



UNIVERSITI PUTRA MALAYSIA

***IN VITRO* MASS PROPAGATION, ANTI-CANCER ACTIVITY
AND PHYTOCHEMICAL ANALYSIS
OF *TYPHONIUM FLAGELLIFORME***

**MOTAHAREH NOBAKHT GALEHPARDSARI
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By

MOTAHAREH NOBAKHT GALEHPARDSARI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of Master of
Science**

July 2009



Specially Dedicated

To

My Father and Mother

Rahim Nobakht and Sosan Saber

**.....who inspired, supported and gave me tremendous courage to
be a well educated person**



Abstract of thesis presented to the Senate of University Putra Malaysia,
in fulfillment of the requirement for the degree of Master of Plant Biotechnology

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Chairman : Associate Professor Mihdzar Abdul Kadir, PhD
Faculty : Agriculture

The study was conducted at the *in vitro* laboratory, Faculty of Agriculture and Biochemistry lab(1), Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor. The main objectives of the study were to determine the anti cancer activity and phytochemical analysis of *Typhonium flagelliforme* between the *in vitro* and *ex vitro* plants.

T. flagelliforme (Araceae) is a medicinal herb which is endowed with curative properties against a variety of illness including injuries, oedema, coughs, pulmonary ailments, bleeding and cancer. A preliminary study has demonstrated *in vitro* anticancer activity of matured field grown plants.



In this study, *T. flagelliforme* was mass propagated *in vitro* and transferred to the natural environment to determine its phytochemical contents and anticancer activity. Plant materials were cultured in Murashige and Skoog (MS) media supplemented with different concentrations of cytokinin (BAP, 0, 1, 5, 10 mg/L) and auxin (NAA, 0, 0.5, 1 mg/L). The effects of these two hormones on the mean number of shoots and the shoot weight were recorded. The combination of 5 mg/mL BAP and 1 mg/mL NAA was found to significantly increase both parameters studied. Dried oven material of *T. flagelliforme* was extracted in the mixture of dichloromethane and methanol (1:1) for the preliminary phytochemical analysis and anticancer activity test. The phytochemical analysis for the major constituents was undertaken using the standard qualitative methods. The plants (*in vitro* and 1- 6 month old *ex vitro* plants) were analysed for the presence of alkaloid, terpenoids, flavonoids and steroids. The presence of alkaloids was detected in 2- 5 month old *ex vitro* plants and strong presence of flavonoids was detected in all the *ex vitro* plants.

T. flagelliforme was evaluated for inhibitory effect on the growth of human breast cancer cell lines (MCF-7), using a microculture tetrazolium (MTT) assay. The growth inhibitory activity was determined by GI₅₀ (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 active against MCF-7 (breast cancer cell line) with the GI₅₀ values of 6.2, 33.1, 14.7, 57.5, 16.6 µg/mL respectively. Meanwhile, the presence of alkaloids and flavonoids were found to be positive in 2-5 months old plants, whereas the six month old plants showed the presence of flavonoids and steroids.



Comparison between the results of anticancer activity within the 6 months of *ex vitro* and *in vitro* indicated that overall *ex vitro* plants have a higher activity except for one month old plants compared with the *in vitro* plants. In contrast phytochemical analysis revealed the presence of two main components (flavonoids, alkaloids) which highly suggested that flavonoids and alkaloids may have contributed to the anticancer potential of this plant.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMBIAKAN *IN VITRO* SECARA BESAR-BESARAN, ACTIVITI ANTI-KANSER DAN ANALISIS FITOKIMIA BAGI *TYPHONIUM FLAGELLIFORME*

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Kajian telah dijalankan di makmal *in vitro*, Fakulti Pertanian dan makmal Biokimia (1) and Makmal Biokimia (1), Fakulti Perubatan dan Sains Kesihatan, Universiti Putra Malaysia, Serdang, Selangor. Objektif utama kajian ini adalah untuk menentukan aktiviti anti kanser dan analisis fitokimia daripada pokok *Typhonium flagelliforme* yang berasal daripada kaedah *in vitro* dan *eks vitro*.

T. flagelliforme (Araceae) merupakan tumbuhan herba yang mempunyai keupayaan untuk mengubati pelbagai jenis penyakit seperti luka, lebam, batuk, radang paru-paru, pendarahan and kanser. Ujikaji saringan telah menunjukkan bahawa aktiviti anti kanser tumbuhan yang matang di ladang dapat dilihat secara sistem *in vitro*. Dalam kajian ini, *T. flagelliforme* telah dibiakkan secara *in vitro* sebelum dipindahkan ke persekitaran semulajadi untuk penentuan kandungan fitokimia dan aktiviti anti-kanser yang dimiliki.



