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Detection and Quantification of Naringenin and Kaempferol in *Melastoma decemfidum* Extracts by GC–FID and GC–MS

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Summary. *Melastoma decemfidum* is a plant species from the Melastomataceae family. The plant was reported to have bioactive flavonoids, which showed antioxidant and cytotoxic activities. Extracts from the leaves of 26 plants were made at room temperature with methanol. Detection and quantification of two of the flavonoids, namely naringenin and kaempferol, in the extracts were carried out by using gas chromatography–flame ion detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS). By optimizing the key experimental parameters, a linear response for the individual target compounds was obtained in the concentration range LOQ from 3.44 to 8.26 μg mL^{-1} (r^2 = 0.9731–0.9772), with LODs from 1.13 up to 2.72 μg mL^{-1} per 1.0 g of crushed leaves, and with repeatability within the relative standard deviation (RSD) of 1.65–1.81%.

Key Words: *Melastoma decemfidum*, flavonoids, naringenin, kaempferol, GC–FID, GC–MS

Introduction

Melastoma decemfidum (synonym *M. malabathricum*) of the Melastomataceae family is a small shrub with the common name of "Singapore rhododendron" [1] and the local name of "senduduk putih". This species is distributed from Madagascar to India and Australia. It is commonly found throughout Malaysia in the lowland and mountain forests, chiefly in open places [2]. The whole plant is used for the treatment of diarrhea, postpartum conditions, hemorrhoids, hepatitis, leucorrhea, swelling, mouth ulcer, toothaches, and sinusitis [3, 4]. Previous phytochemical study on the flowers and leaves [5, 6] of *M. decemfidum* revealed six flavonoids including naringenin (**1**) and kaempferol (**2**) and two triterpenes. Naringenin (**1**) was found to have a significant anticancer effect against MCF7, while kaempferol (**2**) was reported to have anti-inflammatory activity. These phytochemicals were isolated and purified from the air-dried samples by Soxhlet extraction and column chromatography methods, which are both time- and solvent-consuming [5].

Quantitative studies on flavonoids from other plant materials have been reported using high-performance liquid chromatography (HPLC) [7], HPLC coupled with ultraviolet (UV) spectroscopy [8], and combination of HPLC with simultaneous mass spectrometry and diode array detection [9]. Gas chromatography (GC) was found to be the simplest and most efficient method to detect and quantify flavonoids from samples of *Kaempferia parviflora* [10]. Based on this and previous conventional phytochemical study by one of us [5], here we report for the first time the detection and quantification of naringenin (**1**) and kaempferol (**2**) from fresh leaves of *M. decemfidum* using gas chromatography–flame ion detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS).

Experimental

Reference Standards, Solvent, Samples, Extraction

The commercial standards (naringenin and kaempferol) and methanol were purchased from Sigma-Aldrich, Germany. The standard solutions were made at a concentration of 1200 mg mL^{-1}. Twenty-six whole plants of *M. decemfidum* aged 3-4 months each were bought from 26 different nurseries in the southern part of Malaysia, namely Johor and Malacca. Fresh leaves (6.0 g) of each plant were crushed after freezing with liquid nitrogen. Each sample (1.0 g) was macerated with methanol (3.0 mL) and shaken at 150 rpm for 0.5 h. Each extract was filtered through 0.2-μm nylon syringe filters before being injected into the GC system.

Chromatography

GC was performed using a 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). The temperature programmed was 100–275°C at 10°C min^{-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 the carrier gas (helium) was 1.0 mL min^{-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 the Agilent Cerity QA-QC software.

The GC–MS analyses were carried out on a gas chromatograph (HP-7890A, Agilent, USA) equipped with a quadrupole mass spectrometer (Finnigan Trace MS, ThermoQuest CE Instrument, USA) operating in the electrospray ionization (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°C min^{-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 the carrier gas (helium) was 1.0 mL min^{-1}. A split ratio of 50:1 was used. A quantity of 5 μL of the solutions was injected.

Quantitative analyses of naringenin (**1**) and kaempferol (**2**) were carried out using the response factor method [11]. The experiments were carried out in triplicates. The data were subjected to statistical analysis using the SPSS 15.0 software. The analyses involved one-way ANOVA, Pearson correlation, and linear regression.

Results and Discussion

Qualitative Analysis

Individual chromatograms were recorded for all extracts with and without the standards. Examples of chromatograms are shown in *Fig. 1*. Both chromatograms show peaks of naringenin (**1**) and kaempferol (**2**). Based on the chromatograms, the retention times (t_R) of naringenin (**1**) and kaempferol (**2**) were determined as the basis for qualitative identification *(Table I)*.

