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EFFECTS OF EXTRACTION SOLVENTS ON CONCENTRATION OF VALERENIC ACID AND ANTIOXIDANT PROPERTY OF *VALERIANA JATAMANSI* JONES

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ABSTRACT

The effects of extracting solvents (dichloromethane, ethyl acetate and methanol) on the valerenic acid content and antioxidant activity of roots extracts of *Valeriana jatamansi* (cultivated and wild) were investigated. The tested plant extracts contained appreciable amounts of valerenic acid content which affects the antioxidant potential of plant extracts. Higher concentration of valerenic acid and antioxidant activity was obtained using dichloromethane, as compared to ethyl acetate and methanol. The results showed that the concentration of valerenic acid plays an important role in antioxidant activity of *V. jatamansi*. The concentration of valerenic acid was found in the order dichloromethane > ethyl acetate > methanol fractions in different samples of *V. jatamansi*. A similar trend was also found with antioxidant activity of the plant extracts.

KEYWORDS: ***Valeriana jatamansi*, valerenic acid, quantification, HPTLC, antioxidant activity**

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INTRODUCTION

Valeriana jatamansi Jones syn. *Valeriana wallichii* DC belongs to the family Valerianaceae is a perennial herb commonly known as Valerian. Roots of *V. jatamansi* are used as an aphrodisiac, insecticide and in mental disorders[1]. In India, *Valeriana* has long been used in Ayurveda and Unani systems of medicine, which describes its uses in skin diseases, Insanity, epilepsy and snake bite and considered to have remarkable sedative effects in nervous unrest, stress and neuralgia[2]. Valerian is the top ten selling retail herb for herbal supplements in North America and Australia[3]. It has also been prescribed as the perfect herbal tranquillizer, and was used for this purpose in the First World War to treat soldiers suffering from shell shock[4]. Literature survey revealed the presence of flavone glycosides[5,6], iridoids and lignans[7-9] in *Valeriana jatamansi*. Antiinflammatory[10], antispasmodic[11], antioxidant[12], larvicidal[13], antianxiety[14], anti HIV[15], antidiarrhoeal, and bronchodilatory activities[16] of *Valeriana* have been scientifically reported. The plant is also used as cytotoxic[17]. Its essential oil exhibited antimicrobial activity against pathogenic bacteria and also exhibited potent antifungal activity against different human and plant fungal pathogens[18].

Recently, attention has focused on valerenic acid and its derivatives as important sedative components. Total ash, moisture content and qualitative and quantitative analysis of valtrate and acevaltrate in *V. jatamansi* have been carried out by TLC and HPLC[19]. Singh et al.[3] had estimated valerenic acid in *V. jatamansi* and *V. officinalis* by HPTLC using hexane: ethyl acetate: acetic acid (80:20:0.5 v/v) as mobile phase on precoated silica gel $60F_{254}$ aluminium plates. Chlorogenic acid, lignans, flavonoids, valerenic acids and valpotrates were determined by HPLC in various *Valeriana* samples using Phenomenex Luna C_{18} column and gradient elution with a mobile phase consisting of water and 0.05% phosphoric acid[20]. HPTLC method has also been developed for quantification of valerenic acid in *Valeriana jatamansi, Nardostachys jatamansi*, and *Selinum vaginatum*. The separation and quantification of valerenic acid was achieved by using mobile phase of toluene: ethyl acetate: formic acid (80:20:5 v/v) on precoated silica gel $60F_{254}$ aluminum plate[21]. In recent years the demand of *Valeriana* has rise rapidly because of local, national and international interest for home remedies and many herbal preparations. Government of Uttarakhand has formulated a policy to support the commercial cultivation of *V. jatamansi*. The mandate of this policy is to meet out the demand and provide new income opportunity to the farmers. For better commercial value, there is need to standardized the raw herbal material. Therefore, the aim of this work was to determine valerenic acid and antioxidant potential of *Valeriana jatamansi* from cultivated and wild sources and also to determine the effects of extraction solvents on concentration of valerenic acid and antioxidant property.

