SCREENING FOR HIGH KAEMPFEROL CONTENT FROM DIFFERENT SPECIES OF MALAYSIAN MEDICINAL PLANTS

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To my beloved parents, brothers and sister

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ABSTRACT

The flavonoids such as kaempferol that have anti-cancer properties are widely distributed in plants kingdom. The purpose of this study was to screen high kaempferol content in the selected medicinal plants and determine the kaempferol distribution in different plant organ. Kaempferol content in mature leaf of twenty two randomly selected Malaysian medicinal plants were determined using gas chromatography (GC-FID). A commercial kaempferol was used as a standard (positive control). It was discovered that the highest kaempferol content was detected in *Tibouchina semidecandra* (4689.75±654.83 mg/kg) while the mature leaf contained higher kaempferol than young leaf and shoot. However, no kaempferol was detected in young stem of *T. semidecandra*. Therefore, this study suggests *T. semidecandra* as an alternative source for kaempferol.

ABSTRAK

Flavonoid seperti kaempferol bersifat anti-kanser serta mempunyai taburan yang luas di alam tumbuhan. Kajian ini bertujuaan menyaring kandungan kaempferol tertinggi didalam tumbuhan ubatan terpilih dan menentukan taburan kaempferol didalam organ tumbuhan ubatan yang berbeza. Kandungan kaempferol didalam daun matang daripada sejumlah dua puluh dua tumbuhan ubatan Malaysia yang dipilih secara rawak telah disaring menggunakan kromatografi gas bersama pengesan nyalaan ion (GC-FID). Kaempferol komersil dijadikan piawaian (penujuk positif). Hasil kajian menunjukkan kandungan kaempferol tertinggi dikesan didalam *Tibouchina semidecandra* (4689.75±654.83 mg/kg) manakala daun matang mengandungi kaempferol tertinggi berbanding daun muda dan pucuk. Walaubagaimanapun, kaemperol tidak dapat dikesan didalam batang. Oleh itu, kajian ini mengesyorkan *T. semidecandra* sebagai sumber alternatif bagi kaempferol.

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GC-FID	-	Gas Chromatography-Flame Ionized Detector
GC-MS	-	Gas Chromatography-Mass Spectrophotometer
HPLC	-	High Performance Liquid Chromatography
± SEM	-	± Standard Error Mean
SPSS	-	Statistical Package Social Sciences

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Malaysia is among the most biologically diverse countries in the world. There are approximately 12,500 species of flowering plants, including species of valuable and marketable timber and fruit. Plants that produce natural products such as secondary metabolites confer adaptive benefits to plants and human. Important secondary metabolites such as anthocyanin could serve as natural food colorant and shelf life indicator for acidic food (Janna *et al.,* 2006).

Medicinal plant is defined as a plant which has been proven or claimed or thought to have medicinal remedies (Majid *et al.,* 1995). In Peninsular Malaysia, there are about 1,200 species of higher plants which have been reported to have medicinal properties for treatment of various diseases and ailments (Jantan, 1998). However, so far only about hundred have been investigated fully for their medicinal potential (Jamal *et al.,* 2010; Ismail, 2001).

The *Orthosiphon stamineus* extract is widely used in Malaysia as drug for the treatment of diabetes and kidney stones. Traditionally, it was consumed as herbal tea to facilitate body detoxification. More than 50 components including flavonoids, terpinoids and caffiec acid derivatives with different biological activity were successfully isolated. Majority of the flavonoid were obtained from the leaves (Hossain and Ismail, 2011) and the major bioactive compound was rosmarinic acid that is caffiec acid derivatives (Akowuah *et al.,* 2005).

An enormous variety of secondary metabolites such as alkaloid, terpenes, saponines, quinones and polyphenols are synthesized by plants to serve as a chemical defense against herbivores and microbial attack (Wink, 1988), toxicant and repellent for insects, and attractants for pollinators (Close and McArthur, 2002). For example, the *Radopholus similis* (nematode) resistance in *Musa* cultivars (banana) was caused by flavan-3,4-diols and condensed tannin (Collingborn *et al.,* 2000).

Flavonoids are potent antioxidants, free radical scavengers, and metal chelators and lipid peroxidation inhibitor. They have been reported to exhibit a wide range of biological effects, including antibacterial, antiviral, (Hanasaki *et al.,* 1994) anti-inflammatory, antiallergic, (Middleton, and Kandaswami, 1993; Hanasaki *et al.*, 1994; Hope *et al.*, 1983) and vasodilatory actions (Duarte *et al.*, 1993). In addition, flavonoids inhibit lipid peroxidation (LPO) (Salvayre *et al.*, 1988) platelet aggregation, (Tzeng *et al.,* 1991; Robak *et al.*, 1988) capillary permeability and fragility (Torel *et al.*, 1986; Budavari *et al.*, 1989) and the activity of enzyme systems including cyclo-oxygenase and lipoxygenase (Hope *et al.*, 1983; Middleton, and Kandaswami, 1993; Hodnick *et al.*, 1988). For example, baicalin isolated from *Scutellaria baicalensis* that have been used in Chinese and Japenese medicine inhibit HIV-1 replication (Li *et al.,* 1993).

Kaempferol is a flavonoid classified as flavonol that widely distributed group of polyphenolic compounds characterized by a common benzo-pyrone structure. Over 4,000 different flavonoids have been identified and distributed in the leaves, seeds, bark and flowers of plants. For example, the leaves of *Centella asiatica* which belong to *Umbelliferrae* family also known as "Pegaga" in Malay has been reported to contain high kaempferol content besides other flavonoids such as naringin, rutin, quercetin, catechin, apigenin and luteolin (Mohd Zainol *et al*., 2009). Therefore, this study was initiated to screen high kaempferol producing plants in mature leaves because previous study shows that flavonol act as antioxidant in chloroplast of spinach (Hernandez *et al.,* 2009; Takahama, 1984).

1.2 Statement of problem

The potential of high flavonoids producer in Malaysian medicinal plants was not fully explored although extensive studies of bioactive compounds and their total phenolic content in many species have been carried out. Besides that, there was lack of research that focus on identification of kaempferol despite diverse plant secondary metabolites and its wide distribution in plants. In addition, the plant secondary metabolites such as flavonoids vary in type and quantity due to duration of stress exposure and plant species. Therefore, screening of medicinal plants with high flavonoid content particularly kaempferol and its distribution in plant organs will elucidate the biochemical nature of bioactive compounds.

1.3 Objectives of research

The objectives of this study are to:

- screen plant with high kaempferol content from selected Malaysian medicinal plants
- determine distribution of kaempferol in different plant organs of *T. semidecandra*

1.4 Scope of research

The scope of my research includes screening of kaempferol in twenty two randomly selected Malaysian medicinal plants mature leaves using Gas Chromatography with Flame ionized detector (GC-FID). The analysis was done in triplicate and repeated three times. The mature leaf was selected because kaempferol is a flavonoid classified in flavonol group that act as antioxidant in chloroplast (Hernandez *et al.,* 2009) and preliminary study showed that the mature leaf of *Justicia gendarussa* exhibit highest antioxidant activity (Yani, 2009). The other plant organ such as shoot, young leaf and young stem of identified plant with highest kaempferol content was screen for highest organ producer and all the data were analyzed using Statistical Package for the Social Sciences (SPSS).