Table 1. Instrument linearity of flavonoid standards measured using HPLC-MWD1

Footnote under the Table

1 Results were obtained using an HPLC-MWD in room temperature at 290 nm for eriodictyol and 340 nm for apigenin and luteolin. Separation was done using a Reverse-Phase ZORBAX Eclipse XDB-C18 column (150 x 4.60 mm i.d., particle size 5.00 µm) The mobile phases were 4% tetrahydrofuran in acetonitrile (mobile phase A) and 0.2% aqueous phosphoric acid (mobile phase B) at a ratio of 35:65 (A:B). The flow rate was 1.0 mL/min for 15 minutes. Measurements were performed on diluted apigenin, luteolin, and eriodictyol standards

Table 2. Method linearity of flavonoid standards spiked in Indonesian *Plectranthus amboinicus* and measured using HPLC-MWD1

Footnote under the Table

1 Results were obtained using an HPLC-MWD in room temperature at 290 nm for eriodictyol and 340 nm for apigenin and luteolin. Separation was done using a Reverse-Phase ZORBAX Eclipse XDB-C18 column (150 x 4.60 mm i.d., particle size 5.00 µm) The mobile phases were 4% tetrahydrofuran in acetonitrile (mobile phase A) and 0.2% aqueous phosphoric acid (mobile phase B) at a ratio of 35:65 (A:B). The flow rate was 1.0 mL/min for 15 minutes. Measurements were performed on 20 µL of hydrolyzed dried leaves extract of Indonesian *Plectranthus amboinicus* spiked with apigenin, luteolin, and eriodictyol prior to hydrolysis

Table 3. Recovery and precision of flavonoid standards in Indonesian *Plectranthus amboinicus* measured using HPLC-MWD.

Footnote under the Table

1 Results were obtained using an HPLC-MWD in room temperature at 290 nm for eriodictyol and 340 nm for apigenin and luteolin. Separation was done using a Reverse-Phase ZORBAX Eclipse XDB-C18 column (150 x 4.60 mm i.d., particle size 5.00 µm) The mobile phases were 4% tetrahydrofuran in acetonitrile (mobile phase A) and 0.2% aqueous phosphoric acid (mobile phase B) at a ratio of 35:65 (A:B). The flow rate was 1.0 mL/min for 15 minutes. Measurements were performed on 20 µL of hydrolyzed dried leaves extract of Indonesian *Plectranthus amboinicus* spiked with 5.0 µg/mL of apigenin, luteolin, and eriodictyol prior to hydrolysis

2 Obtained by the following formula: (SD/mean) x 100%

3 Based on dried sample weight, results expressed as mean ± standard deviation. Measurements were obtained by HPLC analysis on unspiked samples and subsequently multiplied by the correction factors

4 Horwitz standard deviation obtained by the following equation: 21-0.5\*log (C).

Table 4. Flavonoid quantification and bioactivities of *Plectranthus amboinicus* samples.

Footnote under the Table

1 Based on dried sample weight, results expressed as mean and standard deviation of three measurements, values with different letter annotations denote significantly different measurements based on one-way ANOVA (p-value ≤ 0.05). Results were obtained using an HPLC-MWD in room temperature at 290 nm for eriodictyol and 340 nm for apigenin and luteolin. The mobile phases were 4% tetrahydrofuran in acetonitrile (mobile phase A) and 0.2% aqueous phosphoric acid (mobile phase B) at a ratio of 35:65 (A:B). Separation was done using a Reverse-Phase ZORBAX Eclipse XDB-C18 column (150 x 4.60 mm i.d., particle size 5.00 µm). The flow rate was 1.0 mL/min for 15 minutes. Measurements were performed on 20 µL injections. Final sample solution volume prior to injection (10 mL) was obtained from 0.5 mL extracts or equals to 0.05 g dried leaf weight. nd denotes that the analyte was not detected.

2 TFC denotes total flavonoid content, QE denotes quercetin equivalent., results expressed as mean and standard deviation of three measurements, values with different letter annotations denote significantly different measurements based on one-way ANOVA (p-value ≤ 0,05). Results were inferred using the quercetin calibration curve obtained from colorimetry and based on dried sample weight

3 The IC50 denotes the concentration needed to decrease the concentration of DPPH by 50% or inhibit 50% of α-glucosidase (AGI) activity. Results were obtained through colorimetry. Different letter annotations denote significantly different measurements based on one-way ANOVA (p-value ≤ 0,05)

Figure 1. PCA score plot of Indonesian PA (IPA) and Japanese PA (JPA) obtained from comprehensive extraction. Red circle = IPA Blue circle = JPA . Number representing the order of sample collections from the most non-polar to the most polar solvent gradient (Fraction 1-15 = batch 1 extraction, fraction 16-30 = batch 2, fraction 31-45 = batch 3).

Figure 2. A. OPLS-DA Score plot of NMR data of Indonesian PA (IPA) and Japanese PA (JPA). OPLS-DA B. Loading bi - plot NMR data of Indonesian PA (IPA) and Japanese PA (JPA). Blue circle = JPA Red circle = IPA. Number representing the order of sample collections from the most non-polar to the most polar solvent gradient (Fraction 1-15 = batch 1 extraction, fraction 16-30 = batch 2, fraction 31-45 = batch 3).

Figure 3. Representative 1H NMR spectra containing typical signals for eriodyctiol. See text for detail explanation

Figure 4. Apigenin, luteolin, and eriodictyol content in Indonesian *Plectranthus amboinicus* following hydrolysis at various HCl concentrations for 120 minutes at 85°C (A) and various length of hydrolysis but at 4 M of HCl and 85°C (B). Concentration of apigenin, luteolin, and eriodictyol standards following hydrolysis at different HCl concentrations for 120 minutes at 85°C. Recoveries of apigenin, luteolin, and eriodictyol in the sample matrix at various spiking concentrations (D). Measurements were performed using HPLC-MWD at 290 nm for eriodictyol and 340 nm for both apigenin and luteolin.

Figure 5. Chromatograms of apigenin (1), eriodictyol (2), and luteolin standards (3) at a concentration of 5 µg/mL solvent measured using HPLC-MWD at 290 and 340 nm (A). Indonesian *Plectranthus amboinicus* leaves spiked with 5 µg/mL solvent of apigenin, eriodictyol and luteolin and measured at 290 340 nm (B). Unspiked Indonesian sample leaves measured at 290 and 340 nm (C), and unspiked Japanese sample leaves measured at 290 and 340 nm (D).