



UNIVERSITI PUTRA MALAYSIA

**NUTRITIONAL COMPOSITION, ANTIOXIDANT ACTIVITY AND
ANTICARCINOGENIC EFFECT OF TYPHONIUM FLAGELLIFORME
(NICHOLSON 1029) EXTRACT IN RAT**

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By

THILAKAVATHY A/P KARUPPIAH

**Thesis Submitted in Fulfilment of the Requirements for the
Degree of Master of Science in the Faculty of
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**NUTRITIONAL COMPOSITION, ANTIOXIDANT ACTIVITY AND
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Faculty: Medicine and Health Sciences

Typhonium flagelliforme from the family Araceae is locally known as rodent tuber. It is being used traditionally in Malaysia to treat cancer. The major aim of this study was to investigate the effect of *Typhonium flagelliforme* crude extract on hepatocarcinogenesis in rats induced by diethylnitrosamine (DEN) and 2-acetylaminofluorene (AAF). Besides the *in vivo* study, the plant's nutrient and non-nutrient composition, and its antioxidant activity were also determined. *Typhonium flagelliforme* has carbohydrate (0.5% in leaves-stalks and 27.5% in tubers-roots) as its main constituent. It has high content of vitamin C (106.5 mg/100 g in leaves-stalks and 11.6 mg/100 g in tubers-roots) and potassium (1276.8 mg/100 g in leaves-stalks and 534 mg/100 g in tubers-roots). Alkaloid (0.4 mg/100 g in leaves-stalks and 0.9 mg/100 g in tubers-roots) and catechin (1.5% in leaves-stalks and 0.7% in tubers-roots) were found in this plant. The phytochemicals of *Typhonium flagelliforme* showed a greater antioxidant activity than vitamin E. The effect of *Typhonium flagelliforme* crude extract (0.1 ml/rat) on rat hepatocarcinogenesis was assessed by five different tumour



markers [γ glutamyl transpeptidase (GGT), uridyl diphosphoglucuronyl transferase (UDPGT), glutathione S-transferase (GST), alkaline phosphatase (ALP) and glutathione (GSH)] and histological examinations. Glycyrrhizin [0.005% (w/v)] was used as a comparison to *Typhonium flagelliforme* crude extract. Administration of diethylnirosamine and 2-acetylaminofluorene (DEN/AAF) significantly increased the activities of plasma and liver microsomal GGT ($p < 0.05$), liver microsomal UDPGT ($p < 0.05$), liver GST ($p < 0.05$), liver ALP ($p < 0.001$) and the concentration of liver GSH ($p < 0.01$) compared to control. Supplementation of *Typhonium flagelliforme* crude extract to normal rats did not give any effect towards the tumour markers. The crude extract administered to the DEN/AAF treated rats significantly decreased the activities of plasma and liver microsomal GGT ($p < 0.05$), liver microsomal UDPGT ($p < 0.05$), liver GST ($p < 0.05$), liver ALP ($p < 0.001$) and the concentration of GSH ($p < 0.01$) when compared to the DEN/AAF treated rats. Glycyrrhizin, which was given to the DEN/AAF treated rats reduced all the tumour markers except ALP ($p > 0.001$). Light microscopic examination showed that DEN/AAF caused hepatocytic dysplasia. Transmission electron micrographs showed organelles damage caused by DEN/AAF. No morphological and histological changes were seen on the cells and the organelles when the DEN/AAF treated rats were supplemented with the crude extract. These results suggested that *Typhonium flagelliforme* contained constituents that help in preventing liver cancer and its crude extract administered at this supplemented dose (0.1 ml) to the DEN/AAF treated rats reduced the severity of hepatocarcinogenesis better than glycyrrhizin [0.005% (w/v)].



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**KOMPOSISI NUTRIEN, AKTIVITI ANTIOKSIDAN DAN KESAN
ANTIKARSINOGENIK EKSTRAK *TYPHONIUM FLAGELLIFORME*
(NICHOLSON 1029) PADA TIKUS**

