Effects of rocky desertification habitat on main secondary metabolites of Akebia trifoliata

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Abstract

In recent years, Akebia trifoliata used to restore rocky desertification environment. We first discovered that the medicinal content of A. trifoliata will increase in rocky desertification habitats, but its mechanism of action is not clear. In this study, A. trifoliata was planted in normal habitats and rocky desertification habitats, and changes in the content of secondary metabolites and related enzyme activities were analyzed. The results showed that: (1) the biomass of the roots, stems and leaf of A. trifoliata reduced significantly, but the content of secondary metabolites increased significantly in rocky desertification habitats. It is mainly reflected in the content of tannins in leaves, flavonoids in roots, and total phenols in roots, stems and leaves. (2) A. trifoliata changed the enzyme activities of PAL (Phenylalanine ammonialyase), C4H (Cinnamate-4-Hydroxylase) and 4CL (4-Coumarate: Coenzyme A Ligase), thereby regulated the increase in the content of secondary metabolites in rocky desertification habitat. (3) the content of medicinal components of A. trifoliata increased significantly in rocky desertification habitat. The highest content of oleanolic acid in the roots from July to August, and the highest content of α-hederagenins in the stems in July; (4) principal component analysis showed that the main response index of A. trifoliata secondary metabolites and related enzymes in rocky desertification habitats was total phenols. This study revealed the response mechanism of A. trifoliata secondary metabolites and related enzymes in rocky desertification habitats. It not only provided a new choice for the exploiting of medicinal resources of A. trifoliata, but also provided a new theoretical basis for A. trifoliata to restore rocky desertification environment.

Effects of rocky desertification habitat on main secondary metabolites of *Akebia trifoliata*

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Abstract

In recent years, *Akebia trifoliata* used to restore rocky desertification environment. We first discovered that the medicinal content of *A. trifoliata* will increase in rocky desertification habitats, but its mechanism of action is not clear. In this study,*A. trifoliata* was planted in normal habitats and rocky desertification habitats, and changes in the content of secondary metabolites and related enzyme activities were analyzed. The results showed that: (1) the biomass of the roots, stems and leaf of *A. trifoliata* reduced significantly, but the content of secondary metabolites increased significantly in rocky desertification habitats. It is mainly reflected in the content of tannins in leaves, flavonoids in roots, and total phenols in roots, stems and leaves. (2) *A. trifoliata* changed the enzyme activities of PAL (Phenylalanine ammonialyase), C4H (Cinnamate-4-Hydroxylase) and 4CL (4-Coumarate: Coenzyme A Ligase), thereby regulated the increase in the content of secondary metabolites in rocky desertification habitat. (3) the content of medicinal components of *A. trifoliata* increased significantly in rocky desertification habitat. The highest content of oleanolic acid in the

roots from July to August, and the highest content of α-hederagenins in the stems in July; (4) principal component analysis showed that the main response index of *A. trifoliata* secondary metabolites and related enzymes in rocky desertification habitats was total phenols. This study revealed the response mechanism of *A. trifoliata* secondary metabolites and related enzymes in rocky desertification habitats. It not only provided a new choice for the exploiting of medicinal resources of *A. trifoliata* , but also provided a new theoretical basis for *A. trifoliata* to restore rocky desertification environment.

KEYWORDS

Rocky desertification, *Akebia trifoliata* , Secondary metabolites, Medicinal ingredients

1 | INTRODUCTION

Karst rocky desertification is a process of land degradation caused by human activities, which is widely distributed in Southwest China, especially in Guizhou, and has become one of the three major ecological disasters in China (Wang et al., 2010; Stas et al., 2017). With the aggravation of rocky desertification, a large amount of soil loss leads to insufficient water accumulation, which leads to the decrease of soil water holding capacity and the stability of surface soil aggregates. Therefore, the nutrient content of rocky desertification soil is significantly different from that of normal soil (Sheng et al., 2018; Wang et al., 2018; Ma et al., 2020). In recent years, many scholars have tried to use plants to ecological restoration of rocky desertification, and the effect has only been restored, and its economic value is very small. Therefore, it is very important to find the economic value of vegetation repaired in rocky desertification habitat.

Plant secondary metabolism is the result of interaction between plant and environment in the long-term evolutionary process. Secondary metabolites and related enzymes play an important role in protecting and coordinating plants to adapt to external stress environment (Theis and Lerdau 2003; Ajmal et al., 2011). Secondary metabolites are not only involved in many kinds of plant resistance processes, but also have antioxidant, anti-inflammatory and anticancer effects. They can reduce the incidence of cancer, breast cancer and colon cancer (Romagnolo and Selmin 2012; Bhatia et al., 2011; Nourimand and Mohsenzadeh 2012), For example, polyphenols are a kind of secondary metabolites produced by plant phenylpropane and flavonoid metabolic pathways. They not only participate in plant growth and development, but also give plants the ability to resist ultraviolet, antioxidant and free radicals (Dixon et al., 2005; Skerget et al., 2005). They also have functions of preventing hypertension, hyperlipidemia (Prasain et al., 2010), diabetes (Rodrigo et al., 2011) and anticancer (Araùjo et al., 2011). Flavonoids are natural antioxidants that can directly scavenge oxygen free radicals and reactive oxygen species (Terao 2009), but also prevent osteoporosis and reduce the risk of colon cancer, prostate cancer and breast cancer (Michihara et al., 2012; Priya and Sharma 2013). Tannin is a good free radical scavenger and lipid peroxidation inhibitor (Dicko et al., 2005), with good physiological functions such as astringency, anti diarrhea, antibacterial, antioxidant and antiviral (Frasca et al., 2012). Therefore, the potential economic value of secondary metabolites in plants is very great. The synthesis of these substances is regulated by the phenylpropane pathway in plants, and the main enzymes regulated by it include PAL (Phenylalanine ammonialyase), C4H (Cinnamate-4-Hydroxylase) and 4CL (4-Coumarate: Coenzyme A Ligase) (Hahlbrock and Scheel 2003; Singh et al., 2009).

Akebia trifoliata is one of the economic species selected to control rocky desertification habitat in recent years, and has achieved good results. Its fruit is not only edible, but also can be used to make oil (Jiang et al., 2020). At the same time, it has the functions of promoting blood circulation, anti-inflammatory and diuretic (Jiang et al., 2012). Its extract can significantly inhibit the survival and proliferation of liver cancer cells (Lu et al., 2019). The main medicinal components of *A. trifoliata* are oleanolic acid, α-hederagenins and some secondary metabolites, such as polyphenols, tannins and flavonoids, which affect the synthesis of *A. trifoliata* . For example, drought can increase the content of artemisinin in *Artemisia carvifolia* (Yadav et al., 2014), saikosaponin a and saikosaponin b in *Bupleurum chinense* will increase under moderate water stress (Zhu et al., 2009), and Himalayan vegetation will secrete a lot of polyphenols and alkaloids due to long-term exposure to natural factors such as ultraviolet, drought and strong wind (Bhatia et al., 2011). However, these changes are different in different plants and different stress intensities (Zahir et al., 2014). But so far, there have been no reports on the effects of rocky desertification habitat on the content of secondary metabolites and related enzymes of *A. trifoliata* .

In this study, we compared the changes in the secondary metabolites and related enzymes of *A. trifoliata* in rocky desertification habitats and normal habitats, and attempted to clarify the response mechanism of the secondary metabolites and related enzymes of *A. trifoliata* in rocky desertification habitats. Meantime, it also provides a theoretical basis for the development of *A. trifoliata*medicinal resources.

2 | MATERIALS AND METHODS

2.1 | Experimental site

Ludi village, Shiban Town, Huaxi District, Guiyang City, Guizhou Province China (Huaxi base) and Zhongzhai village, Huajiang Town, Guanling County, Anshun City (Guanling base) were used as experimental sites (one of them was used as a repetition). The annual rainfall is mainly concentrated in April to October. One rocky desertification habitat (> 1000 m^2) is selected in each area, and one normal habitat (non rocky desertification area) is selected as the control (> 1000 m^2). The geographic and meteorological data of each base were from the archives of Guizhou Meteorological Bureau (Table 1). It can be seen from table 1 that Huaxi base and Guanling base had similar environmental factors, which were the two repeated treatments of the experiment. This study focuses on the effects of rocky desertification habitat on secondary metabolites of *A. trifoliata* , and the different performance of plants came from the effects of rocky desertification habitat rather than the different conditions of different bases.

