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Geographical Distribution and Environmental Correlates of Eleutherosides and Isofraxidin in *Eleutherococcus senticosus* from Natural Populations in Forests at Northeast China

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Abstract: Non-wood forest products (NWFPs) derived from understory plants are attracting attention about sustainable forestry development. Geographical distribution and climate correlates of bioactive compounds are important to the regional management for the natural reserves of medical plants in forests. In this study, we collected *Eleutherococcus senticosus* individuals from 27 plots to map the special distribution of concentrations of eleutheroside B, eleutheroside E, and isofraxidin in forests of Northeast China. Compound concentrations in both aerial and underground organs were further detected for relationships with the average of 20-year records of temperature, precipitation, and relative humidity (RH). We found higher shoot eleutheroside B concentration in populations in northern and low-temperature regions ($R = -0.4394$; $P = 0.0218$) and in eastern and high-RH montane forests ($R = 0.5003$; $P = 0.0079$). The maximum-likelihood regression indicated that both RH (*Pr* > Chi-square, 0.0201) and longitude (*Pr* > Chi-square, 0.0026) had positive contributions to eleutheroside B concentration in roots, but precipitation had strongly negative contributions to the concentrations of eleutheroside E (*Pr* > Chi-square, 0.0309) and isofraxidin (*Pr* > Chi-square, 0.0014) in roots. Both geography and climate factors had effects on the special distribution of medical compounds in *E. senticosus* plants in natural populations in Northeast China. The management of NWFP plants at the regional scale should consider effects from climatic geography.

Keywords: ciwujia; Siberian ginseng; *Acanthopanax*; secondary metabolite; Changbai Mountains; Khingan Mountains

1. Introduction

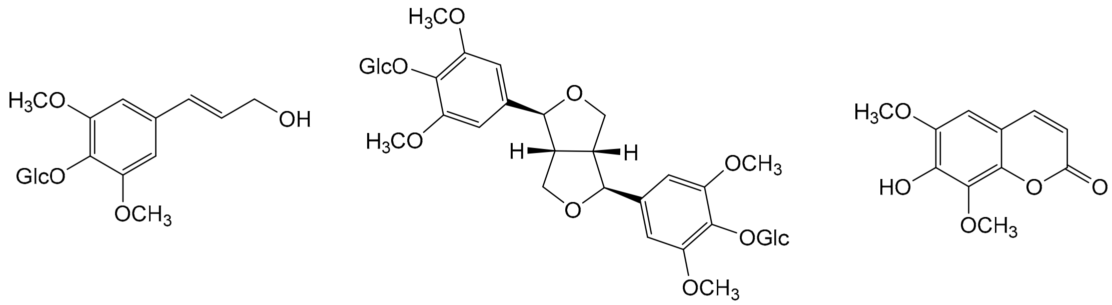
Climate change is expected to drive the shift in structure and composition of forest ecosystems [1]. Forest vegetation is a critical player in land–atmosphere interactions by regulating the energy, water, and carbon cycles [2]. Over the last decades, it was found that growth of temperate forest vegetation is heavily dependent on rising temperature [3,4]. In contrast, the rising (CO_2) consequence of this growth contributed to the increase of daily maximum temperature, which can reinforce the mean temperature increase in temperate forests [2]. Currently, both empirical models and field investigations have intensively detected the change of general vegetation in temperate forest ecosystems in the scenario with increasing temperature. Although plants in the understory plants are sensitive and adaptable to local environment conditions [5–8], their relationship with climatic factors has been less understood.

Many understory plants are of significant values in edible and medicine manufacture, which can be classified into the category of non-wood forest products (NWFPs). NWFPs are defined in this context as all the products other than woods derived from forests, shrubs, tree plantations, and trees outside forests [9,10]. NWFPs can be taken as a potential asset in forest management system generating socioeconomic benefits along the entire value-chain spanning rural development [11]. Increasing needs to touch nature and to consume forest products promote the international trade of NWFPs through countries and interest in NWFPs is growing in Europe and East Asia [9,11–13]. Because of the cover of tree canopy, it is unlikely to directly detect the relationship between NWFP plants and regional climate factors using the model with data about dominant trees. Therefore, it is of strong necessity to make field investigation for functional traits of NWFP plants to establish their responsive dataset. This consumes significant workload and time and much less is known about the response of NWFP plants to regional climate change.

Traditional culture of original stocks to produce NWFPs was conducted on farmland using cloth-shading at high density [14–16]. However, practices are accumulating to cultivate NWFP plants in forests at the understory layer where trees provide necessary shade and soils supply sufficient rhizosphere condition [13,15]. The forest-culture mode can further contribute to the conservation of natural forest ecosystem by fully exploiting the resource of the understory space. Mapping the distribution of natural populations can supply essential information for ecologically sustainable management of NWFP plants [17]. Environmental factors varied with geographical patterns which drive the spatial distribution of phenotype of local plants [5,18]. Therefore, to map the geographical distribution of traits of NWFP plants would benefit the illustration of adaption to location environment and further analyze the response to climate change.

Eleutherococcus senticosus is one of the NWFP species with thorny stem and branch which is also one of the best-known medical plants from the Araliaceae family. *E. senticosus* is also referred to as *Acanthopanax senticosus* as well with so-called names of Siberian ginseng in Europe and America, ciwujia in China, and gasiogapi in Korea [19]. *E. senticosus* is commonly used as an ingredient of folk medicines which have been documented in both Chinese and European pharmacopoeia [20]. Abundant content of multiple bioactive ingredients contributes to the medical value of *E. senticosus*. Eleutheroside B is the main effect compound due to antioxidant, immunomodulatory, and anti-inflammatory activities [21,22]. Eleutheroside E is another bioactive ingredient that has stress-protective activities. The structures of eleutherosides B and E are elucidated as lignans of syringin and acanthoside D, respectively (Figure 1). Isofraxidin (7-hydroxy-6,8-dimethoxycoumarin; Figure 1) has similar effect as eleutherosides and performs better in antifatigue and antistress [21]. Organs of stem, branch, and roots all had records to be detectable for these bioactive compounds [21,22]. Studies of environmental factors on metabolites are scattered and placed by limited attention. It was indicated that temperature in the range of 12–18 °C resulted in the accumulation of total phenolics, flavonoids, and eleutheroside E in *E. senticosus* [3,23]. These results suggest that the trait of metabolites in *E. senticosus* may be distributed with some geographical pattern by the driving of regional environmental factors. However, to the best of our knowledge, relevant evidence is quite scarce for either a geographical map of metabolites or clear relationship with environmental factors.

