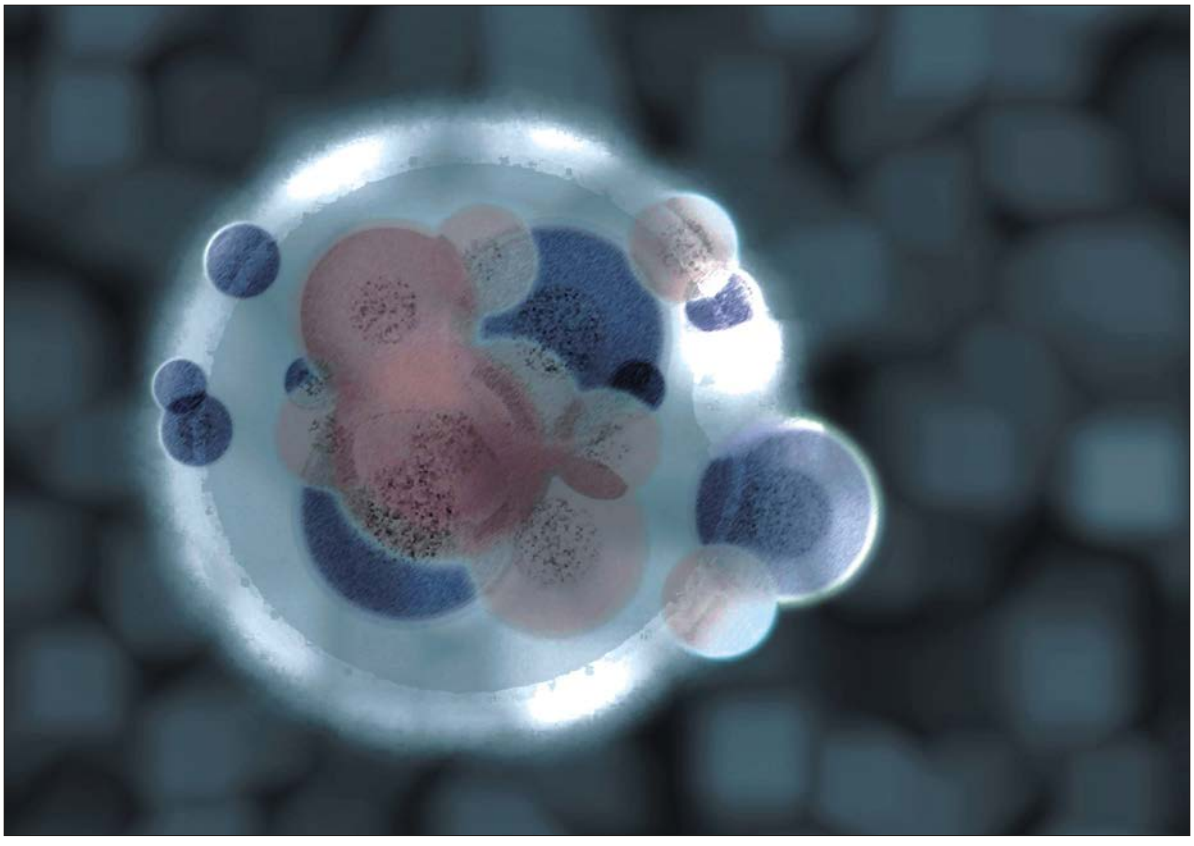


Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 8 (3): 549-555, 2008
ISSN 1727-3048

Evaluation of Some Iranian Wild Species from Valerianaceae as Commercial Sources of Valepotriates

[1]Ebrahimzadeh Hassan, [2]Radjabian Tayebeh, [1]Ekhteraei Tousi Samaneh,
[1]Niknam Vahid and [3]Mozaffarian Vali-O-Allah
[1]School of Biology, University College of Sciences, University of Tehran, Tehran, Iran
[2]Department of Biology, Faculty of Sciences, Shahed University, Tehran, Iran
[3]Research, Education and Extension Organization,
Research Institute of Forests and Rangelands, Tehran, Iran

