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# Altitudinal variation in pharmacologically active compounds of wild and cultivated populations of *Epimedium elatum*

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ABSTRACT

*Epimedium* is a well known genus of Chinese pharmacopoeia, possessing various medicinal properties such as aphrodisiac, antioxidant, immunomodulatory, vasodilatory, hepatoprotective, cardioprotective, antidepressant, anticancerous and antiosteoporosis activities. The active principle has been found to be its flavonoid glycosides, especially Icariin and Icariside-II. In the present study, *Epimedium elatum*, the only species of this genus growing in Indian subcontinent and endemic to Kashmir Himalayas, has been studied for its active principle content at different habitats. The plants were collected from wild populations (W-I W-II &W-III) growing at three different sites of different altitudes and cultivated at low altitudes of Central Kashmir. After two years, the plants from wild populations as well as cultivated populations were collected, shade dried, grinded and prepared for HPLC analysis. The results showed that the content of active principles in leaves vary significantly between plants growing at different habitats. The Icariin and Icariside-II yield (per plant) of wild populations significantly increased with a decrease in altitude of habitat. Cultivated population growing at higher altitude had significantly more yield than all other populations, except the W-III. The content of active principles as a percentage of leaf dry mass increased with increase in altitude. However, the harvest index of wild populations showed a decreasing trend with increasing altitude. The content of active principles as a percentage of dry mass of whole plant was comparable in all the habitats, including the cultivated populations. The present study suggests that *Epimedium* plants cultivated at lower altitudes are equally or more productive in terms of Icariin and Icariside-II content than that of wild plants growing at higher altitudes.



## 1. Introduction

*Epimedium* L. (Berberidaceae) is a genus of about 54 species, distributed chiefly in the Mediterranean region and East Asia (Stearn, 2002; Sun et al., 2005). About 80% of the total species are found in central-southeastern China (Ying, 2001), while only one species (*Epimedium elatum*) is found in Indian subcontinent. It is a well known medicinal genus of Chinese pharmacopoeia (China Pharmacopoeia Committee, 1999), having a long history of use in traditional Chinese medicine (TCM) against many diseases (Tang and Eisenbrand, 1992 Zhu, 1998). Recent pharmacological and clinical studies on various species have confirmed medicinal properties such as estrogenic (Naeyer et al., 2005), antiaging, neuroprotective, antimicrobial (Ma et al., 2011), energy-promoting, memory-enhancing, hypoglycemic, expectorant and antiasthmatic activities (Cai et al., 1998; Lai, 2001; Zhu, 1998); in addition to the activities described below for Icariin and Icarisie-II.

Most of these medicinal properties are attributed to its flavonoid glycosides, such as Icariin, Icariside-II, Epimedin A–C, Sagittoside, etc., (Liang et al., 1996; Liang et al., 1997; Ma et al., 2011). Among them, Icariin is the major pharmacologically active constituent (Shen et al., 2007; Wang and Huang, 2005). Icariin (5-hydroxy-4′-methoxyl-8-prenylflavone-3-O-α-L-rhamnopyranoside-7-O-β-D-glucopyranoside) has been found to possess effective aphrodisiac (Tian et al., 2004; Xin et al., 2003), immunomodulatory (Liang et al., 1997), hepatoprotective (Lee et al., 1995), cardioprotective (Zhang et al., 2000), vasodilatory (Guan et al., 1996), antidepressant (Pan et al., 2005), antiosteoporotic (Ma et al., 2002; Meng et al., 2005), antioxidant and antiapoptotic activities (Wang and Huang, 2005). Because of these medicinal properties, Icariin is a recognized biomolecule that is being commercially exploited as a drug in different formulas and is the quality control standard in many Traditional Chinese Medicine at present (Wang and Lou, 2004). Another impor-

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tant pharmacologically active constituent, Icariside-II (also called baohuoside-1; 5,7,-dihydroxy-4′-methoxyl-8-prenylflavone-3-O-α-L-rhamnopyranoside), has cytotoxic and cytostatic effects on 6 cancer cell-lines (Li et al., 1990). It has a great value in the treatment of chronic inflammatory and autoimmune diseases (Li et al., 1994; Ma et al., 2004). *Epimedium* is regarded as a prized genus because of its well known ethnobotanical uses and experimentally confirmed medicinal properties. The genus is increasingly exploited as a commercial drug either in the raw form as dry leaves and stem, or as a crude extract or as purified compounds in different formulas that are sold in the form of capsules, tablets or sachets (Ma et al., 2011). High international demand for its raw material as well as products has prompted a global need to cultivate its species, in addition to wild collection.

*E. elatum* Morr. and Decne. is a very little known species of this well known genus and is endemic only to Kashmir Himalaya. It is the only representative of this genus in Indian subcontinent, growing wild under the canopy of coniferous forests. The plants are erect, shade-loving, perennial herbs, 50–100 cm tall, with glabrous stem and branches, sub-serrately toothed leaflets and pale yellowish or yellowish white flowers.

