Analysis of quercetin and kaempferol levels in various phase of flowers *Melastoma malabathricum* L.

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Abstract

Melastoma malabathricum L. flowers are part of a plant that contains quercetin and kaempferol. Quercetin and kaempferol are useful for their antioxidant and anticancer properties. The flowering phase affects levels of quercetin and kaempferol. There are four phases of flower blooming: 1st bud phase (K1), 2nd bud phase (K2), 3rd bud phase (K3), and flower bloom (M). It remains to be determined which phase of *Melastoma malabathricum* L. flowering shows the highest levels of quercetin and kaempferol. Quercetin and kaempferol levels were measured using HPLC MS/MS. The measured quercetin levels of K1, K2, K3, and M phases, respectively, were: 19.47 µg/g, 17.78 µg/g, 31.2 µg/g, and 94.32 µg/g. Levels of kaempferol during K1, K2, K3, and M, respectively, were: 140.99 µg/g, 57.28 µg/g, 95.32 µg/g, and 349.37 µg/g.

Introduction

The use of natural ingredients for traditional medicine in Indonesia has been common for centuries. This is evidenced by the old manuscript on palm leaves Husodo (Java), Usada (Bali), Lontarak pabbura (South Sulawesi), documents on Fibre Primbon Charms, Fibre Blend Boreh Wulang nDalem and reliefs of Borobudur depicting people dispensing medicine (herbal medicine) with plants as a raw material.[1]

Melastoma malabathricum L. is one of the plants used as medicine. *M. malabathricum* L. is a plant that grows in the wild. Based on several studies, this plant can be used as an antidiarrheal,[2,3] antioxidant,[4,5] gastroprotective,[6] for burns,[7] antinociception,[8,9] as an antibacterial,[10,11] anticoagulant,[12] antiinflammatory,[9,13] for injuries,[10] as an antivirus,[14] and for cancer.[4,14]

M. malabathricum L. contains saponins, tannins, flavonoids, steroids, and triterpenes.[3,9] Tannins contained in the leaves of *M. malabathricum* include malabathrins A, B C, D, E and F, nobotanin B, D, G, H and J, pterocarinin C, casuarictin, strictinin, and pedunculagin.[15] Flavonoids contained in *M. malabathricum* include α-amyrin,[12,16] quercitrin,[12,16] quercetin,[12,16] patriscabratin,[16] auranamid,[16] kaempferol-3-O-(2",6"-in-Op-trans-kumarol)-β-glucoside,[16] betulinik acid,[12] kaempferol,[16] and narigenin.[16]

Flowers of *M. malabathricum L.* are known to contain quercetin and kaempferol, which exert anticancer and antioxidant effects. Each phase has a different content of quercetin and kaempferol. The anticancer and antioxidant activity of *M. malabathricum L.* depends on the levels of quercetin and kaempferol of *M. malabathricum* L. Unknown levels of quercetin and kaempferol are in the phases of flowers that contain the highest quercetin and kaempferol. This research will examine the levels of quercetin and kaempferol in various phases of *M. malabathricum* L. flowers.

Materials and Methods

Plant material

Purple *M. malabathricum* L. flowers were used. *M. malabathricum* L. plant samples were obtained in the morning before 10:00 am from the area of Mount Kupang Banjarbaru South Kalimantan with 30 28 '39.02 "S and 1140 51' 18.69" E. These plants were identified in the Laboratory of the University Lambung Mangkurat Banjarbaru South Kalimantan, Indonesia. Flowers of *M. malabathricum* L. are divided into four phases, namely bud 1 (K1), where flower buds have no petals on display, bud 2 (K2), where flower buds are starting to show petals, bud 3 (K3), where flower buds have already visible petals that are almost in bloom, and blooms (M), which is a flower that has bloomed completely, as shown in Figure 1. Other materials used were 95% Quercetin (Sigma-Aldrich), 90% kaempferol (Sigma Aldrich), methanol (Merck), acetonitrile (Merck), chloroform (Merck) ethyl acetate (Merck), and aquabidest (PT. Ikhapharmindo Putramas Pharmaceutical Industries).

