

ANTIPROLIFERATIVE EFFECT OF *Dendrophthoe pentandra* EXTRACTS TOWARDS HUMAN BREAST ADENOCARCINOMA CELLS (MCF-7)

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Graphical abstract



Abstract

Dendrophthoe pentandra (DP) is a semi-parasitic plant. Previous studies showed that this plant possessed diverse medicinal properties. The present study was carried out to determine antiproliferative activity of various DP crude extracts. Extracts of petroleum ether, diethyl ether, chloroform, ethyl acetate and methanol, have been evaluated through IC_{50} value, which defined by the concentration of drug that is required for the inhibition of 50% of cell population *in vitro*. The determination of percentage cell viability was conducted by using MTT assay. The screening assay involved the use of malignant cell line MCF-7 (breast cancer cell) whereas the normal cell line was L929 (connective cell). The control drug used was tamoxifen. The ethyl acetate and methanol extracts were found to be more effective against MCF-7 cancer cell lines compared to the others. All extracts showed no remarkable effect against normal cell L929 with IC_{50} values more than 100 $\mu\text{g/mL}$. In contrast to tamoxifen, all extracts showed less cytotoxicity effect towards normal cells. The data obtained from this study provide notable preliminary information on the cytotoxic/antiproliferative effect of different DP extracts and further study should be carried out to isolate the active compound of the most potential DP extracts.

Keywords: *Dendrophthoe pentandra*, extracts, antiproliferative, MCF-7 cells, MTT

Abstrak

Dendrophthoe pentandra (DP) merupakan tumbuhan separa parasit. Kajian terdahulu menunjukkan bahawa tumbuhan ini mempunyai pelbagai ciri perubatan. Kajian ini telah dijalankan untuk menentukan aktiviti antiproliferatif daripada pelbagai jenis ekstrak DP. Ekstrak petroleum ether, dietil eter, kloroform, etil asetat dan methanol DP telah dinilai melalui nilai IC_{50} , yang ditakrifkan sebagai kepekatan drug yang diperlukan untuk merencatkan 50% daripada populasi sel secara *in vitro*. Penentuan peratusan sel yang hidup telah dijalankan menggunakan asai MTT. Untuk asai saringan ini, sel maligna yang terlibat adalah MCF-7 (sel kanser payudara) manakala sel yang normal adalah L929 (sel penghubung). Di antara lima jenis ekstrak DP yang telah digunakan dalam eksperimen ini, ekstrak mentah etil asetat dan methanol lebih berkesan terhadap sel kanser MCF-7. Perbandingan keputusan ini dengan sel normal L929, menunjukkan kesemua lima jenis ekstrak mentah tidak menunjukkan kesan yang luar biasa melalui nilai IC_{50} dengan kesemua nilai melebihi 100 $\mu\text{g/ml}$. Berbeza dengan tamoxifen, semua ekstrak mentah menunjukkan kurang kesan sitotoksik terhadap sel normal. Data yang diperolehi daripada kajian ini menyediakan maklumat awal yang ketara ke atas kesan sitotoksik/antiproliferatif pelbagai ekstrak DP dan kajian lanjut perlu dijalankan untuk mengasingkan sebatian aktif yang paling berpotensi daripada ekstrak DP.

Kata kunci: *Dendrophthoe pentandra*, ekstrak, antiproliferatif, sel MCF-7, MTT

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1.0 INTRODUCTION

Dendrophthoe pentandra (DP) is one the species of *Dendrophthoe sp* in the Loranthaceae family. DP, known as mistletoe, is a semi - parasitic plant that obtains its nutrition by living on the other plants. It is widely distributed in China, Cambodia, India, Indonesia, Laos, Malaysia, Myanmar, Philippines, Thailand, and Vietnam [1]. They are commonly found growing on many different species of host plant. Different species of mistletoe can grow on the same tree or one species of mistletoe can grow on many different trees. It is suggested that mistletoes bioactivities is depend on their host plant since they are semi-parasitic plant [2].