In our previous study (Arief et al., 2015), *E. elatum* Morr. and Decne., has been shown to possess an appreciable content of icarriin and Icarisde-II, the two major pharmalogically active ingredients, thus qualifying for medicinal plant status. The present work has been undertaken to study the active principle content, harvest index and yield of *E. elatum* at different habitats. The main aim was to compare the content of active principle between plants of wild populations growing at higher altitudes with the transplanted populations cultivated at lower altitudes.

## 2. Material and methods

### 2.1. Plant material

*E. elatum* Morr. and Decne. plants were identified on the basis of the relevant literature (Jafri, 1975) and the specimens of Herbarium KASH, Department of Botany, University of Kashmir. A voucher sample was also deposited in the Herbarium KASH.

### 2.2. Collection and cultivation

The plants were identified, morphologically studied and collected from three wild populations (W-I, W-II and W-III), growing at three different sites of different altitudes (W-I: 2730 m; W-II: 2660 m; and W-III: 2350 m asl). The propagules (rhizomes) from wild population W-I were collected in spring, and transplanted and cultivated at lower altitudes of Central Kashmir (C.I at Beerwah, 1754 m and C.II at Srinagar, 1616 m asl) in completely randomized block design. The plants were cultivated in loamy soil having pH 6, under diffused shade in net house and irrigated regularly without any fertilization.

### 2.3. Harvesting, drying and determination of harvest index

After two years, the plants from wild populations as well as cultivated populations were harvested and shade dried at room temperature in well ventilated room. The shade dried parts of the plant were weighed to determine their dry mass per plant. Since leaves are the harvestable part of this plant with regard to the active constituents, the harvest index (HI) was calculated on dry mass of leaves as a percentage of total dry mass, according to the following formula:

$$\text{Harvest index}\,(\%) = \frac{\text{Biomass of harvestable part of plant (leaves)}}{\text{Total biomass of whole plant}} \times 100$$

### 2.4. Extraction

The shade dried aerial parts (leaves) were chopped and grinded into fine powder. 100 g of the powdered material from each sample was extracted thrice with 1 l of 95% ethanol for 24 h at room temperature. All the three extracts (washings) from each sample were mixed and then dried by evaporating the solvent in a thin film rotary evaporator (Heidolph, Hyderabad, India). All extractions were done in duplicate and the subsequent assays were run in triplicate. Qualitative analysis of extract was performed by TLC. The dried extract was run on TLC using solvents chloroform and methanol in 8:2 ratio and co-TLCed with marker compounds, Icariin and Icariside-II.

### 2.5. High performance liquid chromatography (HPLC) analysis

HPLC machine (Thermo Finnigan, Co., Ltd., San Jose, CA, USA), consisting of quaternary P4000 pump, AS3000 autosampler, UV6000LP photo diode array detector and a reverse phase C-18 column (5 μm, Merck, 250 × 4 mm), was used. The machine is fully computer controlled and data is acquired using Chromquest software (Version 4.0).

The dried extract was dissolved in HPLC grade methanol at 12.50 mg ml$^{-1}$. The solution was filtered and the filtrate taken for HPLC analysis. Icariin and Icariside-II were also dissolved in HPLC grade methanol at 0.11 mg ml$^{-1}$ and 0.10 mg ml$^{-1}$ respectively and calibrated on HPLC. HPLC analysis was performed according to protocols given by Liu et al. (2005) and Chen et al. (2007), with some modifications. HPLC analysis was performed at an oven temperature of 30 °C. The mobile phase for Icariin was acetonitrile-water in gradient mode as follows: 0–12 min, 28% acetonitrile; 12–20 min, 28–35% acetonitrile; 20–30 min, 35% acetonitrile; 30–35 min, 35–28% acetonitrile; 35–40 min, 28% acetonitrile. The effluent was monitored at 270 nm and the flow rate was 1.0 ml min$^{-1}$ constantly. Injection volume was 4 μl for marker and 10 μl for samples. Linear calibration curve was obtained for Icariin for concentration ranges of 0.22–1.1 μg. However, the mobile phase for Icariside-II was methanol-water (80:20) and the flow rate was 0.6 ml min$^{-1}$ constantly. Linear calibration curve was obtained for Icariside-II for concentration ranges of 0.12–0.60 μg. Samples were injected in triplicate. The chromatogram was prepared to show both the qualitative and quantitative data of Icariin and Icariside-II. Peak identification was performed by comparison of retention times with the standards. Co-chromatography was also used to confirm peaks. The chromatograms were compared with the calibration curves to quantify the content of Icariin and Icariside-II. The results were computed to give quantity of active principles as a percentage of dry mass of leaf as well as whole plant. The yield of active principles was calculated as the absolute content of Icariin/Icariside-II in aerial parts per plant (mg/plant).

### 2.6. Reagents

Pure standards of Icariin and Icariside-II were a gift from Professor Bao Lin Guo of Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China. The purity of compounds was more than 98% as determined by HPLC.

All organic solvents (ethanol, chloroform, methanol) used for preparation of crude extract and TLC separation were of analytical grade (E. Merck, Mumbai, India), while methanol and acetonitrile used for HPLC was of HPLC grade (E. Merck, Mumbai, India). The water used was fresh ultra-pure distilled water, purified by a Millipore water purification system (Milford, MA, USA).