Forests in Northeast China reserve abundant amount of natural *E. senticosus* populations, which were chosen as the objectives of this study. We conducted a field investigation to collect aerial and below-ground organs to detect contents of eleutheroside B, eleutheroside E, and isofraxidin. The aim of the study was to map the special distribution of these bioactive compounds in forests of Northeast China and further detect the environmental correlates. With regard to the experimental results that eleutherosides accumulate in cooler temperature [3,23], it was hypothesized that metabolite content was higher in the northern part of the research region or in montane areas at higher elevations.



Eleutheroside B   Eleutheroside E   Isofraxidin

Figure 1. Chemical structures of eleutherosides B and E and isofraxidin [24,25].

2. Materials and Methods

2.1. Study Area

Forests in Northeast China were chosen as the study area. Northeast China is referred to including the eastern region of Inner Mongolia Province, Heilongjiang Province, Jilin Province, and Liaoning Province. According to the topography, mountains in Northeast China include Changbai Mountains (eastern montane regions across Jilin and Liaoning Provinces), the lesser Khingan Mountains (mid- to mid-eastern regions of Heilongjiang Provinces), and the Greater Khingan Mountains (northeastern regions in Heilongjiang Province and northwestern regions of Inner Mongolia Province) [26,27]. The regional climate in mountains of Northeast China is characterized by a long and chilling winter, a dry and windy spring and autumn, and a rainy summer with mild temperature. In eastern mountains in Heilongjiang and Jilin Provinces, the mean annual temperature varies between −4.7 and 10.7 °C with an annual precipitation of 866 mm. Regional relative humidity (RH) was averaged to be about 65%. The targeted forests that harbored natural *E. senticosus* populations were mainly dominated by trees of *Abies nephrolepis*, *Alnus mandshurica*, *Betula platyphylla*, *Larix gmelinii*, *Pinus koraiensis*, *Populus davidiana*, and *Quercus mongolica* [28–30].

2.2. Data Collection

Environmental factors were studied with the focus on temperature, precipitation, and relative humidity (RH) [26]. Data about these three parameters were averaged for the historical record of annual amounts during 1996 and 2016 for 27 meteorological stations. Specific locations and topographic information for these stations are listed in Table 1. These stations were chosen because at least one natural *E. senticosus* population was found nearby. During the time from July to late August in 2017, three sub-plots were randomly set around the coordinate of the meteorological station. Each sub-plot had an area of 400 m^2 in a 20 m × 20 m spacing stand, wherein a number of 10 *E. senticosus* individuals were randomly investigated across the stand for height and root-collar diameter (RCD) (Table 1). Thereafter, both aerial and below-ground parts of investigated individuals were harvested. Roots were carefully shoveled from soils and whisked rhizosphere soils. Harvested individuals were transported to the laboratory on ice (0–2 °C) where roots were further carefully rinsed to clean the roots free from soils. Both aerial shoots and underground roots were rinsed by distilled water and prepared for further determination.

Table 1. Plot characteristics of natural *Acanthopanax senticosus* populations in forests in Northeast China.

Plot Number	Latitude	Longitude	Elevation (m)	Slope (°)	Forest Type	CD [1] (%)	Shoot Height (cm)	RCD [2] (cm)
1	49°28′41″	126°46′29″	535	5	Broadleaf-conifer	0.5	161.67 ± 10.41	1.13 ± 0.06
2	49°16′33″	128°53′30″	198	15	Mongolian oak [3]	0.9	168.33 ± 7.64	1.33 ± 0.15
3	48°47′24″	129°25′29″	317	10	Broadleaf-conifer	0.4	193.33 ± 36.17	1.57 ± 0.38
4	48°3′24″	128°59′16″	346	8	Broadleaf-conifer	0.65	203.33 ± 27.54	1.87 ± 0.23
5	47°23′57″	129°32′30″	345	10	Secondary forest	0.2	158.33 ± 16.07	1.77 ± 0.15
6	45°49′58″	130°14′24″	301	15	Secondary forest	0.5	166.67 ± 55.08	1.20 ± 0.35
7	45°43′23″	129°38′38″	164	0	Larch [4]	0.3	178.33 ± 25.17	1.30 ± 0.20
8	47°36′31″	128°30′11″	322	5	Broadleaf-conifer	0.65	196.00 ± 20.81	1.33 ± 0.21
9	47°12′35″	129°41′21″	412	4	Secondary forest	0.45	195.00 ± 21.79	1.43 ± 0.32
10	46°55′57″	128°54′37″	439	10	Broadleaf-conifer	0.7	170.00 ± 5.00	1.20 ± 0.09
11	46°38′35″	128°51′35″	327	13	Broadleaf-conifer	0.7	170.00 ± 10.00	1.68 ± 0.16
12	46°9′10″	128°40′2″	168	5	Secondary forest	0.55	168.33 ± 10.41	1.41 ± 0.12
13	45°22′45″	127°36′18″	363	13	Broadleaf-conifer	0.5	168.33 ± 7.64	1.36 ± 0.21
14	44°49′52″	129°5′47″	674	5	Fir [5]	0.7	197.33 ± 23.69	1.33 ± 0.20
15	44°52′22″	129°8′16″	540	10	Broadleaf-conifer	0.67	150.00 ± 10.00	1.09 ± 0.08
16	43°2′1″	127°59′42″	714	5	Broadleaf-conifer	0.7	176.67 ± 11.55	1.50 ± 0.20
17	42°48′58″	127°54′19″	586	15	Secondary forest	0.7	165.00 ± 21.79	1.33 ± 0.25
18	42°35′48″	127°42′32″	791	3	Secondary forest	0.5	173.33 ± 5.77	1.47 ± 0.29
19	42°2′41″	127°30′44″	839	3	Secondary forest	0.5	160.00 ± 17.32	7.40 ± 5.39
20	42°2′24″	126°43′45″	723	8	Broadleaf-conifer	0.7	186.67 ± 11.55	1.43 ± 0.15
21	41°39′48″	126°28′47″	416	10	Secondary forest	0.7	140.00 ± 0.01	0.87 ± 0.06
22	41°16′35″	126°5′16″	726	15	Secondary forest	0.7	173.33 ± 20.82	1.23 ± 0.23
23	43°52′48″	126°54′16″	305	33	Secondary forest	0.7	196.67 ± 15.28	1.13 ± 0.15
24	44°38′48″	127°27′13″	266	20	Secondary forest	0.7	146.67 ± 5.77	1.27± 0.16
25	45°52′49″	132°08′11″	245	20	Broadleaf forest [6]	0.6	176.00 ± 14.42	1.57 ± 0.15
26	46°53′47″	133°48′57″	398	30	Broadleaf forest	0.6	165.00 ± 15.00	1.60 ± 0.36
27	45°17′22″	129°54′28″	392	25	Broadleaf-conifer	0.6	171.67 ± 12.58	1.43 ± 0.35