TABLE 1 The geographical and meteorological data of different bases

Base	Longitude(E)	Latitude(N)	Altitude/m	Average temperature/	Rainfall/mm
Guanling	105°38'43"	25°38'46"	917	20.90	1318.20
Huaxi	106°40'53"	26°25'54"	1178	20.21	1318.90

2.2 | Experimental materials and design

A. trifoliata was provided by The Institute of Traditional Chinese Medicine, College of Agriculture, Guizhou University. The annual seedlings with strong growth, no diseases and pests, and basically the same growth were selected as the planting materials and planted in early April. Soil samples were taken for physical and chemical properties test (Table 2), and the significance of different habitats in the same base was analyzed. As showed in Figure 1, the normal habitat is planted according to the spacing of 2 m × 3 m, while the rocky desertification habitat was planted according to the actual situation, and was divided into 10 plots. Natural rainfall, did not add any fertilizer, so that its natural growth. Six plants with similar growth were randomly selected from each plot, three of which were used to measure the biomass (every 30 days or so), and the other three were used to measure the secondary metabolites and related enzymes indexes. When sampling, the stems and leaves within 1 m of the upper part of the plant and the root within 20 cm of the soil layer were selected as materials, which were stored in self sealed bags in ice box and quickly brought back to the laboratory for quick freezing in liquid nitrogen at - 80 .

TABLE 2 The soil physical and chemical properties in different habitats

Base	Habitat	Water content/%	Porosity/%	Bulk density/g·cm^{-3}	pH value	Total nit
Guan ling	Rocky desertification	15.52±4.53b	49.66±0.86A	1.30±0.07a	6.32±0.31a	1.18±0.02
	Normal	27.73±5.25a	45.73±0.58B	1.33±0.03a	6.01±0.75a	1.98±0.2A
Hua xi	Rocky desertification	17.63±3.65b	51.25±1.33A	1.28±0.06a	6.25±0.27a	1.03±0.04
	Normal	31.52±4.81a	42.63±0.64B	1.34±0.04a	6.04±0.66a	2.14±0.09

Note: The letters a/b represent 5% significant; the letters A/B represent 1% significant.

FIGURE 1 *A. trifoliata* growing in different habitats

2.3 | Determination of biomass

Separate the plant into three parts: root, stem and leaf. Rinse with water to remove impurities adhering to the plant sample, then rinse with deionized water 2 to 3 times and absorb moisture with absorbent paper. The sample was then treated at 105 °C for 30 minutes and then dried at 70 °C to constant weight.

2.4 | Determination of basic secondary metabolites and enzyme of phenylpropane pathway

The content of tannin was determined by vanillin method; The content of total phenols was determined by Folin ciocalteus method with gallic acid as standard; The content of flavonoids was determined by aluminum nitrate colorimetry, and the standard curve was established with rutin as the standard (Alhaithloul et al., 2019).

The PAL activity was assessed following the method as described by Koukol and Conn (1961). The C4H activity was determined by the method as described by Lamb and Rubery (1975). The 4CL activity was measured using the method as described by Knobloch and Hahlbrock (1975).

2.5 | Determination of medicinal ingredients

The content of oleanolic acid was determined by spectrophotometry (Fu et al., 2019): 5 g dry powder of roots, stems and leaves parts of *A. trifoliata* was precisely weighed and placed in 15 mL of 90% ethanol. Ultrasonic extraction was conducted at 70 for 90 min, and then the filtrate was filtered. Sufficient saturated clarified lime water was added to the filtrate, and the filtrate was stirred and precipitated at 60 for 30 min, and then the filtrate was filtered again. The filter cake was decomposed with 3% dilute sulfuric acid and filtered by suction. The filtrate was extracted twice with 40 mL trichloromethane, and the extraction solution was combined to a constant volume of 50 mL. 2 mL of the extract was added with 5% vanillin glacial acetic acid and 0.8 mL perchloric acid. The absorbance at 545 nm was determined by SPECTROMAX 250 (Molecular devices, USA) at room temperature. The standard curve of oleanolic acid concentration and absorbance was drawn with different concentrations of oleanolic acid as standard samples, and the content of oleanolic acid was calculated, expressed in $mg*g^{-1}$.

A-hederagenins were measured by High Performance Liquid Chromatography (HPLC) (Commission, 2010): (1) Reagent: α-hederagenin reference substance (CFDA, China); Acetonitrile (Tedia, USA); Phosphoric acid (Chengdu Jinshan Chemical Co., Ltd. China); Purified water (Wahaha Group, China). (2) Chromatographic conditions: Water 2695 HPLC instrument; The column was Agilent extend-c18 (4 × 250 mm, 5 μm); The mobile phase was acetonitrile: water: phosphoric acid (45:55:0.1); The flow rate was 1.0 $mL{\cdot}min^{-1}$; Injection volume 10 μL; The column temperature was 25 ; The detection wavelength was 203 nm. (3) Preparation of test solution: 1 g dry powder of roots, stems and leaves parts of *A. trifoliata* was precisely weighed, placed in 100 mL 75% ethanol, weighed, ultrasonicated for 30 min (330W, 50KHz), weighed in cold, added with 75%

methanol, shaken and centrifuged. Using microporous organic filter membrane (0.22 μm) the supernatant was filtered twice to determine the content of α-hederagenins. Repeat 3 times.

2.6 | Data analysis

All data were analyzed with Excel 2010 and SPSS 19.0, and plotted with origin 2019 b. One-way ANOVA and Duncan multiple comparison were used to test the differences between the treatments. In order to comprehensively and systematically evaluate the growth physiological response of*A. trifoliata* in rocky desertification habitat, principal component analysis was conducted on the average values of growth and physiological and biochemical indexes of various organs and parts in the experiment. In order to eliminate the differences between materials, the stress resistance coefficient method was used to evaluate the main response indexes (stress resistance coefficient = measured value under stress / measured value under control).

3 | RESULTS

3.1 | Biomass of *A. trifoliata* in different habitats

As shown in Figure 2, with the growth and development of *A. trifoliata* , the biomass of roots, stems, and leaves will gradually increase. Regardless of the habitat, the root biomass was the highest in the late growth period. In comparison, the biomass of roots, stems and leaf organs in rocky desertification habitats was less than that in normal habitats.

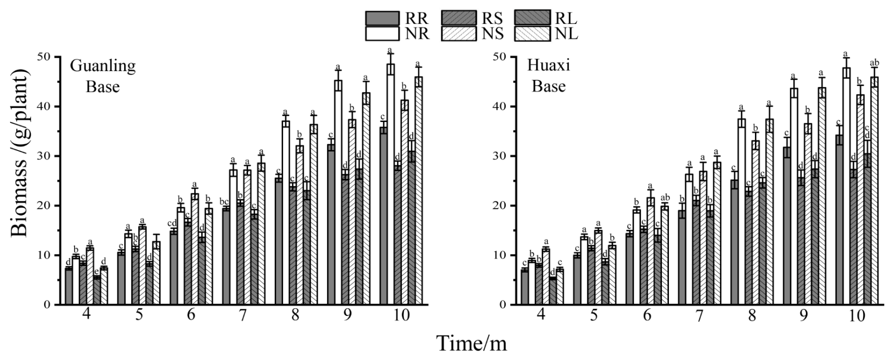

FIGURE 2 Biomass of *A. trifoliata* in different habitats

Note: RR: Root organs of *A. trifoliata* in rocky desertification habitat; NR: Root organs of *A. trifoliata* in a normal habitat; RS: Stem organs of *A. trifoliata* in a rocky desert habitat; NS: Stem organs of *A. trifoliata* in a normal habitat ; RL: leaf organs of *A. trifoliata* in rocky desertification habitats; NL: Leaf organs of *A. trifoliata* in normal habitats.

3.2 | Effect of rocky desertification habitat on the contents of basic secondary metabolites in *A. trifoliata*

Tannin and flavonoid belong to phenolic compounds, which are secondary metabolites of plants. They are good free radical scavengers and lipid peroxidation inhibitors in plants (Dicko et al., 2005). As shown in Figure 3, with the growth and development of *A. trifoliata* , the tannin content in roots, stems and leaves showed an upward trend, and the tannin content in leaves was the highest. At the same time, the tannin content in rocky desertification habitat increased faster than that in normal habitat, especially in leaves. In Guanling base, the difference of roots was the biggest in October, increased by1.35 times, stems was the biggest in October, increased by 1.17 times, leaves was the biggest in August, increased by 1.50 times; In Huaxi base, the difference of roots was the biggest in May, increased by 1.20 times, stems was the biggest in October, increased by 1.14 times, leaves was the biggest in August, increased by 1.63 times. The results showed that the content of tannin in roots, stems and leaves of *A. trifoliata*could be increased in rocky desertification habitat, especially in leaves.

