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BIOLOGICAL ACTIVITY OF *ACHILLEA BIEBERSTEINII* AND *A. MILLEFOLIUM* FROM IRAN

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ABSTRACT

In this study, biological activity of some fractions for *Achillea biebersteinii* and *A.millefolium* (Compositae) was evaluated *in vitro* against the epimastigotes of *Trypanosoma cruzi*, the parasitic agent of Chagas disease. Diethyl ether fractions of both plants were the most active fraction (MLC= 12.5 μMl). The trypanocidal activity seems to be decrease by fractionation using MeOH and water as solvents. The results obtained from biological assay revealed that *Achillea* could be a source of active trypanocidal compounds. In addition, the preliminary phytochemical studies showed that the active fractions were rich of terpenoids and flavonoids.

Key words: *Achillea biebersteinii*, *Achillea millefolium*, Compositae, *Trypanosoma cruzi*

INTRODUCTION

Higher plants are a potential source of new drugs to improve the treatment of American Trypanosomiasis (Ambrozin *et al*., 2004; Coura & Castro, 2002). Currently therapy is unsatisfactory, because a few drugs are available, benzidazole and nifortimox, possess severe side effects and their activity is limited to the acute phase (Nogueda- Torres et al., 2001).

Until recently, Rutacea, Meliaceae, Simaroubaceae and Burceraceae families have been studied in order to find active compounds against *Trypanosoma cruzi*, the causative agent of Chagas disease (Ambrozin *et al*., 2004). The genus *Achillea* (Compositae), generally called Bumadaran in Iran, is well known for medicinal properties such as anthelmintic, anti-inflammatory and anti-microbial effects (Zargari, 1992; Rustaiyan *et al*., 1999). *Achillea millefolium* L.and *A. biebersteinii* Afan. are two of nineteen herbaceous species growing in north of Iran (Huber-Morath, 1989). There is no paper to investigate anti-trypanosoma effect of *Achillea* species, therefore, we decided to examine the activity of some fractions from Iranian *Achillea* on *T. cruzi.*

MATERIALS AND METHODS

Plant materials

Aerial parts of *A. millefolium* and *A. biebersteinii* were collected from KordKooy 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 at the private herbarium of Dr. H. Akhani, housed at the Department of Biology, Faculty of Sciences, Tehran University.

Fractionation of 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 at room temperature for 72 h. This process was repeated on the marc with ethyl acetate, 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 of extracts. The yields of fractionation are described in Table I.

In vitro determination of trypanocidal activity

Trypanocidal assay was performed as described previously. Gentian violet is used as a positive control (MLC = 6.3 μM) (Kiuchi *et al*., 2002).

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Preliminary phytochemical evaluation of the fraction: Standard phytochemical screening tests were carried out for various constituents of the fractions of *A. millefolium* and *A.biebersteinii* according to the methods of Trease and Evans (1983). The presence or absence of saponins, tannins, alkaloids, glycosides, flavonoids and terpenoids were observed using these methods.

RESULTS AND DISCUSSION

In the present study, the trypanocidal activity of 8 fractions (diethyl ether, ethyl acetate, methanol and water extracts) from two species of *Achillea* (Compositae) was evaluated. Table I, summarizes the results obtained from fractionation and biological assay. Preliminary phytochemical tests showed that diethyl ether fractions consist of terpenoids. Flavonoids and terpenoids can also be found in ethyl acetate fractions. Flavonoids, tannins and glycosides were present in the methanol and aqueous fractions. In addition, aqueous fractions consist of saponine components. Table I shows that diethyl ether and ethyl acetate fractions of both plants were active against *T. cruzi*, on the other hand etheric parts of both plants were the most active fraction (MLC= 12.5 µMl). MeOH fraction of *A. biebersteinii* showed anti-epimastigote effect better than *A. millefolium*.

Biological activity seems to be reduced by fractionation using more polar solvents (Table I). Among several fractions, only aqueous fractions of the two plants show no activity at concentrations used. It is possible that the activity of *Achillea* is associated with different constituents from several groups of plant metabolites. For example, until recently, we have reported the isolation of some trypanocidal fravonoids (methoxylated flavonols) and terpenoids (monoterpene aldehydes and triterpenoids) from *D. kotschyi* (Gohari *et al.,* 2003; Saeidnia *et al.,* 2004b) and *D. subcapitatum (*Saeidnia *et al.,* 2004a). Also, some trypanocidal diterpenoids were isolated and identified from *D. komarovi* (Uchiyama *et al.,* 2003; Uchiyama *et al*., 2004). *Achillea* is well known as a source of methoxylated flavonoids (artemetin or salvigenin) and triterpenes (derivatives of ursan or oleanan type) which could be the promising source of biologically active components (Balboul *et al.,* 1997; Wollenweber *et al*., 1981).

Table 1. Yields of extraction and trypanocidal activities for some fractions from *Achillea millefolium* and *A. biebersteinii.*

Species	solvents	yields (%w/w)	MLC* (µg/ ml)
Achillea millefolium	diethyl ether	1.95	12.5
	ethyl acetate	1.00	25.0
	methanol	13.56	100
	water	8.72	>100
A. biebersteinii	diethyl ether	2.5	12.5
	ethyl acetate	2.32	25.0
	methanol	16.64	50
	water	9.18	>100

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