TRYPANOCIDAL COMPONENT OF THE ETHER FRACTION OF SATUREJA MACRANTHA

Soodabeh Saeidnia¹, Ahmad R. Gohari⁎, Abbas Hadjiakhoondi²

¹Medicinal Plants Research Center, Faculty of Pharmacy, Medical Sciences/ University of Tehran, Tehran, Iran
²Department of Pharmacognosy, Faculty of Pharmacy, Medical Sciences/ University of Tehran, Tehran, Iran

ABSTRACT

Ether extract of Satureja macrantha C. A. Mey, one of Iranian species of Satureja belonging to family Lamiaceae, has shown the trypanocidal activity against the epimastigotes of Trypanosoma cruzi, the causative agent of American trypanosomiasis. In this study we aimed to isolate and identify the active component guided by in vitro bioassay. In order to fractionation, column chromatography and Lobar (Si-60) CC were used. Fractions and the separated compound were tested using in vitro anti-epimastigote activity test and then minimum lethal concentration (MLC) determined. All the fractions, consisted of compound 1, have been observed active against epimastigotes of T. cruzi. Compound 1 was isolated and identified as thymol using 1H and 13C-NMR spectra.

Keyword: Satureja macrantha, Lamiaceae, thymol, Trypanosoma cruzi

INTRODUCTION

Several species of Satureja, belonging to the family Lamiaceae, are found in Iran including Satureja macrantha C. A. Mey. (Rechinger, 1986). Satureja plants have been used in traditional medicine as spasmylytic, anti-microbial and diuretic agents. The essential oils of Satureja species have been evaluated as spasmylytic agents and a potent anti-HIV-1 activity (Feresin et al., 2000; Hajhashemi et al., 2000; Bedoya et al., 2001). The essential oil of Satureja species are characterized by the presence of thymol, carvacrol, p-cymene and γ-terpinene (Gohari et al., 2005a).

Chagas disease is caused by the protozoan, Trypanosoma cruzi, and current treatment is unsatisfactory. The drugs are available but possess severe side effects and their activity is limited to the acute phase (Nogueda-Torres et al., 2001). Previously, we investigated the in vitro anti-epimastigote activity of some medicinal plants. Among them the diethyl ether and acetone fractions of Satureja macrantha and S. mutica showed a potent activity against T. cruzi (Saeidnia et al., 2005). Here we report the bioassay guided fractionation of the ether extract to reach the active component.

MATERIAL AND METHOD

Plant Material

Aerial parts of S. macrantha C. A. Mey. were collected from Uroomieh, in north-west of Iran, during the full flowering stage in September 2000. Voucher specimens were deposited at the Herbarium of the Institute of Forests and Rangelands Researches. Plant specimen was identified by Dr. Vali-allah Mozaffarian from the same institute.

Extraction, isolation and structural elucidation

Dried aerial parts of S. macrantha (800 g) were cut into small pieces and successively extracted with diethyl ether at room temperature (for 72 hours) to obtain diethyl ether (30 g) extract.

The diethyl ether extract (20 g) was submitted to silica gel column chromatography (CC) with Hexane: CHCl₃ (8:2, 0:1) and CHCl₃ as eluent, to give eight fractions (A-G). Fraction F (5.76 g) was subjected to Lobar (Si-60) CC with Hexane: EtOAc (19:1, 1:1 and 0:1) to obtain compound 1 (145 mg).

Silica gel 60 F₂₅₄ precoated plates (Merck) were used for TLC. The spots were detected by spraying anisaldehyde- H₂SO₄ reagent followed by heating. 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.

*Corresponding to: Ahmad Reza Gohari, Medicinal Plants Research Center, Medical Sciences/ University of Tehran, PO Box 14155-6451, Tehran, Iran. Tel: +98-21-66950090, Fax: +98-21-66461178, E-mail: goharii@yahoo.com
**In vitro trypanocidal assay**

Epimastigotes of *T. cruzi* (Tulahuen strain) were kept in GIT medium (Wako) supplemented with hemin (12.4 \( \mu \)M, Wako). The epimastigotes in GIT medium (10 \( \mu \)L) were incubated with a test sample (extracts) dissolved in EtOH (5 \( \mu \)L) and autoclaved saline (185 \( \mu \)L). All samples were incubated at 27° C for 24 hours. The movement of epimastigotes was observed under a microscope. We assumed that immobilized organisms were died. The control contained ethanol in the same proportion utilized to dissolve the drugs. Each assay was performed in duplicate. Gentian violet is used as a positive control and its minimum lethal concentration is 6.3 \( \mu \)M (Saeidnia et al., 2005).