[1] CD, canopy density; [2] RCD, root-collar diameter; [3] Dominated by *Quercus mongolica* Fisch.; [4] Dominated by *Larix gmelinii*; [5] Dominated by *Abies nephrolepis* (Trautv.) Maxim.; [6] Dominated by *Populus davidiana*, *Betula platyphylla*, *Q. mongolica* Fisch., and *Acer palmatum*.

2.3. Bioactive Compound Analysis

The methodology of bioactive compound extraction was modified from that of Wu et al. [20]. Samples were oven-dried at 45 °C for 72 h and ground to pass a 60-mesh sieve (0.25 mm). Approximately 1 g of dry powdered plant material was extracted with 25 mL of methanol (70%; v/v) by supersonic for 75 min. The extract was moved to a 2 mL tube for centrifugation at 4000 rpm at 4 °C for 10 min. The supernatant was filtered to pass the 0.45 μm millipore-filter and reserved at 0–2 °C until the analysis using high-performance liquid chromatography (HPLC) (Waters 2695 Separations Module Waters 2998 PDA Detector, Waters Inc., Milford, MA, USA).

Measurement of the bioactive compounds were carried out with the HPLC system using a Welchrom C18 (250 mm × 4.6 mm, 5 μm) chromatographic column. The elution gradient of the mobile phase consisted of chromatographically pure acetonitrile (J&K Scientific Ltd. Headquarters, Beijing, China) and phosphoric acid (0.1%; w/w) at the rate of 1 mL min^{-1}: 10%–15% acetonitrile at 0–12 min, 15–20% acetonitrile at 12–35 min. The column temperature was maintained at 35 °C and the injection volume was 10 μL. The determination wavelength was used at 206 nm.

A weight of 2.502, 2.510, and 2.501 mg of reference substances for eleutheroside B (HPLC ≥ 98%, batch number P14J7F8937), eleutheroside E (HPLC ≥ 98%, batch number W11J7K8869), and isofraxidin (HPLC ≥ 99%, batch number Y12J7S17732) (Yuanye Bio. S&T. Inc., Shanghai, China) was prepared and placed in 10 mL volumetric flasks, respectively. Flasks were added to using methanol to the volume of 10 mL to obtain the references of three compounds. References were used to make the prepared model by regressing known concentrations in a given range to the peak area (Table 2). The chromatogram is shown in Figure 2.

Table 2. Prepared model of linear regression with ranged of known concentrations as independent and the peak area as dependent.

Compound	Regression Model	R^2	Independent Range (μg)
Eleutheroside B	Y = 3,970,604.45x − 1316.93	0.9997	0.0390–2.502
Eleutheroside E	Y = 675,578.96x − 23,980.50	0.9997	0.0390–2.510
Isofraxidin	Y = 6,040,711.41x − 148,307.39	0.9996	0.0195–2.507

Using this regression model, the references were extracted six times in 10 μL for three compounds to calculate the relative standard deviation (RSD). As a result, RSD for the peak area of 0.62%, 0.40%, and 0.32% for eleutheroside B, eleutheroside E, and isofraxidin, respectively, was obtained indicating the precision of the determination can be acceptable. RSD of determination using samples at 0, 4, 6, 8, 10, and 12 h after preparation for eleutheroside B, eleutheroside E, and isofraxidin were 1.19%, 0.88%, and 0.79%, respectively, suggesting samples were stable for12 h. The six repeated analyses of each parameter resulted in RSD ranging from 0.78% to 1.20%, which indicated an acceptable precision of technically repeated measures.

