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GENTIOPICRIN AND SWERTIAMARIN CONTENTS IN *GENTIANA MACROPHYLLA* PALL. ROOTS ALONG ELEVATION GRADIENT IN DONGLINGSHAN MEADOW, BEIJING, CHINA

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Abstract

Quality and quantity of chemical constituents in medicinal plants highly depends on environmental conditions. The contents of active constituents in *Gentiana macrophylla* Pall. may vary among different altitudes of mountain meadows and are affected by environmental factors. The aim of this study was to find the effect of environmental factors on Gentiopicrin and Swertiamarin contents in *G. macrophylla* roots along elevation gradient of Donglingshan meadow. Plant and environmental data were collected from 15 altitudes (50m distance away from each other) along 1600-2301 m elevation gradient by using Braun Blanquet approach. The contents of Gentiopicrin and Swertiamarin were estimated by using High Performance Liquid Chromatography method. Relationship among Gentiopicrin and Swertiamarin contents, soil and other environmental variables was depicted by using Canoco 5 and SPSS software. Regression analysis showed that Gentiopicrin is strongly affected by Elevation, Slope aspect, Soil pH, Soil temperature, Total Nitrogen. Swertiamarin concentration is strongly affected by slope, soil pH and Magnesium. Gentiopicrin contents have statistical significant relation with elevation gradient as compared to Swertiamarin. Insight of important bioactive compounds provided by this study would be helpful for medicine quality control, conservation of *G. macrophylla* and discovery of new drugs.

Key words: HPLC, Qin-Jiao, Quantitative ecology, PCA, Anti-rheumatic.

Introduction

The pharmacopeia of Peoples Republic of China refers the roots of *Gentiana macrophylla* Pall., *G. crassicaulis*, *G. straminea* and *G. dahurica*, as Qin-Jiao. Qin-Jiao has been used as efficient drug to treat jaundice, rheumatism, constipation, pains and hepatitis since 2000 years ago. Iridoids (loganic acid and harpagoside) and secoiridoids (swertiamarin, sweroside and gentiopicroside) are the principle compounds found in Qin-Jiao (Ma & Lin, 2008).

G. macrophylla is native to northwestern China. Its major component, gentiopicroside is used to treat osteoarthritis, rheumatism, ulceration and pains. Due to its vast medicinal usage *G. macrophylla* is vulnerable to serious destruction of habitat and wild population. Preliminary phytochemical analysis depicted the contents of phenolics and flavonoids in the extract to be 26.70 ± 1.5 mg gallic acid equivalent (GAE)/g DW and 10.11 ± 0.8 mg quercetin equivalent/g DW respectively. The phenolics and flavonoids content in the extract was found to be positively correlated to antioxidant activity of the plant extract (Yin *et al.,* 2018). Zhao *et al.,* (2007) used High Performance Liquid Chromatography (HPLC) to determine the gentiopicroside from *G. macrophylla* roots. Su *et al.,* (2012) established a finger print method for the identification of chemical contents from the roots of *G. macrophylla*. Iridoids, sterols, triterpenes and flavonoids have been obtained from its roots (Tan *et al.,* 1996). Isoorientin, vitexin, lutpnarin, saponarin, isovitexin and isoscoparin have been detected in *G. macrophylla* flowers (Olennikov *et al.,* 2015; Jia *et al.,* 2012).

Mountain meadows are treeless areas found on the peaks and upper slopes of mountains with specific environmental conditions such as low temperature, large temperature amplitudes, high wind speed and frequent soil moisture stress (Spira, 1986). Chemical composition and quality of medicinal plants depends upon geographical origin, species in the same territory, cultivation and environmental factors. As a comparison profiling of various species of "Longdan" and "Qinjiao" with their adulterants and applying multivariate statistical analyses to their profiling data sets established the differences among them (Liu *et al.,* 2014). Quality of herbal medicine depends on chemical contents produced by them. Environmental factors affect the accumulation, production, proportion, type and contents of phytochemicals (Pavarini *et al.,* 2012; Liu *et al.,* 2015). Therefore, study of yield and composition of secondary metabolites in relation to environment is very important for sustainable conservation of endangered plant species (Liu *et al.*, 2015). Multivariate statistical analyses of *Tithonia diversifolia* phytochemical data were carried out by Sampaio *et al*., (2016). A significant correlation between metabolites and environmental factors (temperature changes, rainfall) was found. Similarly, root metabolites were found to be strongly affected by soil nutrients (Cu, P, K, Ca, Mg). Results obtained from computational assessment of ecological effect on contents extracted from rhizome and roots of *Sinopodophyllum hexandrum* showed that ecological variable mostly affected the quantity of chemicals but not the type (Liu *et al.,* 2015).

