Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 7 (2): 195-200, 2008
ISSN 1682-3974

Quantification of Valerenic Acid and its Derivatives in Some Species of *Valeriana* L. and *Centranthus longiflorus* Stev.

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Abstract: The present study aims to characterize some Iranian wild species of Valerianaceae with respect to their contents of valerenic acid and its derivatives. Identification of these compounds which are known as reliable markers in Valerianaceae family was achieved using TLC and UV-spectrophotometry methods. Valerenic acid makes substantial contribution to the sedative effect of valerian. Separation of valerenic acid and its derivatives was performed by TLC using a ternary mobile phase of hexane-ethyl acetate-glacial acetic acid (65:35:0.5 v/v) on silica gel $HF_{254+366}$ plates. Spots were detected at 254 and 366 nm and then revelation of them was carried out with HCl-acetic acid reagent followed by anisaldehyde-sulphuric acid reagent spraying at visible light. Quantitative analysis of valerenic acid and its derivatives was performed using UV-spectrophotometry. The calibration curve of authentic valerenic acid was linear in the range of 2-51 mg L^{-1}. Additionally, for a more accurate determination of total valerenic acid derivatives (including valerenic acid, hydroxyvalerenic acid and acetoxyvalerenic acid) extinction coefficient of each compound in ethanol was used. This is the first report for the identification and quantification of valerenic acid derivatives in all organs of *Valeriana sisymbriifolia*, *Valeriana alliariifolia* and *Centranthus longiflorus* in comparison with those of commercial *Valeriana officinalis*. Total valerenic acid derivatives content in different organs of these Iranian wild species ranged from 0.06-1.11% (D.W.). Present results showed that Iranian wild species of *Valeriana* and *Centranthus* DC. may be used as worthy sources of valerenic acid derivatives.

Key words: Valerianaceae, medicinal compounds, TLC, UV-spectrophotometry

INTRODUCTION

Valerianaceae contains about 40 genera and 400 species of almost cosmopolitan distribution, mostly at high elevations (Hidalgo *et al*., 2004; Bell and Donoghue, 2005). Six species of *Valeriana* L. and one species of *Centranthus* DC. grow in Iran (Moussavi-Allashlou, 2001).Valerian root has already been used by the Greek and the Roman physician 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 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). Valerian is the 8th top-selling herbal supplement in North America and in Australia; it is in the top 10 selling retail herbs (Singh *et al*., 2006).

The roots and rhizomes of valerian contain several compounds with demonstrable pharmacological activity. 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). The main non-volatile cyclopentane sesquiterpenes are valerenic acid, acetoxyvalerenic acid, hydroxyvalerenic acid and valerenal. The first two compounds are specific for *V. officinalis*, while the third one is probably an artifact formed during unfavorable storage conditions, e.g. high humidity. Hydroxyvalerenic acid may be produced from acetoxyvalerenic acid by hydration (Bos, 1997). It is yet unclear which group of valerian active substances is responsible for the sedative effect of the plant. In the last two decades, the essential oil and valerenic acid derivatives have become more important, not only from a pharmacological point of view, but also for the quality

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control and standardization of valerian phytomedicines (Hendriks *et al*., 1981, 1985). 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 (Fernández *et al*., 2004), antidepressants (Miyasaka *et al*., 2006), anticoronaryspastic, antihypertensive and antibronchospastic (Circosta *et al*., 2007) agent.

A simple, rapid, cost-effective and accurate thin layer chromatographic method has been developed for separation of valerenic acid (Singh *et al*., 2006). In this study, valerenic acid derivatives 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. and the results compared to those reported in the literature for a commercial cultivar of *V. officinalis*.

MATERIALS AND METHODS

Plant materials: Three populations of *V. sisymbriifolia* were collected from different Iranian geographical locations at north and west regions (locations 1, 2 and 3). Table 1 summarizes the characteristics of the analyzed species, including geographic coordinate, 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.

