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Screening for High Kaempferol Content in Different Species of Malaysian Medicinal Plants

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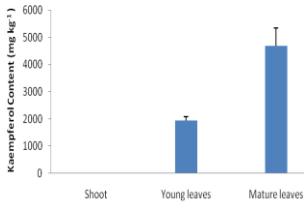
Graphical abstract



Abstract

Kaempferol is a natural flavonol and has been reported to reduce risk of pancreatic cancer and protect against heart disease. Kaempferol also acts as antioxidant that prevents oxidative damage to cell, lipids and DNA. The purpose of this study was to determine kaempferol distribution in the leaves of 20 Malaysian medicinal plants using GC-FID. Results demonstrated that kaempferol was only detected in eight plant species. Of all plants tested, *Tibouchina semidecandra* contained the highest kaempferol concentration. Further assessment showed that mature leaf extracts of *T. Semidecandra* had more kaempferol (4689.75±654.83mg kg^{-1}) than young leaf extracts (1945.04±138.81mg kg^{-1}). However, no kaempferol was detected in shoot extracts. These findings suggest that kaempferol content depends on plant species and physiological state of the plant organs. Thus, *T. semidecandra* could be an alternative source of kaempferol.

Keywords: Kaempferol; flavonol; antioxidant; GC-FID; mature leaf

Abstrak

Kaempferol merupakan flavonol semulajadi yang dilaporkan dapat mengurangkan risiko kanser pankreas dan penyakit jantung. Kaempferol juga bertindak sebagai anti pengoksidaan yang melindungi sel, lipid dan DNA daripada musnah. Tujuan kajian ini dijalankan adalah untuk menentukan kandungan kaempferol dalam 20 jenis pokok herba tempatan menggunakan GC-FID. Kajian menunjukkan bahawa, hanya 8 jenis pokok yang disaring mengandungi kaempferol. *Tibouchina semidecandra* mengandungi kandungan kaempferol yang tertinggi berbanding pokok yang lain. Kajian lanjut menunjukkan ekstrak daun matang pokok *T. semidecandra* mengandungi kandungan kaempferol yang lebih tinggi (4689.75±654.83mg kg^{-1}) daripada daun muda (1945.04±138.81mg kg^{-1}). Walau bagaimanapun, tiada kaempferol dikesan pada pucuk tumbuhan. Hasil kajian mencadangkan bahawa kandungan kaempferol pada tumbuhan bergantung pada spesies pokok dan keadaan fisiologi sesuatu organ tumbuhan. Dengan demikian, *T. semidecandra* boleh dicadangkan sebagai sumber alternatif untuk kaempferol.

Kata kunci: Kaempferol; flavonol; anti pengoksidaan; GC-FID; daun matang

1.0 INTRODUCTION

Nowadays, medicinal plants have become the main source for health supplements and plant-based drug (Balunas and Kinghorn, 2005). It has been proven to have medicinal remedies (Ab. Majid *et al.*, 1995). In Malaysia, many medicinal plants have been used in traditional remedies. For examples, *Aloe vera* (poor digestion), *Morinda citrifolia* (urinary tract infections), *Acorus calamus* (arthritis), *Andrographis paniculata* (diabetes), *Orthosiphon aristatus* (gout), *Eurycoma longifolia* (aphrodisiac) and *Centella asiatica* (hypertension) (Khatun *et al.*, 2011). In addition, several medicinal plants have also been reported to contain medicinal properties such as antioxidant in *Piper sarmentosum* and *Morinda elliptica* (Subramaniam *et al.*, 2003), anticancer compounds in *Melastoma malabathricum* L. (Susanti *et al.*, 2008), antimicrobial activity in *Pereskia bleo*, *P. grandifolia*, *Curcuma zedoaria* and *C. inodora* (Philip *et al.*, 2009), anti-inflammatory and analgestic action in *Justicia gendarussa* (Shikha *et al.*, 2010).