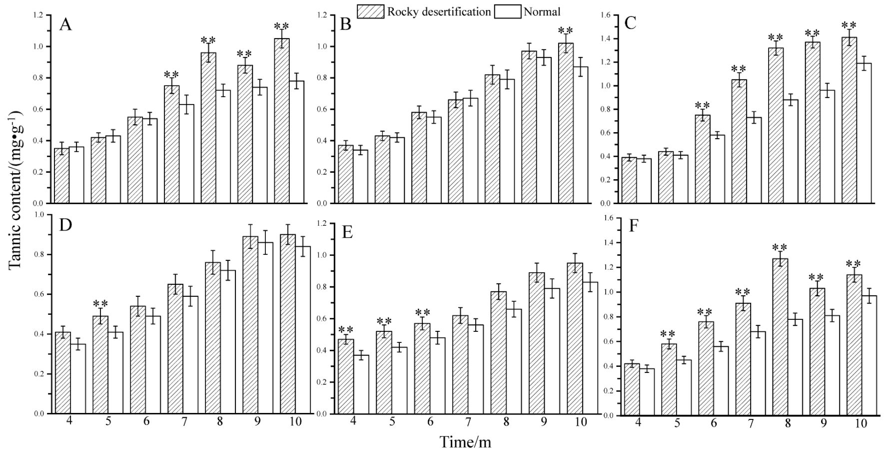

FIGURE 3 Tannin content of *A. trifoliata* in different habitats

Note: A: Root organs of *A. trifoliata* in Guanling base; B: Stem organs of *A. trifoliata* in Guanling base; C: The leaf organs of*A. trifoliata* in Guanling base; D: Root organs of *A. trifoliata* in Huaxi base; E: Stem organs of *A. trifol\iata* in Huaxi base; F: The leaf organs of*A. trifoliata* in Huaxi base. * is 5% significant, ** is 1% significant. The same below.

As shown in Figure 4, with the growth and development of *A. trifoliata* , the content of flavonoids in roots, stems and leaves showed an upward trend. At the same time, the increase rate of flavonoids content in roots and stems of *A. trifoliata* in rocky desertification habitat was faster than that in normal habitat, especially in the roots, while the flavonoids content in leaves was lower than that in normal habitat. In Guanling base, the difference of roots was the biggest in October, increased by 1.35 times, stems was the biggest in July, increased by 1.13 times, leaves was the biggest in September, decreased by 1.19 times; In Huaxi base, the difference of roots was the biggest in October, increased by 1.61 times, stem was the biggest in October, increased by 1.20 times, leaf was the biggest in June, decreased by 0.79 times. In conclusion, rocky desertification habitat can increase the content of flavonoids in roots and stems of*A. trifoliata* , but reduce the content of flavonoids in leaves.

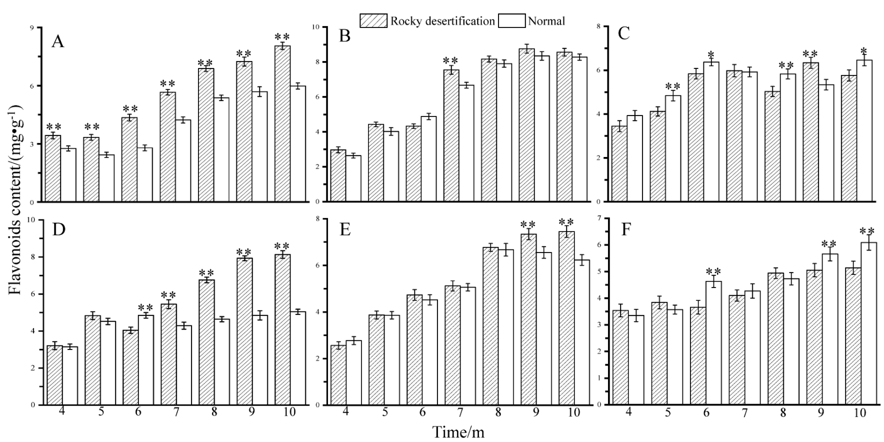

FIGURE 4 Flavonoids content of *A. trifoliata* in different habitats

Phenols are one of the most important secondary metabolites in plants. They are not only one of the components of the plant cell wall, but also can help plants resist the damage caused by various adversities (Ramirez et al., 2001). It can be seen from Figure 5 that with the growth and development of *A. trifoliata*

, the content of total phenol in roots, stems and leaves showed an upward trend, and the content in leaves was the highest. At the same time, the increase rate of total phenol content in rocky desertification habitat was faster, and the content was significantly higher than that in normal habitat, especially in leaves. In Guanling base, the difference of roots was the biggest in October, increased by 1.46 times, stems was the biggest in September, increased by 1.30 times, leaves was the biggest in October, increased by 1.88 times; In Huaxi base, the difference of roots was the biggest in September, increased by 1.52 times, stem was the biggest in October, increased by 1.43 times, leaf was the biggest in September, increased by 1.57 times. In conclusion, rocky desertification can increase the content of total phenols in the root, stem and leaf of *A. trifoliata* .

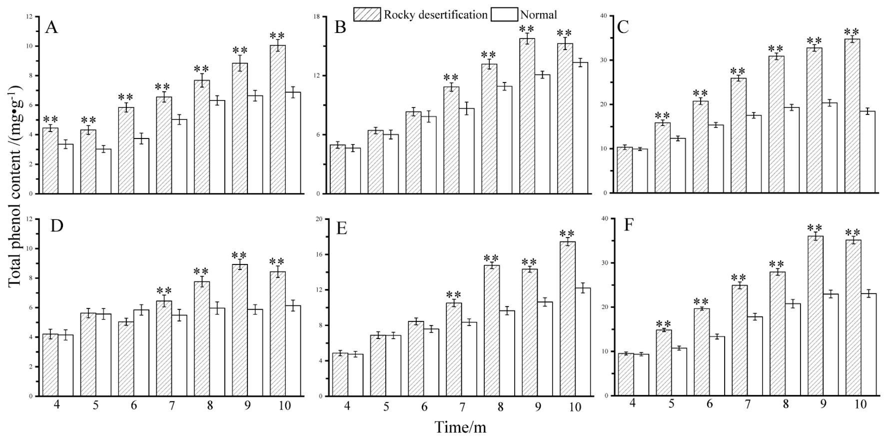

FIGURE 5 Total phenol content of *A. trifoliata* in different habitats

3.3 | Effects of rocky desertification on enzyme activities of phenylpropanoid pathway in *A. trifoliata*

PAL, 4CL and C4H are the three most important enzymes in the phenylpropanoid pathway, and their activities affect the secondary metabolites of the basic phenylpropanoid pathway (Singh et al., 2009). It can be seen from Figure 6 that in the Guanling base, the PAL activity of roots and stems in the rocky desertification habitat first increased and then decreased compared with the normal habitat, while the leaves showed a gradually increasing trend. The difference of roots in September was the biggest, increased by 1.35 times; The difference of stems was the biggest in September, which increased by 6.28 times; The difference of leaves in October was the biggest, increased by 1.28 times. In Huaxi base, the PAL activity of roots in rocky desertification habitat increased first and then decreased compared with that in normal habitat, while that in stems decreased and that in leaves increased. The difference of roots was the biggest in October, increased by 1.20 times; The difference of stems was the biggest in August, which decreased by 0.89 times; The difference of leaves in September and October was the biggest, increased by 1.29 times and 1.33 times respectively. In conclusion, the PAL activity in roots and leaves of *A. trifoliata*could be increased in rocky desertification habitat.

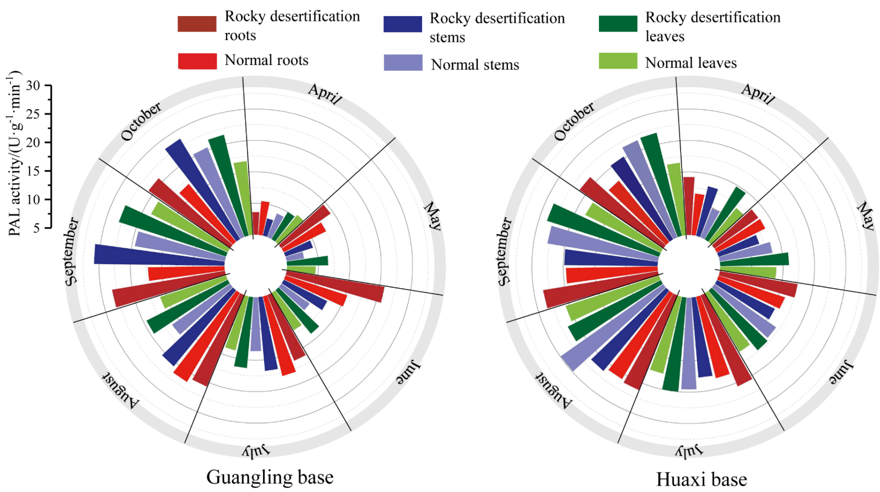

FIGURE 6 PAL activity of *A. trifoliata* in different habitats

It can be seen from Figure 7 that the 4CL activity of roots, stems and leaves in rocky desertification habitat of Guanling base was higher than that in normal habitat, and the difference first increases and then decreases, and the difference was the largest in August, with the increase of 1.23 times in roots, 1.19 times in stems and 1.30 times in leaves; In Huaxi base, the activity of 4CL in roots, stems and leaves in rocky desertification habitat was higher than that in normal habitat, and different parts showed different trends, but the difference of roots, stems and leaves was the largest in April, increased by 1.35 times, 1.29 times and 1.24 times respectively. In conclusion, *A. trifoliata* could significantly increase 4CL activity in roots, stems and leaves in rocky desertification habitat.

