Chemical Composition of and Inhibition of Angiotensin-Converting Enzyme by Senecio samnitum huet

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Abstract

Extracts of Senecio samnitum Huet and derived methyl ester of chlorogenic acid have been shown to inhibit angiotensin-converting enzyme (ACE) by using an in vitro bioassay based on the enzymatic cleavage of the chromophore-fluorophore labeled substrate dansyl-triglycine into dansylglycine, which is quantitatively measured by high-performance liquid chromatography (HPLC). GC/MS and NMR identified compounds present within the studied S. samnitum extracts. The most effective fraction was obtained in ethyl acetate, which gave 52.56 ± 0.23% (SD) inhibition at 300 μg/ml. The major constituent of this fraction, the methyl ester of chlorogenic acid, showed significant ACE inhibition of 56.78 ± 0.25% at a concentration of 82.5 μg/ml.

Keywords: ACE, angiotensin-converting enzyme, Compositae, Senecio samnitum.

Introduction

The large genus Senecio (Compositae) is represented by about 1300 species (Loyola et al., 1985). Pyrrolizidine alkaloids and sesquiterpenes with a furanoeremophilane skeleton are the major components in the Senecio genus (Bohlmann et al., 1986; Urones et al., 1988; Pérez et al., 1991). Senecio species have been used in folk medicine for the treatment of wounds and as antiemetic, anti-inflammatory, and vasodilator preparations (Rose, 1972; Bautista et al., 1991; Pérez et al., 1999). The traditional use of Senecio graveolens (Compositae) for the treatment of mountain sickness and the isolation of dihydroeuparin, a compound with a strong antihypertensive activity, stimulated us to investigate the angiotensin-converting enzyme (ACE) inhibition properties of Senecio samnitum, Huet a species native to southern Italy (Loyola et al., 1985). The primary causative determinants of hypertension remain elusive, but it is generally thought that it develops as a result of disturbances of the body’s blood pressure regulating systems. By exploring the means by which normotension is maintained by the renin angiotensin system (RAS), an understanding of the treatment of hypertension using ACE inhibitors can be achieved. In its classic definition, the RAS maintains blood pressure through angiotensin II, a potent vasoconstrictor (Lee et al., 1993). ACE inhibitors act by inhibiting the conversion of angiotensin I to angiotensin II, and several compounds with this activity (captopril, enalapril, ramipril, etc.) are used clinically, being considered effective and safe for the treatment of hypertension; their antihypertensive effect being enhanced by a low-salt diet. (Abrams et al., 1984). They are also well tolerated and have a good safety profile.

To our knowledge, no previous studies have been undertaken on the phytochemistry and biological properties of S. samnitum Huet. This paper deals with the isolation of compounds from S. samnitum and investigation of the ACE inhibitory properties of n-hexane, dichloromethane, ethyl acetate, and n-butanol extracts and methyl ester of chlorogenic acid of S. samnitum aerial parts.
Materials and Methods

Plant material

The aerial parts of *Senecio samnitum* Huet were collected in the flowering season in Calabria (Italy) in July 2002. The voucher specimen was authenticated by Dr. L. Bernardo (Department of Botany, University of Calabria, Italy) and deposited at the Herbarium of University of Calabria (CLU), Italy, under the reference number 4512.

General experimental procedures

NMR spectra were recorded on Bruker Avance 300 and 400 MHz spectrometers. Electron Ionization Mass Spectrometry (EIMS) (70 eV) analysis was recorded on a Hewlett-Packard 6890N gas chromatograph equipped with a methyl silicone SE-30 capillary column (30 m × 0.25 mm id × 0.25 μm film thickness) and interfaced with a Hewlett Packard 5973N Mass Selective Detector. Si gel (Merck, 200–400 mesh) was used for column chromatography. Thin-layer chromatography analysis was carried out on silica gel GF254 plates (Merck, Italy).

Extraction and isolation

Dried and powdered aerial parts (345 g) of *S. samnitum* were extracted with methanol (15 l) three-times at room temperature, and the solutions were combined and concentrated under reduced pressure to obtain 39.18 g residue. The aerial parts of *S. samnitum* were extracted with methanol (15 l) three-times at room temperature, and the solutions were combined and concentrated under reduced pressure to obtain 39.18 g residue. The methanol extract was dissolved in water and fractionated by liquid-liquid partition with *n*-hexane (2.47 g, 0.72% yield), dichloromethane (1.23 g, 0.36% yield), and ethyl acetate (2.39 g, 0.69% yield), each extract being taken to dryness under reduced pressure.

