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Effects of light intensity on the growth, photosynthetic characteristics, and secondary metabolites of *Eleutherococcus senticosus* Harms

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Abstract

In this investigation, we used the growth, photosynthetic physiological parameters, and targeted metabolite analysis to evaluate the responses of *Eleutherococcus senticosus* in different shading treatments. The results showed that the moderate shading treatment (Z_1) promoted the growth and inhibited photosynthesis of plants. The severe shading treatment (Z_2) inhibited both the growth and photosynthesis of the plants. Besides, Z_1 had no significant effect on the PSII, while Z_2 inhibited the PSII. Most of the eight medicinal metabolites accumulated in the Z_1. The C_6C_1- and C_6C_3-type phenolics accumulated in the Z_1, and the $C_6C_3C_6$-type in the Z_2. In conclusion, the moderate shading treatment accumulated more defensive phenolics; this might be the reason for this shading condition promoting the growth and the accumulation of medicinal metabolites of the plant. The result of this study laid a theoretical foundation for the further study of shading treatments on the secondary metabolism of *Eleutherococcus senticosus*.

Additional key words: chlorophyll fluorescence; effective components; medicinal plant; phenols.

Introduction

Eleutherococcus senticosus (Rupr. Maxim.) Harms, family Araliaceae, is a well-known traditional Chinese herbal plant. The species is widely cultivated in eastern Asia and far western Russia (Zhou *et al.* 2018a). In recent years, *E. senticosus* have become popular in the United States and European countries as a new medicinal plant and dietary supplement. The plant has many pharmacological effects, including antibacterial, anticancer, anti-inflammatory, anti-gout, anti-hepatitis, antioxidant, antipyretic effects; other effects include choleretic, hemostasis, immune stimulation, cholesterol-lowering, and radiation protection (Yi *et al.* 2001, Yoon *et al.* 2004, Park *et al.* 2006, Zhou *et al.* 2018b, Wang *et al.* 2019). *E. senticosus* contains a variety of pharmaceutical compounds, including lignin, coumarins, triterpenoid saponins, flavonoids, vitamins, minerals, and polysaccharides. Because of the lack of standardization for cultivation, some *E. senticosus* appeared in a poor quality at China market. It has been confirmed that 26% of the products did not meet the content of the compounds described on the label (Arouca and Grassi-Kassisse 2013). So, studying the appropriate cultivation conditions of *E. senticosus* has an essential significance for controlling the quality of this medicinal plant.

Light not only provides a source of energy but also acts as a signal for environmental changes to induce various physiological responses in plants (Abidi *et al.* 2013). During the growth of medicinal plants, appropriate light intensity could help plants defend against the inhibition of photosynthesis and abiotic stress. In addition, the material and density of the shading material also affect the quality of medicinal plants (Feijó *et al.* 2009, Kim *et al.* 2016, Oliveira *et al.* 2016). The effects of environmental stress on photosynthesis of medicinal plants include regulating the stomatal size and causing damage to the PSII reaction center (Berry and Downton 1982). Previous studies showed that the effects of shading conditions on photosynthesis of different medicinal plants were different (Wang *et al.* 2017, Tafoya *et al.* 2018, Fan *et al.* 2019).

Shading also changed the synthesis of secondary metabolites in plants. Previous studies showed that the light intensity affected the accumulation of flavonoids, phenolics, alkaloids, and lignin in medicinal plants (Poolman *et al.* 2013, Kong *et al.* 2016, Pan and Guo 2016, Arena *et al.* 2017, Lazzarini *et al.* 2018). Light intensity affects

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Abbreviations: C_i – intercellular CO_2 concentration; E – transpiration rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_v/F_m – maximal quantum yield of PSII photochemistry; GK – control group; g_s – stomatal conductance; P_N – net photosynthetic rate; Q – principal component analysis score; WUE – water-use efficiency (= P_N/E); Z_1 – moderate shading treatment; Z_2 – severe shading treatment.
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synthesis of the secondary metabolites in the medicinal plant by regulation of secondary metabolic pathways. Some studies reported that light intensity regulated the metabolism of flavonoids and phenylpropanoids in medicinal plants (Horváth and Szász 1965, Kitazaki *et al.* 2018). Phenolics are common secondary metabolites in plants. They have anti-infective and antioxidative effects (Lee and Chiu 2015, Kasprzak *et al.* 2018), and also have plant protective properties (Warren *et al.* 2015). Phenolics exist in plants in three skeletal types, including metabolites with C_6-C_1, C_6-C_3, and C_6-C_3-C_6 types. The three types of phenolics are the defensive metabolites through the antibacterial effects (Tugizimana *et al.* 2019). UPLC-MS and GC-MS techniques were also applied to study mechanisms of environmental stress in plant secondary metabolism regulation (Tang *et al.* 2017, Yang *et al.* 2017).

As far as we know, there is no comprehensive study on the growth, photosynthetic characteristics, medicinal compounds, and phenolics of *E. senticosus* under the shading treatment. The artificial cultivation area of the plant increases in China. Understanding how environmental factors affect the growth and photosynthesis and the accumulation of secondary metabolites in *E. senticosus* can be used for the standardized cultivation. We hypothesized that the light intensity effected the growth, photosynthesis, and accumulation of the secondary metabolites of *E. senticosus*. Three shading treatment groups were set up for the purpose and to find the most suitable light intensity conditions. The result can provide the basis for further study on the secondary metabolism of *E. senticosus* under different light intensity.

Materials and methods

Chemicals and reagents: Syringin, eleutheroside E, isofraxidin, hyperoside, rutin, kaempferol, oleanolic acid, and L-phenylalanine were purchased from the Chinese National Institute of Control of Pharmaceutical and Biological Products (Beijing, China). Syringic acid, vanillic acid, *p*-hydroxybenzoic, protocatechuic acid, cinnamic acid, gallic acid, ferulic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, luteolin, genistein, and quercetin were purchased from *ChromaDex Inc.* (Santa Ana, CA, USA). The water used for UPLC-MS/MS was prepared by a *Milli-Q* (*Millipore*, Bedford, MA, USA). Acetonitrile (*J&K Scientific Ltd.*, Beijing, China) was HPLC grade. All other chemicals used in the research were of analytical grade.

Plant materials: The three-year-old *E. senticosus* were obtained from Qitaihe, Heilongjiang Province, China (45°95'N, 131°05'E), and planted in the Botanical Garden of the Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang Province, China (45°72'N, 126°64'E) in April. A month after, seedlings were transplanted in pots of 30-cm diameter (May) containing potting soil (mixed with peat, perlite, and vermiculite):sand (1:1, v/v), and the pH of the soil was 6.5. The maximum water holding capacity of the soil is 50%.

Light intensity treatment: We started the shading treatment on 15 June 2017. The shade shed was placed in the greenhouse (covered with one-layer opaque plastic film) and built with black mesh cloth. Each shed was 3 m long, 1.5 m wide, and 1.4 m high. Then, three shading treatments were set according to the bionic light conditions for wild *E. senticosus*: control group (forest edge, 100%, GK) – no shade; moderate shading group (forest gap, 38.8%, Z_1) – one-layer thick mesh cloth; severe shading group (understory, 16.9%, Z_2) – two layers of thick mesh cloth. The light intensities on sunny days were measured at noon by using a PAR sensor (*6400XT* portable photosynthesis system, *LI-COR Biosciences*, Lincoln, NE) in July and August for the shading treatments, and PPFD were calculated (Table 1S, *supplement*). After the treatment start, the soil pH was maintained at 6.5, and the soil water content was maintained at about 60–75% of the maximum water-holding capacity. Throughout the experiment, tap water was used as the source of irrigation water, and its pH was 8, and the temperature in the greenhouse was controlled at 23–25°C. The experiment was conducted throughout all the plant developmental stage process (from June to August), and each treatment was performed in three replications. Six plants per replications were investigated, and each plant was used for sample collections and analysis. The whole plant was separated into roots, stems, and leaves for metabolic analysis.

Growth parameters: A sampling of plant material was done after two months of shading treatment. Six plants were randomly selected in each treatment group. Measurements of plant height, main stem diameter, crown width, root length, total leaf area, and plant leaf number were performed. The total leaf area was measured with *LI-3100* leaf area meter (*LI-COR Biosciences*, Lincoln, Nebraska, USA). Each sample was measured three times in parallel.

Photosynthetic characteristics: Gas-exchange parameters were measured in a glasshouse from 10:00–13:00 h on 30 July 2017. Weather conditions were normal during the investigation. The net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i) values were provided by the photosynthesis measuring system (*Li-6400XT*, *LI-COR*, USA). Water-use efficiency (WUE) was calculated as P_N/E. Chlorophyll (Chl) fluorescence parameters, including the minimum fluorescence (F_0), maximum fluorescence (F_m), potential photochemical efficiency (F_v/F_0), maximum photochemical efficiency (F_v/F_m) values were provided by the Chl fluorescence system (*PAM-2500*, *Walz*, Effeltrich, Germany). Before the Chl fluorescence parameters were measured, the leaves were dark-adapted for 30 min. Six plants were randomly selected in each treatment group.

The LC-MS analysis of active medicinal ingredients: The samples were ground with a grinder and passed through a 35-mesh sieve. Dry powder samples of 2 g were dissolved in 10 ml of methanol (80%) for 45-min ultrasonic extraction and filtered repeatedly. After centrifugation at

532.88 Pa for 10 min, the supernatant was evaporated to dryness under vacuum. The amount of the solution precipitated with methanol was then 1.0 ml. All samples were filtered through a 0.22-μm diameter microporous filter membrane and injected into the mass spectrometry instrument. Each sample was measured in parallel three times.

The quantitative conditions of determination of eight active compounds were the same as in our previous studies (Xu *et al.* 2019). The UPLC-MS analysis was performed with an *ACQUITY* UPLC system (*Waters Corporation*, Japan) coupled with an *LC-20AD* pump, *SIL-20A* auto-sampler (*Waters Corporation*, Japan). The *ACQUITY UPLC BEH C18* column (1.7 μm, 2.1 × 50 mm) used for UPLC was held at 25°C; injection volume was 10.0 μL; the flow rate was 0.5 mL min^{-1}. Mobile phase A consisted of methanol, while the mobile phase B comprised water. The chromatographic column was eluted with a linear gradient of 25% A for 0–1.5 min, 25–50% A for 1.5–2.0 min, 50% A for 2.0–4.0 min, 50–90% A for 4.0–4.5 min, 90% A for 4.0–4.5 min, 90–25% A for 5.5–6.0 min, and 25% A for 6.0–7.0 min. Mass spectrometric detection was performed using *QTRAP 5500* (*AB SCIEX*, USA) equipped with electrospray ionization (ESI) source. Operation parameters were: cone voltage of 3 kV and ion source atomizing gas 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 eight active compounds are shown in Table 2S (*supplement*). Both MS and MS/MS data were determined in the positive mode, and data were used for multiple reaction monitoring (Fig. 1S, *supplement*).

The targeted metabolic analytical conditions for 13 phenolic metabolites were the same as in our previous studies (Xu *et al.* 2020). Samples were analyzed using an LC system coupled to a QTOF tandem-mass spectrometer *via* electrospray ionization (ESI) interface (*Agilent 6520*, *Agilent Technologies*, Santa Clara, CA, USA). Sample extracts were separated through a reversed-phase on a *Shimpack* LC column (*VP-ODS C18*, pore size 5.0 μm, 2 × 150 mm). The mobile phase comprised of solvent A and solvent B. Solvent A contained 0.04% acetic acid in the water, and solvent B contained 0.04% acetic acid in acetonitrile. The following gradient was adapted a flow rate of 0.5 mL min^{-1}: 0–20 min of 5–95% B; 20–22.1 min of 95–5% B; 22.1–28 min of 5–5% B. Blank measurement with the initial solvent was made after each HPLC run. The injection volume and column temperature were adjusted to 5 μL and 40°C. Optimal MS conditions for positive ion electrospray were: capillary temperature of 350°C; curtain gas pressure of 40 psi; capillary voltage of 3,500 V; fragmentation voltage of 135 V. The instrument was tuned prior to each batch run. A full-scan is ranging between 50–1000 m/z and was conducted with a scan time of 1 s and an interscan delay of 0.1 s in centered mode.

Statistical analysis: All results were executed to the study of variance (*ANOVA*) to determine the significant differences between shading treatments. If one-way *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*., USA). All bar graphs are plotted using *GraphPad 6.0* (*GraphPad Software Inc*., USA). Heat maps of phenol metabolites were drawn using the '*R*' software (https://www.r-project.org/).

Results

Growth parameters: Shading conditions affected the growth of *E. senticosus* (Table 1). The plant height, stem diameter, crown width, root length, leaf number, and leaf area in the Z_1, respectively, increased by 33.8, 43.3, 43.2, 55.7, 24.6, and 34.2% compared with the GK. The plant height, stem diameter, crown width, root length, and leaf area in the Z_2, respectively, decreased by 13.4, 22.9, 20.7, 18.4, and 17.4% compared within the GK, the leaf number increased by 51.8%. The results showed that the Z_1 was more favorable for the growth of *E. senticosus*, while Z_2 inhibited the growth of the plants.

Photosynthetic parameters: Shading also affected the gas-exchange parameters of *E. senticosus* (Table 2). The P_N in the Z_1 and Z_2 was 72.3 and 58.4%, respectively, of that in the GK. The g_s in the Z_1 and Z_2 was 58.3 and 36.5% of that in the GK. The C_i in Z_1 and Z_2 was 105.5 and 121.3%, respectively, of that in the GK. The *E* in the Z_1 and Z_2 was 88.2 and 82.9%, respectively, of that in the GK. Shading also affected the Chl fluorescence parameters of *E. senticosus* (Fig. 1). The F_0 in the Z_1 increased with the shading treatment days (Fig. 1*A*). After 90 d of shading treatment, there was no significant difference between the Z_1 and the GK. The F_0 in Z_2 was significantly higher than that of the GK. The F_m increased with shading treatment days in the Z_1 and was higher than that in the GK on the 90th day (Fig. 1*B*). The F_m in the Z_2 decreased

Table 1. Effect of light intensity on growth parameters of *Eleutherococcus senticosus.* Means (± SD, $n = 6$) followed by *a different small letter* in the same column are significantly different ($p<0.05$). GK – control group; Z_1 – moderate shading group; Z_2 – severe shading group.

Treatment group	Plant height [cm]	Stem diameter [mm]	Crown width [cm]	Root length [cm]	Leaf number	Leaf area [mm^2]
GK	11.612 ± 0.274^a	10.960 ± 0.358^a	32.602 ± 0.456^a	11.588 ± 0.215^a	19.000 ± 0.495^a	14.336 ± 0.378^a
Z_1	15.540 ± 0.351^b	15.707 ± 0.149^b	46.695 ± 0.476^b	18.038 ± 0.385^b	23.667 ± 0.316^b	19.238 ± 0.503^b
Z_2	10.055 ± 0.262^c	8.455 ± 0.202^c	25.863 ± 0314^c	9.462 ± 0.260^c	28.833 ± 0.501^c	11.836 ± 0.241^c

Table 2. Effect of light intensity on the photosynthetic parameters of *Eleutherococcus senticosus.* Means (± SD, n = 6) followed by *a different small letter* in the same column are significantly different (p<0.05). GK – control group; Z_1 – moderate shading group; Z_2 – severe shading group.

Treatment group	P_N [μmol·m^{-2} s^{-1}]	g_s [mol(H_2O) m^{-2} s^{-1}]	C_i [μmol(CO_2) mol^{-1}]	E [mmol(H_2O) m^{-2} s^{-1}]	WUE [mol(CO_2) mol(H_2O)$^{-1}$]
GK	7.566 ± 0.218^a	0.096 ± 0.003^a	235.481 ± 6.381^a	1.532 ± 0.039^a	4.939 ± 0.158^a
Z_1	5.469 ± 0.271^b	0.056 ± 0.003^b	248.306 ± 7.149^b	1.351 ± 0.040^b	4.048 ± 0.112^b
Z_2	4.417 ± 0.214^c	0.035 ± 0.002^c	285.079 ± 5.625^c	1.270 ± 0.036^c	3.005 ± 0.085^c

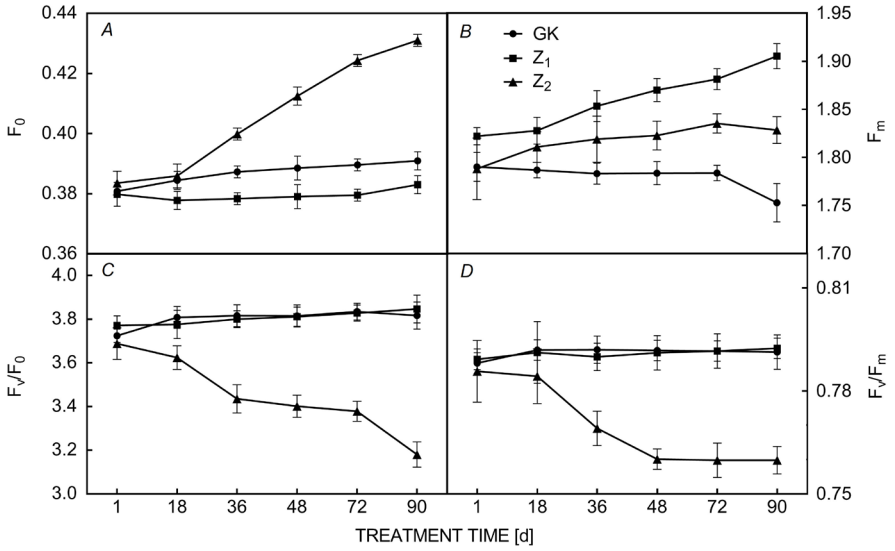

Fig. 1. Effects of different shading conditions on Chl fluorescence parameters of *Eleutherococcus senticosus* (means ± SD, n = 6). F_0, minimum fluorescence (*A*); F_m, maximum fluorescence (*B*); F_v/F_0, latent photochemical efficiency (*C*); F_v/F_m, maximum photochemical efficiency (*D*). GK – control group (●); Z_1 – moderate shading group (■); Z_2 – severe shading group (▲).

on the 20th day and was lower than that in the GK on the 90th day. The F_v/F_0 in the Z_2 was lower than that in the GK, while that in the Z_1 was not distinct from the GK (Fig. 1*C*). There was no difference of F_v/F_m between the Z_1 and the GK, while the Z_2 decreased with the shading treatment days, and was lower than that in the GK and the Z_1 on the 90th day (Fig. 1*D*).

Medicinal compounds: The Q value of principal component analysis was used to illustrate the overall trend in contents of the compounds studied in roots, stems, and leaves under different shading conditions (Fig. 2). The compounds studied showed the same accumulation trend in various organs. The content of the medicinal compounds was the highest in the Z_1.

In the roots, the contents of oleanolic acid, rutin, kaempferol, hyperoside, eleutheroside E, isofraxidin, and syringin in the Z_1, respectively, increased by 44.9, 150.0, 100.0, 60.0, 101.2, 97.8, and 85.2% compared with the GK, while the contents of L-phenylalanine in the Z_1 decreased by 5.4% compared with the GK. The contents of rutin, kaempferol, hyperoside, and eleutheroside E in the Z_2, respectively, increased by 83.3, 157.1, 73.3, and 40.4% compared with the GK. The contents of L-phenylalanine, oleanolic acid, isofraxidin, and syringin in the Z_2, respectively, decreased by 37.8, 19.2, 26.9, and 40.0% compared with the GK (Fig. 3).

In the stems, the contents of rutin, kaempferol, hyperoside, eleutheroside E, isofraxidin, and syringin in the Z_1, respectively, increased by 53.3, 33.3, 42.5, 64.8, 54.9, and 41.5% compared with the GK. The contents of L-phenylalanine, and oleanolic acid in the stems in the Z_1, respectively, decreased by 40.0 and 73.3% compared with the GK. The contents of rutin, kaempferol, and hyperoside in the stems in the Z_2, respectively, increased by 86.7, 93.3, and 66.7% compared with the GK. The contents of L-phenylalanine, oleanolic acid, eleutheroside E, isofraxidin, and syringin in the Z_2, respectively, decreased by 21.0, 37.5, 19.1, 67.3, and 48.3% compared with the GK (Fig. 3).

In the leaves, the contents of L-phenylalanine, oleanolic acid, rutin, hyperoside, kaempferol, eleutheroside E, isofraxidin, and syringin in the Z_1, respectively, increased by 147.7, 100.4, 147.5, 68.0, 43.9, 14.7, 800.0, and 17.4% compared with in the GK. The contents of L-phenylalanine, oleanolic acid, eleutheroside E, isofraxidin, and syringin in the Z_2, respectively, increased by 75.9, 309.3, 78.0; 2,600; and 78.3% compared with in the GK. The contents of rutin, hyperoside, and kaempferol, in the Z_2, respectively, decreased by 68.5, 23.2, and 51.0%, compared with in the GK (Fig. 3).

Phenolic metabolites: Thirteen phenolic compounds were analyzed in the roots of *E. senticosus.* The phenolics can be divided into two categories according to the cumulative

difference under different shading conditions (Fig. 4*A*): in Z_1 – cinnamic acid, ferulic acid, p-coumaric acid, caffeic acid, chlorogenic acid, protocatechuic acid, gallic acid, vanillic acid, p-hydroxybenzoic acid, and syringic acid; in Z_2 – genistein, luteolin, and quercetin. The phenolics can be divided into three categories according to the cumulative difference in the stems under different shading conditions (Fig. 4*B*). The GK group contained protocatechuic acid, gallic acid, vanillic acid, and syringic acid; Z_1 group contained caffeic acid, p-coumaric acid, chlorogenic acid, cinnamic acid, and ferulic acid. Z_2 group contained p-hydroxybenzoic acid, genistein, luteolin, and quercetin. Twelve phenolics were detected in the leaves (Fig. 4*C*). The phenolics can be classified into three categories according to the cumulative differences under different shading conditions. GK contained p-coumaric acid, caffeic acid, chlorogenic acid, and cinnamic acid; Z_1 contained p-hydroxybenzoic acid, protocatechuic acid, vanillic acid, and gallic acid; Z_2 contained quercetin, genistein, and luteolin.

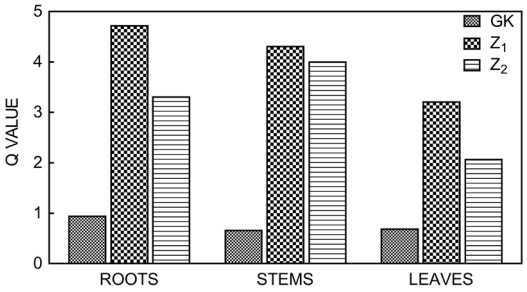

Fig. 2. The overall effects of different shading conditions on the accumulation of secondary metabolites in *Eleutherococcus senticosus* (n = 6). GK – control group; Z_1 – moderate shading group; Z_2 – severe shading group; Q value – principal component analysis score.

