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Cytotoxic effect of *Typhonium flagelliforme* extract

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Traditionally,*Typhonium flagelliforme* is used to treat cancer in Malaysia, People take it as powder or in combination with milk or different types of other herbal extracts as treatment of different type of cancers (Teo and Chang, 1996). The study was conducted to determine anticancer activity of crude extract of 1 to 6 months old *ex-vitro* plantlets and one sample of *in vitro* plants of *T. flagelliforme* against MCF-7 human breast cancer cell lines. The growth inhibitory activity was tested by GI_{50} (the concentration of drugs which inhibited the cell growth by 50%). Extracts of 2, 3, 4, 5, 6 months old field grown plants, was found to be actice against MCF-7 with the GI_{50} values of 6.2, 33.1, 14.7, 57.5, 16.6 µg/ml respectively.

Key words: *Typhonium flagelliforme,* cytotoxic effect, traditional medicine.

INTRODUCTION

During the past decade, medicinal plants have become very significant in global health care. Among the human diseases that can be treated with the use of herbal medicine is cancer, which is probably the most important genetic disease. Drug discovery from medicinal plants has played an important role in the treatment of cancer and indeed, most new clinical application of plant secondary metabolites and their derivatives over the last half century have been applied towards combating cancer (Newman et al., 2003). *Typhonium flagelliforme* is a medicinal herb which belongs to the Araceae (Arum) family, which is commonly found in South East Asia such as Malaysia, Singapore and China, and has been used for decades for the preparation of traditional medicines.

In Malaysia over the last few years, *T. flagelliforme* has been taken traditionally in mixture with other herbal medicines medicines to fight against breast, lung, colon and liver cancers (Chan et al., 2001). The first reported anticancer activity of *T. flagelliforme* was from a study which reported the cytotoxic activity of the extracts of the fresh roots, rhizome, stem and leaves on p338 lymphocyte leukemia cells (Itokawa et al., 1993). While Chan et al. (2001) showed that the polar fraction of the crude extract of this plant showed a weak cytotoxic effect with an IC_{50} of 15 µg/ml.

Lai et al. (2005) studied the different cytotoxic activity in four weeks *in vitro* plantlets and six month old field grown plants which showed better activity in field grown samples. Due to the fact that there is limited information on the suitable harvesting time of *T. flagelliforme* with the highest anticancer activity *in vitro*, this study is probably first of its kind carried out to determine the cytotoxic

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activity against MCF-7 breast cancer cells.

MATERIALS AND METHODS

Plant material

A total of seven samples of consisting *in vitro* and *ex vitro* plants of *T. flagelliforme* were used in this study which has been planted in Laboratory of Agriculture Technology, University Putra Malaysia.

Extraction of the plant material

The whole fresh plants leaves, stem, tuber and roots from 1 to 6 months of *ex vitro* and one sample of *in vitro* were randomly harvested, and carefully washed with tap water until the entire soil and media content was removed and dried in electric oven at 50°C for 3 days. The dried material were cut into small pieces. According to polarity index one solvent system was selected for the extraction namely mixture of methanol (MeOH) and dichloromethane (DCM) at ratio of 1:1. A total of 300 g dried material of *T. flagelliforme* of each sample was used for the extraction, in which the material was soaked for three days at room temperature. The process was repeated several times with the same solvent system until the solvent portion became colourless, the solvent extract was then concentrated under reduced pressure using a rotary evaporator (Büch Rotavapor R-200). After that, the concentrated extract was transferred into conical flasks and the residual solvent was removed. After drying in an oven incubator, dried crude extract was weighted and stored at -20°C for further analysis.

Preparation of extracts for *in vitro* test

A total of 1 g of crude extract of all *ex vitro* and *in vitro* samples was used in the study. Prior to their use, the stocks of samples at 100 mg/ml in dimethyl sulfoxide (DMSO) were prepared and stored at -20°C. Ten-fold serial dilutions (20 µl of each extract in 180 µl RPMI-1640 medium) were then prepared from the stock solutions to obtain the extract concentrations of 1, 0.1, 0.01 and 0.001 mg/ml. These working concentrations were used immediately.