This plant has been used traditionally in the treatment for cough, diabetes, hypertension, cancer, diuretic, smallpox, ulcer, skin infection and after childbirth treatment in Indonesia [3]. DP is traditionally used in cancer treatments in many countries [4]. This has been reported that a complex of perseitol (D-glycero-D-galactoheptitol) and K⁺ ions in a molar ratio of 20:1 was isolated from the leaves originated from Loranthaceae family, which has been traditionally used for the treatment of cancer in Sulawesi Island, Indonesia [5].

According to Artanti and co-workers (2012), DP leaves extract shows significant antioxidant and antidiabetic activity. DP extract has high antioxidant activity with extract exhibit free radical scavenging activity with IC₅₀ less than 50 µg/mL [3]. Quercetin from the flavonoid's group is one of the compounds that has been found in DP that have high antioxidant activity [4]. Flavonoid, alkaloid, tannin and saponin are found out to be present in DP methanol and toxic towards brine shrimp in Brine Shrimp Lethality Test (BSLT) [6]. No extensive research has been done to evaluate antiproliferative activity of different types of DP extracts that could lead to anticancer properties.

Therefore, this current study is conducted specifically to evaluate the antiproliferative activity by using various types of DP extract which are DP petroleum ether, DP diethyl ether, DP chloroform, DP ethyl acetate and DP methanol. From here, the most suitable organic solvent extraction that showed most effective inhibition towards MCF-7 cells could be known.

2.0 EXPERIMENTAL

2.1 Plant Materials

The DP plants were collected and obtained from Pasir Hor, Kelantan, Malaysia. The leaves of DP were identified by the botanist, Dr. Ashraf Mansur from USM, Penang. The harvested fresh leaves of DP were rinsed with tap water and dried in oven at 50°C for 3 days. The dried leaves of DP were blended with homogenizer into fine powder. The powder form of dried DP plant were weighed and kept in airtight container at 20°C until further use.

2.2 Extraction

DP fine powder (500 g) was extracted using five types of solvents; petroleum ether, diethyl ether, chloroform, ethyl acetate and methanol at room temperature for 24 hours. The successive extraction was performed using non-polar solvent to more polar solvent. These types of extraction are followed by increasing order of solvent polarity since different types of solvent polarity could extract different bioactive compound. The residue was filtered using Whatman No. 1 filter paper. The filtrate was then evaporated to dryness using vacuum distillation and rotary evaporator at 40°C. The extracts were transferred to high vacuum apparatus to dry many of the solvents. All the DP extracts were kept at 4°C in refrigerator for further use.

2.3 Cell Cultures

The human breast adenocarcinoma cell line (MCF-7) and connective tissue (L-929) were bought from the American Type Culture Collection (ATCC, USA). Both cell lines were cultured in Dulbeccos Modified Eagles Medium (DMEM); supplemented with 10% of heat-inactivated fetal bovine serum (FBS), 100 IU/mL of penicillin and 100 µg/mL of streptomycin (Pen-Strep) using 25 cm² flasks in humidified incubator (5% CO₂, 37°C). All media and supplement were purchased from Gibco, Life Technologies Ltd. Cells were kept in the logarithmic growth phase by routine passage every 2-3 days using 0.025% Trypsin-EDTA treatment.

2.4 MTT Assay

MTT assay was done to determine the enzyme activity that reduces the MTT yellow colour (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan, giving a purple colour. Cells were seeded 24 hours prior to treatment in a 96-well plate with a density of 5 × 10⁴ cells/well in 100 µL of medium in order to obtain 70% - 80% confluent cultures within 24 hour. Before the growth medium was added, the medium was discarded and the cells were feed with 200 µL growth medium. All types of extract were dissolved in DMSO and followed by a 2 times serial dilution from 0.2 to 100 µg/mL were added to the cells in 200 µL medium. Tamoxifen was used as a positive control while the negative control group was only cells cultured in 200 µL growth medium with addition of DMSO. The final concentration of DMSO used in the corresponding wells did not exceed 1% (v/v). The 96-well plate was incubated for 72 hour at 37°C in a humidified atmosphere with 5% CO₂. At the end of incubation, 50 µL of MTT solution (2 mg/mL MTT in plain culture medium) were added to each well. The plates were then incubated for 4 hours. MTT solution was removed and the purple formazan crystal formed at the bottom of the wells was dissolved with 200 µL DMSO for 30 minutes. The absorbance at 570 nm was read on a spectrophotometric microplate reader. The proportion of surviving cells is calculated as

$$\% \text{ Cell viability} = \frac{\text{Absorbance of extract}}{\text{Absorbance of negative control}} \times 100\%$$

Dose-response curves were constructed to obtain the IC_{50} values. Each concentration is tested in three replicates and derived from at least three independent experiments.