Oleh

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Typhonium flagelliforme dari famili Araceae dikenali sebagai keladi tikus di negara ini. Ia digunakan secara tradisional di Malaysia untuk merawat kanser. Objektif utama kajian ini adalah mengkaji kesan ekstrak kasar *Typhonium flagelliforme* terhadap hepatokarsinogenesis tikus yang diaruh dengan dietilnitrosamin (DEN) dan 2-asetilaminofluoren (AAF). Selain daripada kajian *in vivo*, komposisi nutrien dan antinutrien serta aktiviti antioksidan tumbuhan ini juga telah dikaji. Karbohidrat (0.5% dalam daun-batang dan 27.5% dalam ubi-akar) merupakan konstituen utama *Typhonium flagelliforme*. Ia mengandungi vitamin C (106.5 mg/100 g dalam daun-batang dan 11.6 mg/100 g dalam ubi-akar) dan kalium (1276.8 mg/100 g dalam daun-batang dan 534 mg/100 g dalam ubi-akar) yang tinggi. Alkaloid (0.4 mg/100 g dalam daun-batang dan 0.9 mg/100 g dalam ubi-akar) dan katekin (1.5% dalam daun-batang dan 0.7% dalam ubi-akar) terdapat dalam tumbuhan ini. Sebatian fitokimia *Typhonium flagelliforme* memberi kesan antioksidan yang lebih baik daripada vitamin E. Kesan ekstrak kasar



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LIST OF ABBREVIATIONS

AAF	2-acetylaminofluorene
ALP	Alkaline phosphatase
CDNB	1-chloro-2,4-dinitrobenzene
<i>Ces</i>	<i>Colocasia esculentum</i>
<i>Ct</i>	<i>Coleus tuberosus</i>
DCNB	1,2-dichloro-4-nitrobenzene
DTNB	5'5'-dithio-bis-(2-nitrobenzoic acid)
DEN	Diethylnitrosamine
GGT	Gamma glutamyl transferase
GL	Glycyrrhizin
GSH	Glutathione
GST	Glutathione S-transferase
H&E	Hematoxyline and eosin
HCl	Hydrochloric acid
H ₂ O	Water
H ₂ O ₂	Hydrogen peroxide
H ₂ SO ₄	Sulphuric acid
HPO ₃	Metaphosphoric acid
KCl	Potassium chloride
LH	Unsaturated fatty acid
L·	Lipid radical
LOH	Lipid peroxide



LOO	Peroxyl radical
MARDI	Malaysian Agriculture Research and Development Institute
NaOH	Sodium hydroxide
O ₂	Oxygen
O ₂ [*]	Superoxide
OH	Hydroxyl radical
TEM	Transmission electron microscopy
TFE	<i>Typhonium flagelliforme</i>
UDPGT	Uridyl diphosphoglucuronyl transferase
<i>Xs</i>	<i>Xanthosoma sagittifolium</i>



CHAPTER I

INTRODUCTION

Cancer is a disease, which today remains difficult to cure but is preventable by the administration of one or several chemical compounds. Ministry of Health Malaysia (1995) has statistically reported that malignant neoplasm (45%) is the major cause of death in government hospitals, which is 2.5 times higher than the heart diseases (16%).

Hepatocarcinogenesis or hepatocellular carcinoma or liver cancer is one of the most prevalent and deadly cancers worldwide (Kathryn *et al.*, 1997). The liver is one of the most frequently damaged organs in the body, and it is indeed fortunate that it has an enormous functional reserve. Liver is an important metabolic organ. It is involved in the catabolism of many endogenous substances, and has the capacity to metabolise foreign carcinogens. Not only can reactions therein lead to direct-acting ultimate carcinogens but also additional reactions lead to conjugates which are in transport forms. Where specific tissues or cells have the enzymatic potential to split the active entities from their transport conjugates, cancer may result at such sites.

The sequence of events leading to tumour formation and the resulting cascading effects of the metastases has not been completely elucidated. What had been charted are various steps beginning with an insult to genetic cellular material (genotoxic event; known also as “initiation”) and then to production of abnormal DNA non-genotoxic event: known as “promotion”). The transformation of the altered cell may lead to the proliferation of cells with invasive (malignant) or non-invasive (benign) qualities. From a confined region of tissue, cancer spreads to other tissues (developing secondary tumours or metastases), and finally, to an ultimate cascading effect of the tumourous cells (Nagy and Attaway, 1992).

Through animal studies, measuring tumour marker enzymes can identify the presence of cancer quantitatively by biochemical or immunochemical means in tissue or body fluids. This is further validated by histological examination for diagnosing neoplasia. Electron microscopy has made visible for the first time those cellular structures that are the morphological bases of metabolic process.

Human are exposed to large numbers of carcinogenic chemicals and other carcinogenic stimuli (i.e., ultraviolet light, radon, x-rays, etc.) in their daily life. Small amounts of many naturally occurring mutagens and carcinogens are ingested in our normal diet, and it is not known whether a lifetime of eating small amounts of these dietary carcinogens can cause or contribute to cancer in some people. In addition to the carcinogens and mutagens in our diet, we also ingest large numbers of naturally occurring anti-mutagens and anti-carcinogens. Epidemiological studies indicate that

dietary factors play an important role in the development of human cancer, and attempts to identify these naturally occurring dietary carcinogens and anti-carcinogens should lead to new strategies for cancer prevention.