MATERIALS AND METHODS

Plant Material and reagents

V. jatamansi (Cultivated) roots were collected from Herbal Garden of Herbal Research and Development Institute and wild roots from Mandal forest. Both samples were collected from three places of same population with nearly same maturity for the estimation of valerenic acid and antioxidant property. The plant materials was washed with tap water and cut in small pieces and spread over glass plate to dryness. The dried samples were grinded through pulverizer and particles passed through sieve were taken for extraction and analysis. Standard valerenic acid was procured from Sigma Aldrich (Germany) and all other chemicals used were HPLC grade.

Extraction and preparation of samples solutions

Hundred milligrams of powdered roots of *V. jatamansi* were extracted in 15 ml of dichloromethane, ethyl acetate and methanol separately three times. Each extracts from same sample were combined and the solvent was removed to dryness under vacuum. The dried extracts were dissolved with 2 ml methanol to make 50 mg/ml solution. All extracts were filter through 0.45µm syringe filter and used for analysis. Accurately 15 µl of each sample was applied to TLC plate.

Preparation of standard solutions

A stock solution of valerenic acid (100µg/l) was prepared in methanol. Different volumes (4, 6, 8 and 10 µl) of the stock solution equivalent to 400, 600, 800 and 1000 ng were applied to the TLC plates. The calibration curve, correlation coefficient and regression equation were obtained using WinCATS software and data were arranged in tabular form.

Instrumentation and chromatographic conditions

The standard and sample solutions were applied on precoated 20×10 cm silica gel 60 F_{254} plate in the form of bands with 100 µl syringe using automatic sample applicator (Linomat 5). Samples were applied to the plate as 6 mm band, 8 mm apart from Y and 15 mm from X axis using N_2 gas. The slit dimension was 5 × 0.30 mm and scanning speed was 20 mm/s. The plate was developed in a twin trough chamber saturated with mobile phase. The mobile phase consisted of petroleum ether and ethyl methyl ketone (8:2). After development, the plate was dried with the help of dryer and observed under UV chamber. The well developed bands of valerenic acid in standard and *V. jatamansi* samples were scanned at 254 nm in absorption mode with CAMAG TLC scanner controlled by WinCATS software. The source of radiation was deuterium lamp emitting a continuous UV spectrum in the range of 190-400 nm.

Antioxidant activity

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of *V. jatamansi* extracts was determined according to the Zhishen et al.[22] with slight modifications. The working solutions (10, 20, 50, 70, 100 µg/ml) of the extracts were prepared in methanol. Ascorbic acid was used as standard in 1-100 µg/ml. Briefly, 1 ml of DPPH solution (0.1 mM in methanol) was mixed with 3 ml of sample extracts and standard solutions separately. The mixture was shaken and kept for 30 minutes at room temperature. The decrease of solution absorbance due to proton donating activity of components of extracts was determined at 517 nm using Elico SL-159 UV-Vis spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. DPPH (3 ml of 0.1mM) and Methanol (1 ml) was used as blank. The DPPH radical scavenging activity was calculated using the following formula: DPPH Radical Scavenging Activity (% inhibition) = $[(A_0–A_1/A_0) \times 100]$, where A_0 is the absorbance of the blank, and A_1 is the absorbance of extract mixed with DPPH. IC_{50} value (inhibitory concentration at which DPPH radicals where scavenged by 50%) was obtained by interpolation from linear regression analysis.

RESULTS AND DISCUSSION

High performance thin layer chromatography (HPTLC) was used for the estimation of valerenic acid (structure in Figure 1) from the roots of *V. jatamansi* (cultivated and wild source). The results are presented in Table 1. The rf value and correlation coefficient for valerenic acid was found 0.36 and 0.998, respectively. The chromatogram of standard and samples were shown in Figure 2. The band of valerenic acid in the samples was confirmed by comparing rf value of standard valerenic acid. The qualitative results confirmed the presence of valerenic acid in all the fractions of the plant while the quantitative

results are different (Table 1), representing 0.47% (cultivated)-0.72% (wild). The Dichloromethane fraction of cultivated *V. jatamansi* contained highest amount of valerenic acid, than ethyl acetate and methanol fractions. Similar trend was found in wild *V. jatamansi*. Considering source of plant samples, the concentration was found higher in wild source as compared to cultivated (Table 1).