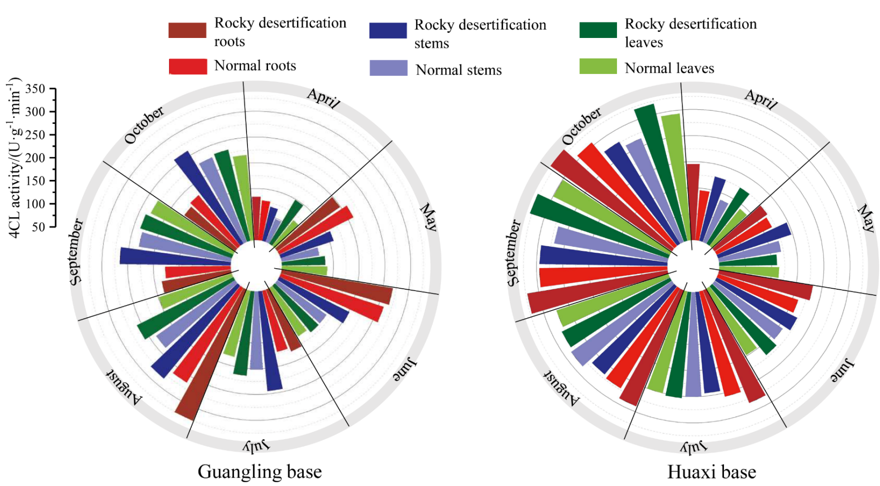

FIGURE 7 4CL activity of *A. trifoliata* in different habitats

It can be seen from Figure 8 that in the Guanling base, the C4H activity in the roots, stems and leaves of the rocky desertification habitat first increased and then decreased compared with the normal habitat. The difference of roots was the biggest in August, increased by 1.21 times, stems was the biggest in September, increased by 1.30 times, leaves was the biggest in October, decreased by 0.93 times. In Huaxi base, the C4H activity in the roots of the rocky desertification habitat showed a trend of first increasing and then decreasing compared with the normal habitat. The difference was the largest in September, which increased by 1.23 times. The C4H activity of stems in rocky desertification habitats was lower than that in normal habitats.

The difference was the largest in August, which decreased by 0.75 times, and the changes in leaves were more frequent. The difference was the largest in October, which decreased by 0.92 times. In conclusion, the C4H activity in roots of*A. trifoliata* increased significantly in rocky desertification habitat, but there was no significant change in stems and leaves.

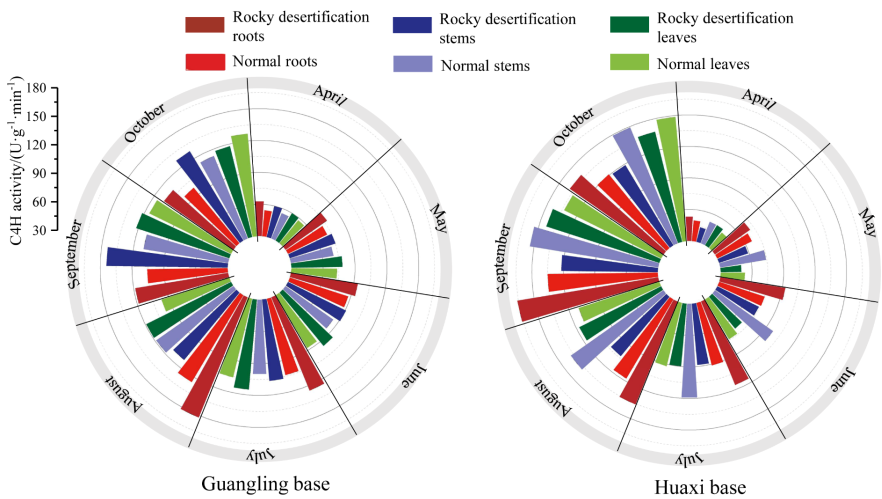

FIGURE 8 C4H activity of *A. trifoliata* in different habitats

3.4 | Effect of rocky desertification habitat on medicinal ingredients content in *A. trifoliata*

It can be seen from Figure 9 that with the growth and development of*A. trifoliata* , the oleanolic acid content in roots, stems and leaves first increased and then decreased, and the content of oleanolic acid in roots > leaves > stems. At the same time, the increasing rate of oleanolic acid content in rocky desertification habitat was faster, and the content was significantly higher than that in normal habitat. In Guanling base, the difference of roots was the biggest in October, increased by 1.45 times, the difference of stem in October was the largest, increased by 1.92 times, and the difference of leaves in June was the largest, increased by 1.26 times; In Huaxi base, the difference of root was the biggest in September, increased by 1.17 times, stem was the biggest in October, increased by 1.69 times, leaf was the biggest in June, increased by 1.25 times. In conclusion, the content of oleanolic acid in roots, stems and leaves of *A. trifoliata* could be increased in rocky desertification habitat.

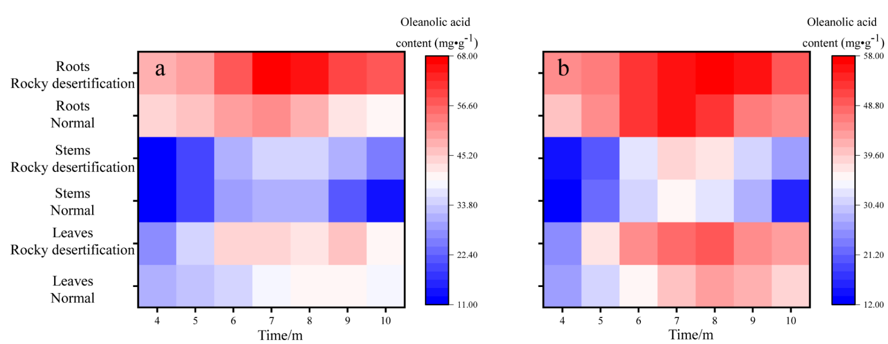

FIGURE 9 Oleanolic acid content of *A. trifoliata* in different habitats

Note: a: Guanling base; b: Huaxi base. The same below.

It can be seen from Figure10 that with the growth and development of *A. trifoliata* , the content of α-hederagenins in roots and stems showed a trend of first increasing and then decreasing, while in leaves it fluctuated frequently and irregularly, and the content of α-hederagenins in stems > leaves > roots. At the same time, the increasing rate of oleanolic acid content in rocky desertification habitat was faster, and the content was significantly higher than that in normal habitat. In Guanling base, the difference of root was the biggest in July, increased by 4 mg·g^{-1}, stem was the biggest in October, increased by 13 mg·g^{-1}, leaf was the biggest in June, increased by 6 mg·g^{-1}; In Huaxi base, the difference of root was the biggest in May, increased by 3 mg·g^{-1}, stem was the biggest in September, increased by 4 mg·g^{-1}, leaf was the biggest in August, increased by 10 mg·g^{-1}. In conclusion, the content of α-hederagenins in roots, stems and leaves of *A. trifoliata* could be increased in rocky desertification habitat.

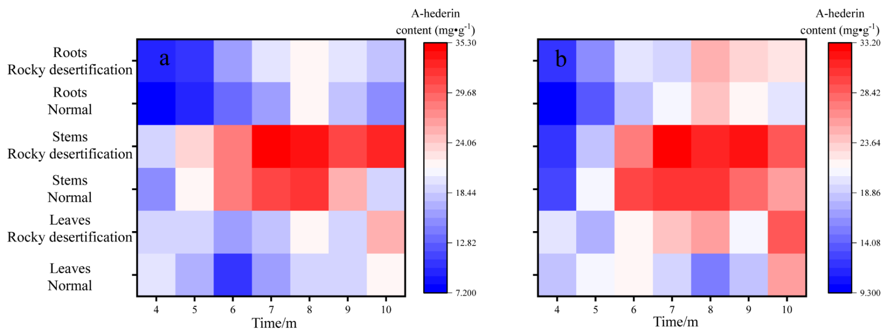

FIGURE 10 A-hederagenin content of *A. trifoliata* in different habitats

3.5 | Comprehensive analysis of secondary metabolites of *A. trifoliata* in rocky desertification habitat

Principal component analysis can transform many original indexes into comprehensive indexes for analysis, which can comprehensively reflect the response degree of plants to rocky desertification habitat. Principal component analysis of secondary metabolites of *A. trifoliata* from two bases was carried out according to the principle of eigenvalue greater than 1 (Figure 11). The main components of Guanling base are oleanolic acid and total phenol; The main components of Huaxi base are flavonoids and total phenols. Comprehensive analysis showed that the main response factor of secondary metabolites of *A. trifoliata* in rocky desertification habitat was total phenol.