ACE inhibition test

Inhibition of ACE is currently considered to be a useful therapeutic approach in the treatment of high blood pressure. Detection of inhibition of ACE by using an *in vitro* ACE-inhibition assay is an effective screening method in the search for new antihypertensive agents (Hansen et al., 1995). This method is based on the ACE-catalyzed cleavage of the chromophore-fluorophore labeled substrate dansyltriglycine into dansylglycine, which is quantitatively measured by HPLC. Solutions of inhibitors were made by dissolving 1 mg of test extract and 250 μg of methyl ester of chlorogenic acid in 1 ml HEPES assay buffer to obtain the final concentration of 330 μg/ml of extracts and 82.5 μg/ml of pure compound. Inhibitors extracted using ethanol were dissolved in HEPES assay buffer containing 10% ethanol. For these studies, a commercially available angiotensin I–converting enzyme preparation from rabbit lung (EC 3.4.15.1) has been used. The ACE solution (25 μl) was preincubated in microtiter plates with a test or control solution (25 μl) for 5 min at 37°C. The enzyme reaction was started by adding a combined solution (25 μl) of the substrate dansyltriglycine (7.86 mM), and the internal standard, dansyl-l-glutamine (0.353 mM). At the end of the incubation time (chosen by the plotting of a calibration curve), the reaction was stopped by adding a solution of 0.1 N Na₂EDTA (50 μl). The cleavage product (dansylglycine) and unreacted substrate (dansyltriglycine) were separated and quantified by reversed-phase HPLC with UV detection at 250 nm.
Instrumentation

HPLC Perkin Elmer Series 410 LC Pump. Injector, Perkin Elmer 20 µl loop. Detector, Perkin Elmer UV/VIS LC290 spectrophotometric. Solvent system, ALTECH SN 1250-99, part no. 288215 BIN II 43, HYPER SIL ODS 5 u lot no. 5002.150 mm × 4.6 mm SN:1250-99. Mobile phase, isocratic system-10 mM NaH₂PO₄ buffer (pH 7) (1.56 g NaH₂PO₄ in 900 ml of water; adjust the pH to 7.0 and make up to 1 liter. Acetonitrile (88.12), flow rate 2 ml/min, run time 30 min. Linear calibration curve for dansylglycine was plotting from 0.2 to 25 µg/ml. All materials were purchased from Sigma (London, UK).

The decreased concentration of dansylglycine in the test reaction compared with the control reaction was expressed as percentage inhibition and calculated from the equation:

\[
\text{Inhibition (\%)} = 100 - \frac{(\text{dansylglycine})_T}{(\text{dansylglycine})_C} \times 100
\]

where \(T\) = test reaction and \(C\) = control reaction.

All experiments were carried out in triplicate. The therapeutic drug captopril was used as positive control.

Tannin test (eliminating false positives)

Extracts inhibiting ACE by 50% or more were subjected to the gelatin salt block test to eliminate false positives brought about by the presence of tannins. The tannin test was performed by extracting 5 g of dry plant material with 50 ml of water, ethanol (96%), or acetone. After evaporation of the solvents, the extracts were redissolved in 13 ml of hot water (90–100°C) and allowed to cool to room temperature. Two drops of 10% NaCl were added to “salt” out any nontannin compounds, which could cause a false positive reaction. After vacuum filtration, 3 ml of filtrate was added to each of four test tubes. The following solutions were then added to the test tubes: 4–5 drops of 1% gelatin solution; 4–5 drops of 1% gelatin + 10% NaCl solution; and 3–4 drops of 10% ferric chloride. For a negative control, water and no extract was used.

The test was considered negative if there was no precipitation in tubes 1 and 2 or if 3 showed no color formation and positive if there was precipitation in tubes 1 and 2 and color formation in 3 (either blue-black for hydrolyzable or brownish-green for condensed tannins). Senecio samnitum extracts produced a negative gelatin salt block test. All materials were purchased from Sigma.

