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## Detection of Naringenin and Kaempferol in *Justicia gendarussa* Leaf Extracts by GC-FID (Pengesanan Naringenin dan Kaempferol dalam Ekstrak Daun *Justicia gendarussa* oleh GC-FID)

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### ABSTRACT

*Justicia gendarussa* methanolic leaves extract from four different locations in Southern region of Malaysia and distribution of two flavonoids, naringenin and kaempferol in plant organs (young and mature leaves) were determined using gas chromatography-flame ionization detector (GC-FID) analysis. Naringenin and kaempferol contents were detected and quantified in leaves extract. The results indicated that mature leaves contained more naringenin and kaempferol compared to young leaves. The highest concentration of naringenin and kaempferol were recorded in mature leaves from Skudai and Muar regions which were 507.692 and 1226.964 mgkg<sup>-1</sup>, respectively. Data analysis showed that naringenin content was directly proportional to the amount of kaempferol in the leaf extracts. In conclusion, this study suggested geographical variation among plant samples and physiological stage of organ parts could contribute to variation in flavonoids concentration in a plant species.

**Keywords:** Flavonoids; GC-FID; *Justicia gendarussa*; kaempferol; naringenin

### ABSTRAK

Ekstrak daun *Justicia gendarussa* menggunakan metanol daripada empat lokasi berbeza di wilayah selatan Malaysia dan taburan dua sebatian flavonoid, naringenin dan kaempferol dalam organ-organ tumbuhan (daun muda dan tua) telah ditentukan dengan menggunakan analisis Gas Kromatografi -Pengesanan Pengionan Nyala (GC-FID). Kandungan naringenin dan kaempferol telah dikesan dan ditentukan dalam ekstrak daun. Keputusan kajian menunjukkan bahawa daun tua mengandungi lebih naringenin dan kaempferol berbanding daun muda. Kepekatan tertinggi naringenin dan kaempferol telah dicatatkan pada daun tua dari kawasan Skudai dan kawasan Muar yang masing-masing adalah 507.692 dan 1226.964 mgkg<sup>-1</sup>. Analisis data menunjukkan kandungan naringenin adalah berkadar terus dengan amaun kaempferol pada ekstrak daun. Kesimpulannya, kajian ini mencadangkan variasi geografi dalam kalangan sampel tumbuhan dan tahap fisiologi bahagian organ boleh menyumbang kepada variasi kepekatan flavonoid pada spesies tumbuhan.

**Kata kunci:** Flavonoid; GC-FID; *Justicia gendarussa*; kaempferol; naringenin

### INTRODUCTION

*Justicia gendarussa* or known as Gendarussa is a member of Acanthaceae family. This species is distributed in many countries including Indonesia, Sri Lanka, India and Malaysia. *J. gendarussa* plant is an erect, branched, smooth undershrub, 0.8 - 1.5 m in height with long leaves (7 to 14 cm) having acute tips, small flowers, terminal pinkish spikes with purple spots (Thomas & Yoichiro 2010). The root and leaves extracts of *J. gendarussa* have been used traditionally to treat many ailments such as chronic rheumatism, inflammations, bronchitis, headache, arthritis, vaginal discharges, dyspepsia, eye disease and fever (Janarthanam & Sumathi 2010). Leaves and stem extracts of *J. gendarussa* were reported to possess anticancer, anti-inflammatory, antioxidant, antibacterial, antifungal, antiangiogenic, antiplatelet and hepatoprotective activities (Ayob et al. 2014, 2013; Krishna et al. 2010a, 2010b; Periyanyagam et al. 2009; Sharma et al. 2011; Subramanian et al. 2012). Phytochemical analysis

on leaves of *J. gendarussa* has showed the presence of flavonoids, alkaloids, amino acids, triterpenoid saponins, aromatics amines, triterpenoid (stigmaterol, lupeol and 16-hydroxy lupeol) (Bambang Prajogo et al. 2009; Chakravarty et al. 1981; Mustafa et al. 2010).