Table 1
Effect of extracting solvents on concentration of Valerenic acid in V. jatamansi

Samples	Solvent used for extraction	%Valerenic acid (dry weight basis)
V. jatamansi (Wild)	Dichloromethane	0.72
	Ethylacetate	0.68
	Methanol	0.65
V. jatamansi (Cultivated)	Dichloromethane	0.68
	Ethylacetate	0.54
	Methanol	0.47

Figure 1
Chemical structure of valerenic acid

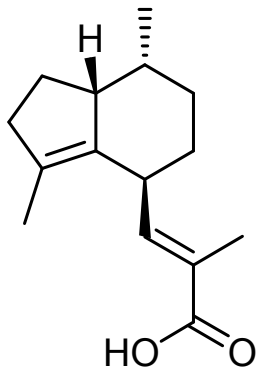
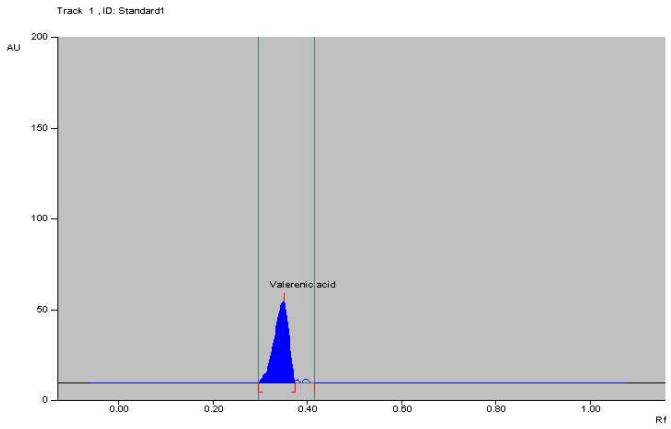
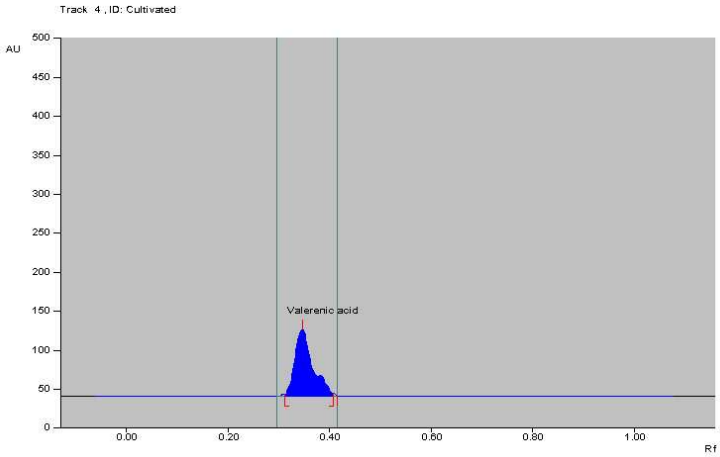


Figure 2

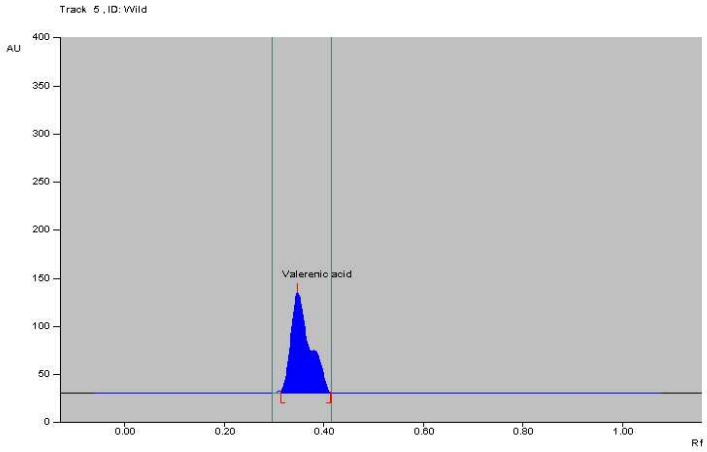
Chromatograms of (a) valerenic acid (b) cultivated V. jatamansi and (c) wild V. jatamansi dichloromethane fraction