Abstract: The aim of the present study was to characterize some Iranian wild species of Valerianaceae with respect to their contents of valepotriates. Identification of these compounds which are known as reliable markers in Valerianaceae family was achieved using TLC and UV-spectrophotometry methods. Valepotriates makes substantial contribution to the sedative effect of valerian. Separation of these compounds was performed by TLC using a ternary mobile phase of toluene-ethyl acetate - methyl ethyl ketone (80:15:5 v/v) on silica gel $HF_{254+366}$ plates. Separated bands were observed in ultraviolet light at 254 nm. For bands revelation, 2, 4-dinitrophenylhydrazine (DNPH) solution spray was used at visible light. For the quantitative determination of valepotriates a standardized product (Valmane®) was used to plot two calibration curves. One calibration curve was drawn in the concentration range of 5-156 mg L^{-1} for determination of valtrate + acevaltrate amounts. The second one was linear in the range of 5-52 mg L^{-1} for didrovaltrate quantification. The qualitative and quantitative determinations were carried out in all organs of *Valeriana sisymbriifolia* Vahl, *Valeriana alliariifolia* Adams and *Centranthus longiflorus* Stev. (Iranian wild plant species) and commercial *Valeriana officinalis* L. sample. Results showed that didrovaltrate amounts (dry weight basis) ranged from 0.13% in inflorescence of *V. alliariifolia* to 3.51% in leaf of *C. longiflorus*. Also acevaltrate + valtrate contents varied from minimum 0.06% in stem of *V. sisymbriifolia* to maximum 10.85% in root of *V. alliariifolia*. Valepotriates contents in these species were compared to those of commercial *Valeriana officinalis* L. root and rhizome samples (didrovaltrate 1.99-3.23%, valtrate + acevaltrate 5.1-9.26%).

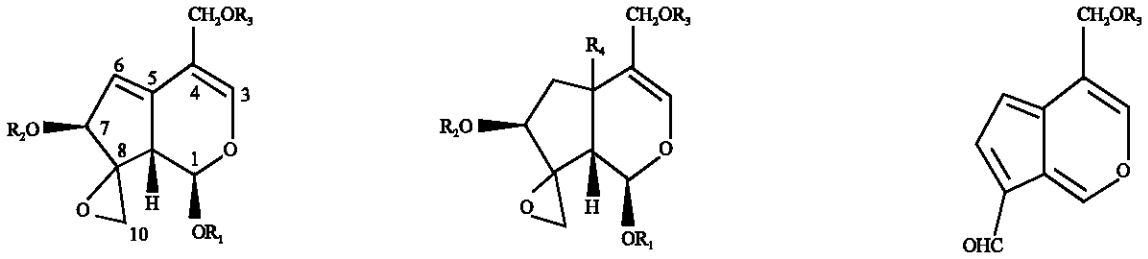
Key words: Valtrate, acevaltrate, didrovaltrate, *Valeriana* L., *Centranthus* DC.

INTRODUCTION

Valerianaceae contains about 350 species of almost cosmopolitan distribution, mostly at high elevations (Bell and Donoghue, 2005). Six species of *Valeriana* L. and one species of *Centranthus* DC. grow as wild populations in different geographical areas of Iran (Moussavi-Allashlou, 2001). The roots and rhizomes of valerian (*V. officinalis* L., in broad sense) contain several compounds with demonstrable pharmacological activities. These include the essential oil and its sesquiterpenoids (valerenic acid derivatives), epoxy iridoid esters (valepotriates) and their decomposition products such as baldrinal and homobaldrinal, amino acids (arginine, GABA, glutamine, tyrosine) and alkaloids (Upton *et al*., 1999). Valepotriates belong to the iridoid group, which contains monoterpenes characterized by a cyclopenta-[c]-pyranoid skeleton. Iridoids occur mainly in glycosidic form in a wide range of plant families. Valepotriates are very specific iridoids, not glycosdidic and lipophilic esters of triols derived from iridane (Fig. 1). Valepotriates are common in Valerianaceae plant family and considered to be one of the main groups responsible for the sedative activity of valerian preparations. Based on their chemical structures, valepotriates can be divided into four main groups; namely dienes (e.g., valtrate and acevaltrate), monoenes (e.g., didrovaltrate), valtrate-hydrines and desoxy monoenes (Bos *et al*., 2002).

Valerian root has already been used by the Greek and the Roman physicians as a diuretic, anodyne and spasmolytic agent. In the 17th century it was used to treat epilepsy. Its current use as a mild sedative dates back to

Corresponding Author: Tayebeh Radjabian, Department of Biology, Faculty of Sciences, Shahed University, Tehran-Qom Freeway, Opposite to Imam Khomeini Holy Shrine, P.O. Box 18155-159, Tehran, Iran
Tel: + 98 021 55277549 Fax: + 98 021 55277639



	R_1	R_2	R_2		R_1	R_2	R_3	R_4		R_3	
1	Iv	Iv	Ac	Valtrate	Iv	Ac	Iv	H	Didrovaltrate	Ac	Baldrinal
2	Ac	Ac	Iv	Isovaltrate						Iv	Hamobaldrinal
3	Aiv	Iv	Ac	Acevaltrate							