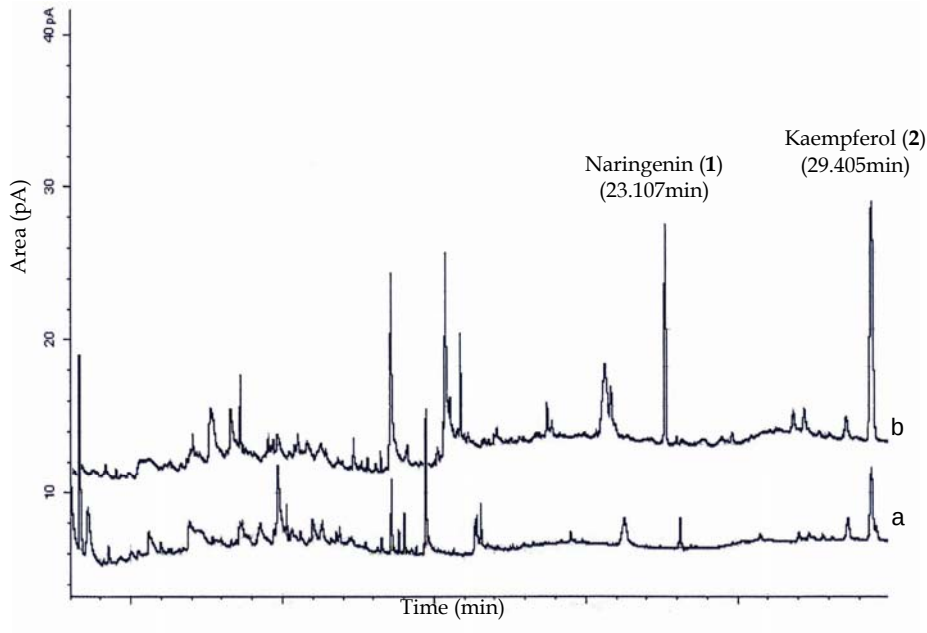

Fig. 1. Chromatograms of *M. decemfidum* extracts: (a) non-spike and (b) spike

Table I. Retention times (t_R) of naringenin (**1**) and kaempferol (**2**) in the extracts

Compound	t_R (min)
Naringenin (**1**)	23.035 ± 0.077
Kaempferol (**2**)	29.280 ± 0.143

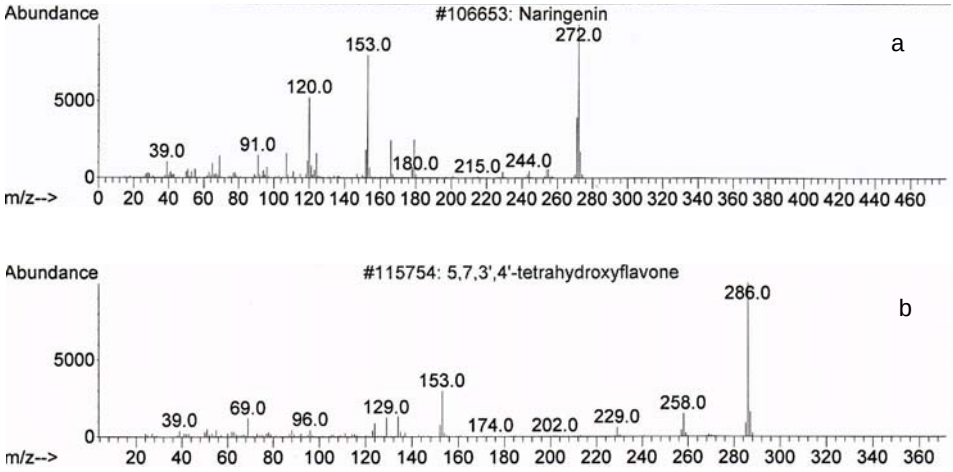

Fig. 2. MS spectra of (a) naringenin (**1**) and (b) kaempferol (**2**) from the extracts

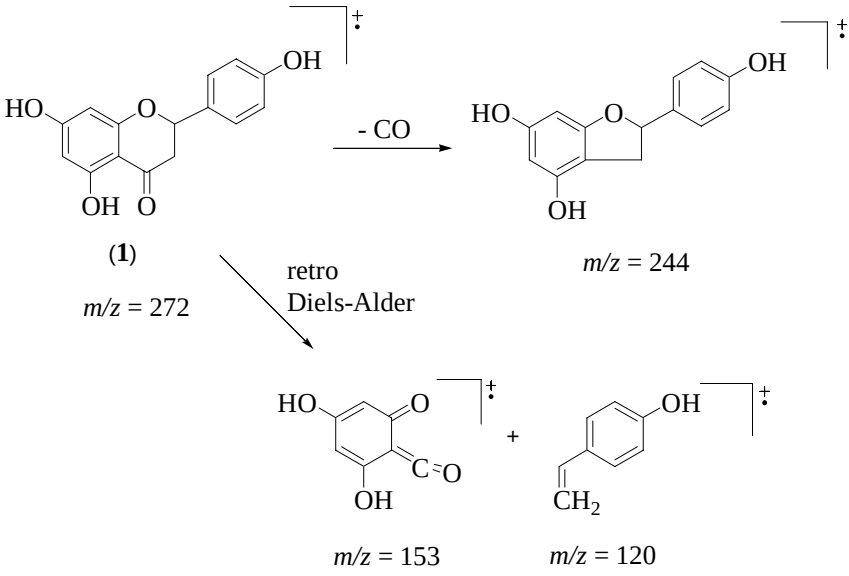

Fig. 3. Fragmentation pattern of naringenin (**1**)

