Trypanocidal Activity of Some Medicinal Plants Against the Epimastigotes of *Trypanosoma cruzi*

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Abstract

Background: Some of medicinal plants are a potential source of new drugs to improve the treatment of Chagase disease whose treatment is still a challenge. Here in this screening, the *in vitro* trypanocidal activity of some fractions for 16 medicinal plants, collected from the northern part of Iran, has been reported.
Methods: Aerial parts of the plants were dried carefully and followed by extraction with hexane and methanol, successively, by maceration at room temperature. Different concentrations of the plant extracts in ethanol were investigated against the epimastigotes of *T. cruzi*. The movement of epimastigotes was observed under a microscope. We assumed that immobilized organisms were died. The negative control contained ethanol in the same proportion utilized to dissolve the drugs. Each assay was performed in duplicate together with gentian violet as a positive control.
Results: Results show that hexane extracts of *Rubus hyrcanus* and *Salvia sclerae* have been observed the most activity against the epimastigotes of *T. cruzi* (MLC = 12.5 µg/ml). None of methanol fractions shows trypanocidal activity except *Salvia sclerae* (MLC = 50 µg/ml). *Echium amoenum, Satureja macrantha, S. atropatana* and *Stachys laxa* did not display activity lower than 100 µg/ml in both hexane and methanol extracts.
Conclusions: Some of Iranian medicinal plants (*Salvia sclerae, Marrubium vulgare* and *Rubus hyrcanus*) could be the promising source of active components against the epimastigotes of *T. cruzi* and need to further phytochemical and pharmacological studies.

Keywords: Trypanocidal activity, *Salvia sclerae, Rubus hyrcanus*

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Introduction

Some of medicinal plants are a potential source of new drugs to improve the treatment of Chagase disease whose treatment is still a challenge and leading to approximately 400,000 deaths per year. Chagas disease is caused by the flagellete protozoan *Trypanosoma cruzi* (Trypanosomatina) [1]. Trypomastigotes ingested by the insect differentiates in to the proliferative epimastigote and then to metacyclic trypomastigotes. The latter form undergoes differentiation in to 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 drugs are available, benznidazole and nifortimox, possess severe side effects and their activity is limited to the acute phase [3].

Literature reviews show that until recently, the *in vitro* trypanocidal activity of herbal extracts belonging to the families Meliaceae and Rutaceae has been evaluated. Among them, leaves of *Conchocarphus heterophyllus* and branches of *Trichilia ramalhoi* were the most active [1]. The extracts of the plants used in traditional Chinese medicine were tested *in vitro* with the epimastigote of *T. cruzi,* of which, *Lithospermum erythrorhizon*, *Saussurea lappa*, *Melia toosendan* and *Cinnamomum cassia* showed the greatest inhibitory activity of 100% [4]. Crude extracts (in the concentration 500 µg/ml) of *Bertholletia excelsa* stem barks were tested and showed significant *in vitro* trypanocidal activity against trypomastigote of *T. cruzi* [5]. Also, crude hydro alcoholic extracts and several fractions of 13 plants from Brazilian Atlantic Rain Forest were tested against epimastigote and trypomastigote forms of *T. cruzi* and show a promising *in vitro* activity [6].

Previously, we reported the trypanocidal activity of some Iranian medicinal plants and isolated many active constituents of them. The most effective of those plants was *Dracocephalum kotschyi* and *D. subcapitatum* [7, 8, 9]. In this work, we assessed the effect of several extracts obtained from 16 plants, used in Iranian traditional medicine, against the epimastigotes of *T. cruzi*, the causative agent of chagas disease.

Experimental

Plant materials

All the plants were collected from northern part of Iran, including west Azerbyjan (near Uroomieh), Gilan (Ganjeh), Ghazvin (Telegan mountains), Golestan (National Park) and Mazandaran (Savad Kuh) provinces) in May and June (2004) during full flowering stage and identified by Dr. A. R. Naghinejad. The voucher herbarium specimens were deposited at the herbarium of Medicinal Plants Research Center housed in Faculty of Pharmacy, Tehran University of Medical Sciences.

