**Analysis of Kava (*Piper methysticum*) Extract Using High Resolution UHPLC-MS and UHPLC-MS/MS**

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Figure 1. Total ion chromatogram of kava extract (10 µg/mL) analyzed on a Shimadzu 9030 UHPLC-Q-ToF mass spectrometer (resolving power 30,000) with positive ion electrospray. A 22-min gradient from 5% to 95% methanol containing formic acid (0.1%) in water (also containing 0.1% formic acid) was used for the UHPLC separation with a Waters Cortecs C18 (2.1 × 150 mm, 1.7 µm) column.

**Table 1.** Proposed and confirmed components corresponding to the peaks in the UHPLC-HRMS chromatogram in Figure 1 of Kava (*Piper methysticum*) extract.

| **Peak number** | **Retention time****(min)** | ***m/z*****[M+H]+** | **ΔM****(ppm error)** | **Proposed ID****Molecular Formula****(CAS)****Confidence**3 | **Chemical Structure** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- |
| 1 | 9.53 | 259.0882 | 7.7 | d4-DaidzeinC15H6D4O4486-66-8Reference standard |  | HRMS supports molecular formula.MS/MS supports structure via reference standard.Retention time matches that of reference standard. D4-daidzein was included as an injection standard |
| 2 | 10.97 | 279.1200 | 9.4 | 11-Hydroxy-12-methoxydihydrokavainC15H18O5-Tentative |  | HRMS supports molecular formula. MS/MS supports structure where the major fragment ions are also observed in other kavalactones. This structure is reported ion the literature [1]. |
| 3 | 11.53 | 201.2392 | - | Unknown | - | The observed peak is a background noise that was also observed in the solvent. When the chromatogram was aligned with the XCMS online, the fold difference between the peak in the extract and the blank was insignificant |
| 4 | 12.35 | 293.1364 | 6.6 | 11,12-DimethoxydihydrokavainC16H20O5-Partial  |  | The HRMS support molecular formulae. The structure is partially supported by the MS/MS via manual interpretation. It maybe another kavalactone that is not reported in the literature. There is no literature data for 11,12-Dimethoxydihydrokavain |
| 5a | 13.59 | 289.1050 | 7.1 | 10-methoxyyangoninC16H16O577900-32-4Partial |  | The HRMS support molecular formulae. The structure is partially supported by the MS/MS via manual interpretation. It maybe another kavalactone that is not reported in the literature. There is no literature data |
| 5b | 13.67 | 275.0895 | 6.9 | MethysticinC15H14O5495-85-2Reference standard |  | HRMS supports molecular formula.MS/MS supports structure via reference standard.Retention time matches that of reference standard |
| 6 | 13.79 | 277.1052 | 6.8 | DihydromethylsticinC15H16O519902-91-1Reference standard |  | HRMS supports molecular formula.MS/MS supports structure via reference standard.Retention time matches that of reference standard |
| 7a | 14.32 | 263.1260 | 6.7 | 5,6,7,8-TetrahydroyangoninC15H18O4-Matched |  | HRMS supports molecular formula. MS/MS supports structure via manual interpretation where the major fragment ions are also observed in other kavalactones. The MS/MS matches that reported in the literature. [2]. |
| 7b | 14.48 | 231.1001 | 6.5 | KavainC14H14O3500-64-1Reference standard |  | HRMS supports molecular formula.MS/MS supports structure via reference standard.Retention time matches that of reference standard |
| 8 | 14.86 | 233.1159 | 5.6 | 7,8-DihydrokavainC14H16O3587-63-3Reference standard |  | HRMS supports molecular formula.MS/MS supports structure via reference standard.Retention time matches that of reference standard |
| 9a | 15.33 | 259.0947 | 6.9 | YangoninC15H14O4500-62-9Reference standard |  | HRMS supports molecular formula.MS/MS supports structure via reference standard.Retention time matches that of reference standard |
| 9b | 15.33 | 262.1135 | 6.8 | Yangonin-d3C15H11D3O4500-62-9Reference standard |  | HRMS supports molecular formula.MS/MS supports structure via reference standard.Retention time matches that of reference standard. This compound was used as an internal standard. |
| 10a | 16.35 | 229.0844 | 6.5 | DesmethoxyyangoninC14H12O315345-89-8Reference standard |  | HRMS supports molecular formula.MS/MS supports structure via reference standard.Retention time matches that of reference standard |
| 10b | 16.61 | 315.1205 | 6.9 | Flavokavain AC18H18O53420-72-2Reference standard |  | HRMS supports molecular formula.MS/MS supports structure via reference standard.Retention time matches that of reference standard |
| 11 | 17.02 | 285.1101 | 7.0 | Flavokavain BC17H16O41775-97-9Reference standard |  | HRMS supports molecular formula.MS/MS supports structure via reference standard.Retention time matches that of reference standard |
| 12 | 19.49 | 393.2832 |  | unknown | - | The observed peak is a background noise that was also observed in the solvent. When the chromatogram was aligned with the XCMS online, the fold difference between the peak in the extract and the blank was insignificant |
| 13 | 20.76 | 270.3142 |  | unknown | - | The observed peak is a background noise that was also observed in the solvent. When the chromatogram was aligned with the XCMS online, the fold difference between the peak in the extract and the blank was insignificant |

