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Research Article

Comparative Estimation of Major Iridoid Glucosides from Different Parts of *Incarvillea emodi*

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Incarvillea emodi (Bignoniaceae) is a rich source of bioactive iridoid glucosides. This plant contains two major iridoid glucosides: plantarenalloside, a neurotropic compound, and boschnalloside, a strong antibacterial compound. Here, in this study we have developed a simple and fast HPLC-DAD method for the total comparative estimation of these two major iridoids from different parts of *Incarvillea emodi*. A linear calibration curve ($r^2 = 0.999$) for both iridoid glucosides in varying range (15.6–500 $\mu\text{g/ml}$) is obtained. The limit of detection (LOD) and limit of quantification (LOQ) for plantarenalloside were 11.4 ng and 38 ng and for boschnalloside were 22.8 ng and 76 ng, respectively. The shoots, roots, and flowers of *Incarvillea emodi* have a combined presence of 7.66, 1.22, and 6.99 percent of these iridoid glucosides on dry weight basis. In shoots, plantarenalloside shows complete dominance (6.78%) over boschnalloside (0.88%), and a reversal of this trend was observed in case of flowers where boschnalloside shows complete dominance (6.12%) over plantarenalloside (0.87%). The roots contain 1.19% and 0.03% of both iridoids, respectively.

1. Introduction

Incarvillea emodi (Bignoniaceae) is a wild flowering plant found mainly at high altitudes of the western Himalayas. This plant has attracted little scientific attention in the past because of its sparse availability due to erosion of natural habitat. Dwindling habitat and lack of concern for preservation through cultivation and other means make it cited as endangered species. In our laboratory, chemical investigation of the whole plant has shown it to be a major source of two naturally occurring bioactive iridoid glucosides, namely, plantarenalloside (1) and boschnalloside (2). Iridoids exhibit a wide range of bioactivities including hepatoprotective, cardiovascular, antihepatotoxic, chlorectic, hypoglycemic, anti-inflammatory, antispasmodic, antitumor, antiviral, immunomodulator, and purgative activities [1–5].

The iridoids isolated from this plant are bioactive compounds with neurotrophic [6] and antibacterial [7] activity. In the present study, a new and rapid high pressure liquid chromatography (HPLC) method with diode array detector was reported for the quantification and

determination of these iridoid glucosides in plant organs. Previously plantarenalloside has been shown to have ten-fold dominance over euphroside in *Penstemon rydbergii* (Scrophulariaceae) [8]. Here in case of *Incarvillea emodi* (Bignoniaceae) plantarenalloside has had dominance over boschnalloside in case of shoots, but in flowers it was otherwise. In recent years, there has been an increased interest towards the development of new HPLC method for estimation and quantification of iridoids [9–13]. In the present study we have developed a simple fast and accurate LC-PDA method for the quantification of major iridoid glucosides plantarenalloside and boschnalloside (Figure 1) from different parts of *Incarvillea emodi*.

2. Experimental

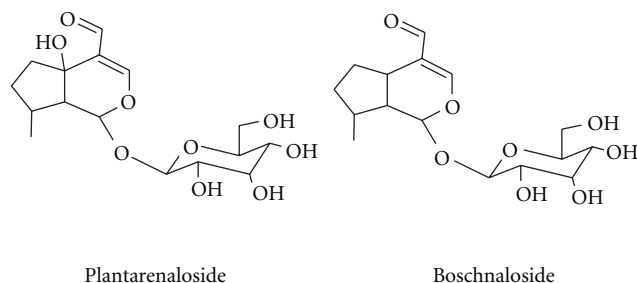
2.1. Plant Material and Reagents. The plant material was collected during February 2010 (flowering season) from the experimental farm of the Institute of Himalayan Bioresource Technology where it was domesticated by Dr. D. Dhyani of Floriculture Division. A voucher sample was deposited in

TABLE 1: Showing linear regression equation and LOD and LOQ of iridoids.

Iridoids	Regression equation	r^2	Test range	LOD	LOQ
Plantarenalioside	$Y = 6888x - 8609$	0.999	15.6–500 $\mu\text{g/mL}$	11.4 ng	38 ng
Boschnalioside	$Y = 7776x - 8498$	0.999	15.6–500 $\mu\text{g/mL}$	22.8 ng	76 ng

TABLE 2: Repeatability and reproducibility of interday and intraday precision ($n = 6$).