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Extraction

M. malabathricum L. flowers were dried at room temperature, then mashed. The simplicia obtained was then extracted using the maceration method with 96% ethanol and dried with a dirotary evaporator, followed by freeze-drying.

Determination of quercetin and kaempferol

Quercetin and kaempferol levels were measured by HPLC, using a column with Hypersil Gold specifications (50 mm × 2.1 mm × 1.9 µm). The HPLC Equipment used was ACCELLA artificial type 1250 (Thermo Scientific) consisting of a vacuum degasser, quartenary pump, thermostatically controlled autosampler and a personal computer using the program x-calibur 2.1. Mobile phase A consisted of 0.1% formic acid in aquabidest, while phase B consisted of 0.1% formic acid in Acetonitrile. A linear gradient at a rate of 300 mL/min with mobile-phase settings was used as follows: a) 0-0.6 min 15% B, (b) 2-3.5 min 100% B, (c) 4.5 min 15% B. The injection volume in the LC was 2µL. The column was controlled at 30°C and autosampler compartment was set at 10°C.

Prior to analysis, each calibration standard was infused directly at a rate of 5 mL/min. The MS/MS Triple Q (quadrupole)

mass spectrometer TSQ QUANTUM ACCESS MAX from Thermo Finnigan with an electrospray ionization (ESI) source was controlled by the software TSQ Tune operated in negative mode. Calibration standards 285 kaempferol with precursor ion m/z obtained ion transitions of 239, 229, and 255 m/z, while standardization with quercetin 301 precursor ion m/z obtained ion transitions of 179, 245, and 272 m/z.

Selected reaction monitoring (SRM) was performed using the following settings: 301>179 m/z for quercetin and 285>229 m/z for kaempferol. ESI ionization conditions are as follows: spray voltage 3 kV; evaporation temperature of 270°C; capillary temperature, 300°C; a sheath gas nitrogen pressure of 40 psi; and an Aux gas pressure of 10 psi with argon gas. Chromatogram standards and samples were measured using LC-Quan software; results were then transferred to Excel software.

Results and Discussion

The chromatogram of each sample can be seen in Figures 2-6. The chromatograms show the average concentration of each sample (Table 1). Table 1 shows that the highest levels of quercetin and kaempferol are in phase M, while the lowest levels of quercetin and kaempferol are found in K2.

Quercetin and kaempferol are compounds that act as antioxidants and also as anticancer compounds. The concentrations of quercetin and kaempferol affect its activity. Each phase of *M. malabathricum L.* flowering is characterized by different levels of quercetin and kaempferol. The results of the research show that the highest levels of quercetin and kaempferol are found in blooming flowers. This is in contrast with research by Janna et al. in 2006[17] showing that levels of anthocyanins in *M. malabathricum* L. flowers are the highest in flower buds about to bloom and all petals. The research results on rose flower xhybrida conducted by Schmitzer *et al*.[18] showed that the highest levels of quercetin are found in flower buds.

The anticancer effects of quercetin depend on its concentration. At a concentration of 1–20 µM, quercetin stimulates proliferation, while at concentrations of 50–200 µM, the compound exerts pro-apoptosis effects, reducing the number of living cells.[19] Kaempferol is well known for its cancer-fighting properties and has been linked with the prevention of breast cancer, ovarian cancer and prostate cancer. Kaempferol consumption also appears to reduce the risk of pancreatic and lung cancer.[20] Kaempferol at a concentration of 1-10 µM stimulates proliferation by increasing the synthesis of DNA. At a concentration of 20-90 µM, the compound inhibits cell growth and DNA synthesis.[21] Knowing the concentrations of quercetin and kaempferol allows us to predict their effects. Also, the

Table 1. Average levels of quercetin and kaempferol in various phases of flowering and fruiting.