Further confirmation on the existence of (**1**) and (**2**) in the extracts was obtained by examining the mass spectra *(Fig. 2)* of both peaks. The MS of compound (**1**) showed a molecular ion at *m/z* 272, which was in agreement with the molecular formula $C_{15}H_{12}O_5$. Removal of CO from the parent ion gave the ion at *m/z* 244, while retro Diels–Alder fragmentation afforded radical cations at *m/z* 153 and 120. The MS spectrum of (**2**) exhibited a molecular ion peak at *m/z* 286, consistent with the molecular formula $C_{15}H_{10}O_6$. Removal of CO and retro Diels–Alder fragmentation of the parent ion gave radical cations at *m/z* 258 and 153, respectively. *Fig. 3* shows the fragmentation pattern for (**1**).

Method Validation and Quantitative Analysis

In this study, the analytical performance of the method was evaluated via the linearity range, LOQ, and LOD. The relative standard deviation (RSD) was taken as a measure of the precision. The validation was performed for 26 samples. The results on LODs and LOQs, as well as the linear dynamic range for all target compounds (**1** and **2**), are given in *Table II*.

Table II. Results from analysis of kaempferol and naringenin from *M. decemfidum* leaf

	Naringenin (1)	Kaempferol (2)
Mean (μg mL^{-1})	0.103	0.730
Precision (%RSD)	1.6544	1.8127
Range (μg)	0.053–0.351	0.524–1.480
Linearity (*r*)	0.9731	0.9772
LOD (μg)	1.136	2.729
LOQ (μg)	3.4424	8.2696
Relative retention time	1.031	1.011

The LODs estimated with the aid of 3:1 signal-to-noise (S/N) ratio criteria were found to be within 1.136–2.729 μg of all the studied samples. The LOQs, calculated as the S/N = 10, were 3.4424–8.2696 μg for 1.0 g of crushed sample leaves. These values indicate that the current method is sensitive enough to determine (**1**) and (**2**) in *M. decemfidum*. The precision of the method under optimized conditions (i.e., sample weight 1.0 g of crushed leaves; 3.0 mL sampling vial, extraction time 0.5 h at room temperature, sample injection 5 μL) was determined by analyzing the samples in triplicate, and the result was 1.6544–1.8127%. This shows that GC and GC–MS

analyses are reliable techniques for the detection and quantification of naringenin (**1**) and kaempferol (**2**) for the plant under study.

Flavanoid Distribution in the Leaf Extracts

Twenty-six plants of the same species of *M. decemfidum* bought from different nurseries were evaluated for the presence of flavonoids (**1**) and (**2**). Naringenin (**1**) was detected in 16 leaf samples (61.5%) of *M. decemfidum*. The maximum concentration of (**1**) was detected in sample 20 (0.3840 ± 0.004 μg mL^{-1}) and significant difference at $p < 0.05$ only in the case of four samples (15.38%). The average concentration of (**1**) in all samples was 0.1107 ± 0.133 μg mL^{-1}.

Twenty leaf samples (76.9%) displayed the existence of (**2**), with the highest concentration detected in sample 25 (1.4728 ± 0.009 μg mL^{-1}) with significant difference at $p < 0.05$ for all samples except samples 16 and 17 (7.69%). The average concentration of kaempferol (**2**) in all samples was 0.7246 ± 0.490 μg mL^{-1}. *Fig. 4* shows the distribution of both flavonoids in the 26 samples.