Discussion

The growth of plants is closely related to their living environment (Hammerschmidt 2004). Light intensities affected plant growth, photosynthesis, and the accumulation of secondary metabolites (Feijó *et al.* 2009, Kong *et al.* 2016, Pan and Guo 2016). Different species have different morphological responses to light intensity (Aleric and Kirkman 2005, Zhu *et al.* 2018, Khan *et al.* 2020). The results of this study showed that the plant height, stem diameter, crown width, root length, and leaf area of *E. senticosus* were higher in Z_1 and lower in Z_2. This is similar to the previous research (Feijó *et al.* 2009). Interestingly, we found that the leaf number increased with decreased light intensity, which may be the morphological change because *E. senticosus* adapted to the shading environment (Kim *et al.* 2019, Zhou *et al.* 2019). The reason why Z_1 promoted the growth of *E. senticosus* might

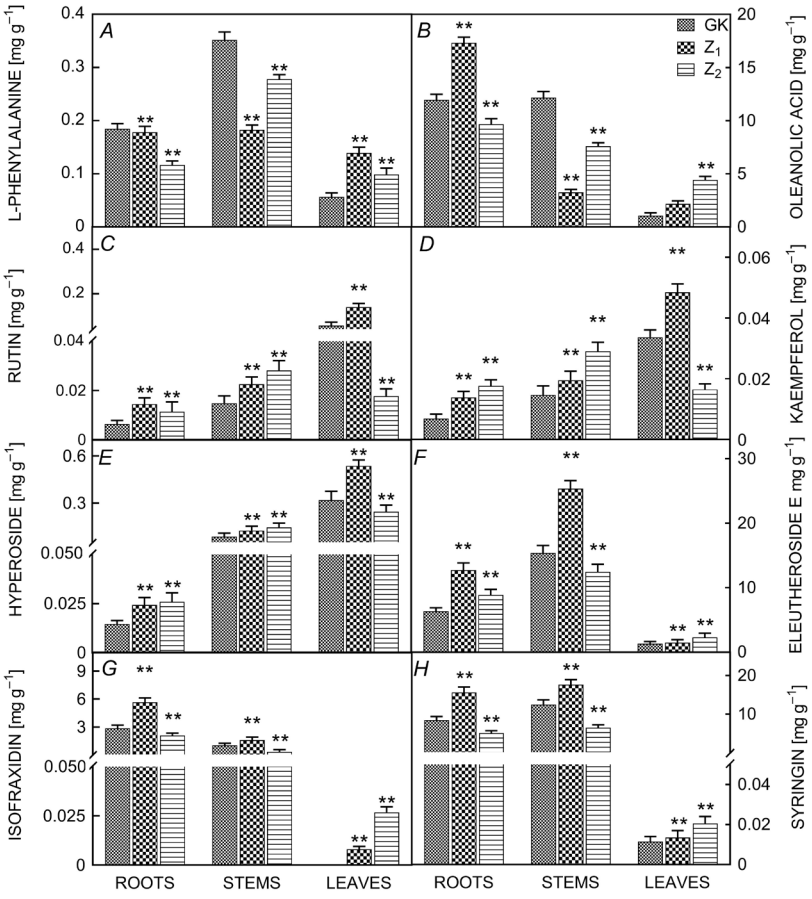

Fig. 3. The accumulation of eight secondary metabolites in different shading conditions in *Eleutherococcus senticosus*. GK – control group; Z_1 – moderate shading group; Z_2 – severe shading group. The bar represents the standard deviation (n = 6). ** indicates significant difference between the treatment group and the control group in the same medicinal part (p<0.01).

