**Contents of flavonoid compounds in** ***Dendrobium officinale* Kimura et Migo determined by** **QuEChERS-HPLC-MS/MS: method validation and influencing factors**

**In total: 9 pages**

**Tables: 5**

**Figures: 1**

**Table S1 Sample informations of 47 *Dendrobium officinale* samples**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **No.** | **Origin** | **Cultivation method** | **Years of growth** | **Processing** | **Collecting time** |
| S1 | Jinhua,Zhejiang (ZJ- Jinhua) | Imitative wild cultivation | 2 years and less | Dry stems | Collecting in harvest period |
| S2 | Jinhua,Zhejiang (ZJ- Jinhua) | Imitative wild cultivation | More than 2 years | Dry stems | Collecting in harvest period |
| S3 | Jinhua,Zhejiang (ZJ- Jinhua) | Imitative wild cultivation | More than 2 years | Dry stems | Collecting in harvest period |
| S4 | Jinhua,Zhejiang (ZJ- Jinhua) | Imitative wild cultivation | More than 2 years | Dry stems | Collecting in harvest period |
| S5 | Jinhua,Zhejiang (ZJ- Jinhua) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in harvest period |
| S6 | Jinhua,Zhejiang (ZJ- Jinhua) | Facility-aided cultivation | More than 2 years | Dry stems | Collecting in harvest period |
| S7 | Jinhua,Zhejiang (ZJ- Jinhua) | Facility-aided cultivation | More than 2 years | Dry stems | Collecting in harvest period |
| S8 | Jinhua,Zhejiang (ZJ- Jinhua) | Facility-aided cultivation | More than 2 years | Dry stems | Collecting in harvest period |
| S9 | Lishui, Zhejiang (ZJ-Lishui) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in non-harvest period |
| S10 | Lishui, Zhejiang (ZJ-Lishui) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in non-harvest period |
| S11 | Lishui, Zhejiang (ZJ-Lishui) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in non-harvest period |
| S12 | Lishui, Zhejiang (ZJ-Lishui) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in non-harvest period |
| S13 | Lishui, Zhejiang (ZJ-Lishui) | Imitative wild cultivation | 2 years and less | Dry stems | Collecting in non-harvest period |
| S14 | Lishui, Zhejiang (ZJ-Lishui) | Facility-aided cultivation | More than 2 years | Dry stems | Collecting in non-harvest period |
| S15 | Lishui, Zhejiang (ZJ-Lishui) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in non-harvest period |
| S16 | Lishui, Zhejiang (ZJ-Lishui) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in non-harvest period |
| S17 | Lishui, Zhejiang (ZJ-Lishui) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in non-harvest period |
| S18 | Lishui, Zhejiang (ZJ-Lishui) | Imitative wild cultivation | More than 2 years | Dry stems | Collecting in non-harvest period |
| S19 | Jinhua,Zhejiang (ZJ- Jinhua) | Facility-aided cultivation | 2 years and less | Slices | Collecting in harvest period |
| S20 | Jinhua,Zhejiang (ZJ- Jinhua) | Imitative wild cultivation | 2 years and less | Slices | Collecting in harvest period |
| S21 | Jinhua,Zhejiang (ZJ- Jinhua) | Imitative wild cultivation | 2 years and less | Dry stems | Collecting in harvest period |
| S22 | Jinhua,Zhejiang (ZJ- Jinhua) | Imitative wild cultivation | More than 2 years | Dry stems | Collecting in harvest period |
| S23 | Jinhua,Zhejiang (ZJ- Jinhua) | Facility-aided cultivation | 2 years and less | Fengdou | Collecting in harvest period |
| S24 | Jinhua,Zhejiang (ZJ- Jinhua) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in harvest period |
| S25 | Mangshi,Yunnan (YN-Mangshi) | Imitative wild cultivation | 2 years and less | Dry stems | Collecting in harvest period |
| S26 | Mangshi, Yunnan (YN-Mangshi) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in harvest period |
| S27 | Mangshi, Yunnan (YN-Mangshi) | Facility-aided cultivation | 2 years and less | Fengdou | Collecting in harvest period |
| S28 | Mangshi, Yunnan (YN-Mangshi) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in harvest period |
| S29 | Mangshi, Yunnan (YN-Mangshi) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in harvest period |
| S30 | Jinhua,Zhejiang (ZJ- Jinhua) | Imitative wild cultivation | More than 2 years | Dry stems | Collecting in harvest period |
| S31 | Jinhua,Zhejiang (ZJ- Jinhua) | Imitative wild cultivation | 2 years and less | Fengdou | Collecting in harvest period |
| S32 | Jinhua,Zhejiang (ZJ- Jinhua) | Imitative wild cultivation | 2 years and less | Fengdou | Collecting in harvest period |
| S33 | Wenzhou,Zhejiang (ZJ-Wenzhou) | Imitative wild cultivation | 2 years and less | Slices | Collecting in harvest period |
| S34 | Wenzhou,Zhejiang (ZJ-Wenzhou) | Facility-aided cultivation | 2 years and less | Fengdou | Collecting in harvest period |
| S35 | Taizhou,Zhejiang (ZJ-Taizhou) | Imitative wild cultivation | 2 years and less | Dry stems | Collecting in harvest period |
| S36 | Quzhou,Zhejiang (ZJ-Quzhou) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in harvest period |
| S37 | Hechi, Guangxi (GX-Hechi) | Imitative wild cultivation | 2 years and less | Fengdou | Collecting in harvest period |
| S38 | Hechi, Guangxi (GX-Hechi) | Imitative wild cultivation | More than 2 years | Fengdou | Collecting in harvest period |
| S39 | Huoshan, Anhui (AH-Huoshan) | Imitative wild cultivation | 2 years and less | Fengdou | Collecting in harvest period |
| S40 | Huoshan, Anhui (AH-Huoshan) | Imitative wild cultivation | 2 years and less | Dry stems | Collecting in harvest period |
| S41 | Quzhou,Zhejiang (ZJ-Quzhou) | Imitative wild cultivation | 2 years and less | Dry stems | Collecting in non-harvest period |
| S42 | Quzhou,Zhejiang (ZJ-Quzhou) | Imitative wild cultivation | 2 years and less | Dry stems | Collecting in non-harvest period |
| S43 | Quzhou,Zhejiang (ZJ-Quzhou) | Imitative wild cultivation | 2 years and less | Dry stems | Collecting in non-harvest period |
| S44 | Taizhou,Zhejiang (ZJ-Taizhou) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in non-harvest period |
| S45 | Taizhou,Zhejiang (ZJ-Taizhou) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in non-harvest period |
| S46 | Taizhou,Zhejiang (ZJ-Taizhou) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in non-harvest period |
| S47 | Taizhou,Zhejiang (ZJ-Taizhou) | Facility-aided cultivation | 2 years and less | Dry stems | Collecting in non-harvest period |