Extractions of the plants

Aerial parts of the plants were dried and reduced to small pieces, followed by extraction with hexane and methanol, successively, by maceration (two time for each solvent and 24 h for each time) at room temperature. Then the solvents evaporated under reduced pressure to obtain the concentrated extracts and dried under vacuum in order to give dried powder of the extracts.

Evaluation of anti- epimastigote activity

Epimastigotes of *T. cruzi* (Tulahuen strain, prepared from Kyoto University, Japan) were

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kept in GIT medium (Wako Pure Chemical Industrial, Ltd, Osaka, Japan) 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). Test samples contained different concentrations of the plant extracts prepared using serial dilution (2.5–200μg/ml). All samples were incubated at 27 ˚C for 24 h. The movement of epimastigotes was observed under a microscope. We assumed that immobilized organisms were dead. The control contained ethanol in the same proportion as used to dissolve the extracts. Each assay was performed in duplicate. Minimum lethal concentration (MLC, concentration on which, all epimastigotes were dead) of each fraction was performed. Gentian violet (MLC = 6.3 μM) was used as a positive control [8, 9].

Results and Discussion

The results of *in vitro* trypanocidal activity of some Iranian medicinal plants are summarized in table 1. In this study, we prepared two different extracts (hexane and methanol) of each plant except two species of *Achillea*. Because, the hexane extract of those mentioned plants was too waxy and not dissolved in safely amounts of DMSO (bellow 1%) for epimastigotes as a co-solvent, we tried to prepare ethyl acetate extract.

Table 1. Comparative results of trypanocidal activity from different extracts of the plants against the epimastigotes of *T. cruzi*.

NO	Plant name	Family	Hexane MLC (μg/ml)	MeOH MLC (μg/ml)	AcOEt MLC (μg/ml)
1	*Achillea talagonica*	Asteraceae	-	200	50
2	*Achillea tenuifolia*	Asteraceae	-	200	50
3	*Tanacetum polycephalum*	Asteraceae	50	200	-
4	*Rubus hyrcanus*	Rosaceae	12.5	>200	-
5	*Rubus discolor*	Rosaceae	50	200	-
6	*Onosma bulbotrichum*	Boraginaceae	50	>200	-
7	*Echium amoenum*	Boraginaceae	200	100	-
8	*Marrubium vulgare*	Lamiaceae	25	100	-
9	*Stachys laxa*	Lamiacaeae	100	100	-
10	*Thymus kotschyanus*	Lamiaceae	50	100	-
11	*Leonurus cardiac*	Lamiaceae	50	100	-
12	*Salvia sclerae*	Lamiacaeae	12.5	50	-
13	*Satureja mutica*	Lamiacaeae	50	100	-
14	*Satureja atrapatana*	Lamiacaeae	100	100	-
15	*Satureja macrantha*	Lamiacaeae	100	200	-
16	*Satureja spicigera*	Lamiacaeae	50	100	-

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Results show that none of methanol fractions displayed trypanocidal activity except *Salvia sclerae*. Hexane extracts of *Rubus hyrcanus* and *Salvia sclerae* have been observed the most activity against the epimastigotes of *T. cruzi* (MLC = 12.5 µg/ml). Hexane fraction of *Marrubium vulgare* was active with MLC = 25 µg/ml. Hexane extracts of *Tanacetum polycephalum*, *Rubus discolor, Onosma bulbotrichum, Thymus kotschyanus, Satureja mutica* and *S. spicigera* showed effects with minimum lethal concentration at 50 µg/ml. Ethyl acetate fractions of *Achillea* species were effective as well. Among the 16 plants, *Stachys laxa, Echium amoenum, Satureja macrantha* and *S. atropatana* did not represent activity in any fraction tested.

There are a few reports about the presence of pentacyclic triterpenes like oleanolic acid and ursolic acid in the *Rubus chingii* and *Salvia sclarea* extracts [10, 11]. Also there are some reports representing trypanocidal activity of ursan skeleton [12, 13], so it seems that trypanocidal activity of hexane extracts of *Salvia sclarea* and *Rubus hyrcanus* is some part due to the presence of these triterpene acid components.

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