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RESEARCH ARTICLE

Altitudinal and Seasonal Variation in Bioactive Compound Aconitine in *Aconitum violaceum*, a Threatened Medicinal Plant of Indian Himalayan Region

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Abstract

Aconitum violaceum Jacq. (Ranunculaceae) is an important medicinal plant of the Indian Himalayan Region shares its position with threatened plant species. The present study was carried out to investigate the altitudinal and seasonal variation in content of bioactive compound aconitine in different plant parts of *Aconitum violaceum* Jacq. Plants were collected in three seasons from five populations across the altitudinal gradients. Morphological features were, in general, negatively correlated with an increase in the altitude. Maximum plant height (26.9 ± 3.1 cm) was observed in populations from lower altitude (Hemkund I, 3650 m asl), whereas, the minimum value (14.2 ± 2.9 cm) was recorded from the relatively higher altitude (Hemkund V, 4400 m asl). Maximum vegetative growth was observed in month of September. The aconitine content of the plant ranged between 0.081 to 0.99% (on dry wt. basis, in different parts of the plant). The result indicated that the aconitine content was found to be significantly higher in tubers as compared to other parts of the plant e.g. stem, leaves, buds, at every altitude. The biosynthesis of aconitine in tubers was found to be highest in the month of October (0.99% on dry wt. basis). The maximum aconitine content reported in the leaves was highest in the month of August, which is the pre-flowering season of the plant, however, aconitine content decreases significantly during month of October. Effect of altitude on aconitine content was also analyzed. A positive correlation was observed between the aconitine content and the altitude of growing sites.

Introduction

Various factors, such as age of the plant, season, microbial attack, grazing, radiation, competition and nutritional status, have an impact on the secondary metabolite profile in higher plants (Harborn, 1982). A factor rare assessed is the altitude of the growing site. Many environmental parameters like precipitation, mean temperature, soil, wind speed, low and high temperature extremes, duration of snow cover, length of the vegetation period, and the intensity of radiation under clear sky conditions differ between low and high altitude sites in temperate zones (Korner, 1999). The increased solar radiation at higher altitudes and the enhance UV-B radiation in particular are assumed to have a negative impact on plant life. Moreover an increase in the content of phenolic compounds and caratenoids with

rising altitude has been postulated as a response to increasing UV radiation (Korner, 1999). The current findings showed the effect of seasonal and altitudinal variation on aconitine content of the *Aconitum* violaceum.

Aconitum violaceum Jacq. member of family Ranunculaceae is an important medicinal plant. Pertinent in subalpine and alpine areas of Indian Himalayan Region at 3500-4000 m elevations, the plant shares its position with threatened plant species of IHR (Chaudhary and Rao, 1998; CAMP, 2003). Aconitum species are the rich sources of diterpene alkaloids and flavonoids. Tubers of the plant are the natural source of alkaloid aconitine, a neurotoxin which attributes to the medicinal properties of the plant (Anonymous 1988). The crude extract of underground parts possess antipyretic and analgesic properties and traditionally been used in renal pain, rheumatism, high fever, allergy, boils, cuts, wounds, edema, treatment of snake and scorpion bites, contagious infections, disorder of gall bladder and inflammation of the intestines (Miana *et al.*, 1971; Kirtikar and Basu, 1984; Ameri, 1998; Chauhan, 1999; Pandey, 2006; Bhattarai *et al.*, 2010). The tubers are also used for tonsillitis, sore throat, gastritis, debility, antioxidative and anti-inflammatory (Kunwar *et al.*, 2010; Uprety *et al.*, 2010). Recently, the antiproliferative activity of the isolated alkaloids of *Aconitum*, were evaluated against human tumor cell lines, ovarian and colon adenocarcinoma (Gao *et al.*, 2006; Dall'Acqua *et al.*, 2008).

Earlier, the plant was used to prepare traditional medicine by local people in small quantities, but commercialization of plant based drugs in recent years has increased the demand and consequent exploitation of the plant. Due to increased demand from pharmaceutical industries, uncontrolled collection and lack of organized cultivation, plant is going depleted in natural habitat. Therefore, to protect the natural germplasm and to collaborate with rising demand of the plant material the present study was undertaken to ascertain the best harvest period to get the maximum yield of the bioactive molecule from the *Aconitum violaceum*.

