Antiproliferative Activities of *Dianella nemorosa* Lam. Leaves Methanol Extract Against HCT-116, C2C12 and 293A Cell lines

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ABSTRACT: *Dianella nemorosa Lam. (Liliaceae) is a medicinal plant traditionally used in Papua for treatment of cancer, injury, fracture, inflammation and also for antiseptic. The aim of this study was to evaluate antiproliferative activities of methanol extract of D. nemorosa leaves against HCT+116 (colorectal cell line), C2C12 (adherent mouse myoblast cell line) and 293A (primary embryonal human kidney transformed with human adenovirus type 5 DNA). Powder of leaves was extracted using methanol. Antiproliverative activities were determined by cell proliferation reagents WST-1 assay for 1h, 2h, and 4h after 72h incubation. The result showed that methanol extract of D. nemorosa leaves showed remarkable antiproliferative activities against HTC-116 cell line with IC50 values of 199.31 µg/ml (1h), 197.87 µg/ml (2h) and 161.12 µg/ml (4h). The activities against C2C12 cell line resulted in the IC50 values of 405.51 µg/ml (1h), 435.12 µg/ml (2h) and 394.38 µg/ml (4h), while the IC50 values for 293A cell line were 580.81 µg/ml (1h), 442.21 µg/ml (2h) and 366.74 µg/ml (4h), respectively. Those results indicated that methanol extract of D. nemorosa leaves possess potential antiproliferative activities against HCT-116, C2C12 and 293A cell lines. Further study is necessary to investigate the inhibitory mechanism of methanol extract of D. nemorosa leaves on HCT-116, C2C12, and 293A cell lines.*

Keywords *: Dianella nemorosa Lam, cancer cell, HCT-116, C2C12, 293A*

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ABSTRAK: *Dianella nemorosa* Lam. (Liliaceae) adalah salah satu jenis tumbuhan yang secara tradisional di Papua digunakan untuk pengobatan kanker, luka, inflamasi dan juga sebagai antiseptik. Penelitian ini bertujuan menguji aktivitas antiproliferatif ekstrak metanol dari daun *D. nemorosa* terhadap sel kanker HCT-116 (*colorectal cell line*), C2C12 (*adherent mouse myoblast cell line*) dan 293A (*primary embryonal human kidney transformed with human adenovirus type 5 DNA*). Serbuk daun diekstraksi dengan menggunakan metanol. Aktivitas antiproliferatif diuji dengan menggunakan uji reagen proliferatif WST-1 selama 1, 2 dan 4 jam setelah inkubasi 72 jam. Hasil penelitian menunjukkan bahwa ekstrak metanol daun *D. nemorosa* memiliki aktivitas antiproliferatif terhadap sel kanker HCT-116 dengan nilai IC_{50} sebesar 199.31µg/ml (1jam), 197.87µg/ml (2jam) dan 161.12µg/ml (4h), untuk sel kanker C2C12 IC_{50} sebesar 405.51µg/ml (1jam), 435.12µg/ml (2jam) dan 394.38µg/ml (4jam), sedangkan sel kanker 293A nilai IC_{50} sebesar 580.81µg/ml (1jam), 442.21µg/ml (2jam) dan 366.74µg/ml (4jam). Dari hasil penelitian ini mengindikasikan bahwa ekstrak metanol daun *D. nemorosa* memiliki aktivitas antiproliferatif terhadap sel kanker yang diuji. Penelitian lanjutan masih diperlukan untuk mengetahui mekanisme penghambatan pada sel kanker HCT-116, C2C12 dan 293A.

Kata kunci : *Dianella nemorosa* Lam, sel kanker, HCT-116, C2C12, 293A

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INTRODUCTION

Cancer or malignant disease is one of the major causes of death in humans. The malignant neoplasm is the third (12.4%) leading cause of death worldwide. The first (30%) being cardiovascular disease, and the second (18.8%) being infectious diseases, which include HIV/AIDS. The total number of cases of cancer in 2000 to 2020 is estimated to increase by 73% in the developing countries (1,2).

Plants may be an alternative to currently used anticancer agents, because they are a rich source of bioactive compounds. Since many of them are largely free from adverse effects and have excellent pharmacological actions. The plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, and phenolic compounds, which have been screened and indicated anticancer, antioxidant, antiinflammatory, and antimicrobial activity (3,4,5,6,7).

Plants from tropical regions such as medicinal plant from Papua region are considered to be one of the potential sources for the screening of anticancer agents. In West Papua and Papua, it has been estimated may be contains 20.000-25.000 species of plant and only little of this species used for medicinal plant. Some of Papua medicinal plants remain to be assessed for possible cytotoxic activities such as *Phaleria macrocarpa*, *Pandanus conoideus*, and *Myrmecodia pendans* (8,9,10,11).