Fig. 1: Structure of some valepotriates and their decomposition products (Bos *et al*., 2002). Ac: Acetyl, Iv: Isovaleryl, Aiv: Acetoxyisovaleryl

the 18th century. Nowadays, valerian preparations are used primarily to treat light forms of neurasthenia and emotional stress. Further indications are disturbances in falling asleep and cramping pains in the gastro-intestinal tract, as a consequence of tension (Bos, 1997). Recently several clinical studies have presented some biological activities of valerian root extracts and introduced the plant as anti-HIV (Murakami *et al*., 2002), sleep aid (Francis and Dempster, 2002; Fernández *et al*., 2004), tranquilizer (Houghton, 1999), antidepressants (Andreatini *et al*., 2002; Miyasaka *et al*., 2006; Hattesohl *et al*., 2008), anticoronaryspastic, antihypertensive and antibronchospastic (Circosta *et al*., 2007) agent. So far, some important medicinal phytochemicals (valerenic acids, essential oils, valepotriates and etc.) have been quantitatively estimated by various analytical methods in different organs of *Valeriana* L. species (Gao and Björk, 2000; Silva *et al*., 2002; Navarrete *et al*., 2006) and commercial preparations of valerian (Shohet *et al*., 2001). In this study, some valepotriates were detected by Thin Layer Chromatography (TLC) and quantified by UV-spectrophotometry in different organs of *V. sisymbriifolia* Vahl., *V. alliariifolia* Adams and *Centranthus longiflorus* Stev. The results were compared to those of a commercial cultivar of *V. officinalis*. To our knowledge the present research is the first more detailed phytochemical study on some Iranian populations of wild species in Valerianaceae.

MATERIALS AND METHODS

Plant materials: Three ecotypes of *V. sisymbriifolia* were collected from different Iranian geographical locations at north and west regions (locations 1, 2 and 3). Table 1 shows the characteristics of the analyzed species, including geographic coordinate and altitude and harvesting time. Organ samples including root, rhizome, stem, leaf and inflorescence were separated and dried at 20±3°C in a ventilated dryer for 12-15 h. All samples were powdered and stored at -20°C, until analysis. The dried roots and rhizomes of *V. officinalis* were prepared from a valerian grower.

Table 1: Characteristics of the analyzed species

Species	Locality	Herbarium No.
V. sisymbriifolia 1	Prov. Mazandaran: Kelardasht area, just after Roudbarak, on the crevices of rocks near the road, 2000 m, May, 2006	Tousi and Zarrei 701[a]
V. sisymbriifolia 2	Prov. Mazandaran: Chalus road, after Kandavan tunnel, Siah-Bisheh, mts in from of the village, 2400 m, May, 2006	Tousi and Zarrei 702[a]
V. sisymbriifolia 3	Prov. Qazvin: Siallan monntains, 3800 m, Aug. 2006	Mozzaffarian 87596 (TARI)
V. alliariifolia	Prov. Azarbayejan: Soluk water-fall, Aug. 2006	Tousi 87595 (TARI)
C. longiflorus	Prov. Azarbayejan: Khoy, Razy, Aug. 2006	Zarre 36771 (TUH)
V. officinalis	Prov. Tehran: field grown samples	Tousi 707[a]

[a]: Indicates these vouchers were deposited in Central Herbarium of Shahed University

Sample preparation for valepotriates qualitative test (Spot test): This test is specific for valepotriates. Carefully powdered plant organ samples (0.2 g), were shaken with 5 mL dichloromethane (DCM) for 1 min in a laboratory test tube. After 5 min standing, samples were filtered and filter papers were washed with another 2 mL of DCM fraction. The final collected filtrate solution was dry evaporated under nitrogen flow for the minimum time necessary to remove the solvent. The residue was dissolved in 0.2 mL of methanol. A volume (3 mL) from a 1:1 mixture of cold acetic acid and hydrochloric acid was added to 0.1 mL aliquot of the methanolic extract. After shaking, presence of valepotriates was revalidated by the detection of a blue color within 15 min (Upton *et al*., 1999). The *in vivo* spot test was carried out with some transverse sections of root and collar of *V. sisymbiifolia*. For this test, we prepared very thin transverse sections of these organs. Then the HCl-acetic acid reagent was added to these sections

(Violon *et al*., 1983). After the emergence of blue color that was characteristic of valepotriates existence in samples, they were rinsed with distilled water until acid completely removed.

