A SEARCH FOR BETAINE COMPOUNDS WITHIN THE IRANIAN ACHILLEA

S. Saeidnia†, A. R. Gohari† and N. Yassa‡

† Department of Pharmacognosy, Faculty of Pharmacy, Medical Sciences University of Mazandaran, Sari, Iran
‡ Department of Pharmacognosy, Faculty of Pharmacy, Medical Sciences University of Tehran, Tehran, Iran

ABSTRACT

In order to determine the presence or variation of betaine components within Achillea plants, 5 different species of Achillea viz., A. millefolium, A. tenuifolia, A. conferta, A. biebersteinii and A. talagonica, were collected from different regions of Iran and screened semi-quantitatively on TLC using typical reaction with dragendorff reagent. Among them, only above ground parts of A. talagonica showed presence of choline as the main nitrogen containing constituent. Choline was isolated from flowering heads of A. talagonica and identified with spectroscopic data of NMR.

Key words: Achillea, compositae, betaines, achilleine, choline, quaternary amine

Of the about 100 species for Achillea (compositae), distributed in the north hemisphere, 19 species are grow in Iran (Huber-Morath, 1989; Bremer, 1994). This genus is a group of hard to distinguish species and subspecies (Rustaiyan et al., 1998; Ochir et al., 1991). Fifty years ago, Miller and Chow (1954) isolated and identified a quaternary amine, achilleine, from A. millefolium L. which was found to reduce the clothing time of blood. Betaines, bipolar amino acid derivatives of achilleine such as betaine, stachydrine and choline are similar to the human biliary salts so they may contribute to the cholagogic effects (Blumenthal et al., 2000). Achillea, named Bumadaran in Persian, has been used traditionally as a chologogue agent, anti- rheumatic pain and for reducing fever (Zargari, 1992).

There is only one report on distribution of betaines in A. millefolium group, growing in Austria (Mehlfuhrer et al., 1997). Therefore this study was carried out which describes presence or variation of betaines within some species of Achillea in Iran.

Achillea samples (3-5 individuals for each species) were collected during full flowering stage (July) and then autumn season (October) from different regions of Iran. Vouchers of A. talagonica Boiss.: 2001, A. tenuifolia Lam.: 1999, A. conferta DC.: 2000 are deposited in the Herbarium of the Faculty of Pharmacy, Mazandaran University of Medical Sciences and identified by I. Mehregan and M. Kamalinejad. Other vouchers of A. millefolium L.: 2000, A. biebersteinii Afan.: 2000 are deposited in the private Herbarium of Dr. H. Akhani housed in the Department of Biology, Faculty of Sciences, Tehran University and identified by Dr. H. Akhani.

Above and underground parts of air- dried flowering plants (each 100g) were extracted with 50% MeOH by percolation at room temperature and then hot water consecutively (Mehlfuhrer et al., 1997). After removal the solvents under reduced pressure, the residues were dried under freeze dryer to obtain powdered crude extracts.

In order to find betaines in the samples, TLC (silica gel GF254, Merck) pre-coated plates (20 x 20) were used and CH$_3$Cl$_2$: MeOH: conc. NH$_3$ (5:4:1) utilized as mobile phase. About 10 mg of all extracts and authentic samples of betaines were dissolved in 1 ml of distilled water and applied on TLC. Betaines were detected as purple and red spots by spraying dragendorff reagent (Wagner and Blant, 1996).

Crude extract (1g) from aerial parts of A. talagonica were dissolved in Methanol 50% in order to subject to paper chromatography (Whatman:3mm chromatographic paper) in the descending chromatocab using BAW (n-butanol: acetic acid: water, 4:1:5) as eluent. Only one line was detected as purple after spraying of dragendorff reagent. That line was carefully separated from other lines, cut into small pieces and put in Methanol 50% and then hot water to release from papers. The extracted residue was concentrated for further purification and chromatographed again over silica gel 60 (Merck) column chromatography with CH$_3$Cl$_2$: MeOH (5:3) containing 0.5 part of acidic water (pH from 2 to 3) as eluent to obtain the pure nitrogen containing compound. Monitoring of paper and column chromatography was performed using TLC (see screening on TLC).

$^1$H and $^13$C- NMR was recorded in C$_6$D$_5$N on a Bruker 500MHz and a Varian 400 MHz (in D$_2$O). TMS used as internal standard. $^1$H – NMR (pyridine- d$_5$) δ: 3.89 (2H, m, H-1), 4.28 (2H, m, H-2), 3.47 (N- methyl groups, 9H,S). $^{13}$C- NMR (pyridine- d$_5$) δ: 56.24 (C-1), 68.25 (C-2), 53.92 (3x N- methyl group, C-3).

* Address for correspondence: Dr. Soodabeh Saeidnia, Faculty of Pharmacy, Medical Sciences University of Mazandaran, Salman Farsi St, Sari, Iran, PO Box: 48175-861, Tel: +98-151-3259802, Fax: +98-151-3254060, E-mail: soodabehsaeidnia@hotmail.com
By TLC screening, some individuals of 5 different *Achillea* species were tested regarding to presence or variation of betaine components (betaine, achilleine, choline and stachydrine). Methanol 50% and hot water extracts of aerial parts (stems, flowers and leaves) and under ground parts (roots) of plants were analyzed separately. Qualitative evaluation was performed after using dragendorff reagent. Results showed that all individuals belonging to *A. talagonica* (section: *Santolinoidea*) showed qualitatively identical betaines reaction. In these samples, only choline represented the major component. The flowering heads showed considerable amount of choline, but there was not any kind of betaine components in the under ground parts of *A. talagonica*. Except *A. talagonica*, betaines could not be detected in all extracts (either MeOH 50% or hot water extracts of under and aerial parts) for different samples of Iranian *Achillea* even in sect. *Millefolium* (*A. millefolium* and *A. bieberstainii*) and another species of sect. *Santilinoidea* (*A. conferta*).

From air-dried aerial parts of *A. talagonica* a quaternary amine compound was isolated. This component gave a typical reaction after treatment by reagent and detected as a purple spot in the same Rf compare to choline. The NMR spectral data of this compound in D2O showed good agreement with those of reported by Mehlfuhrer et al. (1997). Although, the previous study reported the presence of at least one betaine type compound in each species belonging to *Millefolium* group, our results indicated that betaines are not widespread within all species of *Achillea*,

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**REFERENCES**


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