Flavonoids are a group of polyphenolic compounds that ubiquitously found in plants and common constituents in the human diet (Hollman and Katan, 1999). Flavonoids act as natural antioxidant and free radical scavenger in barley, *Hordeum vulgare* var. *nudum* (Okawa *et al.*, 2001). Flavonoids have a wide range of biological effects, including antibacterial activity (Cushnie and Lamb, 2005), antiallergic, antiartherogenic, anti-infammatory, antithrombotic, antioxidant, and cardioprotective (Manach *et al.*, 2005). For example, rosmarinic acid in *Orthosiphon stamineus* serves as anti-diuretic agent. Other flavonoids such as naringenin,

and kaempferol can inhibit HeLa cell proliferation (Sarju *et al.*, 2010).

Kaempferol, a flavonol that widely distributed group of polyphenolic compounds is characterized by a common benzopyrone structure. Leaf extract of *Centella asiatica* has been reported to contain high kaempferol and other flavonoids such as naringin, rutin, quercetin, catechin, apigenin and luteolin (Mohd Zainol *et al.*, 2009). Kaempferol plays important roles in antioxidant activity (Park *et al.*, 2006), cytotoxicity (Sarju *et al.*, 2010) and anticancer against pancreatic cancer cells (Zhang *et al.*, 2008). Therefore, this study was conducted to screen plants with high kaempferol content from selected local medicinal plants and determine distribution of kaempferol in different plant organs of high kaempferol plant lines using GC-FID.

■2.0 MATERIALS AND METHODS

2.1 Reference Standards, Solvent, Samples, Extraction

The commercial standard (kaempferol), and methanol were purchased from Sigma-Aldrich, Germany. The standards solutions were at concentration 1200 $mgmL^{-1}$. Twenty medicinal plants aged 11-12 months were bought from local nursery at Pulai region. The fresh leaves (6.0 g) of each plant were crushed with liquid nitrogen. Each sample (1.0 g) was macerated with methanol (3.0 mL) and shaken at 150 rpm for 0.5 hour. Each extract was filtered through 0.2 μm nylon syringe filter before injected into gas chromatography system.

2.2 Chromatography

GC was performed using a gas chromatograph (HP-6890N, Agilent USA) equipped with an HP-5 fused silica capillary column (30.0 m × 0.32 mm i.d., film thickness 0.25 μm) (Sarju *et al.*, 2012). The temperature programmed was 100-275°C at $10°Cmin^{-1}$ with 1.0 min hold at 100°C and 17 min hold at 275°C. The injector temperature was 275°C. The flow-rate of carrier gas (helium) was 1.0 $mLmin^{-1}$. A split ratio of 50:1 was used. A quantity of 5 μL of the solutions (extracts and standards) was injected. The chromatographic data were recorded and processed using Agilent Cerity QA-QC software.

The GC-MS analyses were carried out on a gas chromatograph (HP-7890A, Agilent USA) with quadrupole mass spectrometer (Finnigan Trace MS, ThermoQuest CE Instrument, USA) operating in EI mode at 70 eV. An HP-5 MS column (30.0 m × 0.32 mm i.d., film thickness 0.25 μm) was used. The temperature programmed was 100-275°C at $10°Cmin^{-1}$ with 1.0 min hold at 100°C and 17 min hold at 275°C. The injector temperature was 275°C. The flow-rate of carrier gas (helium) was 1.0 $mLmin^{-1}$. A split ratio of 50:1 was used. A quantity of 5 μL of the solutions (extracts and standards) was injected.