Iridoids	Amount	Intraday variability		Interday variability	
		Mean	(% RSD)	Mean	(% RSD)
Plantarenalioside	125 $\mu\text{g/mL}$	818338	0.26	816007	0.54
	250 $\mu\text{g/mL}$	1647020	0.42	1644255	0.59
	500 $\mu\text{g/mL}$	3475039	0.62	3449617	0.84
Boschnalioside	125 $\mu\text{g/mL}$	924169	0.43	919175	0.55
	250 $\mu\text{g/mL}$	1851650	0.28	1860019	0.28
	500 $\mu\text{g/mL}$	3923137	0.88	3906005	0.54

FIGURE 1: Iridoid glucosides of *I. emodi*.

the herbarium section of the IHBT Palampur (PLP-5963). The plant material, roots, shoots, and flowers were dried in an oven at $40 \pm 5^\circ\text{C}$. The dried plant material was then subjected to grind to form powder which was then stored in closed containers at room temperature $25 \pm 5^\circ\text{C}$.

2.2. Isolation of Iridoids and Extraction. The iridoid glucosides were extracted from 80% aqueous methanol and further isolated from the partitioned butanolic fraction of extract of the shoot portion of *Incarvillea emodi* and by performing Silica gel (60–120 mesh size) column chromatography and their structures were established by ^1H - and ^{13}C -NMR as reported earlier in our previous report [14] and comparison with the literature data [15, 16]. All the solvents HPLC grade and analytical grade were purchased from S. d. Fine-Chem Ltd. (India).

2.3. HPLC Analysis. HPLC analysis was performed in Waters HPLC system with 2998 PDA detector and 2707 autosampler. The separation was achieved with Phenomenex Synergi MAX-RP 80A column ($4.6 \times 250\text{ mm}$, 4 micron) at 30°C . A linear isocratic solvent system of methanol:water (60:40 v/v) was used at a constant flow rate of 1 mL/min. In isocratic system, the concentration of the solvents remains constant throughout the run. The injection volume was 10 μL and the compounds were detected at 247 nm.

2.4. Sample Preparation. Standard stock solutions (internal standard) of the plantarenalioside and boschnalioside were prepared at a concentration of 1.0 mg/mL with HPLC grade water. For sample preparation, 100 mg of powdered form of shoot root and flowers were extracted in 25 mL volumetric flask with 5 mL of double distilled water using sonicator for 1 min and filter the extract. Again add 3 mL distill water and again extract the residue followed by filtration. Combine the filtrate and then allow it to lyophilize to get powder form. The lyophilized powdered extract was then finally dissolved in 5 mL of HPLC grade water prior to HPLC analysis. All the extracts and standard stock solutions so made were finally filtered through 0.22 μm pore size, Merck filter for HPLC analysis.

2.5. Calibration Curve and Linearity. Six different concentrations of both plantarenalioside and boschnalioside ranging from 15.6 $\mu\text{g/mL}$ to 500 $\mu\text{g/mL}$ were prepared from the standard stock solution. HPLC analysis was done on 10 μL injection volume of each concentration in duplicate. The calibration curve was plotted by peak area versus concentration. The results are shown in Table 1.

2.6. Limit of Detection and Quantification. The limit of detection (LOD) and limit of quantification (LOQ) of plantarenalioside and boschnalioside were determined by signal to noise (S/N) ratio of three and ten, respectively, for the lowest detectable amount of both analytes. Results were given in Table 1.

2.7. Repeatability and Reproducibility. The measurements of interday and intraday variability were utilized to assess the repeatability and reproducibility of the developed method. The intra-day variability was performed under optimal conditions by means of six replicates of mixed standard. The percentage relative standard deviation RSD of both compounds was also calculated. The inter-day variability was examined for three days by performing six replicates each day. The precision of the method was determined at three different concentrations and results are given in Table 2.