Sample preparation for TLC analysis: Freshly powdered plant organs including root, rhizome, stem, leaf, inflorescence (0.2 g) were extracted with 5 mL dichloromethane (DCM) for 1 min in a laboratory test tube. After 5 min standing, samples were filtered and filter papers washed with 2 mL DCM fraction. The final collected filtrate was dry evaporated on a water bath at 40°C. The residue was collected in 0.2 mL DCM and transferred into a small sample vial. A standardized sample of valerenic acid (HPLC grade, Fluka) was extracted with 5 mL DCM for 1 min in a laboratory test tube and processed as mentioned above for plant material. One aliquot (10 μL) from all prepared samples was spotted in 5 mm band width on the same silica gel $HF_{254+366}$ plate (Merck, Germany). Separation was achieved using a ternary mobile phase containing hexane: ethyl acetate: glacial acetic acid (63: 35: 0.5 v/v). Distance of development was 10 cm. Spots were detected at 254 and 366 nm and then revelation of them was carried out with HCl-acetic acid reagent followed by anisaldehyde-sulphuric acid reagent spraying at visible light (Upton *et al*., 1999).

Sample preparation for UV-spectrophotometry: Each 0.2 g plant sample was first extracted with 5 mL DCM then evaporated. After volume adjustment to 1 mL with DCM, organic acids were transferred into 2 mL 2% NaOH. The alkaline aqueous phase was acidified up to pH~2 using 1% HCl and the organic acids (including valerenic acid derivatives) retransferred into 1 mL petroleum ether: diethyl ether (2:1) organic phase (Bos, 1997). The obtained organic phase was divided into two equal fractions and processed as follow:

- Fraction 1 was used to determine valerenic acid derivatives content using their respective aliquot extinction coefficients (ε) found in the literature reference (Bos, 1997). Thus a volume of 0.5 mL was dry evaporated then the residue collected in 1 mL ethanol. After absorbance measurement at 212 nm (hydroxyvalerenic acid), 217 nm (acetoxyvalerenic acid) and 218 nm (valerenic acid), each valerenic acid derivatives content (Ci) was determined using the following formula:

$$C_i = \frac{Abs}{\varepsilon}$$

- Fraction 2 was used to determine the total valerenic acid derivatives (including valerenic acid, hydroxyvalerenic acid and acetoxyvalerenic acid) content using a 10 points calibration curve. Each calibration curve point corresponded to a dilution obtained from the valerenic acid concentration stock solution. The stock solution was prepared using the valerenic acid standardized sample. Thus, a

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 mountains, 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

0.5 mL of fraction 2 was dry evaporated then collected in 1 mL methanol and its absorbance measured at 225 nm.

Calibration curve: Stock solution (51 mg L^{-1}) of valerenic acid was prepared in methanol. Ten points from different valerenic acid concentrations in the range of 2-50 mg L^{-1} were obtained. The regression equation for valerenic acid at 225 nm was y = 0.0125+0.019x (y is absorbance and x defines concentration) and the correlation coefficient (r) was 0.9993.

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: The data obtained in our laboratory represent the average±SE of triplicate determinations. Data obtained from UV-spectrophotometry were subjected to one-way ANOVA. Post hoc multiple comparisons between species for valerenic acid derivatives contents were made using Tukey´s test. Significance was reported starting at the 0.05 level.

RESULTS AND DISCUSSION

Valerenic acid with R_f = 0.49 appeared following application of the HCl-acetic acid reagent as a very faint violet colored band in visible light and as a weak fluorescent band under 366 nm. Subsequent application of anisaldehyde-sulphuric acid reagent (Wagner *et al*., 1984), valerenic acid appeared as a strong violet band (Fig. 1).

Table 2 shows R_f values and colors of separated spots on TLC plates for rhizome extracts of *V. officinalis*. Valerenic acid derivatives spots order arrangement and colors were similar to those reported by the literature references (Wagner *et al*., 1984; Singh *et al*., 2006; Upton *et al*., 1999). 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 sesquiterpenes and some other components present in different organs of Iranian wild species of Valerianaceae and commercial *V. officinalis*. Our qualitative TLC results confirmed the presence of valerenic acid derivatives in all organs of studied plants. After sample preparation, absorbance of methanolic and ethanolic extracts, were determined at 212, 217, 218 and 225 nm. Valerenic acid, hydroxyvalerenic acid and acetoxyvalerenic acid contents were calculated using their respective extinction coefficients.

Total valerenic acid derivatives content (Table 4) was determined through the regression equation of the calibration curve for valerenic acid at 225 nm based on the assumption that the extinction coefficients of these compounds are almost similar. In Fig. 2, variation of valerenic acid derivatives content is shown for each plant organ.