Sample preparation for TLC analysis: Freshly powdered of plant organs including root, rhizome, stem, leaf, inflorescence (0.2 g) were extracted with 5 mL DCM for 1 min in laboratory test tubes. After 5 min standing, samples were filtered and filter papers were washed with 2 mL DCM fraction. The final collected filtrate solutions were dry evaporated on a water bath at 40°C. The residues were collected in 0.2 mL DCM and transferred into small sample vials. One Valmane dragee (as standard product) containing 80% didrovaltrate, 15% valtrate and 5% acevaltrate, was extracted with 5 mL DCM for 1 min in a laboratory test tube and other extraction steps were continued as mentioned above method for plant materials, except in the final step that the residue was collected in 0.2 mL methanol and then transferred into a small sample vial. One aliquot (10 μL) from all prepared samples was spotted in 10 mm band width on the silica gel $HF_{254+366}$ plates (Merck, Germany). Separation was achieved using a ternary mobile phase containing toluene - ethyl acetate - methyl ethyl ketone (80:15:5 v/v). Separated bands were detected at 254 nm and then revelation of them was carried out with 2, 4-dinitrophenylhydrazine (DNPH) solution spray at visible light (Bos *et al*., 2002).

Sample preparation for UV-spectrophotometry: Each 0.2 g dry powdered plant sample was first extracted with 5 mL DCM and then dry evaporated. Residues were recovered into 1 mL methanol. All methanolic extract samples were used to determine the valepotriates amounts using a 10 points calibration curve at 207 nm (for didrovaltrate) and 254 nm (for valtrate + acevaltrate).

Calibration curve: For the quantitative determination of valepotriates, a standardized product (Valmane®) was used to plot two calibration curves. Stock solutions of valepotriates were prepared in methanol. One calibration curve for the determination of valtrate+acevaltrate amounts was in the concentration range of 5-156 mg L^{-1}. The second calibration curve for didrovaltrate quantification was also linear in the range of 5-52 mg L^{-1}. The regression equations for valtrate + acevaltrate at 254 nm and didrovaltrate at 207 nm were $y = 0.0064x + 0.0084$ and $y = 0.0186x + 0.0093$, respectively (y is absorbance, x defines concentration). All lab works have been performed during Autumn-Winter 2006 at the Plant Physiology laboratories of University of Tehran and Shahed University (Tehran, Iran).

Data analysis: All values are expressed as mean±SE of triplicate determinations. Data obtained from UV-spectrophotometry were subjected to one-way ANOVA. Post hoc multiple comparisons between species for valepotriates content were made using Tukey's test. Significance was reported starting at the 0.05 level.

RESULTS

Qualitative determination: The emergence of blue color due to interaction of valepotriates with HCl-acetic acid reagent in different cells of root and collar transverse sections is shown in Fig. 2. Results obtained from TLC detection showed that some valepotriates were also in rhizome samples of Iranian wild species of Valerianaceae and commercial *V. officinalis*. Didrovaltrate with $R_f = 0.53$ and acevaltrate with $R_f = 0.49$ were appeared after application of the DNPH solution spray as colored bands at visible light (Fig. 3). Table 2 shows R_f values and colors of separated bands on TLC plates for rhizome extracts of *V. officinalis*. The order arrangement and colors of these

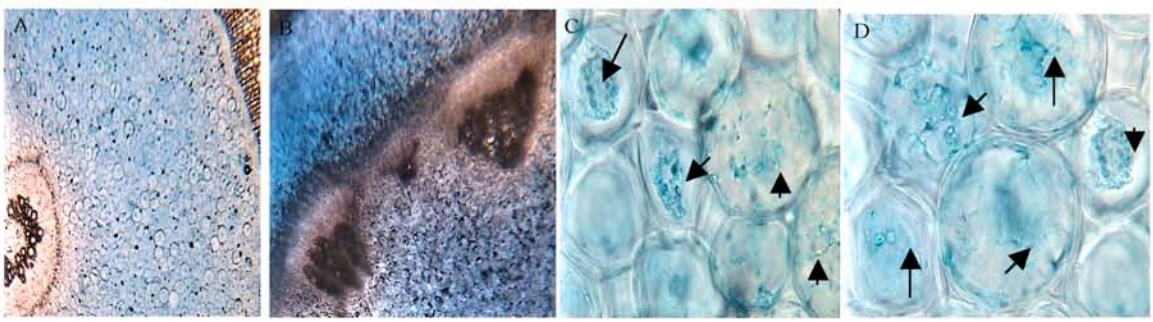


Fig. 2: Valepotriates are characterized as blue droplets in parenchyma cells in transverse sections of root (A, C and D) and collar (B) due to interaction with HCl- acetic acid reagent (magnification 100x; A and B, 400x; C and D)

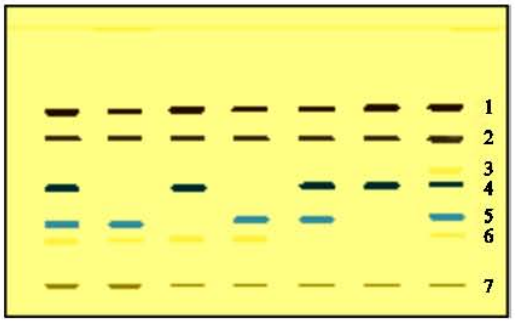


Fig. 3: TLC plate viewed main valepotriates and their decomposition products of rhizome samples at visible light with DNPH reagent. From left to right: *V. sisymbriifolia* 2 (lane 1), *V. sisymbriifolia* 3 (lane 2), *C. longiflorus* (lane 3), *V. alliariifolia* (lane 4), *V. sisymbriifolia* 1 (lane 5), Valmane (lane 6), *V. officinalis* 2 (lane 7). From top to bottom: No. 1: Valtrate (R_f = 0.53), No. 3: Homobaldrinal (R_f = 0.50), No. 4: Acevaltrate (R_f = 0.49), No. 5: IHVD-valtrate (R_f = 0.31), No. 6: Baldrinal (R_f = 0.23) and No. 7: Spotted band on start line

bands were similar to those of reported by the literature (Bos *et al*., 2002). Values of R_f were similar to slightly different depending on the TLC stationary phase polarity. Table 3 shows the results obtained from the TLC detection of the main valepotriates and their decomposition products present in different organs of Iranian wild species of Valerianaceae and commercial *V. officinalis*.

Quantification of valepotriates: After sample preparation, the absorbance of methanolic extracts were determined at 207 and 254 nm (Fig. 4). Valepotriates contents were determined through the regression equation of the calibration curves for didrovaltrate at 207 nm and

Table 2: TLC data of the most common valepotriates and baldrinals on silica gel $HF_{254+366}$ obtained from *V. officinalis* rhizome

Component	R_f	Daylight	DNPH	254 nm
IVHD-valtrate	0.31	Not detected	Light-blue	-
Acevaltrate	0.49	Not detected	Blue	+
Homobaldrinal	0.50	Yellow	Grey-brown	+
Baldrinal	0.23	Yellow	Brown	+
Didrovaltrate	0.53	Not detected	Brown	-
Valtrate/isovaltrate	0.63	Not detected	Dark-blue	+

+: Existence, -: Absence of each band

Table 3: Comparative determination of the main valepotriates and their decomposition products in different organs of Iranian wild species of Valerianaceae in comparison to *V. officinalis* by TLC

		Components					
Plant	Organ	Valtrate/isovaltrate	Didrovaltrate	Baldrinal	Homobaldrinal	Acevaltrate	IVHD-valtrate[a]
V. sisymbriifolia	Root	+	+	+	-	+	+
(Location 1)	Rhizome	+	+	-	-	+	+
	Stem	+	+	-	-	-	-
	Leaf	+	+	+	-	+	+
	Inflorescence	+	+	-	-	-	-
V. sisymbriifolia	Root	+	+	+	-	+	+
(Location 2)	Rhizome	+	+	+	-	+	+
	Stem	+	+	-	-	-	-
	Leaf	+	+	+	+	-	+
	Inflorescence	+	+	-	-	+	+
V. sisymbriifolia	Root	+	+	+	-	-	+
(Location 3)	Rhizome	+	+	+	-	-	+
	Stem	+	+	-	-	-	-
	Leaf	+	+	-	-	+	-
	Inflorescence	+	+	-	-	+	-
V. alliariifolia	Root	+	+	+	-	-	+
	Rhizome	+	+	+	-	-	+
	Stem	+	+	-	-	-	+
	Leaf	+	+	+	-	+	+
	Inflorescence	+	+	-	-	-	+
C. longiflorus	Rhizome	+	+	+	-	+	-
	Stem	+	+	-	-	-	-
	Leaf	+	+	+	-	-	+
	Inflorescence	+	+	+	+	+	+
V. officinalis	Root	-	+	+	-	+	+
	Rhizome	+	+	+	+	+	+

[a]: IVHD-valtrate: Isovaleroxyhydroxydidrovaltrate, +: Existence, -: Absence of the materials

Table 4: Mean values contents ± SE of some valepotriates (g/100 g DW) in different organs of Iranian wild species of Valerianaceae and commercial *V. officinalis*

Plant	Organ	Didrovaltrate	Valtrate+Acevaltrate
V. sisymbriifolia	Root	1.251±0.001	7.820±0.025
(Location 1)	Rhizome	1.962±0.006	5.338±0.024
	Stem	0.292±0.001	0.061±0.000
	Leaf	1.596±0.003	1.124±0.003
V. sisymbriifolia	Root	1.236±0.003	7.216±0.003
(Location 2)	Rhizome	1.048±0.070	3.294±0.001
	Stem	0.428±0.001	0.193±0.000
	Leaf	1.405±0.003	0.884±0.003
	Inflorescence	0.645±0.002	0.223±0.000
V. sisymbriifolia	Root	1.594±0.003	9.632±0.016
(Location 3)	Rhizome	0.947±0.002	2.651±0.000
	Stem	0.268±0.002	0.080±0.000
	Leaf	0.487±0.001	0.223±0.000
	Inflorescence	0.628±0.001	0.646±0.002
V. alliariifolia	Root	2.477±0.145	10.855±0.712
	Rhizome	1.666±0.017	9.553±0.003
	Stem	0.623±0.000	0.759±0.000
	Leaf	1.343±0.115	2.385±0.170
	Inflorescence	0.131±0.001	1.091±0.005
C. longiflorus	Rhizome	0.326±0.003	0.614±0.003
	Stem	0.441±0.001	0.539±0.001
	Leaf	3.508±0.001	3.591±0.005
	Inflorescence	1.058±0.001	3.386±0.007
V. officinalis	Root	1.994±0.003	5.002±0.000
	Rhizome	3.234±0.211	9.261±0.407

acevaltrate + valtrate at 254 nm. Then the valepotriates total content was compared between plant organs in different studied plant species (Table 4, Fig. 5).

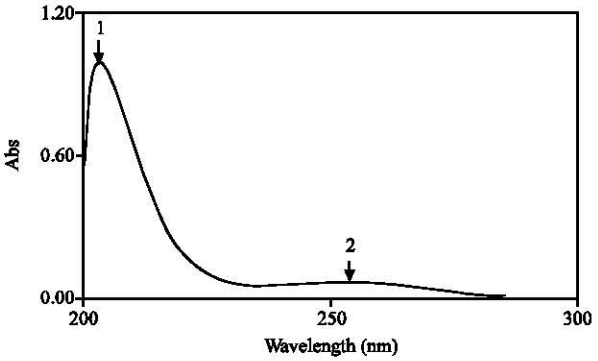


Fig. 4: UV spectrum of *V. officinalis* rhizome methanolic extract, (1) didrovaltrate at 207 nm and (2) acevaltrate + valtrate at 254 nm

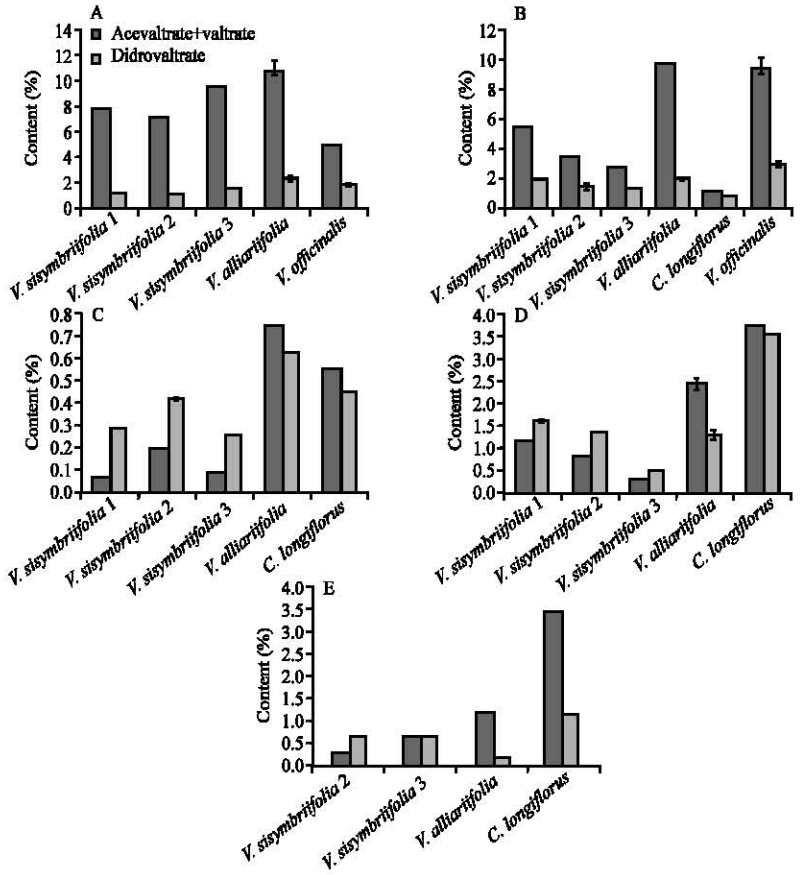


Fig. 5: Comparative analysis of didrovaltrate and acevaltrate + valtrate contents in different organs of the plant samples; (A) Root, (B) Rhizome, (C) Stem, (D) Leaf and (E) Inflorescence. All results are based on dry weight

DISCUSSION

The extraction method of valepotriates from plant raw material is an extremely important process, since these compounds are very unstable. Valepotriates are degraded rapidly, especially in acidic solutions. The main decomposition products of the valepotriates are the yellow-colored baldrinals (Bos *et al*., 2002). Valtrate represents approximately 80-90% of total valepotriates with the remainder consisting of acevaltrate, didrovaltrate and isovaleroxyhydroxydidrovaltrate (IVHD-valtrate).

Present qualitative results from TLC method confirmed the presence of some valepotriates (acevaltrate, valtrate/isovaltrate, IVHD-valtrate, didrovaltrate, baldrinal, homobaldrinal) in studied organs of these plants. By method of TLC with the other stationary and mobile phases, the same components in *V. glechomifolia* (growing in South Brazil) were detected as well (Salles *et al*., 2000). For quantitative assay, Valmane was used. This product, in many parts of Europe, is one of more than 80 products containing valerian preparations which are widely recommended for use as mild sedative agents. Valmane is reported to contain three valepotriates (valtrate, didrovaltrate and acevaltrate) that are claimed to be responsible for the action of this pharmaceutical preparation (Lin *et al*., 1991). Quantitative results showed that didrovaltrate and acevaltrate+ valtrate amounts were ranged from 0.13-3.51% and 0.06-10.85% (dry weight basis), respectively in the Iranian wild species. Valepotriates amounts in these species were compared to those of commercial *V. officinalis* L. root and rhizome samples (acevaltrate+valtrate 5.1-9.26% and didrovaltrate 1.99-3.23%). Results of quantitative survey, in root and rhizome samples, showed higher acevaltrate and valtrate (as diene valepotriates) contents than didrovaltrate (as monoene valepotriates). Meanwhile the amounts of didrovaltrate were higher than acevaltrate and valtrate (significant mean differences, $p<0.05$) in stem and leaf samples of *V. sisymbriifolia* from three different locations. Among the plant organs, roots had the highest mean values of valepotriates contents (acevaltrate+valtrate 8.11%, didrovaltrate 1.71%, $p<0.05$). Root samples of *V. alliariifolia* had higher amounts of valepotriates (acevaltrate+valtrate 10.85%, didrovaltrate 2.47%) than those of the other Iranian wild species and *V. officinalis* (acevaltrate+valtrate 5.01%, didrovaltrate 1.99%). The later species is known as commercial and medicinal source of valepotriates. Among rhizome samples of Iranian wild species, only rhizome of *V. alliariifolia* had comparable amounts of valepotriates (acevaltrate+valtrate 9.55%, didrovaltrate 1.66%) with those of *V. officinalis* (acevaltrate+valtrate 9.26%, didrovaltrate 3.23%). In the leaf and inflorescence samples, the highest amounts (leaf; acevaltrate+valtrate 3.59% and didrovaltrate 3.51%, inflorescence; acevaltrate+valtrate 3.38% and didrovaltrate 1.06%) belonged to *C. longiflorus*. At all, differences were found with respect to the valepotriates composition and content among investigated species and their different populations. On the strength of our results, we can claim that roots of *V. alliariifolia* contain higher amounts of valepotriates than those of *V. officinalis* (Bos, 1997) which are mentioned in European pharmacopoeia. From the result obtained from this study, it seems clear that wild species of *V. alliariifolia* is valuable source of valepotriates.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Z. Bashiri Sadr for the plentiful scientific support about valepotriates and also wish to thank to Dr. Sh. Zarre and Dr. M. Zarrei for collection and identification of the wild plants and Mr. Rashet for kindly providing us with dried rhizome and root samples of *V. officinalis*. This research was supported financially by the Research Councils of University of Tehran and Shahed University (M.Sc. Thesis, Project No. 3923, July, 2006).

REFERENCES

Andreatini, R., V.A. Sartori, M.L. Seabra and J.R. Leite, 2002. Effect of valepotriates (valerian extract) in generalized anxiety disorder: A randomized placebo-controlled pilot study. Phytother. Res., 16 (7): 650-654.

Bell, C.H.D. and M.J. Donoghue, 2005. Phylogeny and biogeography of Valerianaceae (Dipsacales) with special reference to the South American valerians. Organ. Diversity Evol., 5 (2): 147-159.

Bos, R., 1997. Analytical and Phytochemical Studies on Valerian and Valerian Based Preparations. Offsetdrukkerij Ridderprint, Ridderkerk, B.V. (Ed.). Groningen.

Bos, R., H.J. Woerdenbag and N. Pras, 2002. Determination of valepotriates. J. Chromatogr. A., 967 (1): 131-146.

Circosta, C., R. De Pasquale, S. Samperi, A. Pino and F. Occhiuto, 2007. Biological and analytical characterization of two extracts from *Valeriana officinalis*. J. Ethnopharmacol., 112 (2): 361-367.

Fernández, S., C. Wasowski, A.C. Paladini and M. Marder, 2004. Sedative and sleep-enhancing properties of linarin, a flavonoid-isolated from *Valeriana officinalis*. Pharmacol. Biochem. Behav., 77 (2): 399-404.

Francis, A.J.P. and R.J.W. Dempster, 2002. Effect of valerian, *Valeriana edulis*, on sleep difficulties in children with intellectual deficits: Randomised trial. Phytomedicine, 9 (4): 273-279.

Gao, X.Q. and L. Björk, 2000. Valerenic acid derivatives and valepotriates among individuals, varieties and species of *Valeriana*. Fitoterapia, 71 (1): 19-24.

Hattesohl, M., B. Feistel, H. Sievers, R. Lehnfeld, M. Hegger and H. Winterhoff, 2008. Extracts of *Valeriana officinalis* L. s.l. show anxiolytic and antidepressant effects but neither sedative nor myorelaxant properties. Phytomedicine, 15 (1-2): 2-15.

Houghton P.J., 1999. The scientific basis for the reputed activity of Valerian. J. Pharm. Pharmacol., 51 (5): 505-512.

Lin, L.J., G.A. Cordell and M.F. Balandrin, 1991. Valerian-derived sedative agents. 1. On the structure and spectral assignment of the constituents of Valmane using the selective INEPT nuclear magnetic resonance technique. Pharm. Res., 8 (9): 1094-1102.

Miyasaka, L.S., A.N. Atallah and B.G. Soares, 2006. Valerian for anxiety disorders. Cochrane Database Syst. Rev., Issue 4. Art. No. CD004515. DOI: 10.1002/14651858. CD004515. Pub2.

Moussavi-Allashlou, E., 2001. Flora of Iran (Valerianaceae). Research Institute of Forests and Rangelands Publications, Tehran.

Murakami, N., Y. Ying, M. Kawanishi, S. Aoki, N. Kudo, M. Yoshida, E.E. Nakayama, T. Shioda and M. Kobayashi, 2002. New Rev-transport inhibitor with anti-HIV activity from Valerianae Radix. Bioorg. Med. Chem. Lett., 12 (20): 2807-2810.

Navarrete, A., B. Avula, Y.W. Choi and I.A. Khan, 2006. Chemical fingerprinting of *Valeriana* species: Simultaneous determination of valerenic acids, flavonoids and phenylpropanoids using liquid chromatography with ultraviolet detection. J. AOAC. Int., 89 (1): 8-15.

Salles, L., A.L. Silva, S.B. Rech, N. Zanatta and G.L. Poser, 2000. Constituents of *Valeriana glechomifolia* Meyer. Biochem. Syst. Ecol., 28 (9): 907-910.

Shohet, D., R.B. Wills and D.L. Stuart, 2001. Valepotriates and valerenic acids in commercial preparations of valerian available in Australia. Pharmazie, 56 (11): 860-863.

Silva, A.L., S.B. Rech and G.L. Poser, 2002. Quantitative determination of valepotriates from *Valeriana* native to South Brazil. Planta Med., 68 (6): 570-572.

Upton, R., C. Petrone, D. Swisher, A. Goldberg, M. McGuffin and N.D. Pizzorno, 1999. Valerian root, *Valeriana officinalis*, analytical, quality control and therapeutic monograph. American Herbal Pharmacopoeia (AHP) and Therapeutic Compendium.

Violon, C., D. Dekegel and A. Vercruysse, 1983. Microscopical study of valepotriates in lipid droplets of various tissues from Valerian plants. Plant Cell Rep., 2 (6): 300-303.