Piper methysticum Root and Rhizome

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DEFINITION
The article consists of the dried root and rhizome of Piper methysticum G. Forst. (Family Piperaceae). It contains NLT 4.5% of kavalactones, calculated as the sum of methysticin, dihydromethysticin, kawain [1], dihydrokawain, yangonin, and desmethoxyyangonin on the dried basis.

SYNONYMS
None known

POTENTIAL CONFOUNDBING MATERIALS
Piper wichmannii C.DC.
Piper methysticum var. wichmannii (C.DC.) Lebot.

SELECTED COMMON NAMES
English: Kava, kava-kava, kava pepper
Fijian: Yaqona
French: Ava, kawa-kawa
German: Kawapfeffer
Spanish: Kavaka

CONSTITUENTS OF INTEREST
Kavalactones: Kawain [1](kavain), dihydrokawain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin
Chalconoids: Flavokawain A-C

IDENTIFICATION

A. Botanical Characteristics

Macroscopic: Irregular longitudinal and transverse cut pieces of various shapes and thicknesses. The rhizome is 3-20 cm long and 1-10 cm thick. The outer surface of the peeled rhizome is whitish or pale grayish-brown; the inner surface is white to yellowish-white with light and dark brown areas. The fracture is coarsely fibrous and starchy. In a transverse section, the lighter colored sunken pith, surrounded by a radiate xylem crossed by fans of rays, is visible; in some cases, a thin layer of cork may be present. In older rhizomes numerous splits and cavities, resulting from the loss of parenchyma from the central region, are visible. The unpeeled rhizome has a gray to grayish-brown, longitudinally wrinkled outer layer of cork with circular root scars. If not removed, it has a fringe of long filiform lateral roots at the end. The roots are often intertwined in a braid-like formation. The roots are rich in starch.

Microscopic: The entire medullary parenchyma, the medullary rays, and the central cortex are very rich in starch; the parenchyma also contains a fine-grained brown material and some resinous masses. The starch granules are spherical to slightly ovoid, 10-30 µm in diameter, with a central hilum in the form of a cleft or deep split. The primary cortex contains strips of collenchymas, and frequently contains oleoresin excretory cells with greenish-yellow, resinous contents, and contains stone cells covering the phloem. The stone cells have uniform, non-pitted wall thickenings, and are approximately polygonal in shape, with a diameter of about 50 µm. The unpeeled rhizome has a thin-walled cork layer. The center of a transverse section of the rhizome contains primary xylem elements in narrow strips formed mainly by tracheid-like elements, and secondary xylem elements with strands of lignified vascular tissue that extend evenly around the medulla and alternate with broad secondary medullary rays. The secondary vascular elements consist of large xylem vessels with scalariform thickening, groups of thicker-walled fibers with bluntly narrowing ends, and abundant parenchymatous cells with moderately-thickened walls and numerous pits. Additional vascular bundles are scattered throughout the medulla. The phloem contains extremely delicate, thin-walled cells. A transverse section through the radicles occasionally reveals a macrocellular, slightly suberose primary cortex composed of thin-walled polygonal cells; a narrow strip of secondary cortex clearly separated from the primary cortex by a dark brown endodermis; broad medullary rays; and a macrocellular, parenchymatous medulla. There are no calcium oxalate crystals present.

B. Thin-Layer Chromatography
Content of Kavalactones

Assay

**Sample solution:** Sonicate about 1.0 g of *Piper methysticum* Root and Rhizome, finely powdered, in 5 mL of methanol for 10 min, centrifuge, and use the supernatant.

**Chromatographic system**
(See Chromatography <621>, Thin-Layer Chromatography.)

- **Adsorbent:** Use a suitable chromatographic material with an average particle size of 5 μm (HPTLC plates, Si 60 F<sub>254</sub>).
- **Reagent for impregnation:** Dissolve 8 g of caffeine in 200 mL of dichloromethane.
- **Application volume:** 2 μL, as 8-mm bands
- **Relative humidity:** Condition the plate to a relative humidity of about 33% using a suitable device.
- **Developing solvent system:** tert-Butyl ether and cyclohexane (70:30)
- **Developing distance:** 6 cm
- **Derivatization reagent:** Anisaldehyde reagent created by combining 170 mL of ice-cooled methanol mixed with 20 mL of glacial acetic acid, 10 mL of sulfuric acid, and 1 mL of anisaldehyde.