No	Phase	Average content (µg/g)	
		Quercetin	Kaempferol
1	K1	19.47	140.99
2	K2	17.78	57.28
3	K3	31.2	95.32
4	M	94.32	349.37

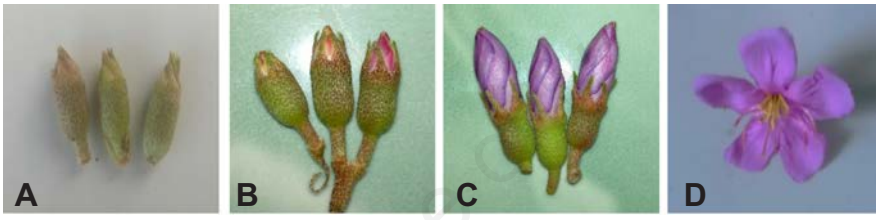

Figure 1. A) Bud 1, B) bud 2, C) bud 3 and D) blooms of *M. malabathricum* L.

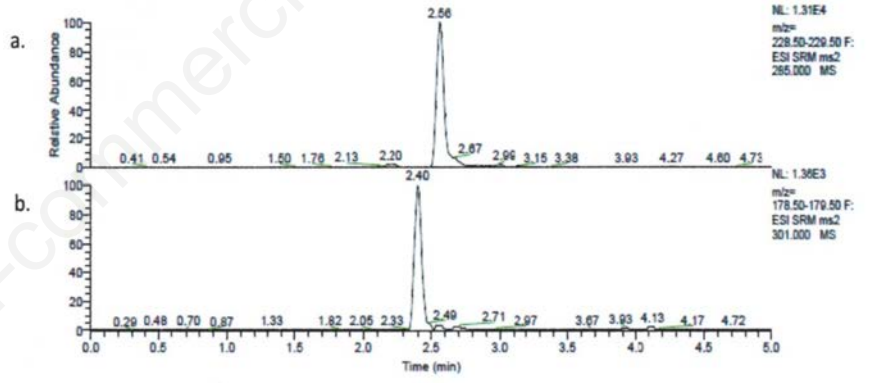

Figure 2. Results of HPLC buds 1 *M. malabathricum* L. (K 1) (a) Quercetin, (b) kaempferol.

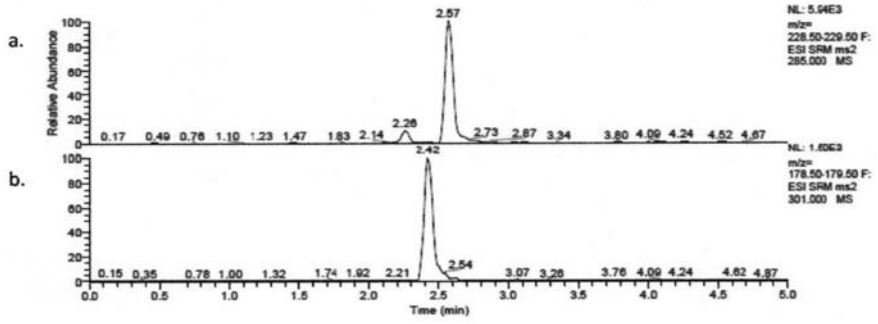

Figure 3. HPLC results for two *M. malabathricum* L. buds (K 2) (a) quercetin, (b) kaempferol.

planning of flower harvest is a very important moment, because the flowering period determines the levels of quercetin and kaempferol.

Conclusions

The highest levels of quercetin and kaempferol are observed during the blossoming phase, with a quercetin concentration of 94.32 µg/g and kaempferol level of 349.37 µg/g.

