0058 - Olive Leaf for Oleuropein by HPLC

**Botanical Name:** *Olea europaea L.*

**Parts of Plant Used:** Leaves

**Uses:** Treatment and prevention of hypertension; as an antioxidant.

**Modes of Action:**
Very few clinical trials have been performed to validate the use of olive leaf extracts. Olive leaf extracts have shown a blood pressure-lowering effect, hypoglycemic activity, and vasodilator effects, but only in in vitro and in vivo tests. Secoiridoids such as oleuropein found in olive leaf support its hypotensive activity.\(^1\)\(^-\)\(^4\) In addition, oleuropein also is a well-known antioxidant.

**Chemical Markers:**
Olive leaf is known to be rich in phenolic compounds. The main phenol identified in olive leaf is oleuropein, a (3,4-dihydroxyphenyl)ethanol ester with ß-glucosylated elenolic acid. Other phenolics identified in olive leaf include vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, gallic acid, syringic acid, tyrosol, hydroxytyrosol, apigenin, kaempferol, quercetin, apigenin-7-glucoside, hesperidin, luteolin-7-glucoside, rutin, verbascoside, oleuropein, demethyloleuropein, and ligstroside. The hexane extract of olive leaf was found to contain hydrocarbons, ester waxes, triglycerides, tocopherols, sterols, linear and terpenic alcohols, and terpenic dialcohols. When the volatile compounds were analyzed by GC and GC–MS, mono- and sesquiterpenes were found to be the main volatile components.\(^2\)\(^-\)\(^9\) Currently, oleuropein is used as a marker compound for quality control of olive leaf extract.

![Oleuropein Structure](image_url)
Methods of Analysis

HPLC is a widely used method for analysis of oleuropein; oleuropein is a phenolic compound and can be detected easily by UV.

Method 1:
The method of Savournin et al.² was used.

Sample Preparation:
Extract 5 g of dried leaves three times with 30 mL of 60% aqueous methanol, sonicate for 15 minutes each time. Filter, combine the solutions, and dilute to 100 mL with the same solvent. Dilute the extract solution (1:5) with mobile phase.

Chromatography:
Column: Waters Symmetry C18, 5 μm, 250 × 3.9 mm, with a SentryGuard Symmetry C18, column, 3.9 × 20 mm.
Mobile phase: Water (adjusted to pH 3 with 0.1 M phosphoric acid)–acetonitrile (adjusted to pH 3 with 0.1 M phosphoric acid).
Gradient: 79:21
Flow rate: 1.0 mL/minute
Injection volume: 20 μL
Detection wavelength: 280 nm

Validation Data:
Linearity: Not available
Accuracy: Not specified
Precision: Less than 2% RSD
Selectivity: Peak identification was determined against standards
Ruggedness: Not specified
Robustness: Not specified
LOD/LOQ. LOD = 0.4 mcg/mL, LOQ = 0.85 mcg/mL

Method 2:
The method of Del Rio et al.¹⁰ was used.

Sample Preparation:
Grind the plant material (leaves, stems, roots, cortex, pith) and shake with dimethyl sulfoxide (150 mg of fresh weight per milliliter) for 1.2 hours.

Chromatography:
Column: Spherisorb ODS-2, 5 μm, 250 × 4.0 mm, with a SentryGuard Symmetry C18 column, 3.9 × 20 mm.
Mobile phase: Solvent A = water–acetic acid (97.5:2.5), solvent B = acetonitrile.
Gradient: 25%B to 95%B in 50 minutes.
Flow rate: 1.0 mL/minute
Injection volume: 10 μL
Detection wavelength: 280 and 353 nm
Column temperature: 30°C
**Validation Data:**
Not specified

**Method 3:**
The method of Ryan et al.\textsuperscript{11} was used.

**Sample Preparation:**
Blend dried samples (about 0.25 g) with methanol–water (50:50) using an Ultra Turrax blender for 20 seconds and let the solution stand for 30 minutes. Filter the samples and extract again using the same procedure, but let stand for 15 minutes. Filter, combine the filtrates, and wash with hexane (5 mL). Use the aqueous phase for HPLC analysis.

**Chromatography:**
Column: Alltech Alltima C18, 5 μm, 150 × 4.6 mm, with a SentryGuard column Symmetry C18, 3.9 × 20 mm.
Mobile phase: Solvent A = water–acetic acid (100:1 vol/vol), solvent B = methanol–acetonitrile–acetic acid (95:5:1 vol/vol/vol).
Gradient:

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>%A</th>
<th>%B</th>
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<tbody>
<tr>
<td>0</td>
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<td>12</td>
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<td>12</td>
</tr>
<tr>
<td>40</td>
<td>88</td>
<td>12</td>
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</tbody>
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Column temperature: 30°C
Flow rate: 0.8 mL/minute
Injection volume: 10 μL
Detection wavelength: 254 nm

**Representative HPLC Chromatogram of Olive Leaf Run by Method 4**

![Representative HPLC Chromatogram of Olive Leaf Run by Method 4](image-url)
References:


