Changes in Growth and Photosynthetic Parameters and Medicinal Compounds in *Eleutherococcus senticosus* Harms under Drought Stress

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**Abstract.** In this investigation, changes in growth and photosynthetic parameters were used to explain the effects of drought stress on morphology and photosynthesis of *Eleutherococcus senticosus*. Liquid chromatography (LC)-mass spectroscopy (MS) was used to determine the content of eleutheroside B, eleutheroside E, isofraxidin, hyperoside, rutin, and kaempferol under different drought stress conditions to explain the effects of drought stress on secondary metabolism of *Eleuthero*. Growth and photosynthetic physiological parameters showed that drought stress could inhibit the growth and photosynthesis of *Eleuthero*. The compounds studied showed the same cumulative trend in various organs of *Eleuthero* under different drought stress conditions, with the highest content in the moderate drought stress group and the lowest in the severe drought stress group. Among them, the content of eleutheroside B was found to be higher in the 5-year-old stem. The content of eleutheroside E was higher in the 3-year root. The content of isofraxidin was highest in the 5-year-old root. The content of hyperoside, rutin, and kaempferol were higher in the 3-year-old leaves. The results show that a wet soil environment was beneficial to growth and photosynthesis of *Eleuthero* and moderate drought stress is conducive to the accumulation of its active ingredients.

*Eleutherococcus senticosus* (Ruper. et Maxim.) Harms is a species of a small woody shrub that belongs to the family Araliaceae (Huang et al., 2011). It is commonly used in China (called *ciwujia*), Korea, Japanese, and Russia (called *Siberian ginseng*). The roots and stems of the plant are recognized as a tonic herb that has a ginseng-like effect (Bucci, 2000). Eleuthero root is considered a pharmacopeia raw material in many countries (Europe, United States, Japan). *Eleuthero* invigorates qi, strengthens the spleen, and nourishes the kidney (Han et al., 2014). Thus, *Eleuthero* may be used for yang deficiency of the spleen and kidney, body weakness and hyperdynamics, poor appetite, aches of the waist and knee, insomnia (Han et al., 2017). *Eleuthero* has anti-inflammatory (Fei et al., 2014; Jiang and Wang, 2015), antioxidant (Kim et al., 2015), antifatigue (Huang et al., 2011; Jiang and Wang, 2015), and anticancer properties (Cichello et al., 2015) and encourages immunomodulation; thus, it has been widely used to treat chronic bronchitis, neurasthenia, hypertension, and ischemic heart disease (Sun et al., 2016). The components of phenolic, triterpenoid sapo- ninis, lignan, coumarins, flavones, polysaccharides, and volatility have been detected in the *Eleuthero* (Huang et al., 2011; Jiang et al., 2006; Li et al., 2016).

The chemical compounds of *Eleuthero* raw materials used in this study are phenolic compounds. Phenols are the most widely distributed metabolites involved in interactions between biology and the environment (Garcia-Calderon et al., 2015). The accumulation of phenols may also affect other secondary metabolite pathways, including alkaloid pathways, because plant defense is a complicated system (Ferreres et al., 2008; Mustafa and Verpoorte, 2007). Phenolic compounds and related pathways can be influenced by exposure of plants to abiotic stresses, such as adverse environmental conditions (Dixon and Paiva 1995; Harb et al., 2010). However, plants have evolved to survive harsh conditions, for example, by using their metabolic capacity to produce a variety of secondary metabolites. Eleutheroside B, eleutheroside E, isofraxidin, hyperoside, rutin, and kaempferol are the main phenols in *Eleuthero* (Baczek et al., 2017; Yang et al., 2012, 2013). Eleutheroside B and E are lignans (Lee et al., 2004), isofraxidin is a coumarin compound (Yamazaki and Tokiwa, 2010), and hyperoside, rutin, and kaempferol belong to the flavonoid group (Baczek et al., 2017). These compounds are derived from shikimic acid (Gamir et al., 2014; Schafellner et al., 1999), the contents of which also seem to increase from water deficit (Becerra-Moreno et al., 2015).

To the best of our knowledge, there is no comprehensive study on the effects of drought stress on *Eleuthero*. The present study focused on photosynthetic physiological parameters and targeted analysis of metabolite features of roots, stems, and leaves under different water-treatments. Collectively, these data will enable assessment of the difference in growth, photosynthetic physiological responses, and secondary metabolism responses to water restriction throughout the plant.

**Materials and Methods**

**Chemicals and reagents.** Eleutheroside B, eleutheroside E, isofraxidin, hyperoside, rutin, and kaempferol were purchased from the Chinese National Institute of Control of Pharmaceutical and Biological Products (Beijing, China). Water used for ultra performance LC-tandem MS (UPLC-MS) analysis was prepared with a Milli-Q water purification system bought from Millipore (Milford, MA). Acetonitrile (J & K Scientific Ltd., Beijing, China) was high-performance LC grade. All other chemicals used in the method were of analytical grade.

**Plant materials.** Seedlings of 3-year-old *Eleuthero* were obtained from Qitahei, Heilongjiang Province, China (geographic coordinates: lat. 45°59′55″N, long. 131°05′E) and...
planted in the Heilongjiang University of Chinese Medicine Botanical Garden, Harbin, Heilongjiang Province, China (geographic coordinates: lat. 45° 72' N, long. 126° 64' E) in February. A month later (March), seedlings were transplanted into 30-cm diameter pots containing a mixture of garden soil and vermicomposting (3:1, w/w); soil pH was 6.50. The experiment was conducted in a glasshouse with temperature ranging from 20 to 30 °C under natural light conditions (day length: 13 h). The climate of Harbin is temperate continental monsoon type. The whole plant was dissected to obtain roots, stems, and leaves for metabolic analysis. The raw materials were dried at 60 °C for 48 h. The experiment was performed in three replications.

**Drought stress treatment.** One month after transplantation (April), the control (GK), moderate (W1), and severe (W2) drought stress treatment was started. The soil moisture of GK, W1, and W2 was maintained at 0.8 to 0.9 g/g, 0.5 ± 0.05 g/g, and 0.3 g ± 0.05 g/g, respectively. The moisture in pot soil was evaluated regularly by measuring the soil water content percentage: 1 g soil from pots of each treatment, as well as the control, was taken, oven-dried, and weight was taken again. Soil water content percentage was calculated by using the formula:

\[
\text{Soil water content percentage} = \frac{\text{Fresh soil weight} - \text{dry soil weight}}{\text{fresh soil weight}} \times 100
\]

This measurement was repeated regularly at 1-d intervals, and water (100–400 mL) was supplied to pots of each treatment during plant development. There were three replicates in each experimental group, and six seedlings in each replicate. The experiment was conducted throughout plant developmental stages (from April to June).

**Determination of growth parameters.** A sampling of plant material was done after 2 months of drought stress treatment. Six plants were randomly selected in each treatment group; measurements of total leaf area (TLA), plant height (PH), and leaf number (LN) were performed. TLA was measured with LI-3100 leaf area meter (LI-COR Bio.

| Table 1. Delustering voltages, collision voltages, and collision chamber emission voltages of the medicinal compounds. |
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| **Analyte** | **Ion pairs (m/z)** | **DP (V)** | **CE (V)** | **CXP (V)** |
| Eleutherose E | 765.3 → 603.1 | 70 | 62 | 23 |
| Rutin | 611.1 → 464.8 | 80 | 20 | 13 |
| Kaempferol | 287.1 → 153.2 | 70 | 51 | 10 |
| Isofraxidin | 223.1 → 206.3 | 60 | 40 | 9 |
| Eleutherose B | 155.0 → 92.9 | 70 | 21 | 17 |
| Hyperoside | 395.1 → 232.1 | 70 | 40 | 17 |

**Determination of photosynthetic parameters.** Photosynthetic parameters were measured using a portable photosynthesis system (Li-6400XT, LI-COR) in a glasshouse from 10:00 AM to 1:00 PM on 30 June 2017. Weather was normal during the investigation. Six plants were randomly selected in each treatment group, and the net photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (E), and intercellular CO2 concentration (Ci) values were read directly using the photosynthesis system.

**LC-MS analysis of the active medicinal ingredient.** Dried leaf, stem, and root of Eleuthero were ground in a mill and passed through a 35-mesh sieve. Approximately 2 g of dry powdered plant material was extracted with 10 mL of methanol (80%) by reflux for 45 min. The extract was repeatedly filtered, and the filtrate was collected. The extract was subjected to centrifugation at 14,000 rpm at 4 °C for 10 min. The supernatant was removed, and the extract was concentrated by evaporation under a vacuum to dryness. Then the precipitate was dissolved with methanol to a volume of 1.0 mL. All samples were filtered through a 0.22-μm diameter micro pore filter membrane, which could be directly injected for LC-MS analysis.

The analysis of compounds is described in our previous studies (Wu et al., 2018); roots and stems were pulverized by using a grinding instrument (MM 400, Retsch, Haan, Germany), and 50-mg tissue aliquots were extracted with 1.0 mL 70% aqueous methanol containing 0.1 mg/L lidocaine for water-soluble metabolites at 4 °C overnight and vortexed three times. The extracts were clarified by centrifugation, combined, evaporated, and then filtered through 0.45-μm nylon membranes (SCAA 104; ANPEL http://www.anpel.com.cn) before LC-MS analysis.

UPLC-MS analysis was performed with a Waters ACQUITY UPLC system (Waters Corporation, Tokyo, Japan) coupled to an LC-20AD pump, SIL-20A autosampler (Waters Corporation). The ACQUITY-UPLC BEH C18 column (1.7 μm, 2.1 mm × 50 mm) used for UPLC analysis was held at 25 °C; injection volume was 10.0 μL, and the flow rate was 0.5 mL/min. Mobile phase A comprised methanol, and mobile phase B comprised water. The column was eluted with a linear gradient of 25% A for 0 to 1.5 min, 25% to 50% A for 1.5 to 2 min, 50% A to 2.0 min, 50% to 4.0 to 4.5 min, 90% A for 4.5 min, 90% A for 4.0 to 4.5 min, 90% to 25% A for 5.5 to 6.0 min, and 25% A for 6.0 to 7.0 min. The chromatograms of six medicinal compounds under a multireaction monitoring mode are shown in Fig. 1.

**MS detection.** MS detection was performed using a QTRAP 5500 (AB SCIEX, Boston, MA) equipped with an Electrospray Ionization source with the following operating parameters: cone voltage of 3 kV and ion source atomization temperature of 500 °C; 25 psi atomizing gas and 20 psi air curtain gas. The ion pair, cluster voltage, collision voltage, and collision chamber injection voltage of six active metabolites are shown in Table 1. Both MS and MS/MS data were determined in the positive mode, and data were used for multiple reaction monitoring. Secondary MS of six active pharmaceutical ingredients is shown in Fig. 2.

**Statistical analysis.** It subjected all results to the analysis of variance (ANOVA) to determine the significant differences between various levels of salt treatment times. If ANOVA was performed, Duncan’s honestly significant
difference (HSD) post hoc tests were conducted to determine the differences between individual treatments (SPSS 22.0; SPSS Inc., Chicago, IL).

**Results and Discussion**

*The changes in growth parameters.* Changes in plant growth parameters are planted responses to drought stress on external morphology (Pellegrino et al., 2010). Changes in PH showed in Fig. 3A. The moderate stress group (W₁) and the severe stress group (W₂) showed apparent differences from the control group (GK). The plant height in W₁ was 57% lower than GK, and in the W₂ group it was 67% lower than GK. The results of LN count is shown in Fig. 3B. Higher LN was found in GK. LN in W₁ was 43.84% lower than GK, and in W₂ it was 32.61% lower than GK. The change of LA was opposite that of LN (Fig. 3C), it was the highest in W₁ and the lowest in the GK.

The results showed that drought stress could significantly inhibit PH and LN of *Eleuthero* and increase its leaf area. Soil water shortage also affects plant growth in many ways. On one hand, plant somatic cell division and differentiation require water participation. When water is absent, the speed of division and differentiation slows down or even stops (Van der Weele et al., 2001).

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**Fig. 2.** Secondary mass spectrum of six active pharmaceutical ingredients. (1) Eleutheroside B, (2) eleutheroside E, (3) isofraxidin, (4) hyperoside, (5) rutin, and (6) kaempferol.

**Fig. 3.** Effect of water stress treatments on stem length, leaf number, and leaf area of *Eleuthero*. The soil moisture of the control (GK), moderate drought stress (W₁), and severe drought stress (W₂) groups was maintained at 0.8 to 0.9 g/g, 0.5 ± 0.05 g/g, and 0.3 ± 0.05 g/g, respectively. n = 6. **Significant difference between the treatment group and the control group (P < 0.01); *significant difference between the treatment group and the control group (P < 0.05).
On the other hand, it affects the transport of substances in plants, and many substances in plants need water as a carrier (Walton et al., 1976). Under drought stress, the transport rate of substances slowed, and the assimilation products could not be readily distributed (Chaves, 1991). Also, drought affects plants’ water absorption (Farooq et al., 2009). In this study, drought stress increased leaf area to a certain extent, which may be a response to adapt to drought conditions.

The factors leading to decline in photosynthetic rate include stomatal and nonstomatal limitations. The stomatal limiting factor was the decrease of Ci because of the decrease of the stomatal opening of leaves, whereas the nonstomatal factor was the decrease and accumulation of CO₂ solubility, which resulted in the decrease of mesophyll photosynthetic capacity. Drought stress can inhibit photosynthesis in these two means (Berry and Downton, 1982). Previous studies (Colom and Vazana, 2001; Rajendrudu et al., 2000) have shown that soil moisture is the main limiting factor for plant photosynthetic parameters. Moderate water deficit does not affect leaves’ stomata opening and therefore does not have a significant effect on \( P_n \), \( g_s \), Ci, and E. In the present study, \( P_n \) and \( g_s \) were less affected under \( W_1 \), and there was no significant difference with the GK. Severe drought stress significantly inhibited the photosynthesis of \textit{Eleuthero} by limiting CO₂ and causing photodamage in medicinal plants (Cornic and Massacci, 1996).

The changes in photosynthetic parameters. When \textit{Eleuthero} was subjected to different drought stress treatments, significant differences in all photosynthetic parameters were observed. The \( P_n \) and \( g_s \) decreased with the increase of drought stress (Fig. 4A and B). There was no significant difference between the GK and \( W_1 \), \( P_n \) and \( g_s \) in \( W_2 \) were significantly lower than GK and \( W_1 \). The transpiration rate (E) was lower in the two drought stress groups (Fig. 4C), and it was lowest in \( W_2 \). The concentration of intercellular carbon dioxide (Ci) showed the opposite trend (Fig. 4D). With worsening of drought stress, the two treatments were significantly higher than the control group, and the highest was in \( W_2 \). This result showed that photosynthetic capability was best in leaves of \textit{Eleuthero} cultivated under GK.

In the present study, the UPLC-MS method was successfully applied for the quantitative analysis of six medicinal compounds in \textit{Eleuthero}. The comprehensive score (Q value) of principal component analysis was used to illustrate the overall trend of the contents of the compounds studied in roots, stems, and leaves under different drought stress conditions (Fig. 5). The compounds studied showed the same accumulation trend in different organs. Higher content of the compounds studied was observed in \( W_1 \). In the roots, the higher content of eleutheroside B, isofraxidin, kaempferol, and hyperoside accumulated in \( W_1 \) (\( P < 0.05 \)). Among them,
the contents of eleutheroside B and isofraxidin in GK were higher than those in W₂, and the contents of kaempferol and hyperoside in W₂ were higher than those in GK. The contents of rutin and eleutheroside E were higher in GK and the lower in W₂. In stems, the contents of eleutheroside B, isofraxidin, hyperoside, kaempferol, and rutin in W₁ were significantly higher than those in other treatments (P < 0.05). Among them, the contents of eleutheroside B, isofraxidin, hyperoside, and kaempferol in GK were higher than those in W₂, and the contents of rutin in W₂ were higher than those in GK. The content of eleutheroside E in GK was higher than that in other treatment groups (P < 0.05). In the leaves, the contents of eleutheroside B, isofraxidin, and eleutheroside E in W₁ were significantly higher than those in other treatments (P < 0.05), and the contents of eleutheroside B and eleutheroside E in GK were higher than those in W₂. Isofraxidin was not detected in GK. The contents of rutin, kaempferol, and hyperoside were the highest in GK and the lowest in W₂ (Fig. 6).

The results showed that the content of eleutheroside B, eleutheroside E, and isofraxidin was higher in roots and stems (Fig. 4A–C), similar to previous research (Kang et al., 2001; Lee et al., 2005), and further confirmed...
the correctness of the Chinese Pharmacopoeia’s roots and stems as the legal medicinal organs of *Eleutherococcus*. Eleutheroside B and isoferaxidin showed the same accumulation trend in different organs under different drought stress conditions, and their content was higher in the W1 group (Fig. 4A and C), which showed that W1 is suitable for the accumulation of two compounds. Drought stress inhibited the accumulation of eleutheroside E in roots and stems. The content of eleutheroside E was higher in GK, in agreement with results of previous studies (Lee et al., 2005; Zhang and Xue, 2008). Eleutheroside E thus has the potential to become a quality control index of *Eleutherococcus* in the Chinese Pharmacopoeia. It has been shown that eleutheroside E possesses an antistress and antifatigue effect (Kimura and Sumiyoshi, 2004; Weng et al., 2007). The results of the present study indicate that the accumulation of eleutheroside E in different organs of *Eleutherococcus* was also high, which is in agreement with results of previous studies (Lee et al., 2005; Zhang and Xue, 2008). Eleutheroside E thus has the potential to become a quality control indicator for *Eleutherococcus*.

The results of the present study show a significant effect of hyperoside, rutin, and kaempferol (all flavonoids) in different parts of *Eleutherococcus*. The content of the three flavonoids was higher in the leaves of *Eleutherococcus* and the content decreased with the soil drought stress. The preceding results indicate that a wet soil environment is suitable for the accumulation of flavonoids in *Eleutherococcus*. This is inconsistent with previous research on *St. John’s wort* and *Hypericum peruvianum* (Caliskan et al., 2017; Gray et al., 2003). This could be explained by the fact that phenylpropanoids and shikimate pathway enzymes reduce their activity under drought stress, as phenylpropanoid metabolism. Plant Cell 10:263–343.

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Conclusions

This investigation provided data on the impact of drought stress on the morphological and photosynthetic characteristics and the medicinal compounds of *Eleutherococcus*. The growth parameters results show that drought stress may inhibit plant growth, and moderate drought stress may significantly inhibit photosynthesis. The medicinal compounds were significantly affected in different parts of *Eleutherococcus* under various water treatments. Moderate drought stress is the most suitable condition for the accumulation of medicinal compounds in *Eleutherococcus*. This study may provide the experimental basis for further study on the metabolic regulation mechanisms of the medicinal components of *Eleutherococcus senticosus* under drought stress.
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