Naringenin and kaempferol are flavonoids that have strong anticancer activity in MCF-7, MDA-MB-231 and MDA-MB-468 breast cancer cells and also HT-29, HeLa and BxPC-3 human cancer cells (Ayob et al. 2014, 2013). There are also possess high antioxidant activity and exhibit inhibitory effects on cholesterol in HepG2 and MCF-7 cancer cells (Cavia-Saiz et al. 2010; Kim et al. 2008; Zhang et al. 2008). For instance, naringenin demonstrated suppressed apoptotic in mouse leukaemia P388 cells while kaempferol has been reported to inhibit cell proliferation, induce apoptosis to pancreatic cancer cells (MIAPaCa-2 and Panc-1) and prevented arteriosclerosis (Kanno et al. 2005; Tu et al. 2007; Zhang et al. 2008). Several flavonoids have been detected in plant extracts of *J. gendarussa* using

high performance liquid chromatography (HPLC) (Mustafa et al. 2010). In addition, a simple and efficient method for detection and quantification of naringenin and kaempferol from fresh crude extracts of *Melastoma decemfidum* using GC-FID has been reported (Sarju et al. 2012). Hence, to the best of our knowledge, this is the first study of the detection and quantification of naringenin and kaempferol from young and mature leaves in *J. gendarussa* from different locations in southern region of Peninsular Malaysia using GC-FID.

## MATERIALS AND METHODS

### PLANT MATERIAL

*J. gendarussa* plants were collected from four different locations in Johor (Skudai, Batu Pahat, Mersing and Muar) and maintained in a glasshouse at Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia. The *J. gendarussa* plant was identified by Dr. Richard Chung Cheng Kong, a senior research officer of the Forest Research Institute of Malaysia (FRIM) and the voucher specimen (PID-100214-06) was deposited at the Herbarium Management Branch, Forest Biodiversity Division, FRIM, Kepong, Selangor, Malaysia.

### STANDARD REFERENCE, SAMPLES, SOLVENT AND EXTRACTION

The commercial standard of naringenin and kaempferol were purchased from Sigma-Aldrich (Subang Jaya, Selangor, Malaysia). Standard solution for each compounds were prepared at concentration of 1 mgmL<sup>-1</sup>. Twenty-four plants of *J. gendarussa* were bought from four nurseries in southern region of Peninsular Malaysia. Fresh of young and mature leaves (5.0 g of each plant) were crushed into a powder in liquid nitrogen. The powdered samples (1.0 g) were macerated in methanol (3.0 mL) and shaken at 150 rpm for 60 min at room temperature. Each extract was filtered through 0.2 µm nylon syringe filter into Agilent's vial prior to GC-FID analysis.

### DETECTION AND QUANTIFICATION OF FLAVONOID

GC-FID and quantitative analyses were carried out according to previously published method (Sarju et al. 2012). 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) was used. The temperature programmed was 100 - 275°C at 10°C min<sup>-1</sup> and 17 min hold at 275°C. Initial temperature was 100°C, injection temperature was 220°C and detector temperature was 270°C. The flow rate of the carrier gas (helium) was 1 mL min<sup>-1</sup>. A split ratio of 50:1 was used. Exactly 5 µL of crude extracts and standards were injected using a microsyringe. The chromatographic data were recorded and processed using Agilent Cerity QA-QC software.

## STATISTICAL ANALYSIS

All experiments were carried out in triplicates. The data was analyzed statistically using SPSS software (SPSS 16.0, SPSS Inc., USA). The analyses involved Independence *t*-test and one-way ANOVA with post-hoc multiple comparisons i.e. Bonferroni test. The correlations were analyzed using Pearson correlation test (Pallant 2007).