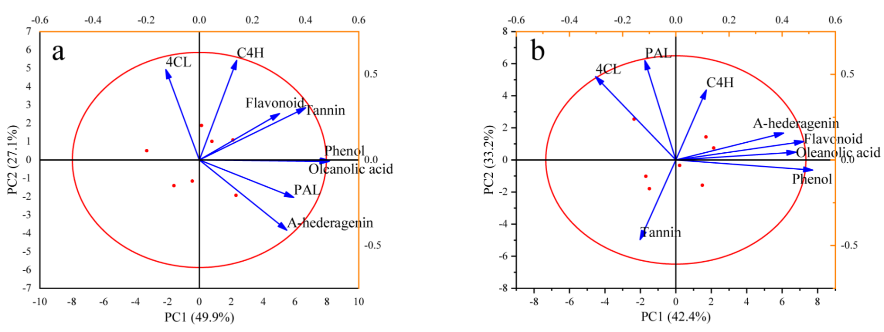

FIGURE 11 Principal component analysis of secondary metabolites of *A. trifoliata* in different habitats

4 | DISCUSSION

Environmental stress can not only inhibit plant growth, but also stimulate the synthesis and accumulation of plant secondary metabolites (Zhang et al., 2020). However, the biggest characteristic of rocky desertification habitat is that it does not keep water, resulting in a large amount of water loss. Thus causing certain drought stress to plants. The biomass of various organs of *A. trifoliata*significantly decreased in the rocky desertification habitat, which indicates that the desertification habitat has caused a certain abiotic stress on *A. trifoliata* , and the metabolic physiological processes of its plants will inevitably change.

4.1 | Effects of rocky desertification on the contents of basic secondary metabolites in *A. trifoliata*

The secondary metabolites such as phenols and flavonoids are closely related to plant stress defense (Sanchez et al., 2011). In this study, we found that rocky desertification could promote the increase of total phenol content in roots, stem and leaf of *A. trifoliata* , which was the same as that of total phenol secretion of Desi ajwain (*Trachyspermum ammi* L.) under drought stress (Azhar et al., 2011). The possible reason is that the increase of total phenol content can help to reduce the water potential in cells, prevent the loss of water in cells; At the same time, phenol can also remove reactive oxygen species produced under drought (Nakabayashi et al., 2015). In this study, it was found that the content of polyphenols in leaves changed the most, which may be due to the fact that leaves are the largest organ of plants exposed to the outside world, and they are most seriously exposed to oxidative stress. Therefore, they secrete a large number of polyphenols, remove reactive oxygen species and free radicals through hydrogen atom transfer mechanism, and are used for the synthesis of antioxidants to resist oxidative damage (Ping et al., 2017).

Tannins and flavonoids belong to the phenolic compounds of plant secondary metabolites, which are good free radical scavengers and lipid peroxidation inhibitors (Dicko et al., 2005). The results showed that the content of tannin in *A. trifoliata* increased rapidly in the early stage and decreased slowly in the later stage. The possible reason is that water shortage in rocky desertification habitat can induce the increase of secondary metabolism related enzyme activities in *A. trifoliata* , so a large number of secondary metabolites are activated. Finally, it is transported to various organelles to repair the damage of membrane system and improve the tolerance of plants to drought stress, which is a self-protection mechanism for plants to cope with stress (Castellarin et al., 2007). The tannin content would slow down and decrease in the later rocky desertification habitat. The possible reason is that with the aggravation of stress, the plant organ damage is serious, the metabolism speed in the body slows down, resulting in the reduction of carbon sources required for the synthesis of phenols. Therefore, the tannin content in the plant does not increase or even decrease. Flavonoids usually have special structures such as hydroxyl, double carbon bond, glycosylation, acylation, methylation, etc., which can effectively resist oxidative damage induced by stress. At the same time, they can also complete antioxidant function by preventing the generation of reactive oxygen species and scavenging reactive oxygen species (Agati et al., 2010; Singanusong et al., 2015). In this study, we found that rocky desertification habitat can improve the content of flavonoids in the root and stem of *A. trifoliata* , which is the same as Zahir et al., (2014) study on water stress in Silybum marianum. The possible reason is the water deficiency caused by rocky desertification, which affects the up regulation of genes related to flavonoid metabolism pathway, such as MYB transcription factor, flavone-3-hydroxylase (F3H) and Flavonol Synthase (FLS) (Ying et al., 2018; Fan et al., 2018), thus increasing the content of flavonoids. However, there is no obvious rule for the change of flavonoids content in *A. trifoliata* leaves in rocky desertification habitat in the two bases. The possible reason is that the flavonoids content in leaves is less affected by rocky desertification habitat, so there is no obvious change rule.

4.2 | Effects of rocky desertification on enzyme activities of phenylpropanoid pathway in *A. trifoliata*

The changes of biological and abiotic factors do not directly affect the synthesis of plant secondary metabolites, but affect the synthesis of secondary metabolites by affecting the expression of key genes and key enzyme activities in secondary metabolism (Havaux and Kloppstech 2001). PAL, C4H and 4CL are key enzymes in phenylalanine metabolism pathway, and their activities are affected by environmental factors such as light, temperature and water (Dupont and Aksnes 2010). This study found that *A. trifoliata* in rocky desertification habitat can improve the activities of pal, C4H and 4CL in roots, 4CL in stems and PAL, 4CL in leaves. Obviously, different enzymes have different responses to rocky desertification habitat, and the changes in different tissues and organs are also different. In rocky desertification habitat, the three enzymes in roots were significantly increased, which may be due to the root as the main organ of plant drought resistance, its flavonoids content is also the most, so its response changes are also the most obvious, especially pal and C4H changes are the most obvious. It can be seen that the appropriate degree of drought stress can promote the accumulation of flavonoids by stimulating the expression and activity of key enzymes involved in flavonoid biosynthesis. This is consistent with the research results of Cheng et al., (2018) that moderate drought stress can promote baicalin accumulation by stimulating the key enzyme activity and expression of Baicalin biosynthesis.

4.3 | Effects of rocky desertification on oleanolic acid and -hederagenin contents in *A. trifoliata*

Oleanolic acid and α-hederagenin are the two most important medicinal components of *A. trifoliata* . They are triterpenoids in terpenoids. They can not only participate in interspecific competition as interspecific sensing compounds (Arimura et al., 2000), but also be used as raw materials for spices, flavorings and cosmetics (Martin et al., 2003), pesticides and industrial raw materials (George et al., 2015), As well as anti-tumor, anti-inflammatory, antibacterial, antiviral, antimalarial, promoting percutaneous absorption, prevention and treatment of cardiovascular disease, hypoglycemic and other biological activities (Chen et al., 2021; Sun et al., 2019). However, their ecological functions, biosynthetic pathways and environmental factors are not very clear. In this study, we found that rocky desertification could increase the contents of oleanolic acid and α-hederagenins in the roots, stems and leaves of *A. trifoliata* . The possible reason is the water stress caused by rocky desertification habitat, which induces the expression of BPW and BPY genes and reduces the expression of BPX genes, resulting in the accumulation of triterpenoid saponins (Yin et al., 2015). This is the same as the result of Chen et al., (2011) which found that moderate drought would increase oleanolic acid in *Prunella vulgaris* L., and the possible reason is that oleanolic acid has antioxidant effect, which can help *A. trifoliata* resist the oxidative damage caused by rocky desertification habitat (Zhao et al., 2013). However, the role of α-hederagenins under drought stress is still unclear, so it needs to be studied. At the same time, this study found that rocky desertification habitat did not affect the distribution of oleanolic acid content in all organs of *A. trifoliata* , which was still root > stem > leaf. However, in Huaxi base, the peak period of oleanolic acid content in roots was delayed to a certain extent, but not in Guanling base. The possible reason is that *A. trifoliata* adapts to rocky desertification habitat stress for a long time in Huaxi base, which causes the peak period of oleanolic acid content to shift. However, the content peak and distribution of α-hederagenins in various organs did not change.

