HPLC Determination of Chicoric Acid in Leaves of *Posidonia oceanica*

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Abstract

In this study, *Posidonia oceanica* (L.) Delile (Posidoniaceae), which is a widely distributed phanerogam in Aegean and Mediterranean coasts, was investigated for the phenolic compounds that have the potential to be used in pharmaceutical sciences. The leaves, separated as young and mature leaves, were extracted and analyzed by high-performance liquid chromatography (HPLC). The younger leaves were found to have higher concentrations of chicoric acid, *p*-coumaric acid, vanillin, and ferulic acid, and the amount of gentisic acid, caffeic acid, and cinnamic acid was found to be higher in the mature leaves. Consequently, the plant might be a source of compounds to be investigated for anti-HIV and immunostimulant (caffeic acid, chicoric acid), antitumor (cinnamic acid, ferulic acid), antioxidant, and antibacterial activities.

Keywords: Chicoric acid, ferulic acid, HPLC analysis, phenolic compounds, *Posidonia oceanica*.

Introduction

*Posidonia oceanica* (L.) Delile (Posidoniaceae), a widely distributed phanerogam in the Mediterranean Sea (Davis, 1984), plays a dominant role in marine ecosystem dynamics such as food and shelter source for several plant and animal species, and also acts as a stabilizer for the sea floor. The plant is endangered because of anthropogenic effects and the accidentally introduced tropical chlorophyte *Caulerpa taxifolia* (Vahl) C. Agardh. Therefore, transplantation studies have been done in order to cultivate the plant. With vegetative transplantation of plagiotropic leaf bundles, successful results were achieved (Molenaar et al., 1993). However, the overall success of this process is low in the long-term due to the difficulty of rooting and anchoring for cuttings as the transplants could not supply their nitrogen requirement (Lepoint et al., 2004).

The plant consists of compounds such as amino acids (arginine-rich in the rhizomes; glutamate-, aspartate-, and serine-rich in the leaves and the roots besides arginine) (Molinier and Pellergrini, 1966; Augier and Santimore, 1979) metallothioneins (Cozza et al., 2006), carbohydrates (Invers et al., 2004), fatty acids (Viso et al., 1993), and sterols (Sica et al., 1984). In addition, the plant has tannin cells, which are specialized in production of phenolic compounds. These phenolic compounds seem to play an important role in the protection of these plants against competitors, predators, and pathogens (Agostini et al., 1998). The phenolic compounds were analyzed previously and various constituents were identified (Serve et al., 1984; Cuny et al., 1995; Agostini et al., 1998; Dumay et al., 2004).

Among the compounds analyzed, chicoric acid and caffeic acid have previously been tested for anti-HIV and immunostimulant activities (King et al., 1999; Kim et al., 2000; Beale Robinson, 2000; Lee et al., 2003; Charvat et al., 2006). On the other hand, cinnamic acid and ferulic acid have been tested for antitumor activity (Nair et al., 1991; Varnavas et al., 1991; Liu et al., 1995; Cardenas et al., 2006) with promising results. Again, obtained from different sources, gentisic acid (Martínez-Tomé et al., 2004), caffeic acid, *p*-coumaric acid (Sousa et al., 2004), ferulic acid (Anselmi et al., 2004), and cinnamic acid (Mansouri et al., 2005) were shown to possess antioxidant activity.

Up to now, very limited studies on *P. oceanica* have been carried out in the pharmaceutical sciences. Aqueous and lipid extracts from the rhizomes of *P. oceanica* were...
found to be active against selected bacteria (Gram+ and Gram−), dermatophytes, and the yeasts (Bernard & Pesando, 1988) besides the antileishmanial activity (Orhan et al., 2006).

The aim of this study was to identify the important phenolic compounds of the plant leaves that could be used in the pharmaceutical sciences and to clarify the distribution of chicoric acid metabolism with an HPLC method.

**Materials and Methods**

**Plant material**

The plant material was collected by scuba diving from Cesme-Izmir, Aegean Sea coasts, in November 1998 at 8 m depth. The plant was identified by Zeki Haznedaroglu at the Department of Pharmaceutical Botany, Faculty of Pharmacy, Ege University (Izmir, Turkey) and voucher specimen are kept at the International IZEF Herbarium (IZEF 5420). The epiphytes were removed by scraping with cotton, and leaves were dried in shade at room temperature. The entire leaves were divided into two groups: young leaves (3–15 cm) mature leaves (15–30 cm).