## RESULTS AND DISCUSSION

### QUALITATIVE ANALYSIS

Phytochemical analysis on methanolic leaves extracts of *J. gendarussa* showed that naringenin and kaempferol were detected in samples collected from four different locations in Johor, Malaysia and distributed in both organ parts i.e. young and mature leaves. Figure 1 shows the chromatogram where two peaks of naringenin and kaempferol and their retention times are listed in Table 1 for qualitative identification. The two compounds were separated within 35.5 min with the baseline separation. Figure 2 shows the chromatograms of untreated mature leaf extract from Muar region (a) and after co-injected with two standards (b). The results indicated that naringenin and kaempferol were successfully detected in the leaf extracts of *J. gendarussa*.

### QUANTITATIVE ANALYSIS OF FLAVONOIDS IN LEAVES EXTRACTS

In this study, distributions of naringenin and kaempferol contents in young leaves (second leaves from shoots) and mature leaves (seven to nine leaves from shoots) from four different locations of *J. gendarussa* have been evaluated. The results indicated that the mature leaves have higher naringenin and kaempferol contents compared to the young leaves. The highest concentration of naringenin and kaempferol were recorded in the mature leaves from Skudai region (507.692 mgkg<sup>-1</sup>) and Muar region (1226.964 mgkg<sup>-1</sup>), respectively (Figure 3). Naringenin content was directly proportional to the kaempferol contents in the leaf extracts. In addition, there was significant difference ( $p < 0.05$ ) of both flavonoids in leaf extracts from four different locations. Both flavonoids contents were affected by different locations and organ parts. The findings suggested that flavonoid contents might be attributed to different in physiological, environmental and geographical, soil and genetic factors (Figueiredo et al. 2008; Kong et al. 2013; Salimon et al. 2010). Previous report demonstrated that kaempferol was not detected by HPLC method (Krishna et al. 2010b). This could be due to the fact that flavonoid such as kaempferol is produced at certain plant parts and development stages of plant (Figueiredo et al. 2008).

Based on flavonoid biosynthetic pathway, naringenin is synthesized at earlier stage and catalyzed by chalcone isomerase (CHI). Naringenin is then further hydroxylated into dihydrokaempferol, which finally converted to

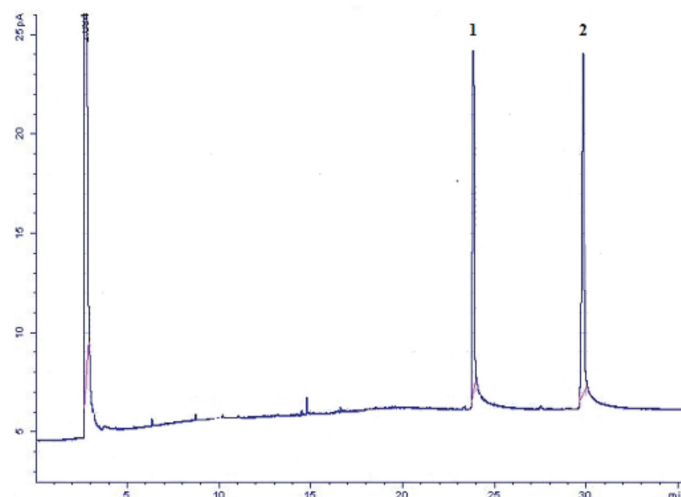


FIGURE 1. GC chromatogram of a standard mixture of two flavonoids. Column: HP-5 (30 m  $\times$  0.32 mm i.d. and df 0.25  $\mu$ m); column temperature program: 100°C (hold 1 min), then ramped to 275°C at 10°C min<sup>-1</sup> and hold for 17 min; injector port temperature: 220°C; detector port temperature: 270°C; carrier gas: helium at 1 mL min<sup>-1</sup>; detector: FID. Peaks: Naringenin (1) and Kaempferol (2)