Although the biomass of roots, stems and leaves of *A. trifoliata*will decrease in the rocky desertification habitat, the content of medicinal ingredients has increased significantly. This not only brings new economic benefits to *A. trifoliata* for restoring rocky desertification habitats, but also further improves the quality of*A. trifoliata* medicinal ingredients.

5 | CONCLUSION

The response of secondary metabolites of *A. trifoliata* to rocky desertification was studied. The results showed that the rocky desertification habitat reduced the biomass of *A. trifoliata* , but significantly increased the tannin content in the leaves, the flavonoid content in the roots, and the total phenol content in the roots, stems and leaves, and reduced the flavonoid content in the leaves; The activities of PAL, C4H and 4CL in roots, 4CL in stems and PAL, 4CL in leaves of *A. trifoliata* in rocky desertification habitat were increased. The content of oleanolic acid and α-hederagenin in the root, stem and leaf of *A. trifoliata* was increased in rocky desertification habitat. In addition, the content of oleanolic acid in the root of *A. trifoliata* was the highest from July to August, which was suitable for picking medicine. The content of α-hederagenin in the stem was the highest in July, which was suitable for picking medicine. The principal component analysis showed that the main response index of secondary metabolites of *A. trifoliata* in rocky desertification habitat was total phenol. The results of this study are of great signifiicance to cultivators and researchers of medicinal plants.

ETHICS STATEMENT

None of the species are endangered, protected, or personally owned. This research was authorized by the Institute of Natural Resources and Ecology.

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None.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Xiaosong Yu: Conceptualization (equal); Funding acquisition (lead); Writing-original draft (lead); Writing-review & editing (equal). **Xiaohong Wang:** Methodology (lead); Visualization (equal); Writing-review & editing (supporting). **Zhi Liu:**Conceptualization (equal); Data curation (equal); Methodology (equal); Writing-review & editing (equal). **Lin Li:** Software (lead); Conceptualization (equal); Visualization (equal); Writing-review & editing (equal). **Ningxian Yang:** Writing-review & editing (supporting). **Mingsheng Zhang:** Conceptualization (equal); Funding acquisition (supporting); Resources (lead); Writing-review & editing (equal).

DATA AVAILABILITY STATEMENT

Data used in this study are available for download in Dryad ("Effects of rocky desertification habitat on main secondary metabolites of Akebia trifoliata"; https://doi:10.5061/dryad.7h44j0ztx).

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REFERENCES

Agati, G., Galardi, C., Gravano, E., Romani, A., Tattini, M. (2002). Flavonoid Distribution in Tissues of Phillyrea latifolia L. Leaves as estimated by microspectrofluorometry and multispectral fluorescence microimaging. *Photochemistry and Photobiology* , *76* (3), 350-360. https://doi.org/10.1562/0031-8655(2002)0760350FDITOP2.0.CO2

Ajmal, S., Perveen, R., Chohan, S., Yasmin, G., Mehmood, M.A. (2011). Role of secondary metabolites biosynthesis in resistance to cotton leaf curl virus (CLCuV) disease.*African Journal of Biotechnology* , *10* (79), 18137-18141. https://doi.org/10.5897/AJB11.2502

Alhaithloul, H. A., Soliman, M. H., Ameta, K. L., El-Esawi, M. A., Elkelish, A. (2019) Changes in Ecophysiology, Osmolytes, and Secondary Metabolites of the Medicinal Plants of Mentha piperita and Catharanthus roseus Subjected to Drought and Heat Stress. *Biomolecules* , 10(1), 43. https://doi.org/10.3390/biom10010043

Araùjo, J. R., Goncalves, P., Martel, F. (2011). Chemopreventive effect of dietary polyphenols in colorectal cancer cell lines. *Nutrition Research* , *31* , 77-87. https://doi.org/10.1016/j.nutres.2011.01.006

Arimura, G. I., Ozawa, R., Shimoda, T., Nishioka, T., Takabayashi, J. (2000). Herbivory-induced volatiles elicit defence genes in lima bean leaves. *Nature* , *406* (6795), 512-515. https://doi.org/10.1038/35020072

Azhar, N., Hussain, B., Ashraf, M. Y., Abbasi, K. Y. (2011). Water stress mediated changes in growth, physiology and secondary metabolites of desi ajwain (*Trachyspermum Ammi* L.). *Pakistan Journal of Botany* , *43* , 15. https://doi.org/10.1093/jpe/rtr019

Bhatia, A., Arora, S., Singh, B., Kaur, G., Nagpal, A. (2011). Anticancer potential of Himalayan plants. *Phytochemistry Reviews* ,*10* (3), 309. https://doi.org/10.1007/s11101-010-9202-0

Castellarin, S. D., Pfeiffer, A., Sivilotti, P., Degan, M., Peterlunger, E., Di, G. G. (2007). Transcriptional regulation of anthocyanin biosynthesis in ripening fruits of grapevine under seasonal water deficit. *Plant Cell and Environment* , *30* (11), 1381-1399. https://doi.org/10.1111/j.1365-3040.2007.01716.x

Chen, X., Chen, B., Shang, X., Fang, S. (2021). RNA in situ hybridization and expression of related genes regulating the accumulation of triterpenoids in Cyclocarya paliurus. *Tree Physiology* , *5* . https://doi.org/10.1093/Treephys/tpab 067

Chen, Y., Guo, Q., Li, L., Li, L., Zhu, Z. (2011). Influence of fertilization and drought stress on the growth and production of secondary metabolites in *Prunella vulgaris* L. *Journal of Medicinal Plant Research* , *5* (9), 1749-1755. https://doi.org/10.2147/DDDT.S10498

Cheng, L., Han, M., Yang, L. M., Yang. L., Sun, Z., Zhang, T. (2018). Changes in the physiological characteristics and baicalin biosynthesis metabolism of Scutellaria baicalensis Georgi under drought stress.*Industrial Crops and Products* , *122* , 473-482. https://doi.org/10.1016/j.indcrop.2018.06.030

Commission, C. P. (2010). Pharmacopoeia of the People's Republic of China. *People's Medical Publishing House* . https://doi.org/10.2753/CLG0009-4609430304

Dicko, M. H., Gruppen, H., Barro, C., Traore, A. S., Berkel, W. J. H., Voragen, A. G. J. (2005). Impact of phenolic compounds and related enzymes in sorghum varieties for resistance and susceptibility to biotic and abiotic stresses. *Journal of Chemical Ecology* , *31* (11), 2671-2688. https://doi.org/10.1007/s10886-005-7619-5

Dixon, R. A., Xie, D. Y., Sharma, S. B. (2005). Proanthocyanidins-a final frontier in flavonoid research? *New Phytologist* ,*165* , 9-28. https://doi.org/10.1111/j.1469-8137.2004.01217.x

Dupont, N., Aksnes, D. L. (2010). Simulation of optically conditioned retention and mass occurrences of Periphylla periphylla. *Journal of plankton research* , *32* (6), 773-783. https://doi.org/10.1093/plankt/fbq015

Fan, M., Jin, L. P., Huang, S. W., Xie, K. Y., Liu, Q. C., Dong-Yu, Q. U. (2008). Effects of drought on gene expressions of key enzymes in Carotenoid and flavonoid biosynthesis in Potato. *Acta Horticulturae Sinica* , *4* (04), 535-542. https://doi.org/10.3724/SP.J.1005.2008.01083

Frasca, G., Cardile, V., Puglia, C., Bonina, C., Bonina, F. (2012). Gelatin tannate reduces the proinflammatory effects of lipopolysaccharide in human intestinal epithelial cells. *Clinical & Experimental Gastroenterology* , *5* (1), 61-67. https://doi.org/10.2147/CEG.S28792

Fu, Z. R., Li, X. F., Zhang, X. X., OUYANG, Y. Z., Wu, M. Y. (2019). Content measurement of oleanolic acid in *Akebia Trifoliata*pericarp by ultraviolet-visible spectrophotometry. *Journal of Jishou University(Natural Sciences Edition)* , *40* (01), 64-68. https://doi.org/10.13438/j.cnki.jdzk.2019.01.016

George, K. W., Alonso-Gutierrez, J., Keasling, J. D., Lee, T. S. (2015). Isoprenoid drugs, biofuels, and chemicals- artemisinin, farnesene, and beyond. *Advance in Biochemical Engineering Biotechnology* ,*148* , 355-389. https://doi.org/10.1007/10_2015_310

Hahlbrock, K., Scheel, D. (2003). Physiology and molecular biology of phenylpropanoid metabolism. *Annual Review of Plant Biology* ,*40* (1), 347-369. https://doi.org/10.1146/annurev.pp.40.060189.002023