If there is one aspect of medical practice that has united the physicians of the world throughout the millennia despite all their diverse opinions, is their reliance on our flora as a staple source of medicinal drugs. Virtually all early civilisations developed the use of plant drugs to a high degree. Traditional medicine is well known for its high nutritional value, as well as, its ability to cure various ailments. Treatments and remedies have varied greatly over the centuries; no fewer than 3000 plant species have been used by the laity to treat cancer (Lewis and Elvin-Lewis, 1977). Most of the early treatments had little or no effect on the disease, but perhaps they lessened suffering either physically or psychologically. Many herbal formulas were used in cancer treatment but none, as far as is known, was recorded as a cure.

In treating cancer, several plants are used in Malaysia traditionally, such as 'akar susun kelapa' (*Tabernaemontana divaricata*), 'akar melur' (*Jasminum sambac*), 'bunga raya putih' (*Hibiscus rosa-sinesis*) and 'ubi bembun' (*Marantha arundinacea*). One of the greatest advantages of herbal medicine as a therapy is that when administered correctly it is completely safe (Zakaria and Mohd., 1994).

Typhonium flagelliforme is a green plant about one foot tall. Its flower ends in a long filament resembling the tail of a mouse, hence its popular lay name rodent tuber. The plant grows readily in soft, damp shady areas. It has been used in traditional medicine for treating different types of malignancy.

Objectives

Even though *Typhonium flagelliforme* has been used traditionally to cure cancer and it is gaining popularity among the scientists, its nutritional and non-nutritional composition has never been reported. *In vivo* studies on cancer using this plant were not done yet. Therefore, the objectives of this study were:

- 1) To determine the nutrient and non-nutrient composition of *Typhonium flagelliforme* such as the proximate analysis, mineral, vitamins, alkaloid, tannin and catechin content.
- 2) To evaluate the antioxidant activity of different parts of *Typhonium flagelliforme*.
- 3) To determine the effect of the crude extract of *Typhonium flagelliforme* in suppressing the process of hepatocarcinogenesis in rats by assessing the body and liver weight, histological examinations (light and transmission electron microscopy), and tumour markers activities such as gamma glutamyl transpeptidase, glutathione S-transferase, alkaline phosphatase, uridyl diphosphoglucuronyl transferase and glutathione S-transferase dependent substrate, glutathione.
- 4) To compare the anticarcinogenic activity of *Typhonium flagelliforme* with glycyrrhizin, one of the drugs used to treat liver cancer.

CHAPTER II

LITERATURE REVIEW

Typhonium flagelliforme

Typhonium is derived from the word Typhon, a mythological giant: the name was given by the ancients to some Aroid. A genus embracing about thirteen species of stove, tuberous, perennial herbs, inhabiting tropical Asia, Australasia, and the Pacific Islands represents the family Araceae. The species known to cultivation are *T. Brownii*, *T. cuspidatum*, *T. divaricatum*, *T. diversifolium Huegelianum*, and *T. trilobatum*. They thrive in light, rich soil, and during the growing season require an abundant supply of water. After the leaves have died down, water must be withheld until growth recommences. The pots containing the tubers can be stored away in any dry, warm place (Nicholson, 1991).

Plants of *Typhonium flagelliforme* (Lodd.) Blume were previously described as *Typhonium divaricatum* (Nicholson and Sivadasan, 1981). In Malaysia and Singapore, *Typhonium flagelliforme* (Plate 1 and 2) is locally known as "rodent tuber" (Neoh, 1992). Characteristic of *Typhonium flagelliforme* are sterile flowers spreading, lower

ones spatulate but upper ones increasingly subulate; spathe pale greenish, extremely narrow and elongate and leaves usually narrowly hastate.

Rare fatty acids i.e. benzenetricanoic acid and benzenetricanoic acid methyl ester, from this species were isolated (Chen *et al.*, 1997). Preliminary tests showed that *Typhonium flagelliforme* suppress cancer cells such as leukaemia, colon carcinoma and melanoma. The extract, which is mixed with honey and drunk immediately, may be taken as single therapy or taken during the course of radiotherapy or chemotherapy. There are apparently no serious long-term side effects (Neoh, 1992).



Plate 1: *Typhonium flagelliforme*. (→) Flower Resembling the Tail of a Mouse.