Bahan tanaman telah dikultur di dalam media Murashige dan Skoog (MS) yang ditambah dengan kepekatan sitokinin yang berbeza (BAP, 0, 1, 5, 10 mg/L) dan auksin (NAA 0, 0.5, 1 mg/L). Kesan daripada kedua-dua hormon keatas bilangan tunas dan berat tunas telah direkod. Kombinasi 5 mg/L BAP dan 1 mg/L NAA telah didapati menunjukkan peningkatan berkesan keatas dua parameter yang dikaji. Bahan *T. flagelliforme* yang telah dikeringkan dalam ketuhar diekstrak dalam campuran dichloromethane dan methanol (1:1) untuk analisis saringan fitokimia dan peperiksaan aktiviti anti-kanser. Analisis fitokimia bagi kandungan utama telah dilaksanakan menggunakan kaedah piawai analisis kualitatif. Pokok tersebut (*in vitro* dan pokok *eks vitro* berusia 1- 6 bulan) telah dianalisis bagi menentukan kandungan alkaloid, terpenoid, flavonoid dan steroid. alkaloid dan flavonoid telah dikesan pada pokok *eks vitro* berusia 2- 5 bulan, dan flavonoid yang kuat telah dikesan pada keseluruhan pokok secara *eks vitro*.

T. flagelliforme telah diuji bagi menentukan kesan perencatan keatas pertumbuhan sel kanser payu dara manusia (MCF-7) dengan menggunakan ujikaji kultur mikro tetrazolium (MTT). Kesan perencatan aktiviti telah ditentukan menggunakan pengiraan GI_{50} (iaitu kepekatan sebatian yang merencat 50% pertumbuhan sel tersebut). Ekstrak daripada tumbuhan yang dibiak di kawasan semulajadi yang berusia 2, 3, 4, 5 and 6 bulan didapati aktif ke atas sel MCF-7 dengan masing-masing nilai GI_{50} adalah 6.2, 33.1, 14.7, 57.5, 16.6 $\mu\text{g/mL}$. Manakala, kehadiran alkaloid dan flavonoids telah dikesan positif dalam pokok berusia 2-5 bulan, sedangkan pokok yang berusia 6 bulan menunjukkan kehadiran flavonoid dan steroid.

Perbandingan di antara hasil kajian daripada aktiviti anti-kanser dalam 6 bulan secara *eks vitro* dan *in vitro* menunjukkan bahawa hampir keseluruhan tumbuhan *eks vitro* mempunyai aktiviti yang lebih tinggi kecuali tumbuhan yang berusia satu bulan berbanding kepada tumbuhan *in vitro*. Walaubagaimanapun, analisis fitokimia telah menunjukkan kedapatan dua komponen utama (flavonoid, alkaloid) yang mana ianya sangat disarankan bahawa flavonoid dan alkaloid menyumbang secara berpotensi kepada aktiviti anti-kanser.



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I certify that an Examination Committee has met on 3rd July 2009 to conduct the final examination of Motahareh Nobakht Galehpardsari on his Master of Plant Biotechnology thesis entitled “*In vitro* Mass Propagation, Anti- Cancer Activity and Pytochemicals Analysis of *Typhonium Flagelliforme* “in accordance with Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the student be awarded the relevant degree.

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DECLARATION

I declare that the thesis is my original work except for quotation and citation which have been duly acknowledged. I also declare that it has not been previously and is not concurrently, submitted to any other degree at Universiti Putra Malaysia or any other institution.

Motahareh Nobakht Galeh Pardari

Date:

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LIST OF ABBRIVATIONS

BAP	6- benzylaminopurine
CRDD	Cancer Research and Drug Discovery
DCM	Dichloromethane
DMSO	Dimethyl Sulfoxide
EDTA	Ethylene_diamine_tetra acetic acid
GI ₅₀	Concentration of drugs which inhibited cell growth by 50%
MCF_7	Breast cancer cell lines
MeOH	Methanol
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide
NAA	α-naphtaleneacetic acid
RMPI 1640	Roswell Park Memorial Institute _1640



CHAPTER 1

INTRODUCTION

Herbal medicines are related to the use of various plant parts like leaves, roots, tubers, stems and even flowers for the medicinal purposes according to World Health Organization (WHO) report in 2003.

Herbal medicines have been used by human beings for over 5000 years (Goldman, 2001), while western medicines have been used for treating diseases for only a few hundred years (Liu and Wang, 2007). In 1817, Friedrich Sertürner isolated morphine from Opium plant and it was the first isolation work which was done in the history of herbal medicine (Samuelsson, 2004).