**Table S2 MS/MS parameters for each target flavonoid (Ionization mode, ESI﹣)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **flavonoids** | **Retention Time (min)** | **Parent ion (m/z)** | **Daughter ion (m/z)** | **Q1 Deflection voltage/V** | **Collision Energy (eV)** | **Q2 Deflection voltage/V** |
| 1 | Naringenin | 4.36 | 271.1 | 151.2\* | 14.0 | 18.0 | 10.0 |
| 119.2 | 18.0 | 25.0 | 24.0 |
| 2 | Quercetin | 4.35 | 301.1 | 151.1\* | 23.0 | 23.0 | 29.0 |
| 179.1 | 22.0 | 17.0 | 19.0 |
| 3 | Rutin | 3.95 | 609.0 | 300.05\* | 22.0 | 40.0 | 23.0 |
| 271.0 | 22.0 | 54.0 | 12.0 |
| 4 | Isorhamnetin | 4.52 | 315.1 | 300.1\* | 12.0 | 23.0 | 14.0 |
| 151.0 | 23.0 | 28.0 | 16.0 |
| 5 | Apigenin | 4.54 | 269.1 | 117.1\* | 10.0 | 34.0 | 23.0 |
| 151.05 | 20.0 | 24.0 | 15.0 |
| 6 | Eriodictyol | 4.19 | 287.1 | 150.9\* | 11.0 | 17.0 | 25.0 |
| 134.95 | 15.0 | 14.0 | 21.0 |
| 7 | Chrysoeriol | 4.56 | 299.1 | 284.05\* | 11.0 | 21.0 | 13.0 |
| 256.05 | 23.0 | 28.0 | 26.0 |
| 8 | Hesperetin | 4.39 | 301.1 | 164.05\* | 11.0 | 24.0 | 17.0 |
| 286.0 | 22.0 | 18.0 | 22.0 |
| 9 | Isoquercitrin | 3.97 | 463.1 | 300.05\* | 24.0 | 30.0 | 10.0 |
| 271.05 | 11.0 | 45.0 | 19.0 |
| 10 | Quercitrin | 4.10 | 447.1 | 300.05\* | 16.0 | 29.0 | 10.0 |
| 301.1 | 17.0 | 22.0 | 21.0 |
| 11 | Luteoloside | 3.92 | 447.1 | 285.0\* | 13.0 | 29.0 | 13.0 |
| 283.85 | 17.0 | 38.0 | 27.0 |
| 12 | Kaempferol | 4.52 | 285.1 | 187.0\* | 11.0 | 26.0 | 10.0 |
| 117.0 | 19.0 | 38.0 | 24.0 |
| 13 | Schaftoside | 3.79 | 563.1 | 353.05\* | 20.0 | 37.0 | 12.0 |
| 383.1 | 20.0 | 36.0 | 18.0 |
| 14 | Apiin | 3.99 | 563.1 | 269.05\* | 22.0 | 32.0 | 13.0 |
| 431.1 | 20.0 | 28.0 | 21.0 |
| 15 | Hyperoside | 3.97 | 463.2 | 300.1\* | 17.0 | 29.0 | 10.0 |
| 270.9 | 17.0 | 46.0 | 18.0 |
| 16 | Naringin | 3.92 | 579.2 | 271.1\* | 22.0 | 34.0 | 19.0 |
| 151.0 | 22.0 | 45.0 | 29.0 |
| 17 | Taxifolin | 3.87 | 303.1 | 285.05\* | 11.0 | 12.0 | 13.0 |
| 125.1 | 22.0 | 20.0 | 22.0 |

