Bioactive Constituents from *Dracocephalum subcapitatum* (O. Kuntze) Lipsky

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From an EtOAc extract of *Dracocephalum subcapitatum*, five flavonoids, calycopterin, xanthomicrol, isokaempferide, luteolin and apigenin, together with five terpenoids, oleanolic acid, ursolic acid, geranial, neral and limonene-10-al, were isolated. Among them, citral and limonene-10-al were the most effective components against epimastigotes of *Trypanosoma cruzi*, the parasitic agent of Chagas disease.

Key words: *Dracocephalum subcapitatum*, Labiatae, Limonene-10-al

Introduction

*Dracocephalum* is a genus belonging to the Labiatae family and is found abundantly in Central Asia, Iran, Turkey and Europe (Rechinger, 1986; Mozaffarian, 1996). These herbaceous plants have been used in traditional medicine for stomach and liver disorders, headache and congestion (Mirheydar, 1995). Recently, some trypanocidal diterpenoids were isolated from *D. komarovi* (Uchiyama et al., 2003; 2004). We have also reported the isolation of trypanocidal flavonoids and terpenoids from *D. kotschyi* (Gohari et al., 2003; Saeidnia et al., 2004b) and their activity test against epimastigotes of *Trypanosoma cruzi*, the parasitic agent of Chagas disease (Nogueda-Torres et al., 2001). Among eight Persian species of this genus *D. subcapitatum* (O. Kuntze) Lipsky is found at limited area in the north-east of Iran (Rechinger, 1986). Literature reviews show that nothing has been reported on phytochemical analysis of this plant. We present here the isolation and identification of the constituents from *D. subcapitatum* and their trypanocidal activity.

Material and Methods

General

Melting points were determined on a Yanagimoto micro melting point apparatus. 1H and 13C NMR spectra were measured on a JEOL JNM-LA500 (500 MHz for 1H and 125 MHz for 13C) spectrometer with tetramethylsilane as an internal standard, and chemical shifts are given as δ values.

Plant material

Aerial parts of *Dracocephalum subcapitatum* (O. Kuntze) Lipsky were collected from the northern part of Khorasan prefecture, Iran in May 2002. Mr. I. Mehragan (Shaheed Beheshti University of Medical Sciences, Tehran, Iran) identified the plant species. A voucher specimen was deposited at the Experimental Station of Medicinal Plants, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan.

Extraction and isolation

Dried aerial parts of *D. subcapitatum* (350 g) were cut into small pieces and successively extracted with EtOAc and MeOH at room temperature overnight to obtain EtOAc (15 g) and MeOH (10 g) extracts. These extracts were tested against epimastigotes of *T. cruzi*. The active EtOAc extract (4 g) was submitted to silica gel column chromatography (CC) with hexane/EtOAc (19:1, 0:1 v/v) and MeOH as eluents to give five fractions (A–E). Among them, fractions B (10 mg), C (400 mg) and D (2.0 g) were active against *T. cruzi*. Fraction B was compound 1. Fraction C was compound 1. Fraction C was fractionated with hexane/CHCl3 (1:1, 2:3 v/v) to afford three parts (C1–C3). Fraction C1...
was compound 1 (20 mg). Fraction C3 was chromatographed twice with hexane/EtOAc (19:1, 9:1 v/v) on silica gel to give compounds 2 (20 mg) and 3 (10 mg). From fraction D, compound 4 (20 mg) and compound 5 (30 mg) were obtained by silica gel CC with hexane/EtOAc (19:1), hexane/aceton (8:2) and then CHCl3/EtOAc (9:1 v/v). Further purification of compound 5 was performed with CHCl3/EtOAc (19:1). The more polar part of fraction D (1.2 g), remaining after the separation of compounds 4 and 5, was chromatographed on silica gel with hexane/EtOAc (6:4) to obtain three parts (D1–D3). From D2 (800 mg), compounds 6 (10 mg), 7 (15 mg) and 8 (30 mg) were isolated on Sephadex LH-20 with CHCl3/MeOH (7:3) as a solvent system. Fractionation of D3 (400 mg) on silica gel (benzene/EtOAc, 3:1) and purification of the separated fractions with Sephadex LH-20 resulted in compounds 9 (35 mg) and 10 (18 mg).