Measurement of total valerenic acid derivatives is more perfect than determination of valerenic acid alone. Additionally, some analytical laboratories calculate total valerenic acid derivatives using specific reference

Table 2: Characteristic thin-layer chromatography of some components in *V. officinalis* roots

Compound	R_f	Visible light	366 nm	254 nm	254 nm (with reagents)
Hydroxyvalerenic acid	0.08	Violet	-	Dark spot	Dark spot
Acetoxyvalerenic acid	0.14	Violet	Dark spot	Dark spot	Dark spot
Valerenic acid	0.49	Violet	Dark spot	Dark spot	Dark spot
Baldrinal	0.57	Yellow	Dark spot	Dark spot	Dark spot
Cryptofauronol	0.64	Purple-violet	Dark spot	Dark spot	-
Patchouli alcohol	0.74	Brownish-blue	Dark spot	Dark spot	Dark spot
Valerenal	0.85	Blue	Dark spot	Dark spot	Dark spot
Valerenone	0.96	Yellow	Dark spot	Dark spot	Dark spot

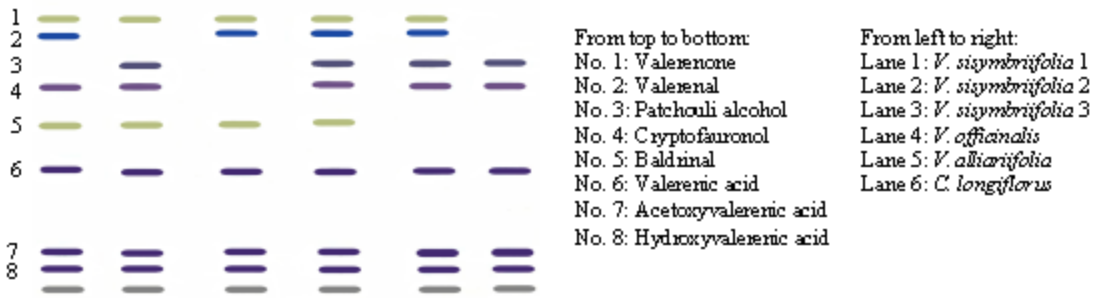

Fig. 1: TLC plate viewed valerenic acid derivatives and other main components at visible light with HCl-acetic acid reagent followed by anisaldehyde- sulphuric acid reagent spraying in plant rhizomes

Table 3: Comparative determination of the main sesquiterpenes and some other components in different organs of Iranian wild species of Valerianaceae in comparison to *V. officinalis* by TLC

		Component							
Plant	Organ	HVA	AVA	VA	BL	CP	PA	VL	VN
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	+	+	+	-	+	+	+	+
(Commercial valerian)	Rhizome	+	+	+	+	+	+	+	+

HVA (Hydroxyvalerenic acid), AVA (Acetoxyvalerenic acid), VA (Valerenic acid), BL (Baldrinal), CP (Cryptofauronol), PA (Patchouli alcohol), VL (Valerenal), VN (Valerenone)