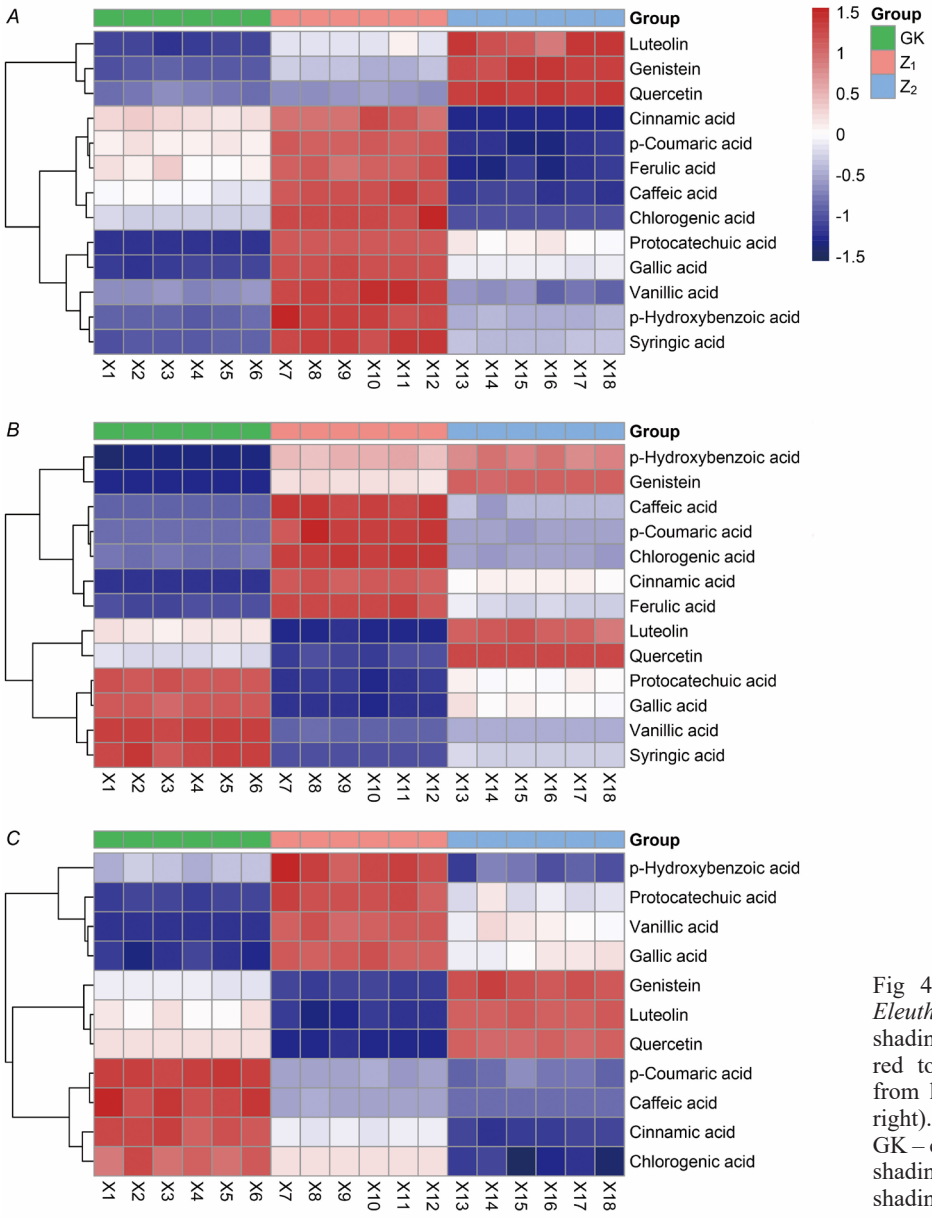

Fig 4. Cluster analysis of phenolics in *Eleutherococcus senticosus* under different shading conditions. The color range from red to blue indicates relative abundance from high to low (color key scale on the right). Roots (*A*); stems (*B*); leaves (*C*). GK – control group (X1–X6); Z_1 – moderate shading group (X7–X12); Z_2 – severe shading group (X13–X18).

be that shade could help plants maintain soil moisture, improve the air humidity around plants, and help plants resist possible water stress, especially in the semiarid area (Yang *et al.* 2019). In this study, we found that shading environment inhibited leaf photosynthesis parameters. Therefore, we believe that the change in photosynthetic parameters is not the reason why Z_1 promoted the growth of *E. senticosus*. However, we believe that the reason for Z_2 inhibiting the plant growth is low light density inhibiting the photosynthesis of *E. senticosus*.

The photosynthetic responses are the indicators of the plant ability to survive and grow in different environments (Gao *et al.* 2019). The effect of the environment on plant photosynthesis is mainly in two aspects, stomatal factors and nonstomatal factors (Jones 1985). The stomatal factors mean that the stomata control the supply of CO_2, which is affected by the number and the size of stomata (Quick *et al.* 1992). The nonstomatal factors are controlled by internal enzyme activity and photosynthetic components (Lal *et al.* 1996). The results of this study show that the P_N, g_s, E, and WUE decreased with decreasing light intensity. This is consistent with the previous study on *Centrosema* (DC.) Benth (Guenni *et al.* 2018). The C_i increased with decreasing light intensity. Previous studies showed that if the changing trend of P_N and C_i is opposite, then the main factor limiting the progress of photosynthesis must be the nonstomatal factor (Flexas and Medrano 2002). Therefore, we believe that shading treatment inhibits the photosynthesis in *E. senticosus* by nonstomatal limitation.