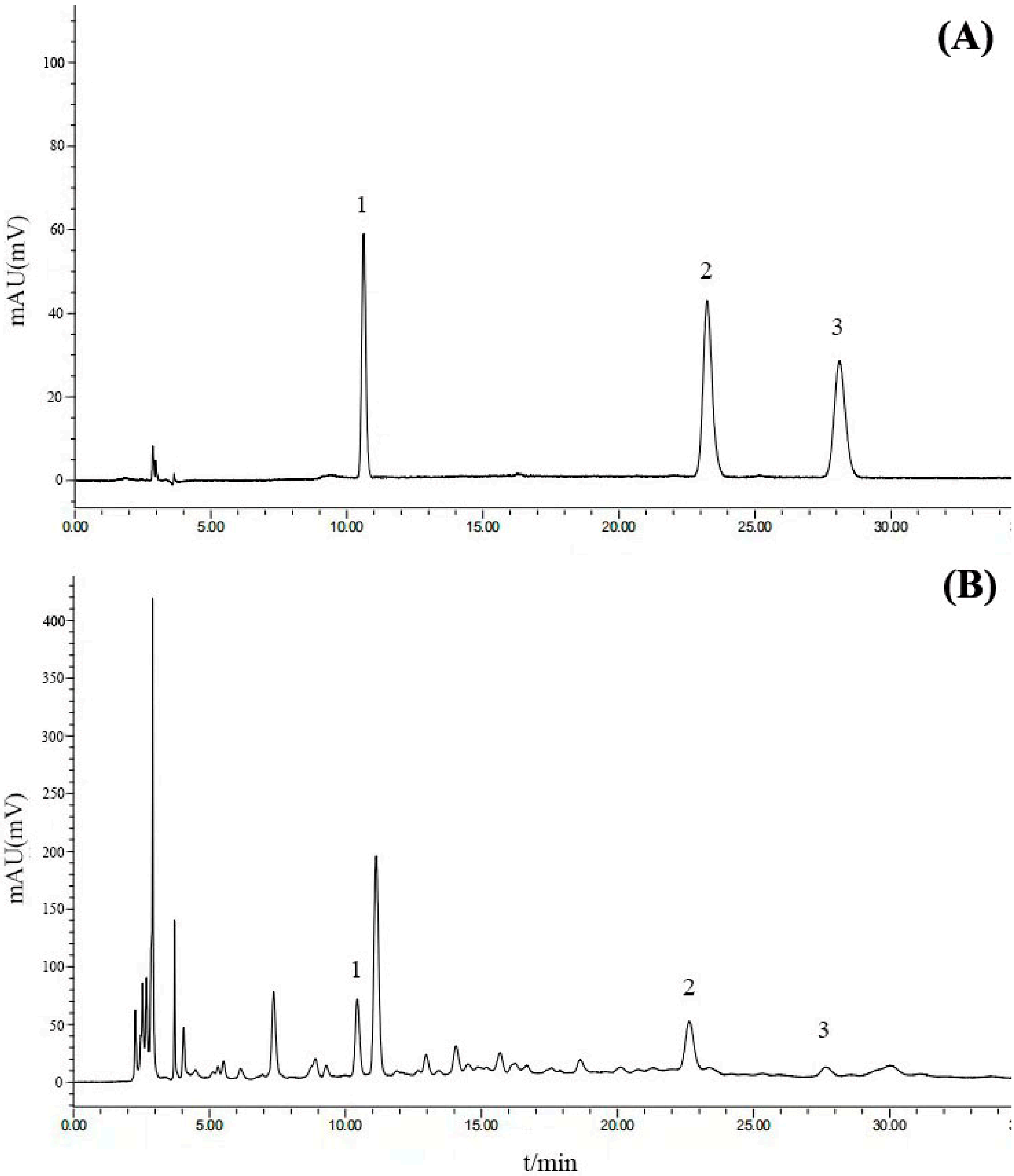


Figure 2. Chromatogram for peaks of eleutheroside B, eleutheroside E, and isofraxidin in *Eleutherococcus senticosus*. (**A**) Peaks of eleutheroside B (1), eleutheroside E (2), and isofraxidin (3) in the reference. (**B**) Peaks of eleutheroside B (1), eleutheroside E (2), and isofraxidin (3) in the tested samples.

2.4. Statistical Analysis

Values of both parameters about regional environment (temperature, precipitation, and RH) and bioactive compounds (eleutheroside B, eleutheroside E, and isofraxidin) were interpolated by kriging using ArcGIS software (V-9.3, Esri China Information Technology Ltd., Beijing, China) to map their spatial distributions. Data about bioactive compounds for one plot of *E. senticosus* population were averaged for the mean from three sub-plots. Data about compound concentrations were calculated and mapped separately in aerial and underground organs.

Statistical analyses were finished using SAS software (ver. 9.4 64-bit, SAS Institute, Cary, NC, USA). All data were checked for the normal distribution and necessary transformation was made to pass the normality test. Pearson correlation was employed twice. Firstly, it was performed to detect the relationship between data about regional climate (temperature, precipitation, and RH) and topographical factors (longitude, latitude, elevation, and slope). Subsequently, the linear correlation was employed again to detect the relationship between any of the unique abiotic factors and bioactive compound concentrations. Thereafter, the stepwise regression was employed to identify the contribution of multiple abiotic variables to concentrations of the three compounds. When the linear regression failed to test the multiple variables, raw data were used for the maximum-likelihood regression. The significance of probability for both Pearson correlation and multiple-variable-regression was accepted at the 0.05 level.

3. Results

3.1. Spatial Distribution of Climatic Factors

The average temperature from 1996 to 2016 presented a geographically descending gradient from the south to the north (Figure 3A). Precipitation was also found to be higher in the southern regions in the study area (Figure 3B). However, RH was lower in the central part of the research area than in the surrounding regions especially the eastern edge and the western and southwestern orientations (Figure 3C).