**Extraction method**

For extraction, 5 g of homogenized leaves were infused for 3 h in 200 ml aqueous ethanol 50% (v/v), in a water bath at 40°C with a reflux system in darkness. The solution obtained was filtered, acidified with 2 N HCl, and extracted with ethyl acetate after the evaporation of the ethanol under vacuum at 45°C. The dry extract was dissolved in mobile phase mixture and examined by HPLC (Cuny et al., 1995).

**Chromatographic analysis method**

A Hewlett Packard 1100 series HPLC (Germany) was used with Merck Hitachi L 400 A UV detector (Germany). The mobile phase was water:acetonitrile:acetic acid (84:14:2) mixture and analytical column was Super Co. Inc. Waters S Spheripor ODS (particle size 5 μm, diameter 4.6 mm, length 250 mm, cat. No. Z226068, England). Flow rate was 1.2 ml/min and the detection was performed at 254 nm (Serve et al., 1984). Analysis was repeated with added standards in order to ensure the results. All reagents were of analytical reagent grade and purchased from Labscan (Ireland) and Merck (Germany).

**Results and Discussion**

With this study, the phenolic compounds of *Posidonia oceanica* of the Turkish coastline was identified and compared in young and mature leaves. All of the reference compounds, chicoric acid, p-coumaric acid, caffeic acid, ferulic acid, cinnamic acid, gentisic acid, and vanillin, were detected in the extracts. The young and the mature leaves consist of the same compounds in different quantities. The young leaves revealed higher concentrations of chicoric acid, p-coumaric acid, vanillin, and ferulic acid, and the amounts of gentisic acid, caffeic acid, and cinnamic acid were higher in the mature leaves. Retention times for major compounds are shown in Table 1 and the concentration of compounds are shown in Table 2.

Distribution of chicoric acid during leaf development has been examined by UV spectrometry (Cariello et al., 1979). With our study, detection of chicoric acid in *Posidonia oceanica* using HPLC was demonstrated for the first time. Our results support the previous work on the distribution of chicoric acid during leaf development and shows that chicoric acid is in higher quantity when the metabolism of the plant is most active, in the young leaves (138.6 μg/g dry weight).

The obtained phenolic compounds are characteristic for the monocotyledonous angiosperms. Compounds like ferulic acid, p-coumaric acid, and vanillin could be analyzed in most of these plants. The occurrence of the components in the leaves was in agreement with previous studies (Serve et al., 1984; Cuny et al., 1995; Agostini et al., 1998). However, the concentrations differ due to the metabolism of the plant in different conditions. Previous studies found ferulic acid (Cuny et al., 1995; Agostini et al., 1998) and p-hydroxy benzoic acid (Serve et al., 1984) as major component, where as in our study, gentisic acid

<table>
<thead>
<tr>
<th>Compound</th>
<th>RT young leaves</th>
<th>RT mature leaves</th>
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<tbody>
<tr>
<td>Gentisic acid</td>
<td>6.47</td>
<td>6.42</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>7.48</td>
<td>7.54</td>
</tr>
<tr>
<td>Chicoric acid</td>
<td>11.82</td>
<td>11.97</td>
</tr>
<tr>
<td>Vanillin</td>
<td>12.57</td>
<td>12.72</td>
</tr>
<tr>
<td>Coumaric acid</td>
<td>13.10</td>
<td>13.18</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>17.93</td>
<td>17.89</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>61.95</td>
<td>62.17</td>
</tr>
</tbody>
</table>

RT, retention time (min).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Young leaves (μg/g dry weight)</th>
<th>Mature leaves (μg/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentisic acid</td>
<td>156.8</td>
<td>395.0</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>10.5</td>
<td>14.4</td>
</tr>
<tr>
<td>Chicoric acid</td>
<td>138.6</td>
<td>38.4</td>
</tr>
<tr>
<td>Vanillin</td>
<td>49.5</td>
<td>9.4</td>
</tr>
<tr>
<td>Coumaric acid</td>
<td>4.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>2.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>1.2</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Table 1. The retention times for the compounds.

Table 2. Concentration of the phenolic compounds.
was the main compound in both young (156.8 μg/g dry weight) and mature (395.0 μg/g dry weight) leaves. Besides these compounds, caffeic acid and vanillin were within the limits of 5–50 μg/g dry weight. The other compounds, coumaric acid, ferulic acid, and cinnamic acid, were detected below the 5 μg/g dry weight limit.

In further studies, the amount of phenolic acids can be analyzed in different parameters, such as depth, hours of the day, season, and location, in order to identify the concentration changes of the plant. The quantities of the compounds in the plant could be compared with terrestrial plants in this point. According to the efficiency of analysis, activity tests of the plant extracts could be performed for anti-HIV, antitumor, and antioxidant activities.

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References