\*: Quantitative ion

**Table S3 Physicochemical properties of flavonoids**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **No.** | **Flavonoids** | **Molecular formula** | **Molecular weight** | **pKa** | **LogP** |
| 1 | Naringenin | C15H12O5 | 272.25 | 7.52±0.40 | 3.19 |
| 2 | Quercetin | C15H10O7 | 302.24 | 6.31±0.40 | 2.08 |
| 3 | Rutin | C27H30O16 | 610.52 | 6.17±0.40 | 1.76 |
| 4 | Isorhamnetin | C16H12O7 | 316.26 | 6.31±0.40 | 1.76 |
| 5 | Apigenin | C15H10O5 | 270.24 | 6.53±0.40 | 2.10 |
| 6 | Eriodictyol | C15H12O6 | 288.25 | 7.49±0.40 | 2.59 |
| 7 | Chrysoeriol | C16H12O6 | 300.26 | 6.49±0.40 | 1.81 |
| 8 | Hesperetin | C16H14O6 | 302.28 | 7.49±0.40 | 2.90 |
| 9 | Isoquercitrin | C21H20O12 | 464.38 | 6.17±0.40 | 1.75 |
| 10 | Quercitrin | C21H20O11 | 448.38 | 6.17±0.40 | 2.17 |
| 11 | Luteoloside | C21H20O11 | 448.38 | 6.10±0.40 | -0.09 |
| 12 | Kaempferol | C15H10O6 | 286.24 | 6.34±0.40 | 2.05 |
| 13 | Schaftoside | C26H28O14 | 564.49 | 5.70±0.40 | -3.06 |
| 14 | Apiin | C26H28O14 | 564.49 | 6.11±0.40 | 0.74 |
| 15 | Hyperoside | C21H20O12 | 464.38 | 6.17±0.40 | 1.75 |
| 16 | Naringin | C27H32O14 | 580.53 | 7.17±0.40 | -0.18 |
| 17 | Taxifolin | C15H12O7 | 304.25 | 7.39±0.60 | 1.82 |

The data is from chemical book website and chemsrc website.

https://www.chemicalbook.com/ProductIndex.aspx, accessed on 03.11, 2022

https://www.chemsrc.com/en/casindex/, accessed on 03.11, 2022

**Table S4 Antioxidant activities of** ***D. officinale* samples**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample** | **DPPH· clearance (%)** | **ABTS+· clearance (%)** | **FRAP/(mg Trolox/100gDW)** |
| S1 | 73.81 | 51.15 | 649.31 |
| S2 | 81.72 | 63.42 | 872.92 |
| S3 | 85.45 | 75.89 | 1093.90 |
| S4 | 84.98 | 96.02 | 1523.41 |
| S5 | 69.27 | 39.62 | 512.92 |
| S6 | 65.31 | 42.35 | 520.13 |
| S7 | 74.04 | 53.56 | 660.46 |
| S8 | 86.03 | 74.11 | 990.95 |
| S9 | 65.77 | 39.94 | 483.41 |
| S10 | 62.05 | 38.05 | 476.20 |
| S11 | 57.39 | 36.37 | 446.69 |
| S12 | 67.87 | 39.73 | 502.43 |
| S13 | 73.34 | 42.87 | 469.64 |
| S14 | 89.06 | 62.47 | 823.74 |
| S15 | 89.64 | 78.83 | 1125.38 |
| S16 | 50.99 | 34.70 | 423.74 |
| S17 | 53.08 | 37.53 | 453.90 |
| S18 | 69.03 | 45.91 | 529.97 |
| S19 | 55.06 | 34.59 | 435.54 |
| S20 | 86.85 | 88.89 | 1285.38 |
| S21 | 65.42 | 39.62 | 548.33 |
| S22 | 71.83 | 42.77 | 531.28 |
| S23 | 56.46 | 37.21 | 436.85 |
| S24 | 81.96 | 54.09 | 726.69 |
| S25 | 88.36 | 60.38 | 693.25 |
| S26 | 75.44 | 45.28 | 579.15 |
| S27 | 49.48 | 28.41 | 383.08 |
| S28 | 43.31 | 23.06 | 316.20 |
| S29 | 35.62 | 16.77 | 277.51 |
| S30 | 78.11 | 48.32 | 646.69 |
| S31 | 87.31 | 56.60 | 780.46 |
| S32 | 78.46 | 45.70 | 660.46 |
| S33 | 75.90 | 44.03 | 582.43 |
| S34 | 54.02 | 27.99 | 436.85 |
| S35 | 86.73 | 82.91 | 1211.93 |
| S36 | 65.08 | 34.49 | 467.02 |
| S37 | 86.73 | 77.78 | 1191.61 |
| S38 | 84.52 | 90.67 | 1382.43 |
| S39 | 86.38 | 63.31 | 936.52 |
| S40 | 85.56 | 72.54 | 1084.07 |
| S41 | 84.63 | 46.44 | 709.64 |
| S42 | 89.52 | 63.73 | 1051.93 |
| S43 | 89.52 | 62.58 | 981.11 |
| S44 | 45.98 | 24.42 | 395.54 |
| S45 | 84.63 | 61.74 | 813.90 |
| S46 | 72.88 | 41.09 | 652.59 |
| S47 | 68.57 | 40.25 | 567.34 |

**Table S5 Relationship between flavonoid contents and antioxidant capacities of *D. officinale***

|  |  |  |  |
| --- | --- | --- | --- |
|  | DPPH | ABTS | FRAP |
| Total flavonoid content | 0.783, *p*<0.05 | 0.894, *p*<0.05 | 0.848, *p*<0.05 |
| Naringenin | 0.521, *p*<0.05 | 0.609, *p*<0.05 | 0.615, *p*<0.05 |
| Quercetin | 0.854, *p*<0.05 | 0.882, *p*<0.05 | 0.871, *p*<0.05 |
| Rutin | *p*>0.05 | *p*>0.05 | *p*>0.05 |
| Isorhamnetin | 0.855, *p*<0.05 | 0.900, *p*<0.05 | 0.873, *p*<0.05 |
| Apigenin | 0.711, *p*<0.05 | 0.849, *p*<0.05 | 0.800, *p*<0.05 |
| Eriodictyol | 0.817, *p*<0.05 | 0.882, *p*<0.05 | 0.837, *p*<0.05 |
| Chrysoeriol | 0.647, *p*<0.05 | 0.772, *p*<0.05 | 0.749, *p*<0.05 |
| Hesperetin | 0.813, *p*<0.05 | 0.886, *p*<0.05 | 0.877, *p*<0.05 |
| Isoquercitrin | 0.305, *p*<0.05 | *p*>0.05 | *p*>0.05 |
| Quercitrin | *p*>0.05 | *p*>0.05 | *p*>0.05 |
| Luteoloside | *p*>0.05 | *p*>0.05 | 0.303, *p*<0.05 |
| Kaempferol | 0.633, *p*<0.05 | 0.730, *p*<0.05 | 0.704, *p*<0.05 |
| Schaftoside | 0.323, *p*<0.05 | 0.451, *p*<0.05 | 0.477, *p*<0.05 |
| Apiin | 0.570, *p*<0.05 | 0.679, *p*<0.05 | 0.707, *p*<0.05 |
| Hyperoside | 0.306, *p*<0.05 | *p*>0.05 | *p*>0.05 |
| Naringin | 0.423, *p*<0.05 | 0.509, *p*<0.05 | 0.517, *p*<0.05 |
| Taxifolin | 0.744, *p*<0.05 | 0.778, *p*<0.05 | 0.742, *p*<0.05 |