(a)



(b)



(c)

A lot of papers were related with the determination of valerenic acid in *Valeriana* by HPLC and HPTLC[20, 3]. In these studies, the HPLC conditions consisting a mobile phase of water and 0.05% phosphoric acid and 2-100% acetonitrile- methanol (1:1) with 0.05% phosphoric acid whereas HPTLC consisted hexane: ethyl acetate: acetic acid (80:20:0.5 v/v). In addition, Hassan et al.[23] used a TLC-UV method to determine the content of valerenic acid in *Valeriana* and *Centranthus longiflorus* Stev. The total valerenic acid derivatives content in wild samples of *V. sisymbriifolia*, *V. alliariifolia* and *C. longiflorus* were found 0.06-1.11%. In present study rf value was observed a little dissimilar to earlier studies which may be due to different mobile phase. TLC method was also used for quantification of valerenic acid content in some brands of valerian pharmaceutical products using ethyl acetate: hexane: methanol (0.5:3:7) as a mobile phase. The amount of valerenic acid in some brands of German valerian products were estimated 0.03-0.16% while in Iranian products it was found 0.05-2.8%[24]. Shohet et al.[25] analyzed thirty one commercial valerian preparations by HPLC for valepotriates, valerenic acid and their derivatives and reported the concentration of valerenic acid and its derivatives between 0.001-0.63%. The present study is in agreement with the work of Hassan et al.[23], Ghafari et al.[24] and Shohet et al.[25], who reported valerenic acid content of *Valeriana* species. Decrease in the absorbance of DPPH in the presence of antioxidants correlates with the free radical scavenging potential of the antioxidant. Dichloromethane fraction of both *V. jatamansi* sample showed significant antioxidant activity with IC_{50} values of 48.02 and 51.35 µg/ml while IC_{50} values for ascorbic acid was 45.89 µg/ml (Table 2). The results indicate that the antioxidant activity of the dichloromethane, ethyl acetate and methanol fractions of *V. jatamansi* are lower than that of ascorbic acid (Table 2). The antioxidant activity of wild samples was stronger as compared to cultivated samples (Table 2). Likewise, on comparison of dichloromethane, ethyl acetate and methanol extracts of both samples, the dichloromethane extract of wild sample exhibited higher radical scavenging property than the cultivated sample. Same trends were also found in ethyl acetate and methanol extracts. None of the samples showed activity as strong as the ascorbic acid (Table 2). However, antioxidant activity of the dichloromethane extract of *V. jatamansi* determined by DPPH method exhibited significant radical scavenging property as compared to other extracts. While comparing with cultivated source, material obtained from wild source has greater free radical scavenging property.

Table 2
Effect of extracting solvents on IC_{50} Value of V. jatamansi

Samples	Solvent used for extraction	IC_{50} Value
Ascorbic acid (Standard)	-	45.89
Valeriana jatamansi (Wild)	Dichloromethane	48.02
	Ethylacetate	52.50
	Methanol	62.24
Valeriana jatamansi (Cultivated)	Dichloromethane	51.35
	Ethylacetate	56.48
	Methanol	65.62

CONCLUSION

The antioxidant effectiveness of the *V. jatamansi* extracts is probably due to a relatively high content of valerenic acid. Extract with higher valerenic acid content showed higher antioxidant property. Both cultivated and wild *V. jatamansi* extracts exhibited significant antioxidant activity. The scavenging activity might be due to the presence of phenolic contents from which valerenic acid may be one of the major active constituents.

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