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Original Article

In vitro Anti-epimastigote Activity of some Iranian Medicinal Plants

Soodabeh Saeidnia[a*], Ahmad Reza Gohari[a], Fumiyuki Kiuchi[b] and Gisho Honda[c]

[a]*Medicinal Plants Research center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.* [b]*Tsukuba Medicinal Plant Research Station, National Institute of Health Sciences, Ministry of Health, Labour and Welfare, Tsukuba, Ibaraki, Japan.* [c]*Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan.*

Abstract

Some medicinal plants are a potential source of new drugs, in order to improve the treatment of Chagas disease whose treatment is still a challenge. In this study, the *in vitro* anti-epimastigote activity of certain fractions of *Achillea biebersteinii, A. millefolium, Satureja mutica* and *S. macrantha* was evaluated. Diethyl ether fractions of *Achillea* species and acetone fractions of *Satureja* species were the most active fractions (MLC=12.5 μg/ml) against the epimastigotes of *Trypanosoma cruzi,* the ethiological agent causing Chagas disease. The trypanocidal activity seems to be decreased by fractionation, using MeOH and water as the solvents. The results obtained from biological assay revealed that *Achillea* and *Satureja* species could be a source of active trypanocidal compounds.

Keywords: *Achillea millefolium*; *Achillea biebersteinii*; *Satureja mutica*; *Satureja macrantha*; Chagas disease; epimastigote; trypanocidal activity.

Introduction

Chagas disease is caused by the flagellete protozoan *Trypanosoma cruzi* (Trypanosomatina), leading to approximately 400,000 deaths per year (1). Trypomastigotes ingested by the insect differentiates into the proliferative epimastigote form that, on reaching the posterior intestine, evolves to metacyclic trypomastigotes. The latter form undergoes differentiation into amastigotes, which after several reproductive cycles transform to trypomastigotes, the form responsible for the dissemination of the infection (2). Current treatment is unsatisfactory, because the only two available drugs, benznidazole and nifortimox, possess severe side effects and their activity is limited to the acute phase (3).

Recently, higher plants belonging to the Rutacea, Meliaceae, Simaroubaceae and Burceraceae families have been studied in an attempt to find active compounds against *Trypanosoma cruzi*, the causative agent of Chagas disease (1). The genus *Achillea* (Compositae) is well-known for medicinal properties such as anthelmintic, anti-inflammatory and anti-microbial effects (4, 5). *Achillea millefolium* L. and *Achillea biebersteinii* Afan. are two of among nineteen herbaceous species growing in the northen parts of Iran (6). The genus *Satureja* (Labiatae) has been used in traditional Medicine for its carminative, tonic, stomachique, diuretic and anti-cancer activities. They are also used for rheumatic pain and asthma (4). *S. mutica* Fisch et C. A. Mey and *S. macrantha* C. A. Mey are two of eight endemic species of *Satureja*

* Corresponding author:
E-mail: soodabehsaeidnia@hotmail.com

growing in north of Iran (7). There is no paper to investigate the anti-trypanosoma effect of these species. In this study it was decided to examine their activity against the epimastigotes of *T. cruzi*.

Experimental

Plant materials

Achillea millefolium and *A. biebersteinii* were collected from Kord Kooy at Golestan State of Iran in August 1999 (during full flowering stage) and identified by Dr. H. Akhani. The voucher herbarium specimens (No.13607 for *A. millefolium* and No. 13606 for *A. biebersteinii*) were deposited in the private herbarium of Dr. H. Akhani, housed at the Department of Biology, Faculty of sciences, Tehran University. *Satureja mutica* and *S. macrantha* were collected in September 2000 from Guilan and West Azarbayjan states in Iran, respectively, and identified by Dr. V. Mozaffarian. The voucher herbarium specimens (TARI- 78411 for *S. mutica* and TARI- 78409 for *S. macrantha*) were deposited at the herbarium of Research Institute of Forests and Rangelands.

Preparing the extracts

Aerial parts of the plants (flowers, leaves and stems) were dried carefully and reduced to powder, followed by extraction three times with diethyl ether via maceration at room temperature for 72 h. This process was repeated on the marc with ethyl acetate (for *Achillea*) or acetone (for *Satureja*), methanol and water, successively, and then the solvents evaporated under reduced pressure to obtain the concentrated extracts. All extracts were dried under vacuum in order to give dried powder extracts.