[1] He X-G, Lin L-Z, Lian L-Z. Electrospray high performance liquid chromatography-mass spectrometry in phytochemical analysis of kava (*Piper methysticum*) extract. *Planta Medica.* **1997**; *63*: 70.

[2] Tang Y, Fields CA. UHPLC-UV method development and validation for determining kavalactones and flavokavains in *Piper methysticum* (Kava). *Molecules.* **2019**; *24*: 1245.

Appendix





Figure 2. Positive ion electrospray HRMS (top panel) and MS/MS of peak 1 with *m*/*z* 259.0882 eluting at 9.53 min





Figure 3. Positive ion electrospray HRMS (top panel) and MS/MS of peak 2 with *m*/*z* 279.1200 eluting at 10.97 min





Figure 4. Positive ion electrospray HRMS (top panel) and MS/MS of peak 3 with *m*/*z* 201.2392 eluting at 11.53 min





Figure 5. Positive ion electrospray HRMS (top panel) and MS/MS of peak 4 with *m*/*z* 293.1364 eluting at 12.35 min





Figure 6. Positive ion electrospray HRMS (top panel) and MS/MS of peak 5a with *m*/*z* 289.1050 eluting at 13.59 min





Figure 7. Positive ion electrospray HRMS (top panel) and MS/MS of peak 5b with *m*/*z* 275.0895 eluting at 13.67 min





Figure 8. Positive ion electrospray HRMS (top panel) and MS/MS of peak 6 with *m*/*z* 277.10523 eluting at 13.79 min





Figure 9. Positive ion electrospray HRMS (top panel) and MS/MS of peak 7a with *m*/*z* 263.1260 eluting at 14.32 min





Figure 10. Positive ion electrospray HRMS (top panel) and MS/MS of peak 7b with *m*/*z* 231.1001 eluting at 14.48 min





Figure 11. Positive ion electrospray HRMS (top panel) and MS/MS of peak 8 with *m*/*z* 233.1159 eluting at 14.86 min





Figure 12. Positive ion electrospray HRMS (top panel) and MS/MS of peak 9a with *m*/*z* 259.0947 eluting at 15.33 min





Figure 13. Positive ion electrospray HRMS (top panel) and MS/MS of peak 9b with *m*/*z* 262.1135 eluting at 15.33 min





Figure 14. Positive ion electrospray HRMS (top panel) and MS/MS of peak 10a with *m*/*z* 229.0844 eluting at 16.35 min





Figure 15. Positive ion electrospray HRMS (top panel) and MS/MS of peak 10b with *m*/*z* 315.1205 eluting at 16.61 min





Figure 16. Positive ion electrospray HRMS (top panel) and MS/MS of peak 11 with *m*/*z* 285.1101 eluting at 17.02 min





Figure 17. Positive ion electrospray HRMS (top panel) and MS/MS of peak 12 with *m*/*z* 393.2832 eluting at 19.49 min





Figure 18. Positive ion electrospray HRMS (top panel) and MS/MS of peak 13 with *m*/*z* 270.3142 eluting at 20.76 min