**Analysis**

- **Samples:** Standard solution A, Standard solution B, and Sample solution

Dip a suitable HPTLC plate in the Reagent for impregnation. Dry at room temperature for 5 min, then heat at 80° for 5 min. Apply the Samples as bands to the plate. Develop the chromatogram in an unsaturated chamber, remove the plate from the chamber, and dry. Treat with Derivatization reagent, heat at 100° for 4 min, and examine under UV light at 366 nm and visible light.

**System suitability:** Under UV light at 366 nm, the chromatogram of Standard solution B exhibits, in the lower half: an orange band corresponding in R<sub>f</sub> and color to the band of kawain in Standard solution A; below the kawain band, with decreasing R<sub>f</sub>, a bright blue fluorescent band and a yellow/orange band due to yangonin and methysticin, respectively; above the kawain band, a pale blue band corresponding to desmethoxyyangonin; one yellowish-green band above the desmethoxyyangonin band; and one bright blue band below the methysticin band. In the upper half of the chromatogram, with increasing R<sub>f</sub>, green, pale yellow, black, white, and orange bands appear.

Under visible light, the chromatogram of Standard solution B exhibits, in the lower half: a red band corresponding in R<sub>f</sub> and color to the band of kawain in Standard solution A; below the kawain band, with decreasing R<sub>f</sub>, a brown band due to methysticin; and above the kawain band, with increasing R<sub>f</sub>, three yellow and one purple bands appear.

**Acceptance criteria:** Under UV light at 366 nm, the chromatogram of the Sample solution exhibits, in the lower half: an orange band corresponding in R<sub>f</sub> and color to the band of kawain in Standard solution A; below the kawain band, with decreasing R<sub>f</sub>, a bright blue fluorescent band and a yellow/orange band due to yangonin and methysticin, respectively; above the kawain band, a pale blue band corresponding to desmethoxyyangonin; one yellowish-green band above the desmethoxyyangonin band; and one bright blue band below the methysticin band. In the upper half of the chromatogram, with increasing R<sub>f</sub>, green, pale yellow, black, white, and orange bands appear.

Under visible light, the chromatogram of Standard solution B exhibits, in the lower half: a red band corresponding in R<sub>f</sub> and color to the band of kawain in Standard solution A; below the kawain band, with decreasing R<sub>f</sub>, a brown band due to methysticin; and above the kawain band, with increasing R<sub>f</sub>, three yellow and one purple bands appear.

- **C. HPLC**

  **Analysis:** Proceed as directed in the Assay for Content of Kavalactones.

  **Acceptance criteria:** The chromatogram of the Sample solution exhibits peaks at the retention times corresponding to the peaks due to methysticin, dihydromethysticin, kawain, dihydrokawain, yangonin, and demethoxyyangonin in the chromatogram of Standard solution B.

**Assay**

**Content of Kavalactones**

- **Mobile phase:** 0.1% Phosphoric acid, acetonitrile, and isopropyl alcohol (64:20:16)
- **Solvent:** Methanol and water (70:30)
- **Standard solution A:** 0.2 mg/mL of USP Kawai RS in toluene
- **Standard solution B:** 50 mg/mL of USP Powdered Kava Extract RS in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

**Sample solution:** Transfer about 750 mg of *Piper methysticum* Root and Rhizome, finely powdered and accurately weighed, to a 50-mL volumetric flask, dilute with Solvent to volume, and sonicate for 60 min. Pass through a membrane filter of 0.45-μm pore size before injection.

