemorosa* may be selective cytotoxic against another cell line. In this study, we assumed that many factor influence cytotoxic activity from methanol

extract of *D. nemorosa* against all cell line was tested. Different method of extraction can result different compounds that have cytotoxic activity. Chloroform extract and ethanol extract of *Erythrina fusca* had different cytotoxic activity against Raji cell line (Zullies dkk, 2006,2007).

The result suggested that in methanol extract of *D. nemorosa* contains a bioactive compounds that have low cytotoxic activity against cell lines tested. These compounds might have high cytotoxic effect against other type of cell cancer. Further study are necessary to be conducted to investigate constituents of their crude extract which could elicit their pharmacological effects can help understanding their structure and activity relantionship and its important to estimated the nature constituens and potency another part of this plant to another cell line.

CONCLUSION

The methanol extract of *Dianella nemorosa* leaves from Papua possessed low cytotoxic activity with IC_{50} values were 1,794 µg/ml, 1,732 µg/ml, 1,719 µg/ml, and 1,049 µg/ml against MCF7, T47D, WiDR and Raji cell line respectively after incubated 24h.

ACKNOWLEDGEMENT

The research was part of the Herbal Research Group. That was supported grant from Health and Medical Cluster 2009, Universitas Gadjah Mada and we also thank to Prof. Dr. Mae Sri Hartati Wahyuningsih, Apt. M.Si, as Coordinator of The Research Group.

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