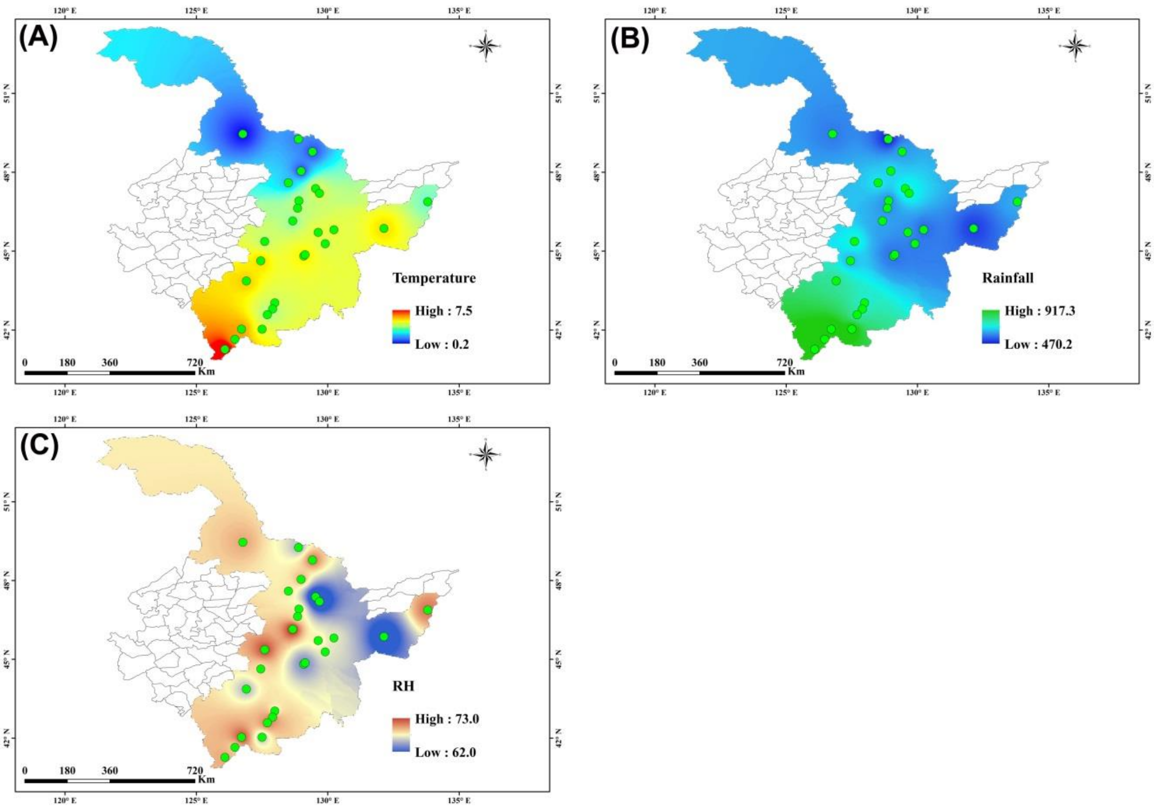


Figure 3. Interpolated mapping of geographical distributions of climatic factors in forests harboring *Eleutherococcus senticosus* populations in Northeast China. (**A**) Geographical distribution of temperature (°C); (**B**) geographical distribution of annual rainfall; (**C**) geographical distribution of relative humidity (RH) (%). Green dots indicate distribution of plots.

3.2. Spatial Distribution of Bioactive Compounds

Eleutheroside B concentration in *E. senticosus* shoot was higher in surrounding areas than in the central part of the study area (Figure 4A). Eleutheroside E concentration was higher in the southern and eastern regions of the study area than in the northern regions (Figure 4B). Shoot isofraxidin concentration was found to be higher in the surrounding areas than in central regions of the studied areas (Figure 4C).

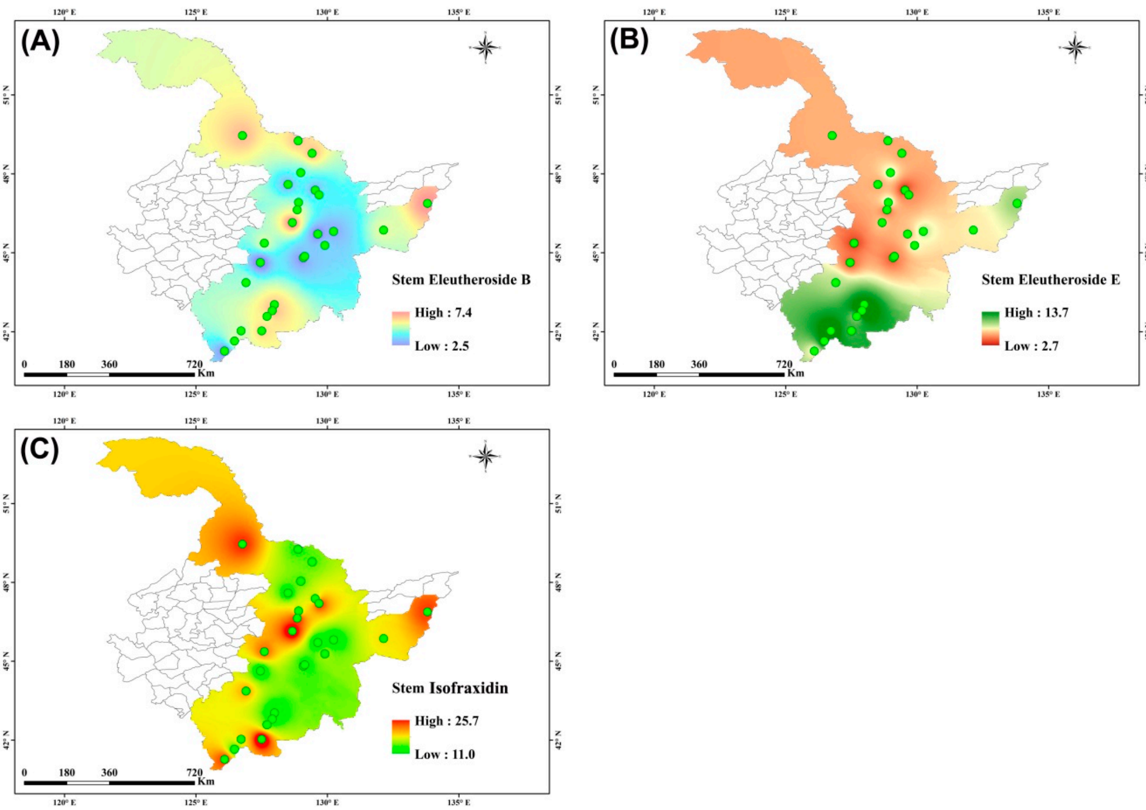


Figure 4. Interpolated mapping of geographical distributions of concentrations of bioactive compounds in aerial organs of *Eleutherococcus senticosus* individuals from populations in Northeast China. (**A**) Geographical distribution of eleutheroside B (mg g^{-1}); (**B**) geographical distribution of eleutheroside E (mg g^{-1}); (**C**) geographical distribution of Isofraxidin (mg g^{-1}). Green dots indicate distribution of plots.

Eleutheroside B and eleutheroside E concentrations in *E. senticosus* roots had similar spatial distribution patterns, which showed alternatively low and high concentrations from the northern part of the study area to the south (Figure 5A,B). These two compounds also showed higher concentrations in the eastern regions. However, root isofraxidin showed another different distribution pattern where the concentration was higher in the two regions near the western and eastern edges than in the central part (Figure 5C).