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

Plant	Organ	Total valerenic acid derivatives	Valerenic acid[a]	Hydroxyvalerenic acid[a]	Acetoxyvalerenic acid[a]
V. sisymbriifolia	Root	0.113±0.005	0.040±0.001	0.042±0.001	0.057±0.001
(Location 1)	Rhizome	0.142±0.010	0.047±0.000	0.049±0.001	0.068±0.001
	Stem	0.171±0.011	0.066±0.002	0.070±0.001	0.094±0.002
	Leaf	0.071±0.010	0.033±0.001	0.036±0.001	0.048±0.001
V. sisymbriifolia	Root	0.116±0.006	0.036±0.001	0.039±0.001	0.052±0.001
(Location 2)	Rhizome	0.144±0.017	0.038±0.001	0.040±0.000	0.053±0.000
	Stem	0.059±0.004	0.029±0.001	0.031±0.001	0.040±0.002
	Leaf	0.093±0.017	0.041±0.002	0.046±0.002	0.059±0.002
	Inflorescence	0.106±0.000	0.033±0.000	0.036±0.000	0.047±0.001
V. sisymbriifolia	Root	0.115±0.014	0.041±0.001	0.043±0.001	0.058±0.002
(Location 3)	Rhizome	0.140±0.019	0.051±0.001	0.054±0.001	0.074±0.001
	Stem	0.085±0.003	0.035±0.000	0.040±0.000	0.051±0.000
	Leaf	0.073±0.000	0.030±0.001	0.031±0.001	0.043±0.001
	Inflorescence	1.110±0.066	0.392±0.006	0.422±0.001	0.571±0.001
V. alliariifolia	Root	0.114±0.009	0.040±0.002	0.044±0.002	0.059±0.003
	Rhizome	0.097±0.009	0.028±0.001	0.030±0.001	0.040±0.001
	Stem	0.101±0.006	0.034±0.002	0.038±0.002	0.050±0.002
	Leaf	0.093±0.001	0.030±0.001	0.032±0.001	0.043±0.001
	Inflorescence	0.163±0.012	0.055±0.000	0.060±0.000	0.080±0.000
C. longiflorus	Rhizome	0.147±0.004	0.063±0.004	0.069±0.004	0.092±0.006
	Stem	0.112±0.012	0.040±0.004	0.042±0.003	0.055±0.005
	Leaf	0.100±0.006	0.036±0.001	0.041±0.001	0.052±0.001
	Inflorescence	0.507±0.004	0.144±0.002	0.153±0.002	0.205±0.003
V. officinalis	Root	0.095±0.004	0.040±0.001	0.043±0.000	0.057±0.001
	Rhizome	0.278±0.020	0.070±0.001	0.073±0.002	0.100±0.002

[a] All ε values were extracted from literature reference (Bos, 1997)

standards, while others calculate the total valerenic acid derivatives content based on the assumption that the extinction coefficients of these compounds are almost similar. However, calculating valerenic acid derivatives content using their respective extinction coefficients provides more accurate results than total valerenic acid derivatives values or valerenic acid quantification alone by plotting calibration curve (Upton *et al*., 1999).