Trypanocidal assay

Epimastigotes (prepared from Juntendo University, Japan) of T. cruzi (Tulahuen strain) were kept in GIT medium (Wako, Tokyo) supplemented with hemin (12.4 µM; Wako). The epimastigotes in GIT medium (10 µl) were incubated with a test sample dissolved in EtOH (5 µl) and autoclaved salin (185 µl). After 24 h of incubation, the movement of epimastigotes was observed under a microscope. We assumed that the immobilized and ball-shaped organisms were dead. Five determinations for minimum lethal concentration (MLC, concentration on which, all epimastigotes were dead) of each compound were performed. The negative control used, contained ethanol in the same proportion utilized to dissolve the compounds. Also, gentian violet was used as a positive control (MLC = 6.3 µM) (Kiuchi et al., 2002).

Results and Discussion

Dried aerial parts (leaves, flowers and stems) of D. subcapitatum were successively extracted with EtOAc and MeOH. Only the EtOAc extract showed in vitro trypanocidal activity (MLC = 50 µM). Therefore, we fractionated this extract and obtained 10 pure compounds (1–10). The identification of these compounds was carried out by comparison of their spectral data (1H and 13C NMR spectra) with previous reports (Table I).

Table I. In vitro activity of the isolated constituents from Dracocephalum subcapitatum against the epimastigotes of T. cruzi.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MLC* [µM]</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Limonene-10-al (1)</td>
<td>3.1 ± 0.3</td>
<td>Saeidnia et al., 2004b</td>
</tr>
<tr>
<td>Geranial (2)</td>
<td>3.1 ± 0.3</td>
<td>Bohllmann et al., 1975</td>
</tr>
<tr>
<td>Neral (3)</td>
<td>3.1 ± 0.0</td>
<td>Bohllmann et al., 1975</td>
</tr>
<tr>
<td>Oleanolic acid (4)</td>
<td>6.2 ± 0.8</td>
<td>Srivastava and Jain, 1989</td>
</tr>
<tr>
<td>Ursolic acid (5)</td>
<td>6.2 ± 0.3</td>
<td>Alves et al., 2000</td>
</tr>
<tr>
<td>Calycopterin (6)</td>
<td>&gt;400</td>
<td>El-Ansari et al., 1991</td>
</tr>
<tr>
<td>Xanthomicrol (7)</td>
<td>&gt;400</td>
<td>El-Ansari et al., 1991</td>
</tr>
<tr>
<td>Isokaempferide (8)</td>
<td>70.0 ± 17.5</td>
<td>Gohari et al., 2003</td>
</tr>
<tr>
<td>Apigenin (9)</td>
<td>30.0 ± 15.0</td>
<td>Wawer and Zielinska, 2001</td>
</tr>
<tr>
<td>Luteolin (10)</td>
<td>&gt;400</td>
<td>Flamini et al., 2001</td>
</tr>
</tbody>
</table>

* Minimum lethal concentrations of the separated compounds; five determinations for each concentration were tested and immobilized organisms were assumed to be dead.

These compounds were limonene-10-al (1), geranial (2), neral (3), oleanolic acid (4), ursolic acid (5), calycopterin (6), xanthomicrol (7), isokaempferide (8), apigenin (9) and luteolin (10).

All the pure compounds were tested against epimastigotes of T. cruzi. The average of five MLC determinations for the active constituents are reported in Table I. Highly methoxylated flavonoids, calycopterin and xanthomicrol, showed no activity even at 400 µM. Among these known compounds, citral (geranial and neral) and limonene-10-al were the most effective components against epimastigotes of Trypanosoma cruzi. In addition D. subcapitatum, D. feotidum and D. kotschyi contain a C10 aldehyde derivate of limonene (Duete et al., 2003; Saeidnia et al., 2004b). Phylogenetic analysis of Dracocephalum species showed that D. subcapitatum has very close relationship to D. kotschyi compared to other species (Saeidnia et al., 2004a). The results obtained from this study, together with our previous research on chemical constituents of D. kotschyi (Gohari et al., 2003; Saeidnia et al., 2004b), showed chemotaxonomical similarity of these two species (D. kotschyi and D. subcapitatum), to support their close phylogenetic relationship.

Acknowledgements

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Flamini G., Antognoli E., and Morelli I. (2001), Two flavonoids and other compounds from aerial parts of \textit{Centaurea bracteata} from Italy. Phytochemistry 57, 559–564.