In Papua, most of the research activities in natural products are still limited to the inventory of herbal medicine information and utilization of various plants and trees, meaning that obtaining scientific proof for their biological activities are still challenging and need more investigation. One of the medicinal plants is *D. nemorosa* Lam. which might be potential for new source of anticancer agent. This plant is commonly known as Tegari, and has local name in Papua is *Pra Kepey* referred to as *War Plant* and members of Liliaceae family (12,13).

This plant grown on tropical areas and produces beautiful flowers with a variety of colours such as blue, purple and white, and commonly planted for ornament and decorative purpose. Scientific publication on *D. nemorosa* is still difficult to obtain and generally only discussing about its taxonomy and ethnobotany. This plant is one of most common medicinal plants used by local community in Tablasupa village, Papua. *D. nemorosa* has been used as an alternative treatment for control of cancer, injury, fracture, inflammation, various of skin diseases and also was used as an antiseptic. Some species of Dianella extracts have potential to be developed as anticancer agent. Methanol extract of *D. nemorosa* leaves contains alkaloid, terpenoid, phenolic compounds and shown that it inhibition in HeLa cell line using MTT assay (14).

Other study reported that several classes of compounds have been isolated and identified including naptoquinone, stypandrol, saponin, plum-bagin, poliphenolic, and alkaloid from different species of Dianella and have shown a wide variety of pharmacological activities, such as antimicrobial, anticancer, antitumor and antiinflammatory activities (15,16,17,18).

Herbal medicine have long been used traditionally to prevent and treat many disease including cancer and thus they are good candidates for the development of anticancer agent. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbals (19, 20).

To our knowledge, a few recorded data for clinical study or for biological activity of *D. nemorosa* Lam. against human cell line. Here, we have attempted to evaluate the antiproliferative activities of *D. nemorosa* leaves methanol extract from Papua against HCT-116, C2C12 and 293A cell lines.

MATERIALS AND METHODS

Preparation of *D. nemorosa* 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 maseration. The Methanol extract was filtered and concentrated using a rotary evaporator and the evaporated do dryness.

Preparation of cell line

HCT-166 cell line obtained from Clinical Oncology Laboratory stock of Kawasaki Medical School, Japan. HCT-116 cell line was grown on McCoy'S 5A medium (Gibco) containing 10% v/v Fetal Bovine Serum (FBS) (Sigma) and presence of 1% w/v of Penicillin-streptomycin (Sigma). C2C12 and 293A line was obtained from Laboratory stock of Department Moleculer and Developmental Biology, Kawasaki Medical School, Japan. C2C12 was grown on DMEM (Sigma) medium containing 10% v/v Fetal Bovine Serum (FBS) (Sigma) and 1% v/v kanamicin (Sigma), and 293A cell line on complete medium containing 10% Fetal bovine serum (FBS), 2mM L glutamine (Gibco), Amino acids (NEAA) (Gibco) and presence of 1%w/v of penicillin-streptomycin (Sigma). The cultures were maintained at 37°C in humidified atmosphere of 5% CO_2.

In vitro assay for antiproliferative activities

The cell suspension 4.0x10^3 cell/ml (100µl) was plated into 96 well microplate (Nunc, Germany) and treated with different concentration of methanol extract *D. nemorosa* leaves, in a serial dilution (500, 250, 125, 62.50, 31.25 dan 15.625, 7.8125 µg/ml). Following treatment, plates were incubated in CO_2 incubator at 37°C for 72h. Medium removed by aspirator and add medium with 10 µl cell proliferation reagent WST-1 for

Figure 1

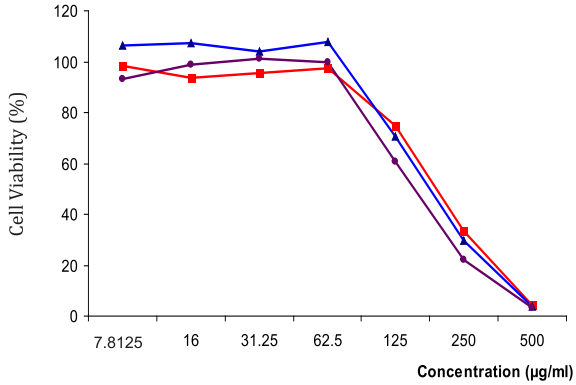

Figure 1. Effect of methanol extract of *D. nemorosa* leaves (µg/ml) on the viability of HCT-116 cell line (%). Antiproliferative activities was measured after incubation for 72h at 37°C with 5% CO_2, and after add WST-1 reagent for 1h (■), 2h (▲) and 4h (●).