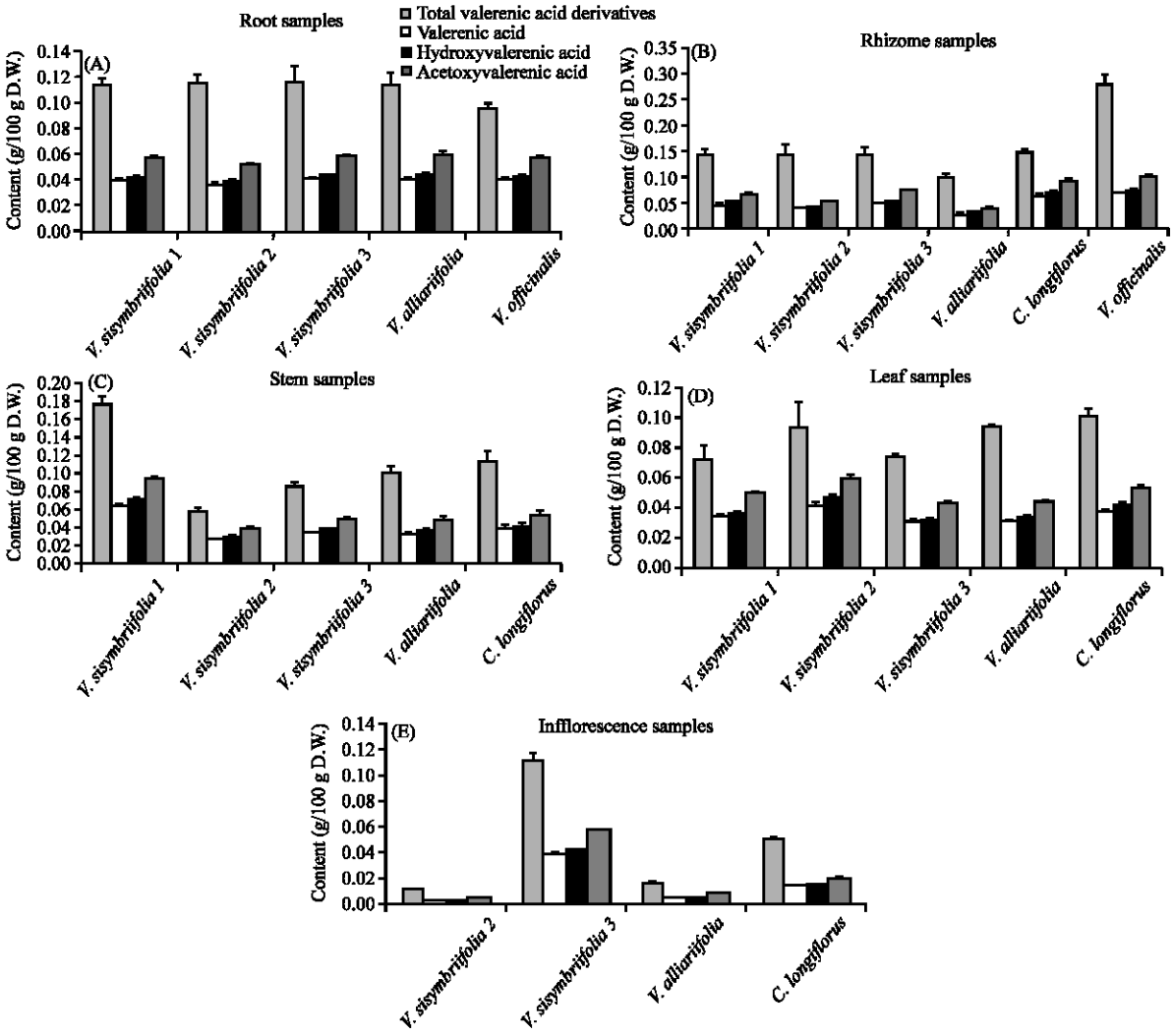

Fig. 2: Comparative analysis of valerenic acid derivatives in different organs of the plant samples; root (A), rhizome (B), stem (C), leaf (D) and inflorescence (E). All results are based on dry weight

Valerenic acid content in commercially available roots from plants of the Anthos cultivar, the current preferred industry cultivar of *V. officinalis* in Australia, was reported to be about 3 mg g^{-1} (dry weight basis) as an average (Wills and Shohet, 2003). A survey of some valerian commercial manufactured products showed considerable variation in concentration of valerenic acid derivatives from <0.01 to 6.32 mg g^{-1} (Li *et al*., 2006). The content of valerenic acid derivatives in *V. officinalis* ranges from 0.05% (in wild plants) to 0.9% (in cultivated strains) (Bos, 1997).

Our quantitative analysis showed that acetoxyvalerenic acid was the dominant fraction in all samples, compared to valerenic acid and hydroxyvalerenic acid. The survey of valerenic acid derivatives content in the studied samples showed a considerable variation from 0.06% in stem of *V. sisymbriifolia* from location 2 to 1.11% in inflorescence of *V. sisymbriifolia* from location 3.

Total valerenic acid derivatives content of roots in *V. sisymbriifolia* plants from locations of 1, 2, 3 and *V. alliariifolia* was 0.11, 0.12, 0.12 and 0.11%, respectively, which showed lower amounts compared to that from roots of Anthos cultivar (0.3%) (Significantly mean difference, $p<0.05$). The same results were found for rhizomes of *V. sisymbriifolia* from locations 1 (0.14%), 2 (0.15%), 3 (0.14%), *V. alliariifolia* (0.10%) and *C. longiflorus* (0.15%) compared to that of commercial *V. officinalis* (0.28%) (Significantly mean differences, $p<0.001$).

In spite of different geographical distribution and habitat altitude for three studied populations of *V. sisymbriifolia*, data did not show any considerable

differences in total amounts of valerenic acid derivatives in their roots and rhizomes. Among the different organs of wild plants, inflorescence of *V. sysimbriifolia* from location 3 (1.11%) and *C. longiflorus* (0.5%) had higher amounts of total valerenic acid derivatives.

Valerenic acid derivatives content of valerian root and rhizome is considered as an essential quality factor regarding the health benefit to consumers (Bos, 1997). Thus, we were very interested to get some comparing data between some Iranian wild species and commercial valerian figuring in the European Pharmacopoeia. Present results showed that, studied Iranian wild species of *Valeriana* L. and also wild species of *Centranthus* DC. may be used as worthy sources of valerenic acid derivatives.

ACKNOWLEDGMENTS

The authors wish to thank to Dr. V. Mozzaffarian and Dr. Sh. Zarre 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 work was supported by University of Tehran and Shahed University and Chemical Industries Institute of the Iranian Research Organization for Science and Technology.

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