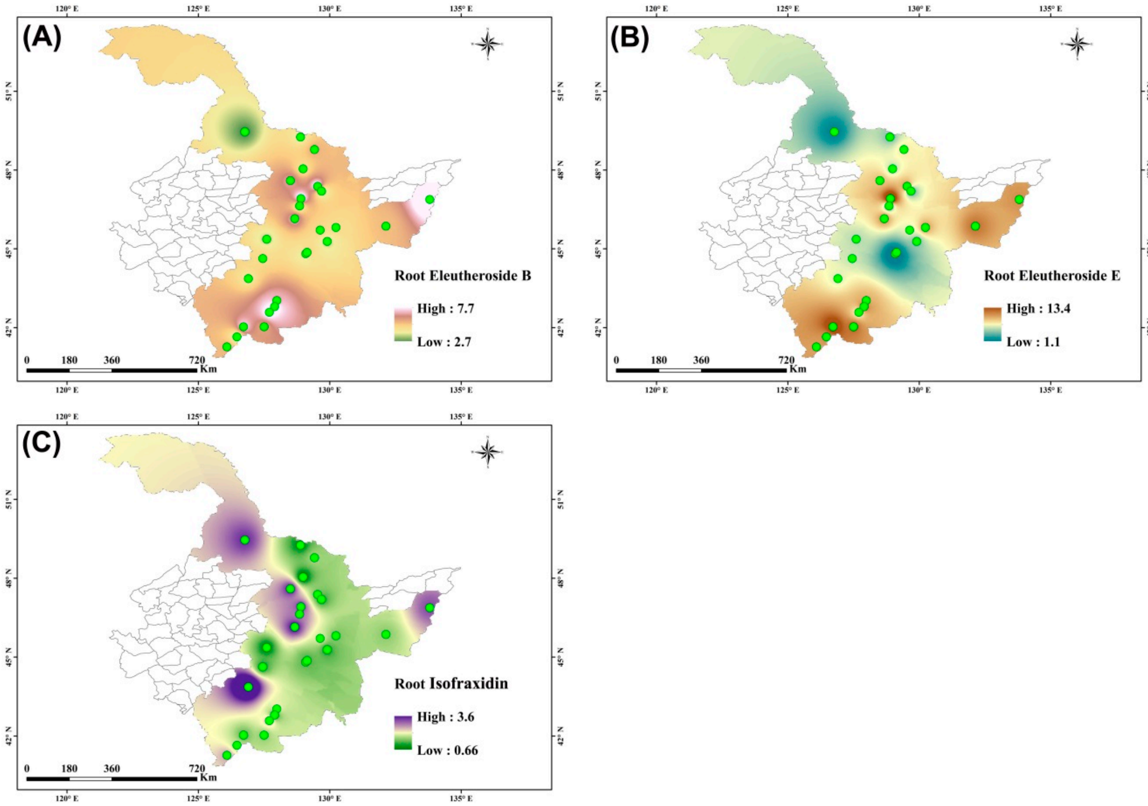


Figure 5. Interpolated mapping of geographical distributions of concentrations of bioactive compounds in underground organs of *Eleutherococcus senticosus* individuals from populations in Northeast China. (**A**) Geographical distribution of eleutheroside B (mg g^{-1}); (**B**) geographical distribution of eleutheroside E (mg g^{-1}); (**C**) geographical distribution of Isofraxidin (mg g^{-1}). Green dots indicate distribution of plots.

3.3. Relationship Between Parameters About Climate and Topography

Both longitude and latitude had a negative relationship with temperature, suggesting temperature tended to decline to the northern or to the eastern orientations in the study area (Table 3). Elevation had a positive relationship with RH, suggesting the high air humidity at high altitude. Longitude also had a positive relationship with elevation, but latitude had a negative relationship with elevation (Table 3). These results suggested that mountains tended to be higher in regions to the southeastern orientation.

3.4. Relationship Between Abiotic Factors and Bioactive Compounds

The Pearson regression indicated that RH was positively correlated to eleutheroside B concentration in shoot (Pearson correlation coefficients: $R = 0.5003$; $P = 0.0079$). Therefore, RH was further found to have a linear relationship with eleutheroside B concentration in shoot (Figure 6A). In contrast, temperature was negatively correlated to eleutheroside B concentration in shoot (Pearson correlation coefficients: $R = -0.4394$; $P = 0.0218$). Another negatively linear regression was found between temperature and shoot eleutheroside B concentration (Figure 6B). RH was positively correlated to eleutheroside B concentration in roots (Pearson correlation coefficients: $R = 0.4155$; $P = 0.0311$) (Figure 6C), but latitude was negatively correlated to eleutheroside E concentration in shoot (Pearson correlation coefficients: $R = -0.5083$; $P = 0.0068$) (Figure 6D).

Table 3. Pearson correlation between parameters about climate and topography.

	Regression Coefficient	Temperature	RH [1]	Rainfall [2]	Longitude	Latitude	Elevation [3]	Slope [4]
Temperature	*R*	1						
	P							
RH	*R*	0.22984	1					
	P	0.2488						
Rainfall	*R*	−0.14057	−0.22209	1				
	P	0.4843	0.2655					
Longitude	*R*	−0.41167	0.14211	−0.11174	1			
	P	0.0329	0.4795	0.579				
Latitude	*R*	−0.78313	−0.25705	0.08392	0.0885	1		
	P	<0.0001	0.1955	0.6773	0.6607			
Elevation	*R*	0.1373	0.52385	−0.05712	0.43327	−0.50833	1	
	P	0.4947	0.0050	0.7772	0.0240	0.0068		
Slope	*R*	0.07157	−0.01393	−0.27039	0.36195	−0.0009	−0.12076	1
	P	0.7228	0.945	0.1726	0.0636	0.9964	0.5485	

[1] RH, relative humidity, transformed by the square divided by 100; [2] transformed by the reciprocal times 100; [3] transformed by the logarithm; [4] transformed by the logarithm of raw data plus one.