Cytotoxic assay

Cells were grown in RPMI-1640 standard culture medium, supplemented with 10% heat inactivated foetal calf serum, 2 mM L-glutamine with 100 IU/ml penicillin and 100 µg/ml streptomycin in a 25 cm² tissue culture flasks, at 37°C, 5% CO_2 and humidified atmosphere. Once the cells reached 80 to 90% confluency, 1 ml of trypsin-EDTA solution was added into the flask. The flask was incubated at 37°C for 10 min to detach the cells. The detached cells were suspended with 3 ml of culture medium.

Using a multi-channel pipette, 180 µl of cells suspension was dispensed into each well of microtitre well plate, except for the wells allocated for blank. These cells were incubated at 37°C (5% CO_2) overnight to allow the cells to attach to the bottom of the wells. Four drug concentrations (1.0, 0.1, 0.01 and 0.001 mg/ml) were obtained from the stock solution (100 mg/ml in DMSO) in the culture medium. 20 µl from various concentrations of drug was added into each well. Each concentration was tested in quadruplicate and the control wells received 20 µl of medium only. The plates were then incubated at 5% CO_2 in an incubator at 37°C for 96 h.

Micro-culture tetrazolium cell viability assay

For many years, the micro-culture 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide Micro-culture tetrazolium (MTT) cell viability assay had been a valuable tool in the drug discovery process. It is based on the methods proposed by Mosmann (1983), and uses a tetrazolium salt to reduce the coloured formazan product by mitochondrial dehydrogenases present only in living cells. It is frequently used and has been shown as suitable for *in vitro* drug screening (Alley et al., 1988).

After 96 h incubation, 50 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) (2 mg/ml in PBS) was added into each well containing 200 µl medium. The plates were incubated for 4 h to let metabolism of MTT to form formazan crystals through cellular mitochondrial dehydrogenase. The MTT medium was aspirated and 100 µl of DMSO was added to each well. The plates were gently shaken to dissolve the formazan crystals. The absorbance of the purple formazan solution was measured at 550 nm using a microplate reader. GI_{50} (the concentration of drugs which inhibited cell growth by 50%), the percentage of cell growth was determined using the formula:

$$\% \text{ of cell growth} = \frac{A_T - A_0}{A_C} \times 100$$

A_T = Optical density of extracts-treated cells after 96 h; A_C = Optical density of control cells after 96 h; A_0 = Optical density on day 0.

RESULTS AND DISCUSSION

The crude extracts of *T. flagelliforme*, from the two to six months old *ex vitro* plants, have been found to have strong inhibitory activity against the growth of MCF-7 cell. *In vitro* and one month old plants did not show significant growth inhibitory effect. These finding were consistent with those of Lai et al. (2005) who reported that *ex vitro* grown plants had higher activity compared with the *in vitro* plants. In contrast to the finding that have been done by Chan et al. (2001), the hexane extract of *T. flagelliforme* displayed poor cytotoxic activity against murine leukaemia cancer cell lines. It shows the superior ability of MeOH and DCM solvent to extract the *T. flagelliforme* components which demonstrated better cytotoxic activity against MCF-7 cancer cell lines.

The GI_{50} value of *ex vitro* plants and *in vitro* plants are shown in Table 1. The values were found to be in the range of 6.2 to >100 µg/ml. The strongest growth inhibition was shown by the two month old *ex vitro* plants with GI_{50} value of 6.2 µg/ml. The second highest activity was shown by the four month old plants followed by six, three, and five month old plants. Both *in vitro* and one months *ex vitro* plants failed to show activity ($GI_{50} > 100$ µg/ml).

Intersting part from our observation showed that *T. flagelliforme* starts flowering from 2 months old which has shown the strongest inhibitory effect against MCF-7 breast cancer cell lines (Figure 1).