The objective of this study was to find out quantities of Gentiopicrin and Swertiamarin in *G. macrophylla* roots at different elevation and the effect of other environmental variables on them in Donglingshan Meadow, Beijing, China.

Materials and Methods

Plant and environmental data: On the basis of presence of *G. macrophylla*, fifteen sampling points (50m away from each other) were established along an elevation gradient of 1600-2301 m of Donglingshan meadow. Three roots samples of *G. macrophylla* were collected from each point and then collated to form one sample for each point (elevation). Plant sample was identified by Prof. Liu Quanru, plant taxonomist in College of life sciences, Beijing Normal University, Beijing, China. Seventy five quadrats were randomly set up (5 at each sampling point). Height and cover for 85 plant species was recorded for calculation of importance values.

Soil samples were collected (20cm in depth) from each point using a soil auger, put in zipper bags and taken to laboratory for processing and chemical analysis. Total nitrogen (TN), total phosphorus (TP), total potassium (TK), magnesium (Mg) and zinc (Zn) concentration was estimated by using inductively coupled plasma emission spectrophotometer, visible spectrophotometer and Atomic absorption spectrophotometer (Ata *et al.,* 2015; Zhang *et al.,* 2011).

Altitude, slope and slope aspect were measured by using GPS and Compass meter. Soil pH and soil temperature were measured by using pH meter and thermometer respectively (Zhang & Chen, 2004; Zhang *et al.,* 2012). Rope test was used to measure soil type (Nyobe *et al.,* 2014). Aspect measurements were standardized by assigning them classes. Disturbance intensity was recognized on the basis of tourists' number, distance from nearest human population and road, grazing, trampling and garbage quantity (Zhang *et al.,* 2011). Its evaluation was done on a scale of 1-5 as follows; 1(no distinct disturbance), 2(less disturbance), 3(medium disturbance), 4(Intense disturbance), 5(very intense disturbance).

Measurement of Gentiopicrin and Sweriamarin contents

Chemicals: Methanol (HPLC grade), Phosphoric acid, Acetonitrile, Ultra high purity water. Standard Gentiopicrin and Swertiamarin (Fig. 1).

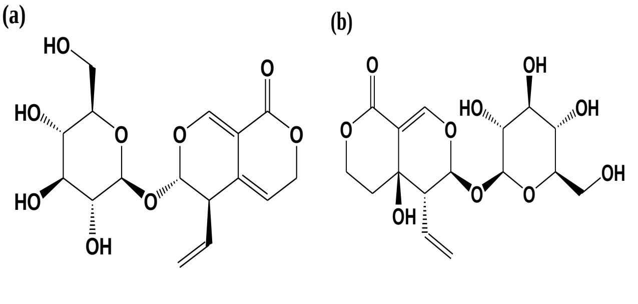

Fig. 1. Chemical structure of (a) Gentiopicrin and (b) Swertiamarin (Mirzaee *et al*., 2017).

Equipment: Supersonic SY-3200 was used to assure complete dissolution of root contents. Rotatory evaporator (Type RE-52AA) was used for dehydration of extracts. HPLC system (Agilent Technology 1200 series Isocratic) provided with a manual sample injector and Zorbax C18 SB-Aq (5µ 4.6*250mm, USA) column was used for bioactive compound separation.