Materials and methods

Plant material collection

A total of five populations across the altitudinal gradients (Hemkund I-3650 m asl to Hemkund V-4400 m asl) were examined regarding morphological features during three different seasons. Populations and seasons were chosen on the basis of availability of plants and their life cycle in the study area, i.e., August: young plant with vegetative growth; September: adult plant with young flowers; October: mature plant with flowers and seeds. The population study was carried out following the belt transect method (Michael, 1990). Three populations were selected from top, middle and lower stands to reach altitudinal range and ten squares (1m x 1m) were determined random, within each stand. Ten individuals/stand were sampled randomly for recording morphological characters (plant height, stem diameter, tuber length and diameter, leaf area).

Daughter tubers of three individuals from each stand were seasonally collected and sprinkled with a systemic fungicide, Bavistin (50% carbendazim, w/v) and brought in polythene bags to the institute. These were washed to remove traces of soil and dried at room temperature (25 °C) for 20 days. The air-dried tubers were powdered in a grinder and made into a composite mixture before analysis (Panday *et al.*, 2008). Extraction of active ingredients was done following Rawat *et al.*, (2013). The powdered samples(1.0 g) were extracted (25 ml x 3; 30 min each) with ammoniacal ether (ether containing 5% v/v, ammonia solution); the residue was then extracted with methanol (25 ml) for 16 h followed by two more extractions for 3 h each.

Column chromatography

Samples were further purified on neutral alumina (Sisco Research Laboratories Pvt Ltd., Mumbai) columns (8 x 2 cm; length and diameter) eluted with 50 ml of ethyl acetate and methanol (7.3 v/v). The elutes were dried in vacuum (30 °C) in a rotary film evaporator, dissolved in HPLC grade methanol (1.0 ml) for further analysis by HPLC.

High performance liquid chromatography

The quantification of aconitine was carried out using a HPLC system (Shimadzu corporation, Japan; Model LC-10 ATVP) in RP-1 Spherisorb column (250 x 4.6 mm id, 5 µm; Merck Darmstadt, Germany), eluted in an isocratic mode with methanol and water (60:40 v/v) containing 0.1% of acetic acid (Panday *et al.*, 2008). The column elutes were monitored using an online UV detector set at 263 nm. The flow rate was 1 ml/min. The peaks were identified on the basis of retention time (9.95 min) and quantification was carried out on peak area basis using a dose–response curve prepared with authentic compounds. Three analyses were done per sample. The lower limit of detection was ~100 ng. Aconitine standard was purchased from Sigma chemicals Co. St. Louis, USA.

Results and discussion

Only a limited number of studies have been conducted for determining the aconitine in *Aconitum violaceum*. Considerable variation is found regarding morphological features of *A. violaceum* among different altitude in all the three months (Table 1). The morphological features, in general, correlated negatively with altitude. Maximum plant height (26.9 ± 3.1 cm) was observed in populations from lower altitude (Hemkund I, 3650 m asl), whereas, the

minimum value (14.2 ± 2.9 cm) was recorded from the relatively higher altitude (Hemkund V, 4400 m asl). The maximum leaf area (3.1 ±0.4 cm^2/plant) was recorded from the comparatively lower altitude (4050 m asl) in the month of September which is vegetative growth period of the plant, whereas, minimum leaf area (0.94 ±0.2 cm^2/plant) was recorded in the month of August at higher altitude (4250 and 4400 m asl). Same trend has been reported for the stem diameter, maximum dia (5.9 ±2.2) was recorded from lower altitude (3650 m asl). However, data on tuber length and diameter indicated that tuber size did not correlate with plant height (Table 1). Indeed, plants with the shortest height had long tubers. The results of morphological features also showed that species performs better (in terms of vegetative growth) at lower altitude. Similar kinds of results have been reported in another important medicinal plant *Podophyllum hexandrum* (Nadeem *et al.*, 2007).