2.3 Quantification of Kaempferol Content

Quantification of kaempferol content was conducted according to previous method (Sarju *et al.*, 2010). Peak area (pA) from the chromatography represented concentration of the flavonoid. The F factor was obtained by using known concentration and peak area of positive standard (naringenin and kaempferol) and external standard n-alkanes. Kaempferol content of each treatment was calculated via formula:

Concentration and area of positive standard
Concentration and area of external standard } Known

$$\frac{[\text{positive standard}]}{\text{Area positive standard}} = F \frac{[\text{external standard}]}{\text{Area external standard}}$$

F = factor for positive standard

$$F = \frac{\text{Concentration positive standard in sample}}{\text{Area positive standard in sample}}$$

F × Area positive standard in sample = Concentration positive standard in sample

2.4 Data Analysis

In this study, all samples were run in three replicates. Hence, the data obtained were analyzed using SPSS for Window software (SPSS 17.0 for Windows Evaluation Version software, SPSS Inc., USA). For comparison concentration of kaempferol among species, statistical significance was established through One-Way ANOVA while the means was compared using One-Way ANOVA with Post Hoc Bonferroni test.

■3.0 RESULTS AND DISCUSSION

3.1 Quantification of Kaempferol

Phytochemical analysis of methanolic leaf extracts from 20 randomly selected Malaysian medicinal plants is shown in Table 1. Retention time of kaempferol standard was recorded at 27.6 minutes (Fig. 1-A). For example, the presence of kaempferol in *Tibouchina semidecandra* was also detected at similar retention time (Fig. 1-B). Out of 20 plant species tested, kaempferol was only detected in eight plant species (Fig. 2). The maximum kaempferol was recorded in leaf extracts of *T. semidecandra* (4689.74±654.83 $mgkg^{-1}$) and the lowest was detected in *Pedilanthus tithymaloides* extracts (1582.27±313.85 $mgkg^{-1}$).

Table 1 Phytochemical analysis of 20 methanolic leaf extracts

No	Family	Scientific name	Local name	Kaempferol Content (mg kg^{-1})
1	Acanthaceae	*Strobilanthes crispus*	Pecah kaca	0.00±0.00
2	Apocynaceae	*Wrightia religiosa*	Anting putri	1853.19±104.66
3	Compositae	*Gynura procumbens*	Sambung nyawa	0.00 ± 0.00
4	Euphorbiaceae	*Acalypha hispida*	Ekor kucing	3696.28±206.52
5		*Acalypha indica*	Kucing galak	0.00±0.00
6		*Pedilanthus tithymaloides*	Lelipan	1582.27±313.85
7		*Phyllanthus amarus*	Dukung anak	0.00±0.00
8	Flacourtiaceae	*Flacourtia rukam*	Rukam	3895.43±810.88
9	Lamiaceae	*Orthosiphon stamineus*	Misai kucing	0.00±0.00
10		*Plectranthus amboinicus*	Bangun-bangun	0.00 ± 0.00
11	Malvaceae	*Hibiscus rosa-sinensis*	Bunga raya putih	0.00±0.00
12	Melastomataceae	*Tibouchina semidecandra*	Senduduk biru	4689.75±654.83

13	Myrtaceae	*Rhodomyrtustomentosa*	Kemunting	3182.67±295.05
14	Piperaceae	*Piper betle*	Sireh	0.00±0.00
15	Rubiaceae	*Morinda citrifolia*	Mengkudu	0.00±0.00
16	Rutaceae	*Ruta angustifolia*	Geruda	0.00±0.00
17	Solanaceae	*Cestrum nocturnum*	Harum sundal malam	2590.79±161.13
18	Zingiberaceae	*Alpinia conchigera*	Lengkuas genting	2355.53±462.57
19		*Curcuma zedoaria*	Temuputih	0.00±0.00
20		*Kaempferia pulchra*	Cekur batik	0.00±0.00

*The value of kaempferol content are means ± SEM (n=3) and the mean difference, p is significant at the 0.05 level