Preparation of roots extract: Dried roots were crushed to make small pieces. One gram of dried root was weighed and put into test tube. Methanol (20ml) was added into it and it was treated with supersonic. Left it to settle for 30 minutes. After 30 minutes, it was again treated with supersonic and left for another 30 minutes. Filtration was done and procedure was repeated twice with the residual. Dehydration of filtrate was carried out with a rotatory evaporator (type RE 52AA) in a Bain Marie at 40ºC. After dehydration, 1ml of methanol was added. Overall, 15 samples of roots extract were prepared.

Preparation of standard solutions: Gentiopicrin (1mg) and Swertiamarin (1mg) was dissolved in 1ml methanol to prepare standard solutions.

Chromatographic conditions of HPLC analysis: The suitable conditions were obtained by linear gradient elution. The mobile phase consisted of 0.25% phosphoric acid, 15% acetonitrile and 85% water at a flow rate of 1ml/min in a gradient elution. Temperature of column was room temperature and UV detection wavelength was 254nm. Firstly, standard solutions were run at the injection volume of 20 µL to obtain standard peaks. Then samples were injected. System restoration time was 15 minutes.

Data analysis: Chromatographic data was obtained by in-built Agilent Chem Station software of HPLC system. Relationship between Gentiopicrin and Swertiamarin contents, soil and other environmental variables was carried out by using PCA package of Canoco 5 software and SPSS.

Results

For the determination of Gentiopicrin and Swertiamarin, various mixtures of methanol, water, phosphoric acid and acetonitrile were tested as flow phase. Tests resulted in 0.25% phosphoric acid, 15% acetonitrile and 85% water as appropriate flow phase. The quantities of Gentiopicrin and Swertiamarin were calculated from the standard curves obtained by High Profile Liquid Chromatography (HPLC). Both compounds showed good linear regression with high correlation coefficient values (peak areas and amount). Linearity was good (r^2>0.99) in all concentrations (1, 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/ml) of standards. Fig. 2 shows the peaks of standards and sample. Concentration of Gentiopicrin and Swertiamarin varies from 0.04 to 0.29 mg/ml and 0.08 to 0.12 gm/ml in all the samples (Table 1).

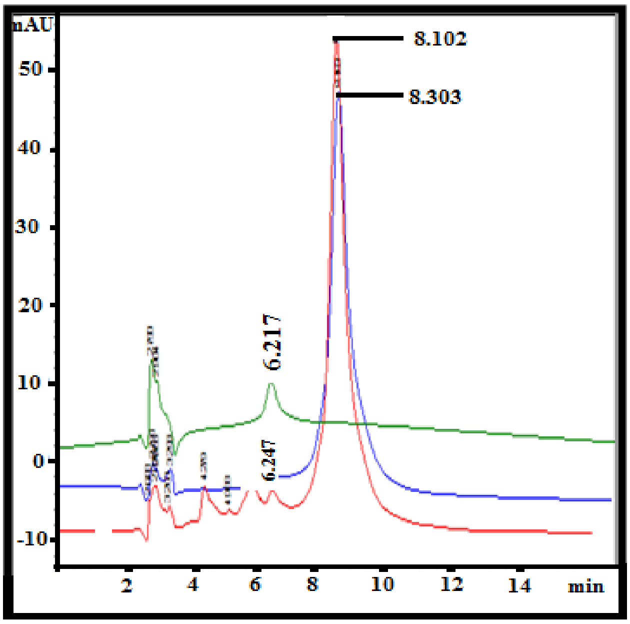

Fig. 2. Chromatographic peaks of Standards and sample (Green colored peak represents Swertiamarin standard (6.217), Blue colored peak represents Gentiopicrin standard (8.303), Red colored peak represents sample).