The bioactive compound, aconitine was determined in four parts of *A. violaceum* (Leaf, bud, stem and tuber) in three different months (August, September and October) using HPLC and expressed in % DW basis (Fig. 1). Aconitine content was significantly higher (0.99% DW) in month of October, which is late flowering stage of the plant, when compared to August and September. The aconitine content in leaf and stem during the vegetative stage (August- September) was higher at every altitude (Table 2). However, during flowering stage (end of September and mid October) aconitine content decreases significantly in leaf and stem. Since secondary metabolites in a plant constantly changes throughout the growth period, the maximum amount of aconitine in leaves are accumulated before flowering, and gradually decline (Maknickiene, 2008). In the present investigation, it is observed that aconitine content increases in tubers, as the plant progresses from vegetative to flowering stage (reverse in the case of leaves; Table 2). Among the four tested parts of the plant, minimum aconitine content was observed in bud. Similar results were observed in *Aconitum* and Lycoctonum (D'yachkovskaya, 1971; Sinam and Devi, 2011). The aconitine content can vary with the species, place of origin, time of harvest, and most importantly the method and adequacy of processing (Chan *et al.*, 1994).

The effect of altitude on aconitine content accumulation has also been carried out. The regression analysis suggested that the aconitine content of the tubers correlated positively with altitude ($y=0.000x - 1.011$, $R^2= 0.766$; Fig. 2). Purohit *et al.*, (1999) analyzed rhizome samples of *Podophyllum hexandrum*, from populations collected between 1800-3800 m in Garhwal Himalaya and reported higher levels (8.26%) of podophyllotoxin in plants growing at relatively high altitude (about 3800 m asl). However, in another study (Nadeem *et al.*, 2007), a correlation between podophyllotoxin levels and altitude could not be established as some populations from lower elevations (1800 m asl) also contained high levels; the authors attributed such differences to genetic variation found in nature. The analyses (in triplicate) of tuber samples of five different populations revealed that aconitine levels ranged from 0.18 to 0.99% (on dry wt. basis). Among the tested plants parts, maximum aconitine content was recorded in tubers of the plant at every altitude (Table 2). The maximum aconitine content was found in tubers collected in month of October from population Hemkund V (4400 m asl), while the minimum value (0.18%) was recorded in samples collected in month of August from population Hemkund I. The results of the present study on *A. violaceum* populations from Hemkund area clearly indicate a positive correlation between aconitine and levels and increasing altitude (Fig. 2). The age of the plant is known to influence the active ingredients content and it has been reported earlier in another important medicinal plant of Himalaya (Sharma *et al.*, 2000). It must be mentioned that in the present study the age of naturally growing stands could not be determined, and therefore tuber samples were collected randomly. The reported variation in aconitine content along the altitude could be due to the genotypic differences. Furthermore, variations may also arise due to the presence of different chemotypes in natural populations and the method of extraction.

Table 1: Morphological characteristics of *Aconitum violaceum* across different seasons and altitudes

S. No.	Altitude (m)/ Location	Season	Morphological features of *A. violaceum*				
			Plant height	Stem diameter (mm)	Root/tuber (cm)		Leaf area/plant (cm^2)
					Length	Diameter	
1	3650/ Hemkund I	August	8 .2 (±1.4)	2.1 (±0.4)	0.8 (±0.2)	0.43 (±0.1)	0.96 (±0.2)
		September	14.6 (±2.3)	4.2 (±1.3)	0.81 (±0.2)	0.56 (±0.2)	2.83 (±0.3)
		October	26.9 (±3.1)	5.9 (±2.2)	0.85 (±0.2)	0.59 (±0.2)	2.83 (±0.3)
2	3850/ Hemkund II	August	6.5 (±1.7)	2.3 (±0.7)	0.65 (±0.2)	0.32 (±0.1)	1.2 (±0.3)
		September	11.2 (±2.4)	3.8 (±1.4)	0.81 (±0.2)	0.49 (±0.2)	2.93 (±0.4)
		October	24.8 (±3.4)	4.6 (±2.1)	0.81 (±0.2)	0.67 (±0.2)	2.9 (±0.3)
3	4050/ Hemkund III	August	6 .2 (±1.5)	2.5 (±0.5)	0.75 (±0.1)	0.46 (±0.2)	1.1 (±0.3)
		September	10.6 (±3.4)	3.9 (±1.2)	0.83 (±0.2)	0.61 (±0.2)	3.1 (±0.4)
		October	16 .2 (±4.1)	5.3 (±1.1)	0.9 (±0.2)	0.83 (±0.2)	3.0 (±0.4)
4	4250/ Hemkund IV	August	6 .2 (±1.1	2.5 (±0.6)	0.8 (±0.2)	0.33 (±0.1)	0.94 (±0.2)
		September	10.3 (±1.9)	3.8 (±0.9)	0.85 (±0.2)	0.49 (±0.2)	1.8 (±0.3)
		October	14.8 (±2.4)	5.6 (±1.4)	0.9 (±0.3)	0.68 (±0.2)	1.86 (±0.3)
5	4400/ Hemkund V	August	5.6(±1.4)	2.1 (±0.3)	0.81 (±0.3)	0.31 (±0.1)	0.94 (±0.2)
		September	11.3 (±2.3)	3.7 (±1.1)	0.9 (±0.2)	0.52 (±0.2)	1.91 (±0.3)
		October	14 .2 (±2.9)	5.2 (±1.9)	0.9 (±0.3)	0.81 (±0.2)	2.14 (±0.3)