At present, people all around the world have accepted the use of herbal medicines as drugs. Although the modern chemically synthesised drugs are typically used in the western and developed countries, medicinal plants have found their roles globally (Dubey *et al.*, 2004). WHO reported that medicinal plants are used by around 80 % of the world population, either directly or as extracts with common liquid such as water or milk (Kayser and Quax, 2007).

According to Voigt (2006), only 14 members of WHO regulated the selling of herbal medicine as drugs in 1988, and this rate significantly increased to 53 countries in 2003. Meanwhile, the sale of herbal medicine such as crude extract or complete product



increased from 15 billion US dollars in 1999 to 23 billion US dollars in 2002 world wide (Dubey *et al.*, 2004).

Secondary metabolites of medicinal plants have been recognized for their roles to work against cancer. Four main classes of herbal medicinal compounds that are currently being used clinically as anticancer agents include alkaloids, epidophyllotoxin, taxanes and camptothecins (Balunas and Kinghorn, 2005).

Cancer is a group of illness specified by uncontrolled growth and spread of abnormal cells which can cause death if it is not properly controlled. In 2007, the American Cancer Society predicted that from more than 12 million new cancer cases diagnosed around the world, 20,000 deaths would be recorded per day. At that time, the National Institute of Health of the USA expected that the overall cost for the treatment of cancer would be around 219.2 billion US dollars according to the report of the American Cancer Society in 2008.

Typhonium flagelliforme from the Araceae family is a rodent tuber plant 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 to fight against breast, lung, colon and liver cancers (Chan *et al.*, 2001). It has been taken as juice extract or in the form of dried powder.



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). Lai *et al.* (2005) studied the different cytotoxic activity between four weeks *in vitro* plantlets and six month old field grown plants which showed better activity in field grown samples. Therefore, it is vital to identify the suitable time for harvesting *ex vitro* plantlets. Although several studies have been conducted to evaluate the medicinal properties of *T. flagelliforme* but the exact chemical contents of the secondary metabolite products such as alkaloids, steroids, terpenoids and flavinoids were not known.

The problems related to *T. flagelliforme* are sensitivity to specific growth condition in the natural environment such as moist and be in the shady area which provided the biggest need to produce the plant *in vitro* in massive amount.

Thus, the aim of the present study was to mass propagate and determine the suitable harvest time of *T. flagelliforme* for bioactivity test. The specific objectives of this study include:

1. To develop a rapid *in vitro* mass propagation technique of *T. flagelliforme*.
2. To determine the suitable harvest time of *in vitro* and *ex vitro* plants for its highest anticancer effect.
3. To determine the contents of various phytochemical constituents of *in vitro* and *ex vitro* plants.



CHAPTER 2

LITERATURE REVIEW

2.1 Medicinal plants

2.1.1 *T. flagelliforme*

Typhonium flagelliforme is a medicinal herb which belongs to the Araceae (Arum) family (Figure 2.1). Most of the plants from this group are monocotyledons which grow in a tropical climate. Nine sub-families and 107 genera exist in this family. *Typhonium* genus includes 25 species (Nicolson and Sivadasan, 1981). *T. flagelliforme*, commonly called Rodent Tuber in South-eastern Asia, is known in Malaysia as keladi tikus (Chan *et al.*, 2001).

Su *et al.* (2000) described the morphology of *T. flagelliforme* as a plant with dark green leave blades, a parallel venation and two to three prominent veins running along the mid-rib of the leaf. The leaf blade is about 9.96 ± 1.39 cm long and 4.06 ± 0.67 cm wide at the widest section, while the leaf petiole is 19.43 ± 2.70 cm in length.

Matured *T. flagelliforme* plant can grow up to 26 cm in height ((Figure 2.1.A). All varieties of this plant produced a single inflorescence consisting of a spadix surrounded

by a long and slender greenish yellow spathe. The spathe is with the length and width of 15.37 ± 1.17 cm long and 1.44 ± 0.07 cm broad at the widest portion. The spadix is divided into four portions: a lower 0.41 ± 0.03 cm pistillate portion, an intermediate 1.48 ± 0.14 cm portion with sterile flowers, a 0.34 ± 0.05 cm staminate portion, and terminated with lemon yellow 12.92 ± 1.25 cm rodent tail-like appendix.

Su *et al.* (2000) also stated that both staminate and pistillate flowers would be less when the plants reach maturity; include having no petals, sepal or bracts. The total number of staminate flowers per spadix ranged from 50 to 65, with an overall mean of 57.00 ± 4.24 per spadix, while the number of pistillate flowers per inflorescences ranged from 70 to 85, with an average of 76.50 ± 2.12 . Sterile flowers portion could be seen as two distinct parts, with the lower part attached with spreading, spatulate, dark-tipped rudiments and the upper part with spreading to deflecting subulate rudiments (Figure 2.1.B).