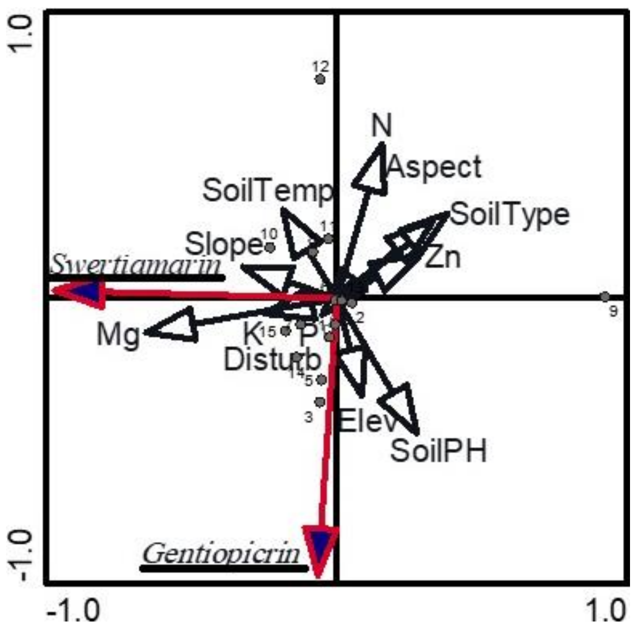

Fig. 3. Principle component analysis; triplot of 2 bioactive compounds, 12 environmental variables and 15 samples.

Principle component analysis (PCA) was run for 15 samples and 12 environmental variables. A relationship between environmental variables and chemical compounds was confirmed. The Monte Carlo permutation test showed that eigenvalues for all axes are significant ($p<0.001$). Eigen values for first and second axis are 0.7016 and 0.2984 respectively. PCA triplot indicated that Gentiopicrin contents are strongly affected by elevation gradient as compared to Swertiamarin (Fig. 3).

Regression analysis showed that Swertiamarin concentration is strongly affected by slope, soil pH and Magnesium (Fig. 4). Gentiopicrin was strongly affected by Elevation, Slope aspect, Soil pH, Soil temperature, Total Nitrogen (Table 2; Fig. 5).

Discussion

Relationship between plant growth, reproduction, development, distribution, diversity and environment is an important aspect of ecological research (Zhang *et al.*, 2011; Naveed *et al.*, 2019). Correlation studies of bioactive compounds and environment have been carried out in cultivated plants and crops (Zhou, 2003; Hayashi & Sudo, 2009). We studied the relationship between quantity of bioactive compounds and environmental factors in natural population. Evaluation of plant products quality is done by using the information on relationship between secondary metabolites and environmental variables. In addition to evaluation of *G. macrophyalla* quality, this study is also helpful to determine the conservation measures of this medicinal plant in its natural population (Zhang, 2005).

Environmental factors affect the bioactive compounds because different environmental conditions affect the metabolism, physiology and accumulation of bioactive constituents leading to produce secondary metabolites differently. There is significant difference between contents of Gentiopicrin and Swertiamarin in the *G. marophylla* root. This result is similar to Lu *et al.*, (2014) for Swertiamarin and Gentiopicroside. Results are also consistent with another study for other species of *Gentiana* (Huang *et al.*, 2013) in which Gentiopicrin contents are affected by elevation. This result is in line with the similar studies for other plant species (Zhang *et al.*, 2011; Ai, 2004). Genetic structure, plant diversity and environment may be the most important reasons for the variation in active compounds contents (Duffy *et al.*, 2009; Nyobe *et al.*, 2014).

Effect of soil pH was negative and significant on the Swertiamarin contents whereas positive and significant on Gentiopicrin contents. It depicts the importance of soil pH in determining the contents of active compounds in *G. macrophylla*. Similar relationship was observed for soil minerals and active compounds in *Glycyrrhiza uralensis* in another study (Zhang *et al.*, 2011).

Among soil nutrients magnesium and total nitrogen significantly affected the contents of Swertiamarin and Gentiopicrin respectively. Magnesium is positively related to Swertiamarin and total nitrogen is negatively related to Gentiopicrin. Whatever are the Gentiana species and site, contents of Gentiopicrin remain higher than Swertiamarin contents in *G. macrophylla*. This result exactly matches to the compounds checked by Nyobe *et al.*, (2014) in another medicinal plant. Soil and other environmental factors strongly affect the phytochemical profile of a plant. Especially, soil fertility has an important role in this regard. Imbalanced nutrients can lead to various environmental stress in the plant which affect the production of bioactive compounds in them. Hence, specific soil nutrients and environmental conditions obtaining the highest quantity of bioactive compounds have been pointed out in our research. Moreover, being a rich source of Gentiopicrin, *G. macrophylla* can be used for further screening and investigation on the positive bioactivity interaction of Gentiopicrin acting as antirheumatic agent in medicinal research.

