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CYTOTOXICITY OF *ACHILLEA TALAGONICA* BOISS. AND *A. TENUIFOLIA* LAM.

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ABSTRACT

In this study, we examined the cytotoxic activity of the ethyl acetate, methanol and aqueous methanol extracts of the aerial parts of two species of *Achillea* using Brine Shrimp Cytotoxicity Assay. Results showed that ethyl acetate and methanol extracts of *A. talagonica* and *A. tenuifolia* had a cytotoxic effect against the larva of *A. salina*. The minimum lethal concentrations of aqueous methanol extracts of both plants were more than 1000 μM. EtOAc extract of *A. talagonica* was the most effective extract (LC_{50} = 413 μM) among all fractions.

Key words: *Achillea talagonica, Achillea tenuifolia,* Compositae, *Artemia salina*

INTRODUCTION

Achillea species have been used in traditional medicine since the Trojan war (Weyerstahl *et al.*,1997). The genus *Achillea* comprises more than 100 species distributed world wide (Bremer, 1994). Many species including *Achillea talagonica* and *A. tenuifolia* are widespread in Iran mainly in north and west parts (Huber-Morath, 1989).

In Persian traditional medicine, the consumption of extracts for *Achillea* species in the treatment of skin inflammation, wound, fever, ulcers and hemorrhoid has been reported (Zargari, 1992). Until recently, we have reported the immunosuppressive activity of the aqueous extract for *A. talagonica* which is endemic species of Talegan mountains (Rezaeipoor *et al.*, 1999). Antifungal activity of *A. tenuifolia* against the *Trycophyton schoenleinii*, *T. mentagrophytes* and *T. verucosum* has been also determined (Amin *et al.*, 2002). Antioxidant activity of the methanolic extract for later plant against linoleic acid peroxidation has been examined and more than 80% (using 40 μg of extract) peroxidation inhibition was shown (Souri *et al*., 2004). There is no report on cytotoxic effect of these species therefore we decided to study the brine shrimp lethality of some fractions for these *Achillea* plants on the larva of *Artemia salina.*

MATERIALS AND METHODS

Plant materials

Aerial parts of *Achillea talagonica* Boiss. and *A. tenuifolia* Lam. were collected from Talegan area and north-west of Tehran (Abiek, Karaj highway), respectively, in July 2001 (during full flowering stage) and identified by I. Mehregan and M. Kamalinejad. The voucher herbarium specimens were deposited in the Herbarium of the Faculty of Pharmacy, Mazandaran University of Medical Sciences .

Fractionation of extracts

Aerial parts (flowers, leaves and stems) of the plants (700 g for *A. talagonica* and 180 g for *A. tenuifolia*) were dried carefully and reduced to small pieces, followed by extraction three times with ethyl acetate by percolation at room temperature for 72 hours. This process was repeated on the marc with methanol and aqueous methanol (50%), 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. The yields of fractionation are described in Table I.

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Brine Shrimp Lethality Assay

The method described by Mongelli *et al*. (1996) was adopted to study the general and cytotoxic activity of the compounds (1996). Water life brand brine shrimp (*A. salina*) eggs were purchased from the Shilat Center (Tehran). The eggs were hatched in a flask containing 300ml artificial seawater made by dissolving distilled water. The flask was well aerated with the aid of an air pump, and kept in a water bath at 29-30 ˚C. A bright light was left on. The nauplii hatched within 48h. The methanol and water extracts were dissolved in normal saline. Tween-80 was used as a co-solvent for dissolving EtOAc and MeOH extracts. Different concentrations were obtained by serial dilution. Solution of each concentration (500 µl) was transferred into clean 24 wells plates via a pipette, and aerated seawater including 10-20 nauplii (500 µl) was added. A check count was performed, and the number alive noted after 24h. The mortality end point of the bioassay was determined as the absence of controlled forward motion during 30 sec of observation. The controls used were tween, seawater and a well-known cytotoxic alkaloid, berberine hydrochloride (LC_{50} = 26 µM). Lethality percentages were determined and LC_{50} calculated based on Probit Analysis with 95% of confidence interval.

RESULTS AND DISCUSSION

In the present study, the cytotoxic activity of 6 fractions (ethyl acetate, methanol and aqueous methanol (50%) extracts) for two species of *Achillea* (Compositae) was evaluated. Results show that EtOAc and MeOH extracts of both *A. talagonica* and *A. tenuifolia* could inactivate the forward motion of the active larvae of *A. salina*. The minimum lethal concentrations of aqueous methanol (50%) extracts of plants are more than 1000 µM. Actually, high polarity extracts of species showed low cytotoxic activity. Biological lethality seems to be reduced by fractionation using more polar solvents (Table I).

It is possible that the activity of *Achillea* is associated with different constituents of terpenoids and/ or methoxylated flavonoids which are frequently found in the *Achillea* species (Viera *et al.,* 1997; Wollenweber *et al*.,1987; Falk *et al.,* 1975; Balboul *et al.,* 1997). Bipolar amino acid derivatives of achilleine such as choline that can be found in aqueous methanol extrat of *A. talagonica* (Saeidnia *et al.,* 2004), and also glycosylated phenolic and flavonoid constituents are no probably resposible for cytotoxic activity of *Achillea*, because the high polar extracts were inactive.

Table I. Yiels of fractionation and LC_{50} of each fraction resulted from Brine Shrimp Lethality Test on *Achillea talagonica* and *A. tenuifolia*.

Plant species	Solvets	Yields(v/v %)*	LC_{50}(µM)
Achillea talagonica	Ethyl acetate	1.3	413
	Methanol	3.6	752
	Water-MeOH (50%)	6.4	>1000
Achillea tenuifolia	Ethyl acetate	5.1	534
	Methanol	7.2	956
	Water-MeOH (50%)	9.3	>1000

* based on dry weight of plant samples

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