Chl fluorescence is related to various reaction processes in photosynthesis. The results showed that with the passing of shading treatment time, the F_0 increased in the Z_2, and decreased in the Z_1 (Fig. 1*A*). It was shown that nonphotochemical energy dissipation reduces F_0 and

the damage to photosynthetic machinery increases F_0 (Zhang 1999). The results of this study indicated that the PSII was damaged in Z_2 (Antal *et al.* 2013). This study showed that F_m increased in the shading treatment group and was higher in Z_1 (Fig. 1*B*). It indicated that the shading treatment promoted electron transport through PSII. The results of the above two parameters are consistent with the previous research on *Aloe vera* L. (Hazrati *et al.* 2016). The results of this study showed that the F_v/F_0 and F_v/F_m were not different between Z_1 and GK. The two parameters decreased in Z_2 with the shading time (Fig. 1*C*,*D*). The results indicate that Z_2 inhibited the light energy conversion efficiency of PSII (Demmig-Adams *et al.* 1996). In short, Z_1 promotes electron transmission through PSII center, which may be one of the reasons for Z_1 promoting the growth of *E. senticosus*. Furthermore, Z_2 damages the PSII center, which is also the reason why the condition inhibits the growth of the plant.

Previous studies showed that syringin, isofraxidin, and eleutheroside E are the active compounds in the roots and stems of *E. senticosus* (Li *et al.* 2006, Xu *et al.* 2017). The World Health Organization used syringin and eleutheroside E as an indicator for the quantitative determination of the plant (World Health Organization 2002). In addition, the 'Chinese Pharmacopoeia' (2010 and 2015 edition), specified isofraxidin and syringin as a quantitative standard for *E. senticosus*, respectively (Committee C. P. 2010, 2015). The results showed that the above-mentioned three active components accumulated more in the roots and stems (Fig. *3F–H*). This verified the using of the three compounds as quality control indicators of *E. senticosus* and coincided with the previous research results (Li *et al.* 2006, Cheng 2009). The result also indicated that the relative quality of *E. senticosus* roots and stems was better in Z_1. Three flavonoids, including rutin, hyperoside, and kaempferol (Fig. 3*C–E*), accumulated in the leaves. This is consistent with previous studies on the active compounds of *E. senticosus* leaves (Chen *et al.* 2002a,b). Our results also indicated that the relative quality of leaves was the best in Z_1. L-phenylalanine is an essential metabolite in the metabolism of shikimic acid. It is a precursor metabolite for the synthesis of lignin, coumarin, and flavonoid compounds. In this study, the accumulation of the L-phenylalanine in various organs was different (Fig. 3*A*); its metabolic regulation mechanism needs further research using enzyme and transcriptomics techniques.

In this study, we found that C_6-C_3-C_6 type phenolics, including genistein, luteolin, and quercetin, accumulated more in Z_2. This result is in accord with previous studies on the effect of light intensities on phenolics in maize. The limited light-induced anthocyanin accumulation in maize was mainly activated due to phenylalanine ammonia lyase (PAL) (Pál *et al.* 2020). The results of a research on ginger also show that low light intensity is conducive to the accumulation of flavonoids. Moreover, different photosynthetic rates at different light intensities may be related to the absence or presence of certain flavonoids (Ghasemzadeh *et al.* 2010). In addition, we also found that the accumulation of flavonoids in active medicinal ingredients is different from that of phenolics. This result is similar to the previous study on lettuce; it may be because the light intensity regulates different pathways of flavonoids production in plants (Pérez-López *et al.* 2018). The C_6-C_1 and C_6-C_3 type phenolics mainly accumulated in Z_1. They have the antibacterial and antioxidant capacity and improve the disease resistance of *E. senticosus* (Dey *et al.* 2005, Chang *et al.* 2007). The result of a previous study is different from this study, which showed that the strong light could promote the synthesis of cinnamic acid in lettuce (Zhan *et al.* 2012). Therefore, we believe that the response mechanism of phenolics to light intensity in different species is different, and the mechanism needs to be explained by further metabolomics research. In summary, most of the phenolics with protective effects accumulated in Z_1, which may be one of the mechanisms how Z_1 promotes the growth of *E. senticosus*.

Conclusions: This study investigated the changes caused by shading treatments on the morphological, photosynthetic characteristics, and secondary metabolites in *E. senticosus*. The growth parameters showed that Z_1 promoted the growth of the plants. Z_1 also promoted the leaf photosynthesis, while Z_2 inhibited photosynthesis. The chlorophyll fluorescence parameters indicated that Z_2 inhibited the PSII activity. Most of the medicinal compounds accumulated in Z_1, which indicated that the relative quality of *E. senticosus* is better in Z_1. Most of the defensive phenolics accumulated in Z_1. In short, Z_1 promoted the growth and the accumulation of medicinal compounds in *E. senticosus* by enhancing photosynthesis and increasing the accumulation of defensive phenolics. This study may provide some experimental basis for further study about the metabolic regulation mechanism of the *E. senticosus* under shade stress.

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