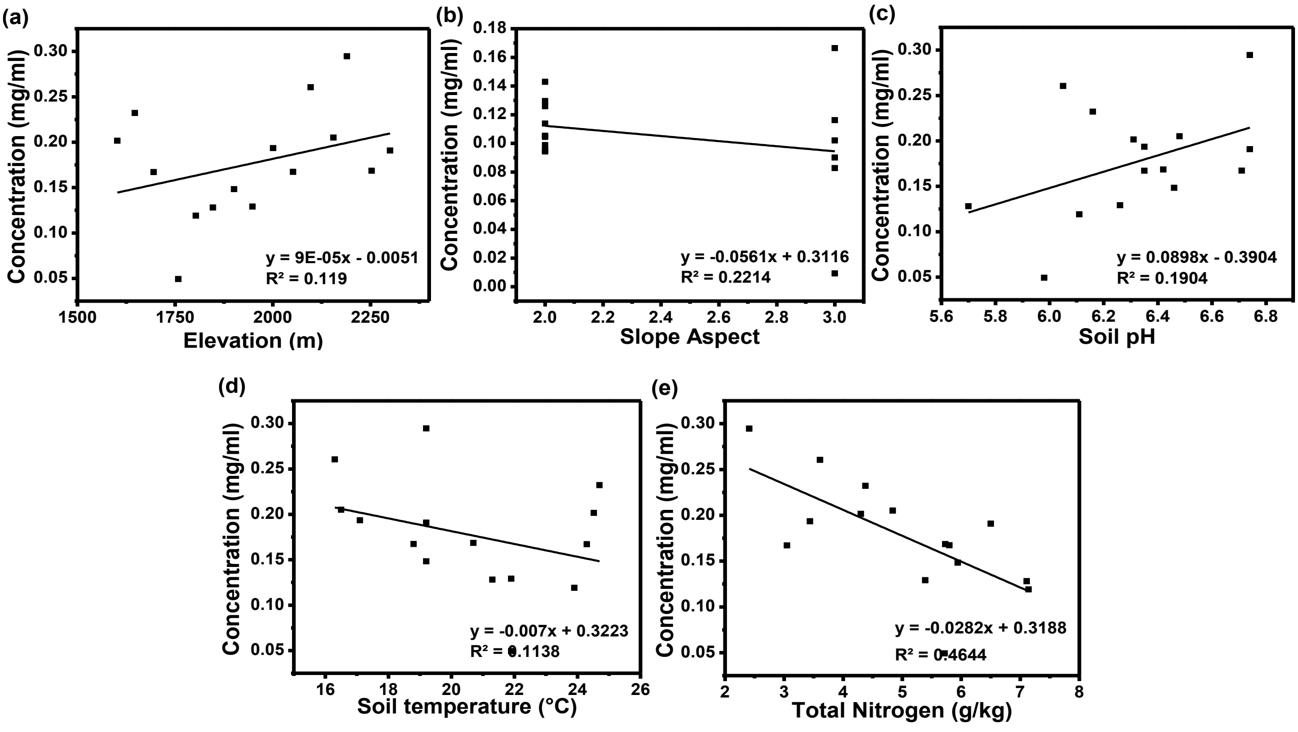

Fig. 4. Relationship between (a) Gentiopicrin contents and Elevation (b) Gentiopicrin contents and slope aspect (c) Gentiopicrin contents and soil pH (d) Gentiopicrin contents and soil temperature (e) Gentiopicrin contents and total nitrogen.