**Chromatographic system**
(See Chromatography <621>, System Suitability.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; L7 (similar to Waters YMCbasic S-5)
Flow rate: 0.6 mL/min
Injection volume: 5 μL

System suitability
Samples: Standard solution A and Standard solution B
Chromatographic similarity: The chromatogram of Standard solution B is similar to the reference chromatogram provided with the lot of USP Powdered Kava Extract RS being used.
Resolution: NLT 1.8 between demethoxyyangonin and yangonin, Standard solution B
Relative standard deviation: NMT 2.0%, Standard solution A

Analysis
Samples: Standard solution A, Standard solution B, and Sample solution
Using the chromatograms of Standard solution A and Standard solution B, and the reference chromatogram provided with the lot of USP Powdered Kava Extract RS (2) being used, identify the retention times of the peaks corresponding to methysticin, dihydromethysticin, kawain, dihydrokawain, yangonin, and desmethoxyyangonin in the Sample solution. The approximate relative retention times are provided in Table 1.

Separately calculate the percentages of methysticin, dihydromethysticin, kawain, dihydrokawain, yangonin, and desmethoxyyangonin in the portion of Piper methysticum Root and Rhizome taken:

\[
\text{Result} = \left( \frac{r_0}{r_s} \right) \times \left( \frac{C_s}{W} \right) \times 5000 \times F
\]

- \(r_0\) = response from the Sample solution
- \(r_s\) = response from Standard solution A
- \(C_s\) = concentration of Standard solution A (mg/mL)
- \(W\) = weight of Piper methysticum Root and Rhizome taken to prepare the Sample solution (mg)
- \(F\) = conversion factors for the analytes as provided in Table 1

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Approximate Relative Retention Time (min)</th>
<th>Conversion Factor (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methysticin</td>
<td>0.80</td>
<td>0.66</td>
</tr>
<tr>
<td>Dihydromethysticin</td>
<td>0.94</td>
<td>1.65</td>
</tr>
<tr>
<td>Kawain [1]</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Dihydrokawain</td>
<td>1.09</td>
<td>1.70</td>
</tr>
<tr>
<td>Yangonin</td>
<td>1.20</td>
<td>0.88</td>
</tr>
<tr>
<td>Desmethoxyyangonin</td>
<td>1.31</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Calculate the content of kavalactones as the sum of the percentages of methysticin, dihydromethysticin, kawain, dihydrokawain, yangonin, and desmethoxyyangonin.

Acceptance criteria: NLT 4.5% on the dried basis

CONTAMINANTS
- **Elemental Impurities—Procedures <233>**

Acceptance criteria
- Arsenic: NMT 2.0 μg/g
- Cadmium: NMT 0.5 μg/g
- Lead: NMT 5.0 μg/g
- Mercury: NMT 0.1 μg/g
**ARTICLES OF BOTANICAL ORIGIN, General Method for Pesticide Residues Analysis <561>:** Meets the requirements

**MICROBIAL ENUMERATION TESTS <61>:** The total aerobic bacterial count does not exceed $10^5$ cfu/g, the total combined molds and yeasts count does not exceed $10^3$ cfu/g, and the bile-tolerant Gram-negative bacteria does not exceed $10^3$ cfu/g.

**TESTS FOR SPECIFIED MICROORGANISMS <62>:** Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*.

**ARTICLES OF BOTANICAL ORIGIN, Test for Aflatoxins <561>:** Meets the requirements

**SPECIFIC TESTS**

- **ARTICLES OF BOTANICAL ORIGIN, Foreign Organic Matter <561>:** NMT 2.0%
- **ARTICLES OF BOTANICAL ORIGIN, Alcohol-Soluble Extractives, Method 1 <561>:** NLT 10.0%
- **ARTICLES OF BOTANICAL ORIGIN, Water-Soluble Extractives, Method 2 <561>:** NLT 20.0%
- **LOSS ON DRYING <731>:**
  - **Sample:** 1 g of *Piper methysticum* Root and Rhizome, finely powdered
  - **Analysis:** Dry the Sample at 105° for 2 h.
  - **Acceptance criteria:** NMT 12.0%
- **ARTICLES OF BOTANICAL ORIGIN, Total Ash <561>:** NMT 8%
- **ARTICLES OF BOTANICAL ORIGIN, Acid-Insoluble Ash <561>:** NMT 1%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **LABELING:** The label states the Latin binomial and the part(s) of the plant contained in the article.
- **USP REFERENCE STANDARDS <11> [3]**
  - USP Aflatoxins RS [4]
  - USP Kawa in RS [1]
  - USP Powdered Kava Extract RS [2]

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