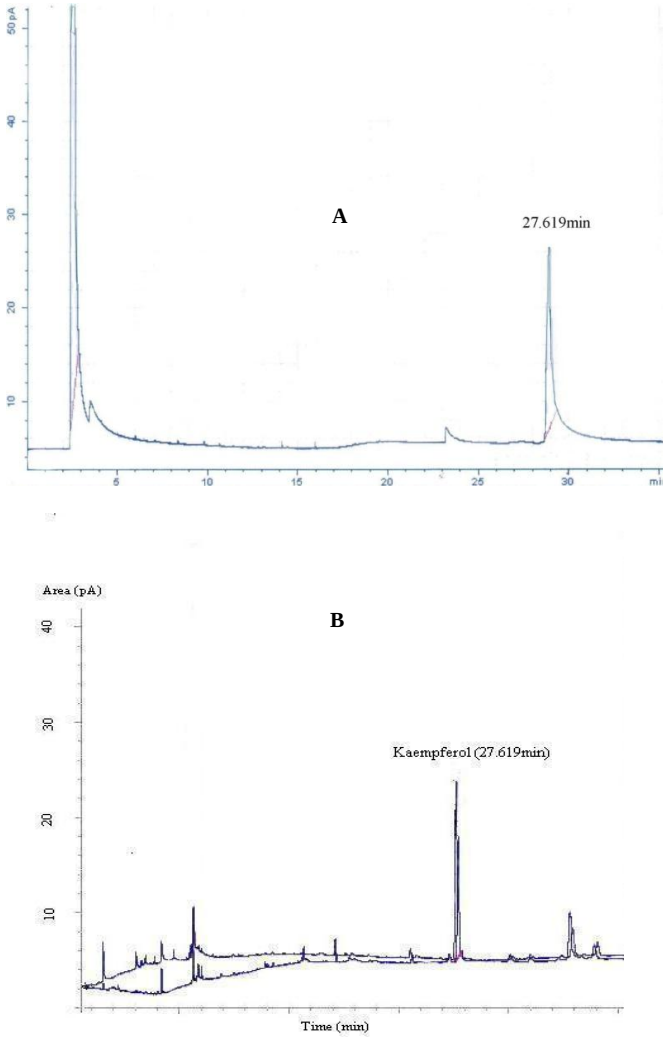


Figure 1 GC-FID chromatograms: A. Kaempferol (standard); B. Leaf extract of *Tibouchina semidecandra* (with spike)

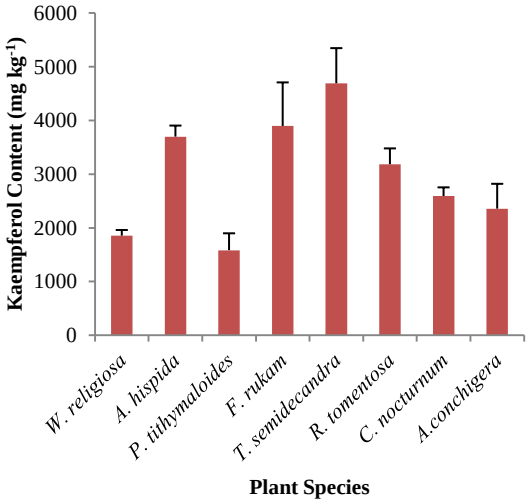


Figure 2 Kaempferol content in eight medicinal plants

However, there was no kaempferol detected in *Gynura procumbens* and a similar finding was also reported by other researches using HPLC (Mustafa *et al.*, 2010). Overall results showed that *T. semidecandra* contained significantly higher kaempferol content than *Wrightia religiosa* and *Pedilanthus tithymaloides* ($p<0.05$). Kaempferol, a flavonol is found abundantly in chloroplast (Hernández *et al.*, 2009) especially in leaves (Yamasaki *et al.*, 1996). Previous finding also indicated that flavonoids content in leaf was influenced by light intensity (Ghasemzadeh *et al.*, 2010).

The precursors for synthesis of all flavonoids including kaempferol are malonyl-CoA and ρ-coumaroyl-CoA. The condensation reaction of one molecule of 4-coumaroyl-CoA and three of malonyl-CoA to naringenin chalcone is catalysed by chalcone synthase (CHS) (Figure 3). In another reaction, flavonol synthase (FLS) catalyses kaempferol formation from dihydrokaempferol (Holton and Cornish, 1995). Flavonol synthase has multiple isoforms. For example, there are five isoforms of FLS detected in *Arabidopsis thaliana*. Therefore, other isoforms of FLS could responsible for synthesis of other flavonols such as myricetin and quercetin. However, only flavonol synthase 1 (AtFLS1) influenced the production of the flavonols, kaempferol and quercetin (Owens *et al.*, 2008). Besides, kaempferol level could also be increased up to 100-fold by silencing the flavonoid 3′5′-hydroxylase (F3′5′h) gene, enzyme that catalyses the conversion of dihydrokaempferol and dihydroquercetin into dihydromyricetin (Rommens *et al.*, 2008).