Figure 2 shows the cellular effects of cancer cells treated with 2 months old plant extracts on MCF-7 breast cancer cells at various concentrations. All extract showed prominent morphological concentration-dependent growth inhibition. The *in vitro* and one month old plants extracts did not produce a significant effect on the growth of MCF-7 cells.

Table 1. Growth inhibitory effect of *T. flagelliforme* on MCF -7 breast cancer cells.

Age of plant	GI_{50} (µg/ml)
In vitro	>100±0.0
First month	>100±0.0
Second month	* 6.2±1.58
Third month	* 33.1±27.83
Fourth month	* 14.7±2.84
Fifth month	* 57.5±13.66
Sixth month	* 16.6±3.31

Values are the mean of 3 separate experiments and errors represent the SD values GI_{50} values were determined by the MTT assay. * = represent significant difference vs. *in vitro* as control ($p \leq 0.05$). One way Anova, Bonferrori's multiple range test.

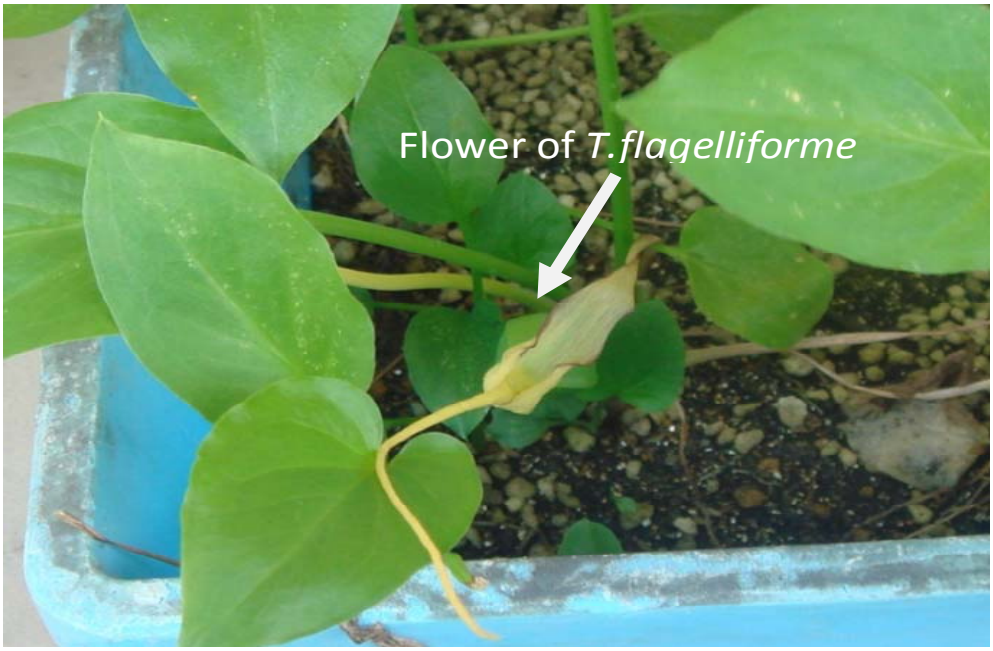

Figure 1. Two (2) months old *T. flagelliforme* plants.

DISCUSSION

Cytotoxicity screening models provide important preliminary data to help select plant extracts with potential antineoplastic properties for future work (Cardellina et al., 1999). The plant *T. flagelliforme* has been traditionally used as a medicine to treat a broad range of diseases, including internal injuries, oedema and cancer. In this study, the crude extracts of *T. flagelliforme* were prepared from one up to six months old *ex vitro* plants to test their activity against MCF-7 cells in order to establish the exact suitable harvest time. The purpose was to obtain a high quality product with the highest bioactivity.

Based on statistical analysis, compared with *in vitro* plants, it was interesting to find out that the 2 to 6 months old *ex vitro* plant showed strong activity to inhibit the growth of MCF-7 cell line (Table 1).