Havaux, M., Kloppstech, K. (2001). The protective functions of carotenoid and flavonoid pigments against excess visible radiation at chilling temperature investigated in Arabidopsis npq and tt mutants.*Planta* , *213* (6), 953-966. https://doi.org/10.1007/s004250100572

Jiang, Y., Du, Y., Zhu, X., Hua, X., Meng, W. W., Hu, J. (2012). Physicochemical and comparative properties of pectins extracted from*Akebia trifoliata* var. Australis peel. *Carbohydrate Polymers* , *87* (2), 1663-1669. https://doi.org/10.1016/j.carbpol.2011.09.064

Jiang, Y. L., Yin, H., Zheng, Y., Wang, D. F., Liu, Z. M., Deng, Y., Zhao, Y. Y. (2020). Structure, physicochemical and bioactive properties of dietary fibers from *Akebia trifoliata* (Thunb.) Koidz. Seeds using ultrasonication/shear emulsifying/microwave-assisted enzymatic extraction. *Food Research International* , *136* , 109348. https://doi.org/10.1016/j.foodres.2020.109348

Knobloch, K. H., Hahlbrock, K. (1975) Isoenzymes of p-coumarate: CoA ligase from cell suspension cultures of Glycine max. *European Journal of Biochemistry* , *52* (2), 311-320. https://doi.org/10.1111/j.1432-1033.1975.tb03999.x

Koukol, J., Conn, E. E. (1961). The metabolism of aromatic compounds in higher plants: IV. Purification and properties of the phenylalanine deaminase of Hordeum vulgare. *Journal of Biological Chemistry* ,*236* (10), 2692-2698. https://doi.org/10.1016/S0021-9258(19)61721-7

Lamb, C. J., Rubery, P. H. (1975). A spectrophotometric assay for trans-cinnamic acid 4-hydroxylaje activity. *Analytical Biochemistry* , 68, 554-561. https://doi.org/10.1016/0003-2697(75)90651-X

Lu, W. L., Yang, T., Song, Q. J., Fang, Z. Q., Pan, Z. Q. (2019).*Akebia trifoliata* (Thunb.) Koidz seed extract inhibits human hepatocellular carcinoma cell migration and invasion in vitro.*Journal of Ethnopharmacology* , *234* , 204–215. https://doi.org/10.1016/j.jep.2018.11.044

Martin, V. J., Pitera, D. J., Withers, S. T., Newman, J. D., Keasling, J. D. (2003) Engineering a mevalonate pathway in Escherichia coli for production of terpenoids. *Nature Biotechnology* , *21* (7), 796-802.

Ma, T., Deng, X., Chen, L., Xiang, W. (2020). The soil properties and their effects on plant diversity in different degrees of rocky desertification. *Science of The Total Environment* , 139667. https://doi.org/10.1016/j.scitotenv.2020.139667

Michihara, S., Tanaka, T., Uzawa, Y., Moriyama, T., Kawamura, Y. (2012). Puerarin exerted antiosteoporotic action independent of estrogen receptor-mediated pathway. *Journal of Nutritional Science &*

Vitaminology . *58* (3), 202. https://doi.org/10.3177/jnsv.58.202

Nakabayashi, R., Saito, K., (2015). Integrated metabolomics for abiotic stress responses in plants. *Curr. Opin. Plant Biol* , *24* , 10-16. https://doi.org/10.1016/j.pbi.2015.01.003.

Nourimand, M., Mohsenzadeh, S. (2012). Physiological responses of fennel seedling to four environmental stresses. *Iranian Journal of Science and Technology* , *1* , 37. https://doi.org/10.1007/s11069-012-0225-2

Ping, Q., Xu, S., Chen, W., He, X. Y., Huang, Y. Q., Wu, X. (2017). Effects of increased O_3 concentration on growth, subcellular structure and reactive oxygen metabolism of turf-type Festuca arundinace. *Chinese Journal of Applied Ecology* ,*28* (12), 3862-3870. https://doi.org/10.13287/j.1001-9332.201712.009

Priya, B., Sharma, A. K. (2013). Anti-cancer potential of flavonoids: recent trends and future perspectives. *Biotechnology* ,*3* (6), 439. https://doi.org/10.1007/s13205-013-0117-5

Prasain, J. K., Carlson, S. H., Wyss, J. M. (2010). Flavonids and age-related disease: Risk, benefits and critical windows.*Maturitas* , *66* , 163-171. https://doi.org/10.1016/j.maturitas.2010.01.010

Ramirez-Tortosa, C., Andersen, O. M., Cabrita, L., Gardner, P. T., Morrice, P. C., Wood, S. G., Duthie, S. J., Collins, A. R., Duthie, G. G. (2001). Anthocyanin-rich extract decreases indices of lipid peroxidation and DNA damage in vitamin E-depleted rats. Free Radical Biology Medicine, *31* (9), 1033-1037. https://doi.org/10.1016/S0891-5849(01)00618-9

Rodrigo, R., Miranda, A., Vergara, L. (2011). Modulation of endogenous antioxidant system by wine polyphenols in human disease. *Clinica Chimica Acta* , 412, 410-424. https://doi.org/10.1016/j.cca.2010.11.034

Romagnolo, D. F., Selmin, O. I. (2012). Flavonoids and cancer prevention: areview of the evidence. *Journal of Nutrition in Gerontology and Geriatrics* , *31* (3), 206. https://doi.org/10.1080/21551197.2012.702534

Sanchez, D. H., Schwabe, F., Erban, A., Udvardi, M. K., Kopka, J. (2011). Comparative metabolomics of drought acclimation in model and forage legumes. *Plant Cell & Environment* , *35* (1), 136-149. https://doi.org/ 10.1111/j.1365-3040.2011.02423.x

Sheng, M., Xiong, K., Wang, L., Li, X., Li, R., Tian, X. (2018). Response of soil physical and chemical properties to rocky desertification succession in South China Karst. *Carbonates Evaporites* , *33* , 15-28. https://doi.org/ 10.1007/s13146-016-0295-4

Singanusong, R., Nipornram, S., Tochampa, W., Rattanatraiwong, P. (2015). Low power ultrasound-assisted extraction of phenolic compounds from mandarin (*Citrus reticulata Blanco* cv.Sainampueng) and lime (*Citrus aurantifolia*) peels and the antioxidant. *Food analytical methods* , *8* (5), 1112-1123. https://doi.org/10.1007/s12161-014-9992-6

Singh, K., Kumar, S., Rani, A., Gulati, A., Ahuja, P. S. (2009). Phenylalanine ammonia-lyase (PAL) and cinnamate 4-hydroxylase (C4H) and catechins (flavan-3-ols) accumulation in tea. *Functional & Integrative Genomics* , *9* (1), 125-134. https://doi.org/10.1007/s10142-008-0092-9

Skerget, M., Kotnik, P., Hadolin, M., Rizner-Hras, A., Simonic, M., Knez, Z. (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chemistry* , *89* , 191-198. https://doi.org/10.1016/j.foodchem.2004.02.025

Stas, S. M., Rutishauser, E., Chave, J., Anten, N. P. R., Laumonier, Y. (2017). Estimating the aboveground biomass in an old secondary forest on limestone in the Moluccas, Indonesia: Comparing locally developed versus existing allometric models. *Forest Ecology & Management* ,*389* (5), 27-34. https://doi.org/10.1016/j.foreco.2016.12.010

Sun, W. T., Qin, L., Xue, H. J.,Yu, Y., Ma, Y. H., Wang, Y., Li, C. (2019). Novel trends for producing plant triterpenoids in yeast.*Critical Reviews in Biotechnology* , *39* (5), 1-15. https://doi.org/10.1080/07388551.2019.1608503

Terao, J. (2011). Dietary flavonoids as antioxidants and beyond antioxidants in target tissues. *Forum of Nutrition* ,*61* (61), 87. https://doi.org/10.1159/000212741

Theis, N., Lerdau, M. (2003). The evolution of function in plant secondary metabolites. *International Journal of Plant Science* ,*164* , 93-102. http://www.jstor.org/stable/10.1086/374190

Wang, L., Wang, P., Sheng, M., Tian, J. (2018). Ecological stoichiometry and environmental influencing factors of soil nutrients in the karst rocky desertification ecosystem, southwest China. *Global Ecology and Conservation* , *16* , e00449. https://doi.org/10.1016/j.gecco.2018.e00449

Wang, S. J., Liu, Q. M., Zhang, D. F. (2010). Karst rocky desertification in southwestern China: geomorphology, landuse, impact and rehabilitation. *Land Degradation & Development* ,*15* (2), 115-121. https://doi.org/10.1002/ldr.592