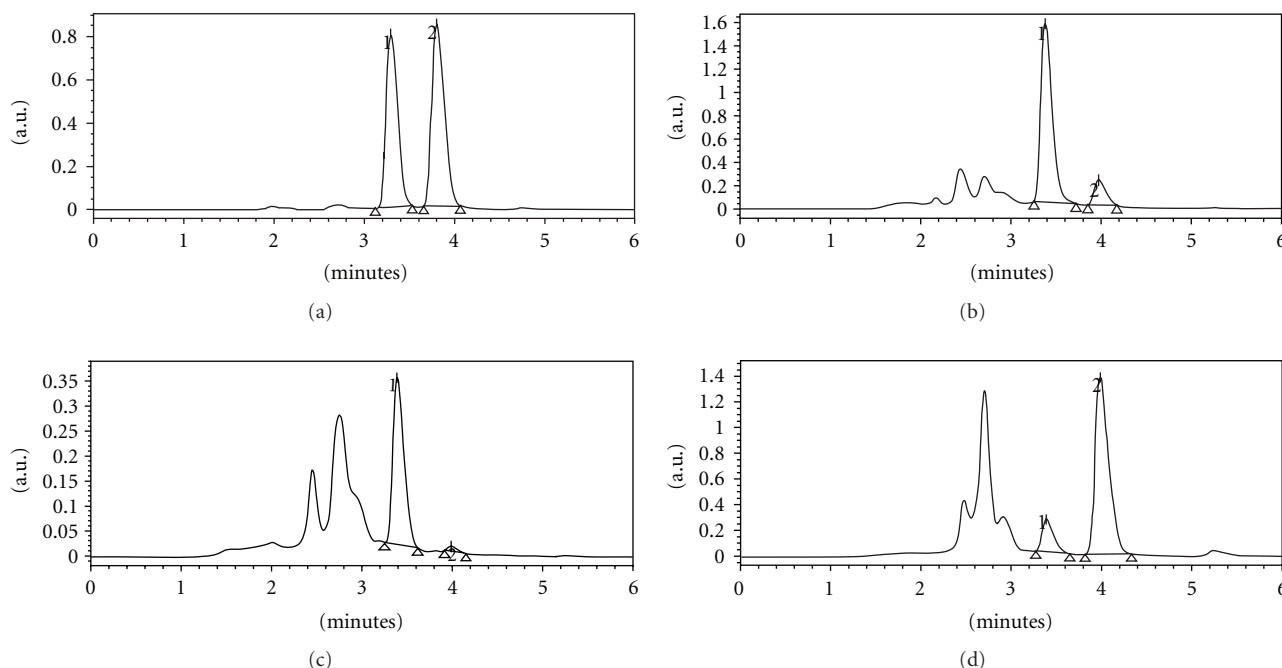


FIGURE 2: HPLC chromatograms (a) mixed standard of both plantarenalloside (1), and boschnalloside (2), (b) shoot portion, (c) root portion, and (d) flower portion.

3. Result and Discussion

In the HPLC method development, the mobile phase using methanol and water in ratio of 60 : 40 v/v was found to have optimal influence in the resolution of the plantarenalloside and boschnalloside in plant samples. The chromatograms of root, shoot, and flower extracts of *Incarvillea emodi* are shown in Figure 2. This method is short, precise, and very suitable for detection and quantification of these iridoids.

The linear regression data for calibration curve of plantarenalloside and boschnalloside shows good linear relationship. The LODs ($S/N = 3$) for plantarenalloside and boschnalloside were 11.4 and 22.8 ng and LOQs ($S/N = 10$) for plantarenalloside and boschnalloside were found to be 38 and 76 ng, respectively. The precision data results for intra-day and inter-day variations of both iridoids were recorded in the range of 0.26–0.88% and 0.28–0.84%, respectively, at three different concentrations levels (125, 250 and 500 $\mu\text{g/mL}$).

The HPLC analysis results show presence of plantarenalloside at concentrations of 6.78%, 1.19%, and 0.87% in shoots, roots, and flowers, respectively, in dry weight basis of the plant powder form, while boschnalloside concentration in the respective plant dried powder form ranged in the order 0.88%, 0.03%, and 6.12%, respectively. This shows that plantarenalloside is the major iridoid in shoots and roots of *Incarvillea emodi* while boschnalloside is found to be in major amount in flowers. Aerial portion of *Incarvillea emodi* (shoots and flowers) contains combined 7% approximately of these two iridoid glucosides.

4. Conclusion

A rapid and sensitive HPLC-DAD method for the determination and quantification of iridoid glucosides, plantarenalloside, and boschnalloside was developed. This is the first ever report on the quantification of these iridoid glucosides, from different parts of *Incarvillea emodi*. The present study shows that shoots contain major amount of plantarenalloside, while flowers contain major amount of boschnalloside. There is a presence of 6.78% of plantarenalloside in shoots and 6.12% of boschnalloside in flowers of *Incarvillea emodi*. This plant shows a very good example of dominance of individual iridoid glucoside plantarenalloside over boschnalloside in shoots and reverses in case of flowers. The method can serve to quantify such iridoid glucosides from other plants of same taxa or larger order while exploring role of these two iridoid glucosides as chemical markers in ecological context.

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