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, 17.78 μg/g, 31.2 μg/g, and 94.32 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), Lontarap pabura (South Sulawesi), documents on Fibre Primbon Charms, Fibre Blend Borch 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 anti diarrheal,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, casuaricin, strictinin, and pedunculin.15 Flavonoids contained in *M. malabathricum* include α-amyrin,12,16 quercetin,12,16 quercetin,12,16 patiscabratrin,16 auranamid,16 kaempferol-3-O-(2″,6″-in-Op-trans-kumarol)-β-glucoside,16 betulinic 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 114°51′18.69″E. These plants were identified in the Laboratory of the University Lambung Mangkurat Banjarmasin 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), acetoniitrile (Merck), chloroform (Merck) ethyl acetate (Merck), and aquabidest (PT. Ikphapharmindo Putramas Pharmaceutical Industries).

**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 2 μL. 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 μL/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 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 *Hybrid* conducted by Schmitzer et al. 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 by increasing the synthesis of DNA. At a concentration of 20–90 μM, the compound inhibits cell growth and DNA synthesis. Knowing the concentrations of quercetin and kaempferol allows us to predict their effects. Also, the

<table>
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<th>No</th>
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<th>Quercetin (µg/g)</th>
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</tr>
<tr>
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<td>17.78</td>
<td>57.28</td>
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<td>3</td>
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<td>31.2</td>
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<tr>
<td>4</td>
<td>M</td>
<td>94.32</td>
<td>349.37</td>
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</table>

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.

**References**