Table 2: Seasonal and altitudinal variation in aconitine content in different parts of *Aconitum violaceum*

S. No.	Altitude (m)/ Location	Season	Aconitine content in different parts of the plants (% on DW basis)			
			Stem	**Bud**	**Leaf**	**Root/tuber**
1	3650/ Hemkund I	August	0.0481	0.001	0.171	0.181
		September	0.0647	0.006	0.189	0.531
		October	0.0081	NA	0.068	0.741
2	3850/ Hemkund II	August	0.0458	0.003	0.1741	0.3132
		September	0.0611	0.007	0.1941	0.5317
		October	0.0141	0.043	0.0634	0.7683
3	4050/ Hemkund III	August	0.0461	NA	0.181	0.3091
		September	0.0671	0.008	0.214	0.6182
		October	0.0114	0.053	0.084	0.797
4	4250/ Hemkund IV	August	0.0491	NA	0.2013	0.641
		September	0.066	0.0087	0.1882	0.8915
		October	0.0414	0.0089	0.0631	0.9318
5	4400/ Hemkund V	August	0.0291	NA	0.1863	0.3414
		September	0.0688	0.0081	0.2091	0.8314
		October	0.0321	0.0089	0.1993	0.9913

Figure 1. Seasonal and altitudinal variation in aconitine content in (A) Stem, (B) Leaf, (C) Bud and, (D) Tuber of Aconitum violaceum.