Figure 2

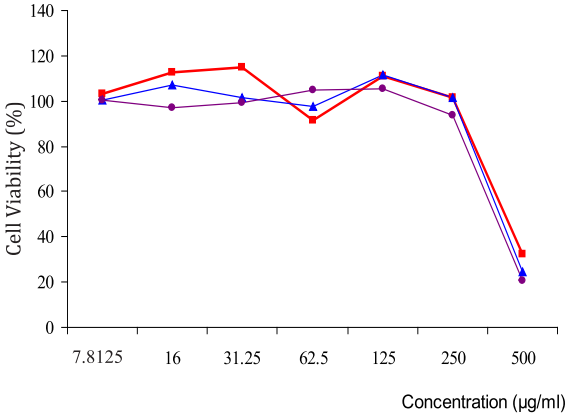

Figure 2. Effect of methanol extract of *D. nemorosa* leaves (µg/ml) on the viability of C2C12 cell line (%). Antiproliferative activities was measured after incubation for 72h at 37°C with 5% CO_2, and after add WST-1 reagent for 1h (■), 2h (▲) and 4h (●).

Figure 3

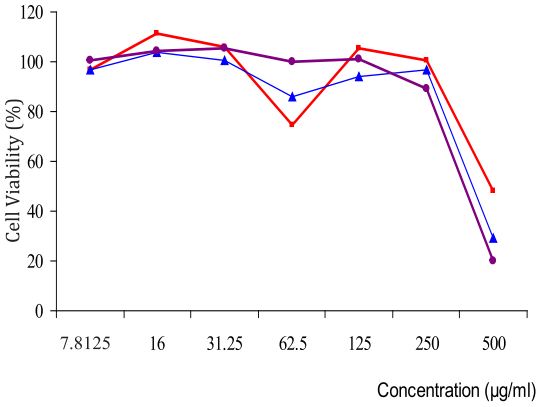

Figure 3. Effect of methanol extract of *D. nemorosa* leaves (µg/ml) on the viability of 293A cell line (%). Antiproliferative activities was measured after incubation for 72h at 37°C with 5% CO_2, and after add WST-1 reagent for 1h (■), 2h (▲) and 4h (●).

1h, 2h and 4h and incubated in CO_2 incubator at 37^0C. The absorbance was read at wavelength of 450nm using ELISA reader type Varioskan Flash (Thermo scientific). The percentage cellular viability was calculated with appropriate control taken into account. The concentration which inhibited 50% of cellular growth (IC_{50}) was determined. The inhibitory rate of cell proliferation was calculated by the following formula:

$$\text{Cell Viability (\%)} = \frac{\text{OD Control - OD treated}}{\text{OD Control}} \times 100$$

RESULT AND DISCUSSION

Cell viability was assayed by determination of the cleavage of tetrazolium salt (WST-1) to formazan by mitochondrial dehydrogenase. This assay is based on the reduction of WST-1 by viable cell producing a soluble formazan salt. The increase in the amount of formazan dye formed is directly correlated to the number of metabolically active cell in culture that was quantified by measuring the absorbance at appropriate wavelenght. In this study, toxicity data are expressed as IC_{50}, a concentration of extracts that cause 50% inhibition or cell death, and was obtained by plotting the percentage cell viability versus concentration of methanol extract sampels (21). The extract that gave a IC_{50} value of 1000µg/ml or less was considered chemoprenventif activities (22).

The WST-1 assay was conducted, serial dilution of methanol extract were prepared in medium culture cell and results of the cytotoxicity test indicate the methanol extract of *D. nemorosa* was cytotoxic activity against HCT-116, C2C12 and 293A cell lines.

D. nemorosa showed antiproliferative activities against HCT-116 cell line with IC_{50} values of 199.31 µg/ml (1h), 197.87 µg/ml (2h) and 161.12 µg/ml (4h), for C2C12 cell line with IC_{50} values of 405.51 µg/ml (1h), 435.12 µg/ml (2h) and 394.38 µg/ml (4h), while for 293A cell line with IC_{50} values of 580.81 µg/ml (1h), 442.21 µg/ml (2h) and 366.74 µg/ml (4h), respectively.

The result showed that methanol extract of *D. nemorosa* leaves possess potentials inhibitory effect or antiproliferative activities against HCT-116, C2C12 and 293A cell lines with increasing time incubation. This plant extract containing bioactive compounds that against HCT-116, C2C12 and 293A cell lines. That is usually regarded as interesting for *in vitro* cytotoxic activity when IC_{50}<1000 µg/ml and its can be used for chemoprenventif agent (22). Based on this study, the extracts showed interesting *in vitro* cytotoxic activity to all cancer cells with various IC_{50} values.