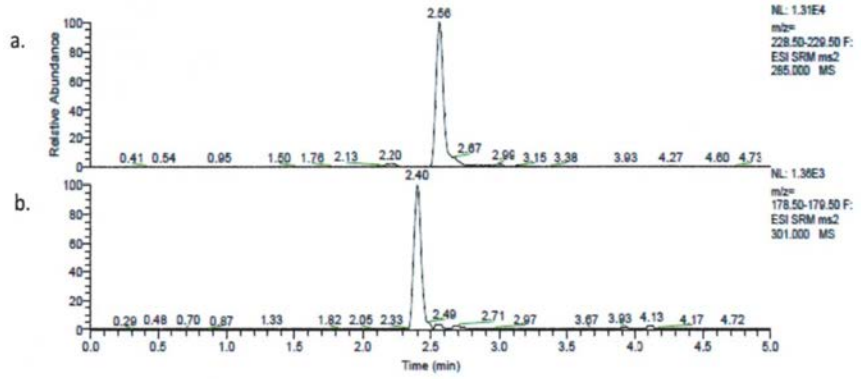

Figure 4. HPLC results for three *M. malabathricum* L. buds (K 3) (a) quercetin, (b) kaempferol.

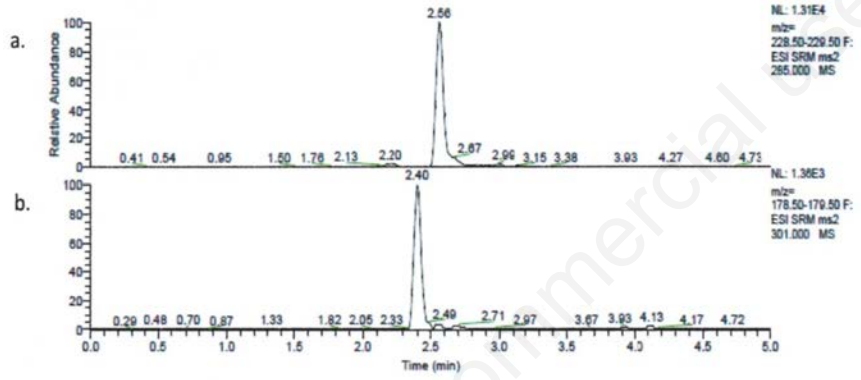

Figure 5. HPLC results for *M. malabathricum* L. flower bloom (M) (a) quercetin, (b) kaempferol.

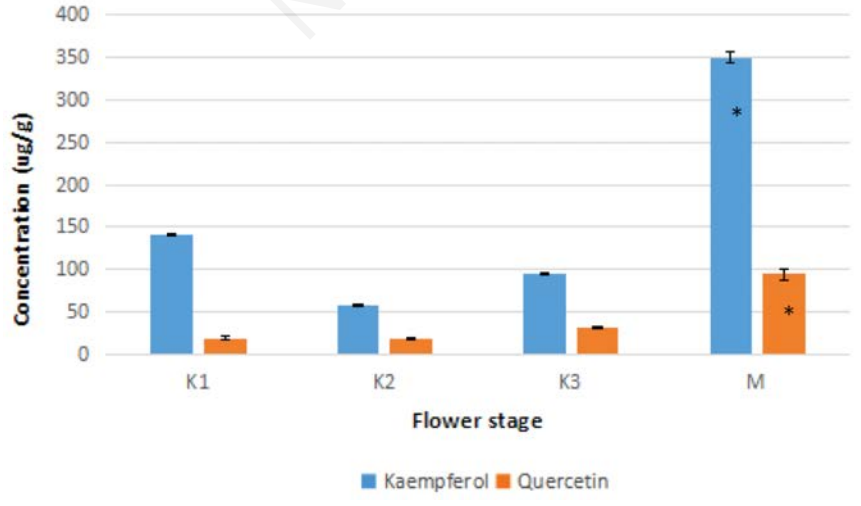

Figure 6. Levels of quercetin and kaempferol in various phases of flowering. *P<0.05

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