Spearman correlation was applied to determine the relationship.

****

**Figure S1** The color of the extract treated by various purification treatments. A: No purification, B: PSA 75 mg, C: PSA 50 mg, D: PSA 25 mg, E: C18 75 mg, F: C18 50 mg, G: C18 25 mg, H: g-MWCNTs 5 mg, I: g-MWCNTs 2.5 mg, J: MWCNTs-COOH 5 mg, K: MWCNTs-COOH 2.5 mg, L: MWCNTs-OH 5 mg, and M: MWCNTs-OH 2.5 mg.

**Specific methods for determining antioxidant activity**

1. DPPH· scavenging assay

1 mL extract was added into 5 mL DPPH solution (0.1 mmol/L) for dark reaction at room temperature for 30 min. Absorbance was measured at 517 nm by UV spectrophotometer, which was recorded as A1. The blank (replaced the sample solution with 1 mL of distilled water) absorbance was also measured at the same time, which was recorded as A0.The DPPH clearance was determined using the equation: DPPH· clearance (%) = (A0-A1)/A0× 100% (Zhang et al. 2017).

2. ABTS+·scavenging assay

A volume of 7 mM ABTS+· solution and 2.45 mM potassium persulphate were mixed thoroughly and kept in the dark for 16 h at room temperature. Then, the mixture was diluted with phosphate-buffered saline (pH 7.0) to an absorbance of 0.70 ± 0.02 at 734 nm before use. The sample extract (0.15 mL) was added to 6 mL of ABTS+· solution and mixed vigorously. The reaction mixture was allowed to stand at room temperature for 6 min, and the absorbance at 734 nm was recorded as A1, and blank (water instead of the sample) was also measured, which was recorded as A0. The ABTS+· clearance was determined using the equation: ABTS+· clearance (%) = (A0-A1)/A0× 100% (Chen et al. 2019).

3. FRAP assay

The FRAP reagent was freshly prepared by mixing acetate buffer (0.3 mol/L, pH 3.6), a solution of 10 mmol/L TPTZ in 40 mmol/L HCl, and 20 mmol/L FeCl3 at 10:1:1 (v/v/v). Added 0.2 mL of sample solution to 5 mL FRAP reagent and mixed thoroughly. After reaction at 37 ℃ for 30 minutes, the absorbance was measured at 593 nm. All solutions were used on the day of preparation. Standard curve was prepared using the different Trolox concentrations and results were expressed in terms of mg Trolox/100 g dry weight (DW) (Jin et al. 2016).

**Reference**

Jin, L., Li, X.B., Tian, D.Q., Fang, X.P., Yu, Y.M., Zhu, H.Q., Ge, Y.Y., Ma, G.Y., Wang, W.Y., Xiao, W.F., Li, M., 2016. Antioxidant properties and color parameters of herbal teas in China. Ind. Crop Prod. 87, 198-209. https://doi.org/10.1016/j.indcrop.2016.04.044.

Meng, Q., Chen, F., Xiao, T., Zhang, L., 2019. Superfine grinding of *Dendrobium officinale*: the finer the better? Int. J. Food Sci. Tech. 54(6), 2199-2208. https://doi.org/10.1111/ijfs.14129.

Zhang, Y., Zhang, L., Liu, J., Liang, J., Si, J., Wu, S., 2017. *Dendrobium officinale* leaves as a new antioxidant source. J. Func. Food. 37, 400-415. https://doi.org/10.1016/j.jff.2017.08.006.