Since it is known that different cell lines might exhibit different sensitivities while treated with different plant extracts, therefore the use of more than one cell line seems necessary for the comprehensive plant extract anticancer activity screening. Cell type have cytotoxic specificity of plant extracts is likely to be due to the presence of different classes of compounds in the extract.

The activities of these extract against HCT-116, C2C12 and 293A cell line might be due to the presence of highly complex compounds that present in *D. nemorosa.* Different compound might influence different biochemical processes or stages in different manners.

In our preliminary study using paper chromatography also showed that the methanol extract of *D. nemorosa* contains phenolic, alkaloid, tannin and terpenoid compounds but it does not contain saponin.

The result showed that natural compounds present in the extract exhibited a dose-dependent inhibitory effect on cell line used in this study. Many compounds from plant such as phenolic, alkaloid, terpenoid and tannin have been shown to have cancer preventive properties. The biological activities of crude extract can be due to a natural mixture of its components, and a single constituent may be have no an activity greater than that of the total extract as a whole (23).

Phenolics compounds have been indicated to have several biological activities such as antioxidant, antimutagenic, anticarcinogenic, anti-in-

flammatory and antimicrobial activities in human cell line (6,24). Phenolic compounds like epigallocatechin gallate, a major constituent of green tea has been shown to have antitumor activity in animal models of colon (25) and skin cancer (26). Polyphenols from red wine inhibited colon carcinogenesis induced by azoxymethane in rats (27). Another study reported that alkaloid tetrandine irreversibly inhibit the proliferation of human colon carcinoma cell by arresting cell in G_1 followed apoptosis and its increase the p53 and p21 expression in wild type p53 HCT-116 cell line (28). and methanol extract *Cataranthus roseus* (L) contains alkaloid, phenolic, flavonoid, steroid and glycoside had significant anti-cancer against HCT-116 (29), other study about another compounds curcumin (group of polyphenol compounds) isolated from *Curcuma longa* induces apoptosis in HCT-116 cell line (30).

Some terpenoid isolated from chloroform and methanolic extract of *Andrographis paniculata* possess cytotoxic activity against cancer cell lines HepG2, and HCT-116 using MTT Assay (31).

Several study reported many compounds from herbal extract or venom extract different cytotoxicity activity on the different cell line. Quercetin and DMSO modulated and changed Bcl-2 gene expression (Apoptosis regulating proteins) during myogenesis on C2C12 cell line (32) and in cytotoxicity tests, Arsenic trioxide (As_2O_3) has anticancer properties and its significantly reduced cell viability in SY-5Y neuroblastoma and 293A embryonic kidney (HEK) and staining with Hoechst 33342 showed occurrence of apoptosis and DNA damage (33).

Other study reported that bupivacaine and lidocaine caused cytotoxic on C2C12, and prevented cell growth and apoptosis, and it's caused cell death in a dose-dependent manner. IC_{50} for bupivacaine and lidocaine were 0.49 and 3.37 mmol/L, respectively (34).

Some venom from snake had cytotoxic activities on mouse myoblast cell line and primary human embryonic kidey like 293A, Egyptian cobra venom *N. haje* has cytotoxic effects, induced apoptosis and necrosis on 293T (primary human embryonic kidney) and C2C12 cell lines (35), while crude venoms from *B. alternatus* and *B. diporus* induced an early and significant decreased in cell viability C2C12 cell line and induce apoptosis process. *B. diporus* venom was significantly more cytotoxic (CC_{50}: 2 µg/mL) than *B. alternatus* (CC_{50}: 5.8 µg/mL) (36).

It assumed that methanol extract of *D. nemorosa* leaves contains phenolic, alkaloid, terpenoid, and tanin and that compounds responsible for antiproliferative activities against HCT-116, C2C12 and 293A cell culture.

In this study, showed that the ability of methanol extract to inhibit proliferation of HCT-116, C2C12 and 293A cell line was estimated by analysing its effect on the growth of the cells. The increase of the extract concentration, followed by the decrease of formazan dye. The formation of formazan dye directly correlates to the number of metabolically active of cell in the culture (21). It is indicated that methanol extract of *D. nemorosa* leaves proved to possess anticancer properties against HCT-116, C2C12 and 293A lines tested. This result supported the WST-1 experiment as shown in figure 1,2,3.

Various of carcinogenic pathways may be involved in HCT-116, 293A and C2C12 therefore, different appro-aches will be needed to prevent and treat such cancer.

Further investigations on compounds responsible for cytotoxic effects in this plant and antiproliferative mechanisms on HCT-116, C2C12 and 293A cell line were needed. Furthermore, an understanding of the mechanism of an anticancer agent is also important for future therapeutic application

CONCLUSION

The methanol extract of *Dianella nemorosa* leaves from Papua possess potential antiproliferative activities against HCT-116, C2C12 and 293A cell lines.

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