

## TRYPANOCIDAL COMPONENT OF THE ETHER FRACTION OF *SATUREJA MACRANTHA*

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### 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 trypanomiasis. 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 <sup>1</sup>H and <sup>13</sup>C- 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 spasmolytic, anti-microbial and diuretic agents. The essential oils of *Satureja* species have been evaluated as spasmolytic 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  $\gamma$ -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 (Nogueada- 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: CHCl<sub>3</sub> (8:2, 0:1) and CHCl<sub>3</sub> 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 F<sub>254</sub> precoated plates (Merck) were used for TLC. The spots were detected by spraying anisaldehyde- H<sub>2</sub>SO<sub>4</sub> reagent followed by heating. <sup>1</sup>H and <sup>13</sup>C NMR spectera were measured on a JEOL JNM-LA500 (500 MHz for <sup>1</sup>H and 125 MHz for <sup>13</sup>C) spectrometer with tetramethylsilane as an internal standard, and chemical shifts are given as  $\delta$  values.

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### *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<sub>3</sub>) 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*

Fractions	Yields <sup>1</sup> (g)	MLC <sup>2</sup> ( $\mu$ g/ml)	Concentrations ( $\mu$ g/ml)					
			6.25	12.5	25	50	100	200
<i>diethyl ether</i>	20	25	++	++	-	-	-	-
fr. A	1.2	12.5	±	-	-	-	-	-
fr. B	1.4	>100	++	++	++	++	++	+
fr. C	2.1	>100	++	++	++	++	++	+
fr. D	3.9	12.5	±	-	-	-	-	-
fr. E	3.8	50	++	++	++	-	-	-
fr. F	5.8	50	++	+	±	-	-	-
fr. G	1.0	>100	++	++	++	++	+	+
gentian violet	(positive control)	6.25	-	-	-	-	-	-
	negative control	-	++	++	++	++	++	++

<sup>1</sup>Based on dry weights.

<sup>2</sup> 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- H<sub>2</sub>SO<sub>4</sub> 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 **1**. Compound **1** was identified as thymol. The <sup>1</sup>H and <sup>13</sup>C NMR spectral data of this component showed a good agreement with those of reference reports.<sup>5</sup> 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<sub>50</sub> value for thymol: 114 ng/ml, for carvacrol: 149 ng/ml) (Tasdemir *et al.*, 2006).

Thymol is a monoterpene, 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**

- Bedoya, L. M., Sanchez, S., Abad, M. J., Bermejo, P. and J. Alcami (2001). Anti-HIV activity of medicinal plant extracts. *J. Ethnopharmacol.*, 77: 113-116.
- Feresin, G. E., Tapia, A. A. and D. A. Bustos (2000). Antibacterial activity of some medicinal plants from San Juan Argentina. *Fitoterapia*, 71: 429-432.
- Gohari, A. R., Hadjiakhoondi, A., Sadat-Ebrahimi, S. E., Saeidnia, S. and A. Shafiee (2005a). Composition of volatile oils of *Satureja spicigera* and *Satureja macrantha* from Iran. *Flav. Frag. J.*, 21: 348-350.
- Gohari, A. R., Hadjiakhoondi, A., Sadat-Ebrahimi, S. E., Saeidnia, S. and A. Shafiee (2005b). Cytotoxic triterpenoids from *Satureja macrantha* C.A. Mey. *Daru*, 13: 177-181.
- Hajhashemi, V., Sadraei, H., Ghannadi, A. R., M. Mohseni (2000). Antispasmodic and anti-diarrheal effect of *Satureja hortensis* L. essential oil. *J. Ethnopharmacol.*, 71: 187-192.
- Noguera- Torres, B., Rodriguez- Paez, L., Ramirez, I. B. and C. W. Ramirez (2001). Trypanocidal activity of isopropyl salicylaldehyde and 4- isopropyl salicylic acid on *Trypanosoma cruzi*. *Rev. Lat. Microbiol.*, 43: 1-6.
- Rechinger, K.H. (1986). *Flora Iranica, Labiatae*. (I. C. Hedge ed.). Akademische Druck Verlagsantalt, Graz, 150, pp: 495-504.
- Saeidnia, S., Gohari, A.R., Kiuchi, F. and G. Honda (2005). Anti-epimastigote activity of some Iranian medicinal plants. *Iran J. Pharmaceut. Res.*, 2: 101-103.
- Seungwon, S. and K. Ji-Hyun (2004). Antifungal Activities of Essential Oils from *Thymus quinquecostatus* and *T. magnus*. *Planta Med.*, 70: 1090-1092.
- Tasdemir, D., Kaiser, M., Demirci, F. and K. H. C. Baser (2006). Essential oil of Turkish *Origanum onites* L. and its main components, carvacrol and thymol show potent antiprotozoal activity without cytotoxicity. In proceedings of the 54th Annual Congress on Medicinal Plant Research, 29 August –2 September 2006, Helsinki, Finland. Abstract i in *Planta Med.*, 72.

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