2.5 Statistical Analysis

All values were expressed as the mean \pm standard deviation. Statistical analyses were evaluated by independent student's T-test using SPSS version 22.1. The data was considered to be statistically significant if the probability $p < 0.05$.

3.0 RESULTS AND DISCUSSION

All of these extracts have been evaluated through IC_{50} value, defined by the concentration of compound that is required to inhibit 50% of cell population *in vitro*[7]. Inhibition of cell proliferation that was induced by certain agents or solvents that cause cell death can be simplified as antiproliferative activity. The requirement for extract to be considered having good antiproliferative activity is an IC_{50} value $\leq 20 \mu\text{g/mL}$, as stated by the National Cancer Institute (NCI). In this study, that range value was used as a rough reference point for evaluating the activity of the DP extracts. The determination of percentage of cell viability was conducted by using MTT assay.

For this screening assay, malignant cell lines involved was MCF-7 (breast cancer cell) whereas the normal cell line was L929 (connective cell). The positive control drug used was tamoxifen (TAMO). The negative control used was DMSO. The antiproliferative activity of DP was carried out purposely to select the most effective suppression of breast cancer cell growth when treated with various types of DP extracts. Only DP extract that showed most effective inhibition towards the MCF-7 cancer cell line was selected for further research studies.

Figure 1 summarized all the values as reported above for effect of various types of DP extracts toward MCF-7 cells. Within the five types of DP extracts that have been tested in this experiment, crude extract of DP ethyl acetate and DP methanol were more effective against MCF-7 cancer cell lines with IC_{50} $14.42 \pm 0.34 \mu\text{g/mL}$ and $17.70 \pm 0.21 \mu\text{g/mL}$, respectively. Results showed that there was less significant on MCF-7 antiproliferative activity with IC_{50} $82.33 \pm 2.61 \mu\text{g/mL}$, $89.70 \pm 0.41 \mu\text{g/mL}$ and $101.57 \pm 1.29 \mu\text{g/mL}$ when treated with DP chloroform, petroleum ether and diethyl ether, respectively. This can be said that different types of extract could reveal different therapeutic effects towards MCF-7 breast cancer cells.

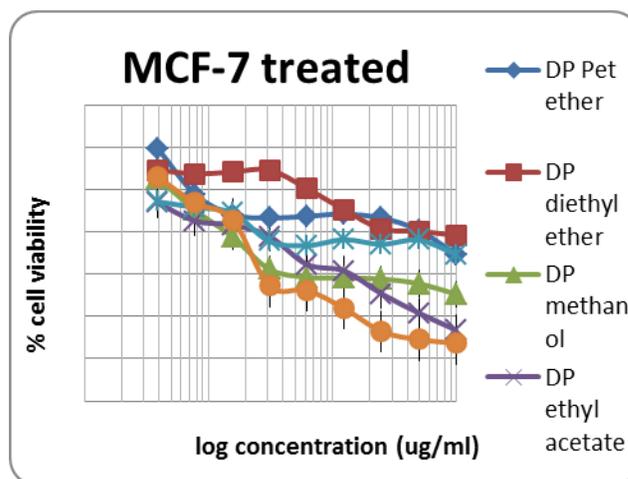


Figure 1 Antiproliferative activity of various extracts of DP on MCF-7 breast cancer cell. Each point showed the percentage of viable cells compared to positive control, Tamoxifen (average) and negative control, DMSO. Each point represented as mean \pm S.D, $n = 3$

There are other researchers reported that ethyl acetate extract of leaves of *Memecylon edule* could inhibit the gastric cancer cell growth in dose dependent manner which is the cytotoxicity was more towards the NUGC and MKN-74 gastric cancer cells, compared to GES-1 normal gastric cells [8]. Ethyl acetate extract of *Phellinus linteus* that grown on ginseng exhibited more enhanced antiproliferative activity on HT-29 human colon carcinoma cells than *Phellinus linteus* alone or even ginseng alone [9]. Methanolic extract of *C. concinna* Hance roots significantly reduced cell viability of two oral cancer Ca9-22 and CAL 27 cell lines in dose responsive manner [10]. The effectiveness of ethyl acetate extract and methanol extract are synchronized with findings from current study which also showed that DP ethyl acetate and DP methanol showed potency as antiproliferative agent towards MCF-7 breast cancer cell.