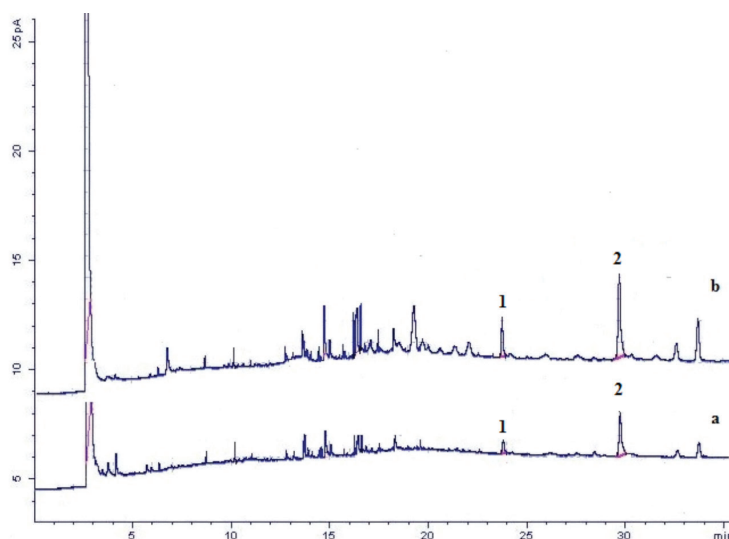


FIGURE 2. Chromatogram of naringenin and kaempferol contents in (a) mature leaves extract from Muar (control) and (b) co-injected with standards (naringenin and kaempferol)

TABLE 1. Retention times,  $t_R$  of naringenin and kaempferol on HP-5 column

Peaks	Compounds	Retention time, $t_R$ (min)
1	Naringenin	23.865
2	Kaempferol	29.828

kaempferol (flavonol) by flavonol synthases (FLS) (Forkmann 1991). It is suggested that naringenin is converted into kaempferol completely in mature leaves. Besides that, kaempferol (flavonol) can be induced when leaves are exposed to UV-B radiation (Lake et al. 2009; Mazid et al. 2011). Kaempferol and naringenin are stored

in vacuole to avoid harmful effect against cells (Roytrakul & Verpoorte 2007).

Flavonoids including flavone, flavanone, flavanol, chalcone, isoflavone and isoflavanone are produced *via* shikimate and acetate-malonate biosynthetic pathway (Markham 1982). Most secondary metabolites including flavonoids and their glycosides are produced when tissues and organs are produced during plateau phase (Bar-Peled et al. 1993). Additionally, accumulation of secondary metabolites undergo during stationary phase of cell growth kinetics. Interestingly, many enzymatic activities are actively synthesized during this phase because secondary metabolites are more actively synthesized when carbon is no longer needed in large quantities for primary metabolites (Bourgaud et al. 2001).

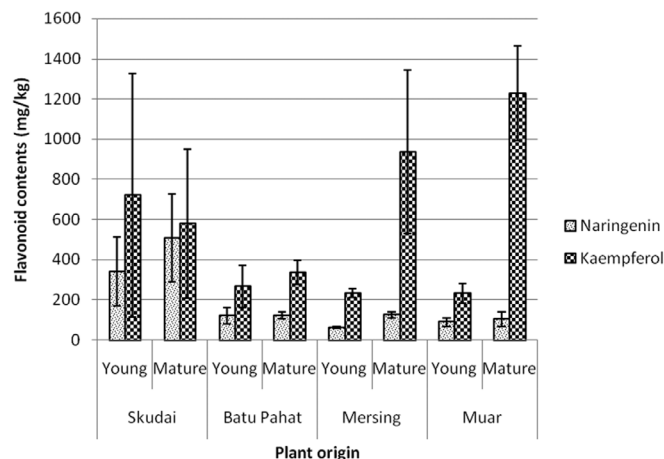


FIGURE 3. Naringenin and kaempferol contents in young and mature leaves extracts of *J. gendarussa* extracts from four different locations

### CONCLUSION

*J. gendarussa* with high naringenin and kaempferol contents were successfully screened by GC-FID. Both compounds are found to be highly accumulated in mature organs i.e leaves. Further investigation such as plant tissue culture approach could be applied to optimize flavonoids content *in vitro* plants by manipulating medium composition and hormone concentration.

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