**RESULTS AND DISCUSSION**

In this report, the trypanocidal activity of ether extract and 7 fractions of *Satureja macrantha* were evaluated in order to receive one of main active component. Diethyl ether extract of the plant showed an activity with MLC = 25 \( \mu \)g/ml. Fractionation of the extract with silica gel column chromatography (Hexane: CHCl\(_3\)) resulted in seven sections A-G and the activity of each fraction was tested *in vitro* against the epimastigotes of *T. cruzi*. Table 1 summarizes the results obtained from fractionation and biological assay.

Table 1. Yields of fractionation and trypanocidal activities from *Satureja macrantha*

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Yields(^1) (g)</th>
<th>MLC(^2) ((\mu)g/ml)</th>
<th>Concentrations ((\mu)g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6.25</td>
<td>12.5</td>
</tr>
<tr>
<td>diethyl ether</td>
<td>20</td>
<td>25</td>
<td>++</td>
</tr>
<tr>
<td>fr. A</td>
<td>1.2</td>
<td>12.5</td>
<td>±</td>
</tr>
<tr>
<td>fr. B</td>
<td>1.4</td>
<td>&gt;100</td>
<td>++</td>
</tr>
<tr>
<td>fr. C</td>
<td>2.1</td>
<td>&gt;100</td>
<td>++</td>
</tr>
<tr>
<td>fr. D</td>
<td>3.9</td>
<td>12.5</td>
<td>±</td>
</tr>
<tr>
<td>fr. E</td>
<td>3.8</td>
<td>50</td>
<td>++</td>
</tr>
<tr>
<td>fr. F</td>
<td>5.8</td>
<td>50</td>
<td>++</td>
</tr>
<tr>
<td>fr. G</td>
<td>1.0</td>
<td>&gt;100</td>
<td>++</td>
</tr>
<tr>
<td>gentian violet</td>
<td>(positive control)</td>
<td>6.25</td>
<td>-</td>
</tr>
<tr>
<td>negative control</td>
<td></td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

\(^1\)Based on dry weight.
\(^2\)Minimum Lethal Concentration; ++: moving normally (same movement with the negative control), +: apparently less active than negative control, ±: most of epimastigotes are immobile but a few are still moving, -: all are dead (ball shaped) or immobile.

Some of those fractions have been observed effective on the epimastigotes. TLC monitoring of the fractions by anisaldehyde- \( \text{H}_2\text{SO}_4\) reagent showed a significant orange spot in all the active proportions so the fraction F was selected to continue the isolation process based on the dry weight of fraction and intensity of the orange spot. Lobar (Si-60) CC (Hexane: EtOAc) of the fraction F afforded the compound I. Compound I was identified as thymol. The \(^1\)H and \(^13\)C NMR spectral data of this component showed a good agreement with those of reference reports. Until recently, the essential oil of Turkish *Origanum onites* L. (Lamiaceae) and the main components, carvacrol and thymol show potent antiprotozoal activity without cytotoxicity. Both compounds have retained the stronger trypanocidal activities as observed for the oil. (IC\(_{50}\) value for thymol: 114 ng/ml, for carvacrol: 149 ng/ml) (Tasdemir et al., 2006).

Thymol is a monoterpane, previously isolated from *Satureja* species, with an *in vitro* cytotoxic effect against the larva of brine shrimp (Gohari et al., 2005b). This compound, obtained from volatile oil of *Thymus* plants, exhibited synergism with ketoconazole against *Trichophyton rubrum*, with highest susceptibility regard to these oils (Seungwon et al., 2004). The essential oil of *Satureja* plants and *S. macrantha* as well, are characterized by the presence of thymol, carvacrol, \(p\)-cymene and \(\gamma\)-terpinene (Gohari et al., 2005a). Although, it seems that the volatile oil of *S. macrantha* should be effective against the epimastigotes of *T. cruzi*, the amount of thymol in that oil is not so considerable (6.2%). Because, the oil of *S. macrantha* is enriched of spathulenol and vanillin (Gohari et al., 2005a and b).
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REFERENCES


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