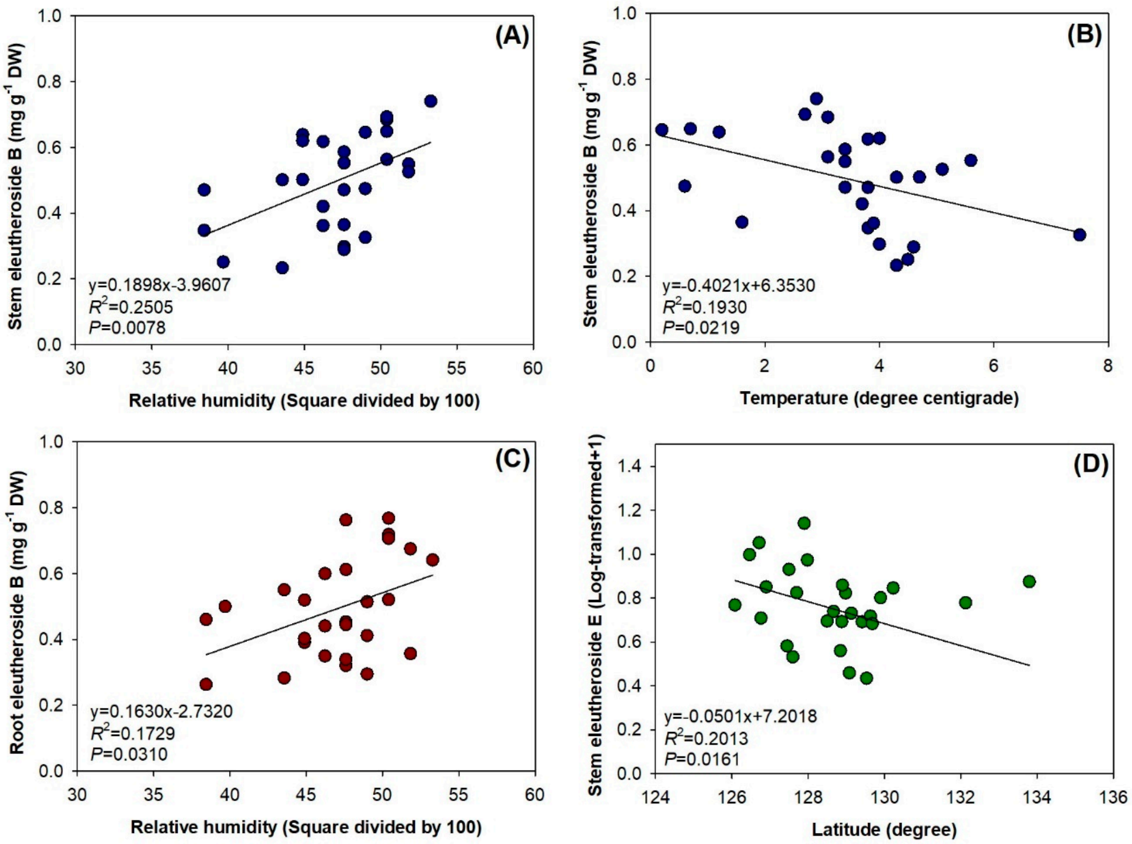


Figure 6. Linear correlation between abiotic factors and concentrations of bioactive compounds in *Eleutherococcus senticosus* individuals from populations in Northeast China. (**A**) Regression between relative humidity and shoot eleutheroside B concentration; (**B**) regression between temperature and shoot eleutheroside B concentration; (**C**) regression between relative humidity and root eleutheroside B concentration; (**D**) regression between latitude and shoot eleutheroside E concentration. R^2 and P values are coefficients from linear regression models.

3.5. Regression of Multiple Varialbles of Abiotic Factors with Bioactive Compounds

Stepwise regression indicated that temperature and latitude had negative contributions to eleutheroside B concentrations in *E. senticosus* shoot (Table 4). Although latitude also had a negative contribution to eleutheroside E concentration in shoot, the contribution of temperature to shoot eleutheroside E concentration was positive.

Table 4. Stepwise regression of temperature and latitude factors on eleutheroside B and E concentrations in *Eleutherococcus senticosus* stems.

Variable	Estimate	Standard Error	Type II SS [1]	*F* Value	Pr > *F*
		Stem eleutheroside B			
Intercept	28.06726	7.82155	18.16931	12.88	0.0015
Temperature	−0.91374	0.23469	21.38854	15.16	0.0007
Latitude	−0.43905	0.15775	10.93055	7.75	0.0103
		Stem eleutheroside E [2]			
Intercept	4.39383	0.87811	0.44527	25.04	<0.0001
Temperature	0.07197	0.02635	0.13269	7.46	0.0116
Latitude	−0.07435	0.01771	0.31342	17.62	0.0003

[1] SS, sum of squares; [2] Data about stem eleutheroside E were log-transformed before use in the regression model.

The maximum-likelihood regression indicated that both RH and longitude had positive contributions to eleutheroside B concentration in roots, but precipitation had a strongly negative contribution to the eleutheroside E concentration in roots (Table 5). Temperature and elevation had positive contributions to isofraxidin concentration in shoot, while the contributions from RH and slope were negative. Temperature, RH, and longitude all had positive contributions to isofraxidin concentration in roots, but precipitation had a negative contribution.

Table 5. Maximum-likelihood regression of variables about climate and topography on eleutheroside B and E concentrations in roots and isofraxidin content in stem and root in *Eleutherococcus senticosus* plants.

Variable	DF	Estimate	Wald Chi-square	*Pr* > Chi-square	Estimate	Wald Chi-square	*Pr* > Chi-square
		Root eleutheroside B			Root eleutheroside E		
Intercept	1	**−56.9266**	**4.72**	**0.0298**	−53.51	1.11	0.2929
Temperature	1	−0.208	0.48	0.4897	−0.0061	0	0.9916
RH	1	**0.1708** [1]	**5.4**	**0.0201**	0.1321	0.86	0.3546
Rainfall	1	−8.0786	0.4	0.5247	**−53.2192**	**4.66**	**0.0309**
Longitude	1	**0.5154**	**9.05**	**0.0026**	0.6154	3.42	0.0643
Latitude	1	−0.2855	1.46	0.2271	−0.2281	0.25	0.6191
Elevation	1	1.0146	0.44	0.5092	−2.4571	0.68	0.4103
Slope	1	−0.1232	0.03	0.8630	0.622	0.2	0.6537
Scale	1	1.1264			2.1869		
		Stem isofraxidin			Root isofraxidin		
Intercept	1	16.0687	2.45	0.1173	1.6175	1.74	0.1866
Temperature	1	**2.1062**	**9.67**	**0.0019**	**0.3094**	**14.64**	**0.0001**
RH	1	**−0.6087**	**4.47**	**0.0346**	**0.1628**	**22.43**	**<0.0001**
Rainfall	1	−1.2718	0.9	0.3438	**−0.5127**	**10.22**	**0.0014**
Longitude	1	0.0076	0	0.9706	**0.0485**	**3.85**	**0.0496**
Latitude	1	0.0009	0	0.9546	−0.0027	2.07	0.1499
Elevation	1	**0.7587**	**4.74**	**0.0295**	0.0266	0.41	0.5219
Slope	1	**−2.2329**	**3.89**	**0.0486**	−0.0963	0.51	0.4762
Scale	1	5.9059			0.705		

[1] Values in bold font indicate estimates that have significant contribution to dependent variables.