Evaluation of anti-epimastigote activity

Epimastigotes of *T. cruzi* (Tulahuen strain) were kept in GIT medium (Wako) supplemented with hemin (12.4 µM, Wako). The epimastigotes in GIT medium (10 µL) were incubated with a test sample (extracts) dissolved in EtOH (5 µL) and autoclaved saline (185 µL). All samples were incubated at 27˚C for 24 h. The movement of epimastigotes was observed under a microscope. It was assumed that immobilized organisms had been killed. The control contained ethanol in the same proportion utilized to dissolve the drugs. Each assay was performed in duplicate. Gentian

Table 1. Yields of extraction and trypanocidal activities for some fractions from *Satureja* and *Achillea* species.

Plants extract	yield (%w/w)	MLC* (µM)	concentration (µM)					
			6.25	12.5	25	50	100	200
Satureja mutica								
diethyl ether	8.29	25	++	+	-	-	-	-
acetone	12.02	12.5	+	-	-	-	-	-
methanol	11.06	>200	++	++	++	++	++	±
water	5.80	>200	++	++	++	++	+	±
Satureja macrantha								
diethyl ether	*4.44*	50	++	++	++	-	-	-
acetone	10.50	12.5	±	-	-	-	-	-
methanol	6.11	> 200	++	++	++	++	++	±
water	6.80	> 200	++	++	++	++	++	+
Achillea millefolium								
diethyl ether	1.95	12.5	+	-	-	-	-	-
ethyl acetate	1.00	25	+	±	-	-	-	-
methanol	13.56	50	++	++	++	+	-	-
water	8.72	> 200	++	++	++	++	+	±
Achillea biebersteinii								
diethyl ether	2.50	12.5	++	-	-	-	-	-
ethyl acetate	*2.32*	25	+	+	-	-	-	-
methanol	*16.64*	50	++	++	+	-	-	-
water	*9.18*	> 200	++	++	++	++	+	±
Gentian violet (positive control)		6.25	-	-	-	-	-	-
Negative control		-	++	++	++	++	++	++

• Minimum Lethal Concentration; ++: moving normally (same movement with the negative control), +: apparently less active than the negative control, ±: most of epimastigotes are immobile but a few are still moving, -: all have been killed (ball shaped) or immobilized.

violet was used as a positive control and its minimum lethal concentration was found to be 6.3 µM (8-10).

Results and discussion

In the present study, the trypanocidal activity of 16 fractions (diethyl ether, acetone, methanol and water extracts) from *Achillea* and *Satureja* species was evaluated. Table 1 summarizes the results obtained from fractionation and biological assay and shows that the diethyl ether and ethyl acetate fractions of both *Achillea* plants were active against the epimastigote of *T. cruzi.* MeOH fraction of *A. biebersteinii* shows a better trypanocidal effect than *A. millefolium*. Among several fractions of *Achillea*, only aqueous extracts did not show any activity at concentrations used in this study. Table 1 shows that the diethyl ether and acetone fractions of both *Satureja* plants were active against *T. cruzi.* Diethyl ether fractions of *S. mutica* showed a better trypanocidal effect than *S. macrantha*. Trypanocidal activity seems to be enriched by fractionation, using acetone as a solvent, and all the epimastigotes were completely eliminated. Only methanol and aqueous fractions of the plants did not show any activity at concentrations used in this study. Detailed explanation of the observed activity of these fractions must await the results of the ongoing phytochemical studies and *in vivo* bioassay of the isolated compounds against the parasite.

In the concept of efforts to improve the therapy of Chagas disease, higher plants appear to be a potential source of new drugs, with high activity and low toxicity. A broad spectrum of chemical classes of substances could show activity against the parasite. (11). It is possible that the activity of the Labiatae family could be associated with the terpenoids and flavonoids (12, 13). *Achillea* is well-known as a source of methoxylated flavonoids, which could be the main cause of biological activity against the *Trypanosoma cruzi* (13, 14).

In conclusion, the Iranian species of *Satureja* and *Achillea* (especially non-polar fractions) could be promising sources of active components against the epimastigotes of *T. cruzi*.

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