Results and discussion

Previous studies showed that many plant-derived compounds have resulted in ACE inhibition: hydrolyzable tannins, phenylpropanes, proanthocyanidins, flavonoids, xanthones, fatty acids, and terpenoids (Nyman et al., 1998). Since a survey of the literature revealed that no studies on the potential angiotensin-converting enzyme inhibition activity of extracts and compounds isolated from Senecio samnitum had been undertaken, the aim of this study was to investigate the ACE-inhibition activity, according to the method described by Elbl and Wagner (1991), which was later modified by Hansen et al. (1995).

In order to identify the compounds present within the Senecio samnitum extracts, GC/MS analysis was performed. Senecio samnitum extracts were diluted to a final volume of 1 ml with methanol (approximately 1 mg/ml). One microliter of each solution was injected into the gas chromatograph and analyzed with a quadrupole mass spectrometric detector. The compounds detected are not new, but they are reported for the first time in Senecio samnitum. A total of 19 compounds were detected in the n-hexane, dichloromethane, and ethyl acetate extracts of the studied Senecio samnitum. The results from these analyses are summarized in Table 1, where indication of the sample from which each component has been identified is reported.

The current work showed the ACE inhibitory property of different extracts of Senecio samnitum. This may imply that in vivo, these extracts may have a hypotensive effect. The methanol extract of the aerial parts of Senecio samnitum showed a good ACE inhibition of 72.56 ± 0.12% at 330 µg/ml. The most effective fraction

| Table 1. Compounds present within Senecio samnitum Huet extracts and identified by GC-MS. |
|---|---|
| Extracts | Identified compounds |
| n-Hexane extract | • Docosanoic acid  
• Octadecanoic acid  
• Hexadecanoic acid  
• 9,12-Octadecadienoic acid  
• a-Amyrin  
• b-Amyrin  
• Vitamin E  
• Stigmasta-5, 22-dien-3-ol (3β, 22E)  
• (22R,24S)-22,24-Dimethylcholesterol |
| Dichloro methane extract | • Benzenacetic acid, 4-hydroxy  
• 1,2-Benzenedicarboxylic acid, dibutyl ester  
• 10-Demethylsqualene  
• Seneconine  
• Seneciphylline  
• Integerrimine |
| Ethyl acetate extract | • Propanedioic acid, dimethyl ester  
• Butanedioic acid, dimethyl ester  
• Butanedioic acid, hydroxy-, dimethyl ester  
• 9,12,15-Octadecatrienoic acid, methyl ester |
Table 2. ACE inhibitory of Senecio samnitum Huet extracts (tested concentrations 330 μg/ml) and methyl ester of chlorogenic acid (tested concentration 82.5 μg/ml).

<table>
<thead>
<tr>
<th>Extracts/compound</th>
<th>Inhibition % ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total MeOH</td>
<td>72.56 ± 0.12</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>N.A.</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>N.A.</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>52.56 ± 0.23</td>
</tr>
<tr>
<td>Methyl ester of chlorogenic acid</td>
<td>56.78 ± 0.25</td>
</tr>
</tbody>
</table>

The values represents means of two different experiments under standard assay conditions described in the text ± SD. Captopril (38 nM) used as positive control. N.A., not active.

of the total methanol extract was that obtained with ethyl acetate, which gave 52.56 ± 0.23% inhibition at 330 μg/ml.

It was possible to isolate and identify the main component of this extract, which is the methyl ester of chlorogenic acid (9.8% of ethyl acetate extract), not isolated in other Senecio species. This compound showed significant ACE inhibition of 56.78 ± 0.25% at a concentration of 82.5 μg/ml. This is greater than that reported previously for the chlorogenic acid, which was 4% at 330 μg/ml (Lacaille-Dubois et al., 2001). All results are shown in Table 2.

We have not tested senecionine, which is a member of pyrrolizidine alkaloids known to be hepatotoxic, but it is easily removed from extracts of S. samnitum by dichloromethane (Bah et al., 1994; De Vivar et al., 1996).

In conclusion, the methanol and ethyl acetate extracts of Senecio samnitum have been shown to cause inhibition of angiotensin-converting enzyme; further studies will be useful in order to demonstrate possible therapeutic relevance of S. samnitum extracts.

Acknowledgments
The authors wish to thank Dr. L. Bernardo of the Botany Department of University of Calabria, Italy, for the identification and collection of the plant material and Dr. V. Filippelli for English revision of manuscript.

References