4. Discussion

Our results were agreeable to our hypothesis that eleutheroside B concentration in *E. senticosus* shoot tended to be higher in the northern part of the study area and lower in the southeast part. Pearson correlation clearly indicated a negative relationship between temperature and shoot eleutheroside B concentration. Our results were different from those of Shohael et al. [31], where eleutheroside B concentration increased from 18 to 24 °C with an undetectable level at 12 °C. It may be roughly concluded that eleutheroside B concentration increased with temperature in Shohael et al., which appeared to disagree to our results [31]. However, two aspects of methodology existed in the former study to generate the difference. Firstly, materials in Shohael et al. were used as somatic embryos, which were fragile and sensitive to exogenous environment [31], but ours were employed as mature individuals from the natural population. Furthermore, the temperature in Shohael et al. varied within the range of indoor conditions from 12 to 30 °C [31], but the real temperature for natural populations was as low as 0–7 °C with annual chilling periods. It was indicated that the temperature at 20 °C was suitable for the development of embryos [14], but this temperature may occur only in summer for some natural populations. However, other botanic studies revealed that low temperature can induce the synthesis of anthocyanin in several plant species [15,18]. In addition, Schmidt et al. studied the climatic influence on kale (*Brassica oleracea*) and found that the concentration of flavonoids grew in cooler temperatures ranging 0.3–9.6 °C [32].

It was surprising that the geographical change of shoot eleutheroside B concentration had no relationship with the single factor of latitude unless with the involvement of temperature as another independent variable in the stepwise regression. Thus, both geography and Pearson correlation clearly revealed that temperature declined along the latitude gradient from the south to the north. These results together suggest that the special distribution of eleutheroside B concentration in *E. senticosus* stem was shaped by the geographical distribution of temperature along the latitude gradient. On the other hand, we also found a positive relationship between RH and shoot eleutheroside B concentration. This part of results contradicted those in Guo et al., where RH was negatively correlated with betulin and lupeol concentrations in *B. platyphylla* trees [26]. However, our results concur with those findings about forest crops in the understory [23,33]. Two possible explanations may be responsible for our results. The increase of RH can induce the synthesis of secondary metabolites through depressing vapor pressure deficit [23]. Otherwise, higher RH may induce the synthesis of eleutheroside B concentration as a response to the stimulated disease explosion [33].

In contrast to the relationship between temperature and shoot eleutheroside B concentration, temperature was found to be positively correlated with shoot eleutheroside E concentration with decreasing latitudes. This coincided with the higher eleutheroside E concentration in *E. senticosus* individuals in the southwestern part of the study area and the lower one in the northern part. This special distribution matched that of regional temperature. Again, our results about shoot eleutheroside E concentration disagreed with those in Shohael et al., where *E. senticosus* embryos showed decreasing eleutheroside E concentration with the increase of temperature from 12 to 24 °C [31]. This part of the results concurs with those in Guo et al., where the concentration of secondary metabolites in *Scutellaria baicalensis* plants was positively correlated with temperature across mainland China [29]. However, our results about shoot eleutheroside E concentration failed to support the hypothesis because there was a negative relationship with latitude. Because the single factor temperature failed to have any relationship with shoot eleutheroside E concentration, we surmise other abiotic factors, such as light quality and ultraviolet (UV) light [34], may have contributed to the spatial pattern of eleutheroside E distribution.

Results about eleutheroside B concentration in *E. senticosus* roots support the other part of our hypothesis. The maximum-likelihood regression indicated that both RH and longitude contributed to the positive effect on root eleutheroside B concentration. In addition, RH was positively correlated with elevation, which was further positively correlated with longitude. Therefore, these results together suggest that *E. senticosus* individuals tended to have high accumulation of eleutheroside B concentration in roots in populations in moist forests distributed in altitudes of mountains in the eastern regions. The eastern alp in our study was mainly accounted for by Changbai mountains, where it was reported that the decline of temperature with the increase of elevation controlled the evapotranspiration, which resulted in the rising RH along the elevation gradient [35]. The high RH in the eastern mountains may have promoted the accumulation of eleutheroside B in roots.

Although we found the negative relationship between rainfall and eleutheroside E concentration in roots, no relationship was further detected between rain and other climate or topographical parameters. We surmise that abundant rainfall favored the condition for *E. senticosus* growth and controlled the synthesis of eleutheroside E in roots. This part of the results concurred with those about medical herbs in South Africa [36] and Brazil [37]. A study on *Euclea undulata* Thunb. var. *myrtina* reported that concentrations of epicatechin and 7-methyl-juglone in roots were depressed in the rainy season for the population in the winter rainfall area. The study on *Tithonia diversifolia* revealed that change of inorganic-element concentrations in soils determined the concentration of secondary metabolites in roots.

Factors of slope and temperature had strong negative and positive contributions (estimates of about ± 2.0) to the isofraxidin concentration in stem, respectively. These two factors had no relationship with each other; therefore, the two contributions were separated. The positive effect of temperature on isofraxidin concentration coincided with that on eleutheroside E, which may share the same mechanism that had been discussed in the preceding paragraphs. The slope in our study ranged between 0° and 33°. In another study, Wei et al. studied the response of *Aralia elata*, the species from another genus in Araliaceae, to different slopes and found that nearly all plant parameters decreased since the slope increased up to 9° [13]. The positive correlation between elevation and shoot isofraxidin concentration resulted from strong UV-light-induced compound synthesis and accumulation [34]. The strongly negative correlation between rainfall and isofraxidin concentration in roots concurred with the findings about root eleutheroside E concentration. Positive contributions of temperature, RH, and longitude to root isofraxidin concentration indicated that *E. senticosus* individuals in natural populations in regions at high elevation with an annual temperature of about 5 °C had the highest levels of root isofraxidin.

5. Conclusions

In this study, 27 plots were investigated to detect the special distribution and environmental correlates of eleutheroside B, eleutheroside E, and isofraxidin in individuals from natural *E. senticosus* populations in forests of Northeast China. Populations in northern regions around the Greater Khingan Mountains tended to have a higher concentration of eleutheroside B in aerial organs, which was driven by the lower temperature therein. Another region of forests that harbored *E. senticosus* individuals with higher concentration of eleutheroside B concentration in shoots was located at the eastern mountains with high elevation and high humidity. Concentrations of eleutheroside B and isofraxidin were higher in the southern and eastern edges of the study area, where higher temperature drove the concentrations in aerial organs and lower rainfall drove those in roots. Therefore, the spatial distribution of bioactive compounds in medical plants in the understory can be driven by regional climate factors. The management of non-wood forest product plants at the regional scale should consider effects from climatic geography.

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