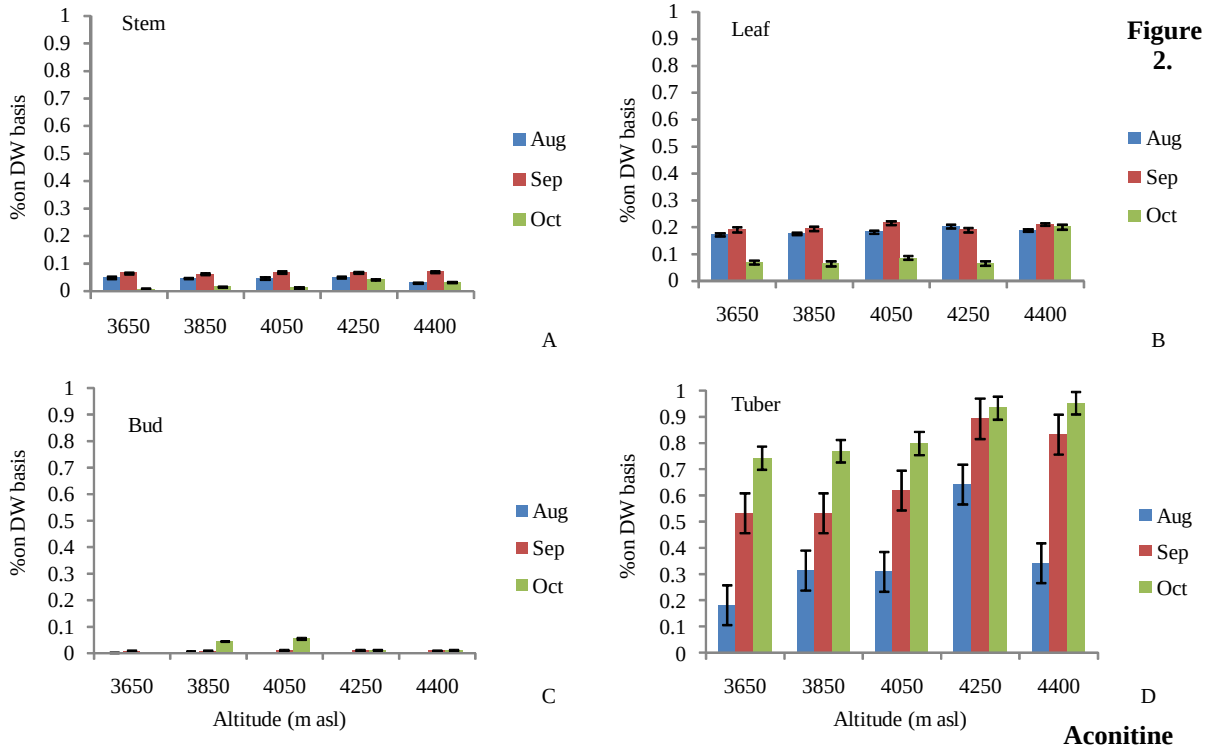
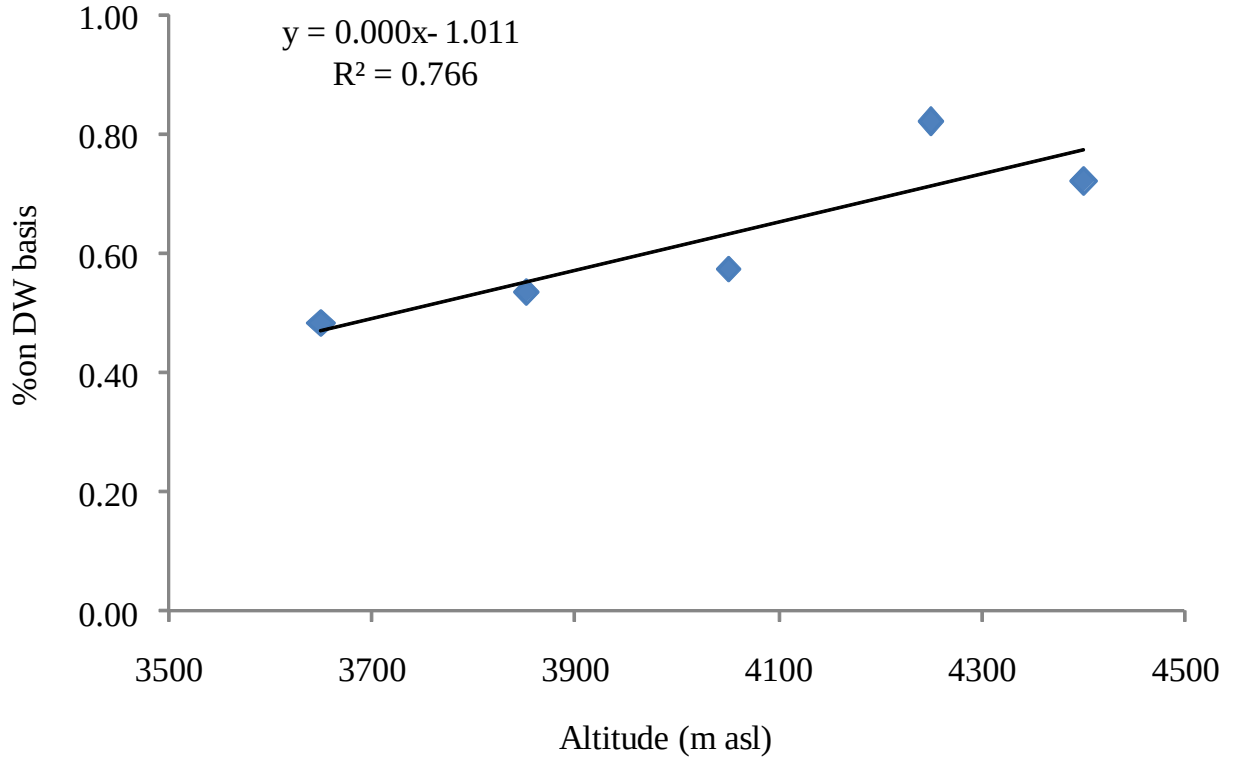


Figure 2. Aconitine content in tubers of Aconitum violaceum collected from five different populations growing at various altitudes.



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