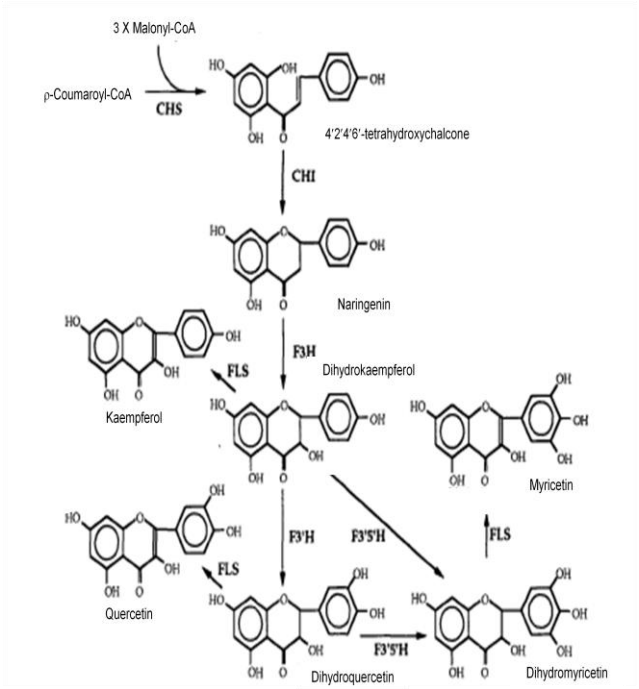


Figure 3 Anthocyanin and flavonol biosynthetic pathway (Adapted from Holton and Cornish, 1995)

3.2 Distribution of Kaempferol from Organ Parts

Distribution of kaempferol in different type of organs of *T. semidecandra* is shown in Figure 4. Results showed that mature leaves (4689.75±645.828 mg kg^{-1}) contained more kaempferol than young leaves (1945.04±138.81mg kg^{-1}) ($p<0.05$). Vacoule is the largest storage compartment of flavonoids in a mature plant cell (Jauh *et al.*, 1999). The highest kaempferol in mature leaf as compared to other organs such as young leaf could be due to high accumulation of flavonoids in vacuole. On contrary, no kaempferol was detected in shoot.

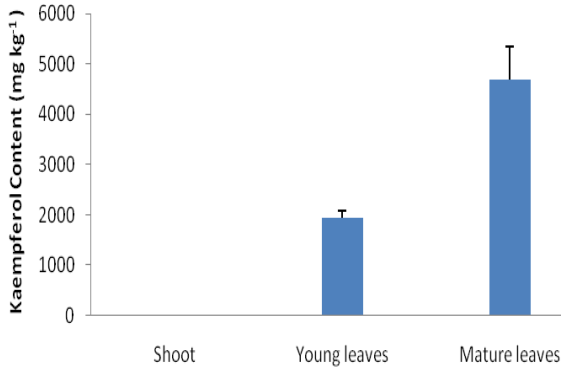


Figure 4 Distribution of kaempferol content in different type of organs

■4.0 CONCLUSION

Among 20 Malaysian medicinal plants tested, *Tibouchina semidecandra* contained the highest kaempferol content and thus potentially become an alternative source of natural antioxidant and anticancer compound. Kaempferol was also found to be highly accumulated in mature organ such as in mature leaves as compared to younger leaves. Therefore, future works such as screening of high kaempferol plant lines and *in vitro* propagation of *T. semidecandra* could optimize kaempferol production for commercial uses.

Acknowledgement

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