According to Nobakht et al. (2010), alkaloids and flavonoids are the major phytochemicals found in *T. flagelliforme*. Of all the *ex vitro* plants, the two and four month old plants contained the highest amounts of the phytoconstituents (alkaloids and flavonoids). It is interesting to note these phytochemicals are commonly associated with various pharmacological activity a natural products (Cragg and Newman, 2005).

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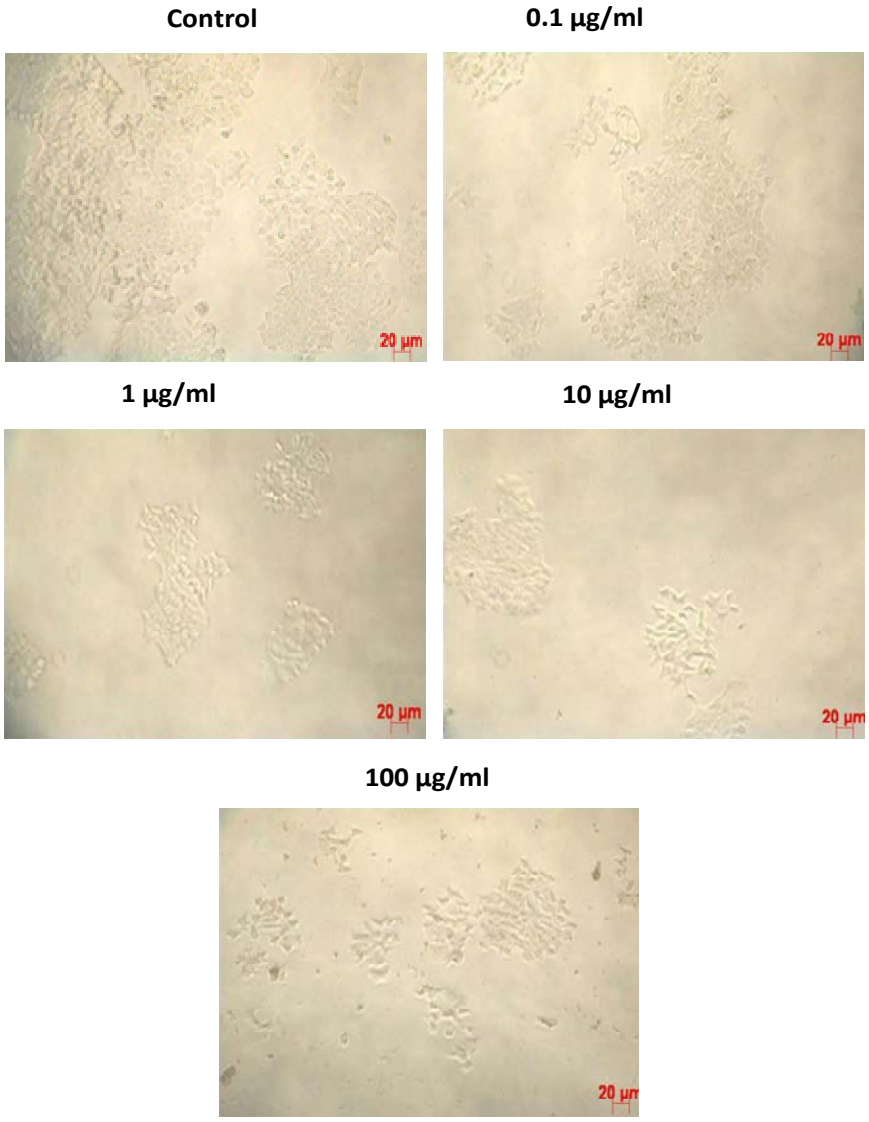

Figure 2. MCF-7 cells at 96 h incubated with 0.1 to 100 µg/ ml crude extracts of *T. flagelliforme* collected from two months old plants.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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