By comparing to other types of DP extract, the DP aqueous extracts exhibited a decrease of viability in breast cancer cell line, T47D cell lines in a concentration dependent-manner where the IC_{50} value was found out to be 1.2% [11]. To relate with efficiency of DP that could having the antiproliferative activity, flavonoids content of DP can inhibit growth of brine shrimp, *Artemia salina* Leach species as anticancer activity assay, *in vivo* [11]. Furthermore, the above results could be encouraging as evidence for the use of DP for cancer traditional/alternative medicine since brine shrimp lethality test (BSLT) is usually used for preliminary screening for bioactivity including for anticancer activities [6].

Figure 2 summarized all the values as mentioned above for effect of various types DP extracts toward L929 cells. By comparing the results with normal cell L929, all five types of DP extract showed no remarkable effect as IC₅₀ value where all the values were more than 100 µg/mL. This could be said that it is acceptable range of IC₅₀ value as the methanol extract of *O. indicum* exhibited good antiproliferative activity toward HeLa cells with IC₅₀ 3.87 µg/mL without affecting the proliferation of normal cells such as Vero and MDCK cells where the IC₅₀ values were > 99 µg/mL [12].

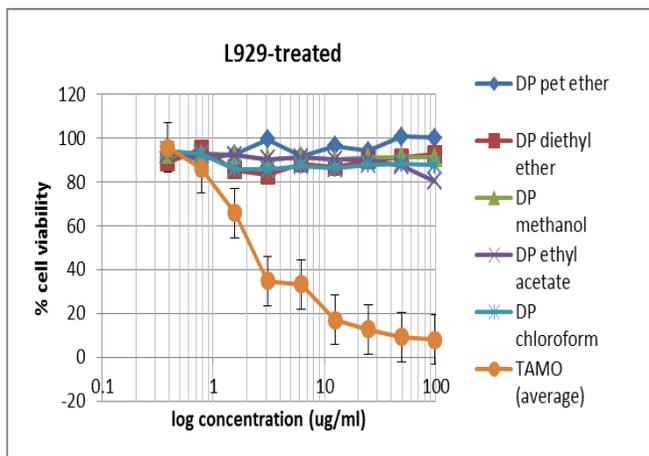


Figure 2 Antiproliferative activity of various extracts of DP on L929 normal cell. Each point showed the percentage of viable cells compared to positive control, Tamoxifen and negative control, DMSO. Each point represented as mean, n = 3

On the other hand, tamoxifen which have been studied intensely particularly on the breast cancer incidence and it was approved by the Food and Drug Administration (FDA) in order to be used for reducing breast cancer occurrence [13]. Meanwhile, this positive drug control showed great inhibitory effect where lesser value obtained as the IC₅₀ value is 3.02±0.70 µg/ml towards MCF-7 breast cancer cells and 6.98±0.76 µg/ml towards L929 normal connective cells. Growth of some cancer cells may initially be attenuated by tamoxifen but become resistant to continued treatment and the cells can continue to grow [14]. Undesired side effects have affects healthy normal cells of L929 when given treatment of tamoxifen. This proves that the tamoxifen is not only toxic against cancerous cell line but also toxic against non-malignant cell lines. In contrast to tamoxifen, various types of DP extracts showed less cytotoxicity effect towards normal cells.

4.0 CONCLUSION

In conclusion, it could be suggested that DP methanol extract and DP ethyl acetate extract could inhibit MCF-7 cell growth throughout all treatment period.

Further investigation on the isolation of both extracts and mechanism of cell death via apoptosis pathway are currently being performed.

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