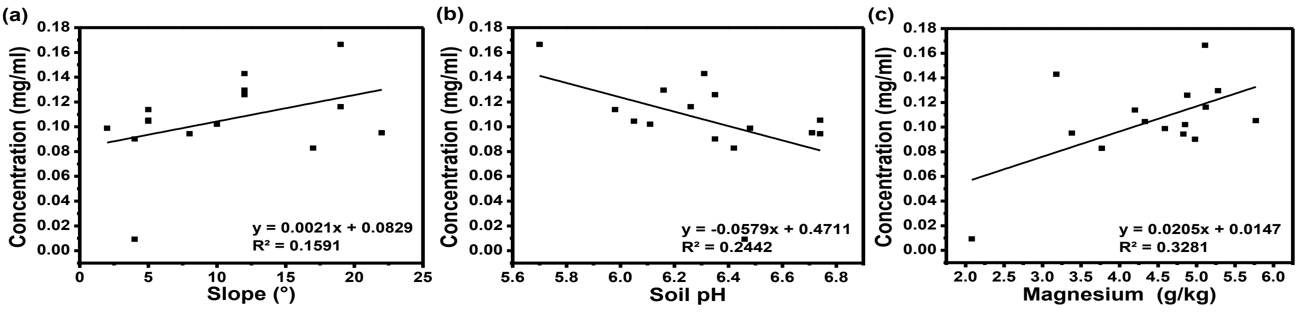

Fig. 5. Relationship between (a) Swertiamarin contents and slope (b) Swertiamarin contents and soil pH (c) Swertiamarin contents and magnesium.

Table 1. Gentiopicrin and Swertiamarin contents in G. *macrophyalla* roots.

Sample (Elevation (m))	Gentiopicrin			Swertiamarin		
	R_{time}	Concentration (mg/ml)	Area (a.u.)	R_{time}	Concentration (mg/ml)	Area (a.u.)
1 (2300)	8.135	0.1908	2782.1	6.445	0.0943	439.5
2 (2253)	8.13	0.1685	2353.7	6.241	0.0828	258.8
3 (2190)	8.122	**0.2946**	4789.3	6.261	0.1053	611
4 (2155)	8.105	0.205	3068.2	6.571	0.0988	510.7
5 (2097)	8.084	0.2604	4129	6.241	0.1045	564.8
6 (2052)	8.102	0.1673	2325.8	6.24	0.0951	466.7
7 (2001)	8.087	0.1935	2838.9	6.348	0.1259	928.4
8 (1948)	8.078	0.1291	1588.1	6.795	0.1162	776.8
9 (1901)	8.087	0.1483	1959.8	6.479	0.0093	410.7
10 (1847)	8.129	0.128	1566.9	6.307	**0.1664**	1556.1
11 (1803)	8.315	0.1191	1393.8	6.437	0.102	558.2
12 (1758)	8.264	0.0493	42.4	6.424	0.1138	740.1
13 (1695)	8.279	0.167	2322.9	6.537	0.0901	374.2
14 (1647)	8.712	0.2322	3594.5	6.305	0.1296	983.4
15 (1602)	9.034	0.2016	2994.7	6.378	0.1429	1176.5

Table 2. Quantity of soil minerals present in the soil collected from vicinity of *G. macrophyalla*.

Samples (Elevation (m))	Total nitrogen	Total phosphorus	Total potassium	Magnesium	Zinc
1 (2300)	8.135	0.1908	2782.1	6.445	0.0943
2 (2253)	8.13	0.1685	2353.7	6.241	0.0828
3 (2190)	8.122	0.2946	4789.3	6.261	0.1053
4 (2155)	8.105	0.205	3068.2	6.571	0.0988
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Conclusion

Early research on *Gentiana* species was focused on its ethnobotanical and folk uses as cure to joint pain related diseases. After biochemical fingerprinting, Gentiopicroside along with many other compounds was characterized with respect to biological activity. Environment plays basic role in the constituent development of medicinal plants. Correlations between bioactive compounds and environmental variables established in this study can be used for quality control as well as preservation and conservation of *G. macrophyalla*.

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