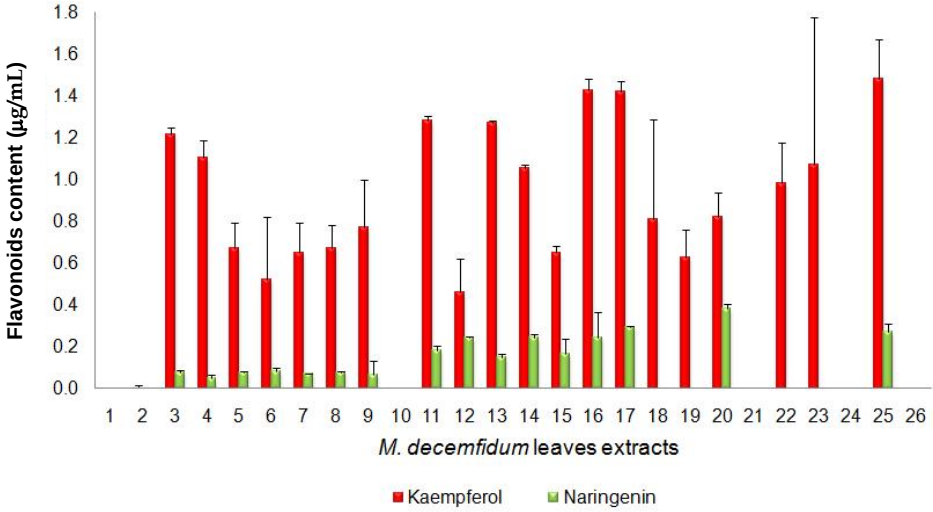

Fig. 4. Distribution of naringenin (**1**) and kaempferol (**2**) in 26 extracts of *M. decemfidum*

Pearson correlation analysis was carried out to determine the relationship of (**1**) and (**2**) in terms of the concentration pattern. The results showed that the concentrations of (**1**) and (**2**) were positively correlated and significant at the 0.01 level (two-tailed). The correlation between (**1**) and (**2**) in the leaf extracts was 0.469, suggesting that the concentration of both flavonoids

is directly proportional. Hence the presence of (**2**) could indicate the presence of (**1**) and vice versa. *Fig. 5* displays the linear regression between naringenin (**1**) and kaempferol (**2**).

The rapid preparation of samples combined with a short GC analysis time offers an efficient method for quality control of herbals or products claimed to have originated from *M. decemfidum*. The ability to detect and quantify the bioactive flavonoids of (**1**) and (**2**) in the leaves of *M. decemfidum* could lead to isolation of high-flavonoid-producing plant lines. Development of the extraction and determination of these compounds in vivo (leaf, stem, fruit, and root) and manipulation of their production by addition of elicitors in culture can be exploited further for large-scale production of these medicinally important compounds [12].

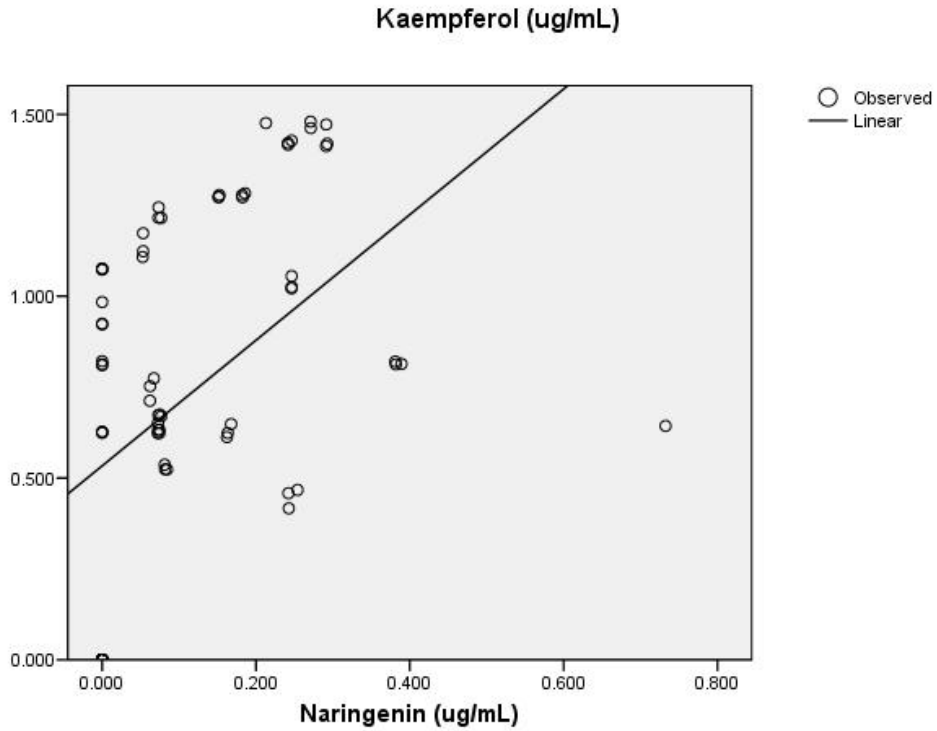

Fig. 5. Pearson correlation between naringenin (**1**) and kaempferol (**2**) in terms of concentration pattern

Conclusion

This paper presented the use of GC–FID and GC–MS in the detection and quantification of important flavonoids from the plants extracts of *M. decem-*

fidum. The GC analysis for an extract was completed in a short time, and hence this rapid method can be used to detect and quantify the same flavonoids from other plant species.

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