Yadav, R. K., Sangwan, R. S., Sabir, F., Srivastava, A. K., Sangwan, N. S. (2014). Effect of prolonged water stress on specialized secondary metabolites, peltate glandular trichomes, and pathway gene expression in Artemisia annuaL. *Plant Physiolgy and Biochemistry* , *74* , 70-83. https://doi.org/10.1016/j.plaphy.2013.10.023

Ying, Z., Tang, N., Huang, L., Zhao, Y., Tang, X., Wang, K. (2018). Effects of salt stress on plant growth, antioxidant capacity, glandular trichome density, and volatile exudates of schizonepeta tenuifolia Briq.*International Journal of Molecular Sciences* , *19* (1), 252. https://doi.org/10.3390/ijms19010252

Yin, J., Liang, T., Wang, S., Zhang, M., Xiao, J., Zhan, Y., Li, C. (2015). Effect of drought and nitrogen on betulin and oleanolic acid accumulation and OSC gene expression in white birch saplings.*Plant Molecular Biology Reporte* , 33, 705-715. https://doi.org/10.1007/s11105-014-0778-1

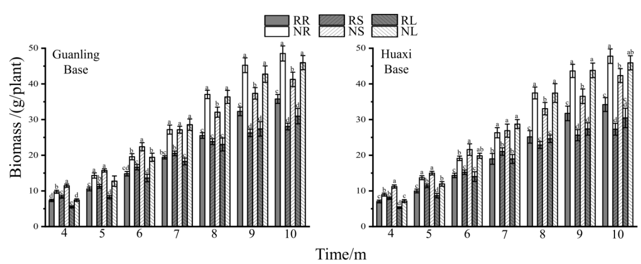
Zahir, A., Abbasi, B. H., Adil, M., Anjum, S., Zia, M., Ihsan, U. H. (2014). Synergistic effects of drought stress and photoperiods on phenology and secondary metabolism of silybum marianum. *Applied Biochemistry & Biotechnology* , *174* (2), 693-707. https://doi.org/10.1007/s12010-014-1098-5

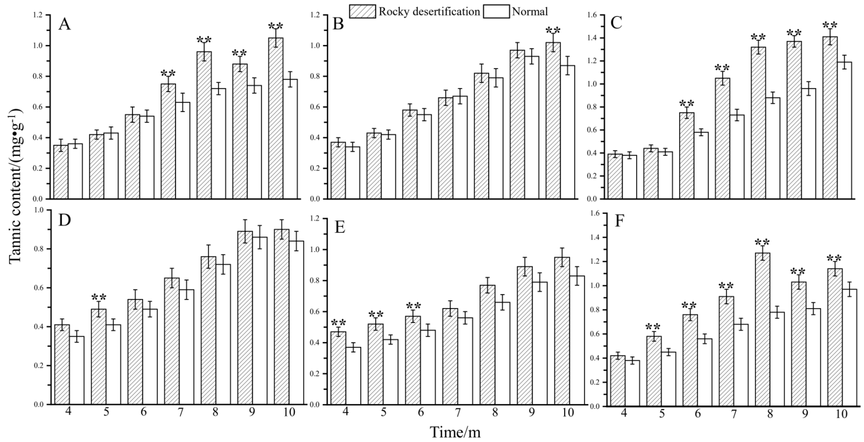
Zhang, W. J., Wang, S., Kang, C. Z., Lv, C. G., Guo, L. P. (2020). Pharmacodynamic material basis of traditional Chinese medicine based on biomacromolecules: a review. *Plant methods* , 16(1), 1-28. https://doi.org/10.1186/s13007-020-00571-y

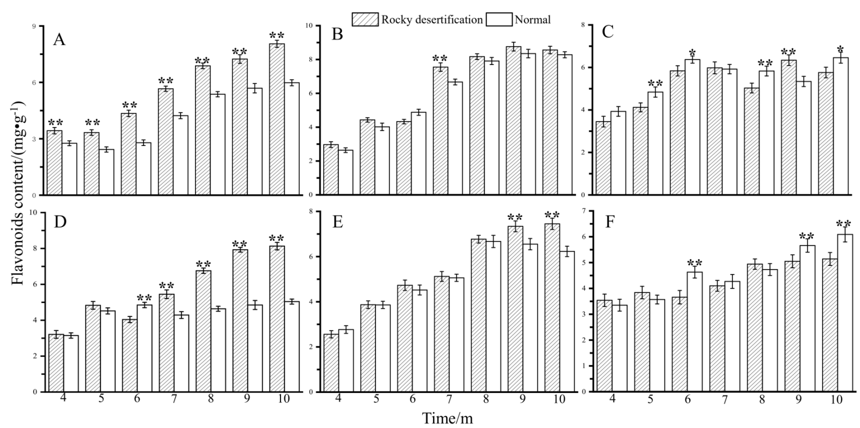
Zhao, Z. J., Song, Y. G., Liu, Y. L., Qiao, M., Zhai, X. L., Xiang, F. N. (2013). The effect of elicitors on oleanolic acid accumulation and expression of triterpenoid synthesis genes in Gentiana straminea.*Biologia Plantarum* , *57* (1), 139-143. https://doi.org/10.1007/s10535-012-0260-6

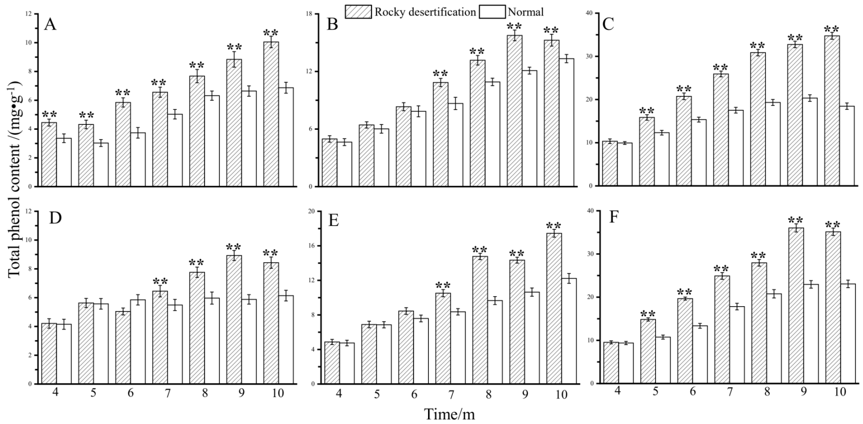
Zhu, Z. B., Liang, Z. S., Han, R. L., Xin, W. (2009) Impact of fertilization on drought response in the medicinal herb Bupleurum chinense DC.: growth and saikosaponin production. *Industrial Crops & Products* *29* (2):629. https://doi.org/10.1016/j.indcrop.2008.08.002

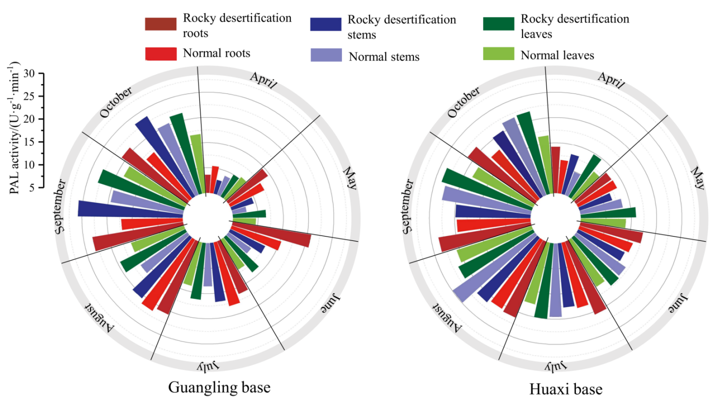

Rocky desertification
Normal

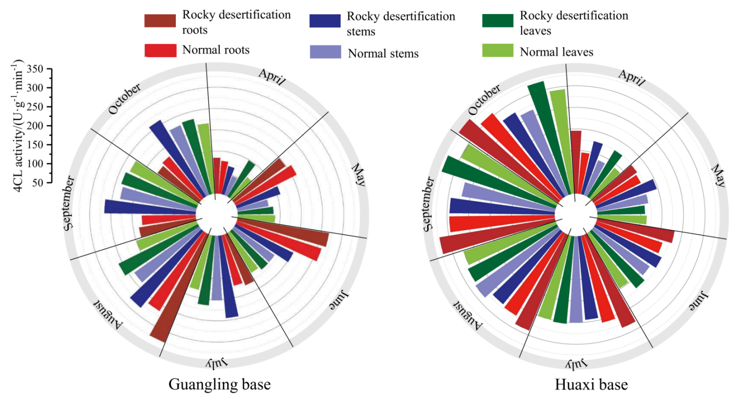

RR
NR
RS
NS
RL
NL
Guanling Base
Huaxi Base
Biomass /(g/plant)
Time/m

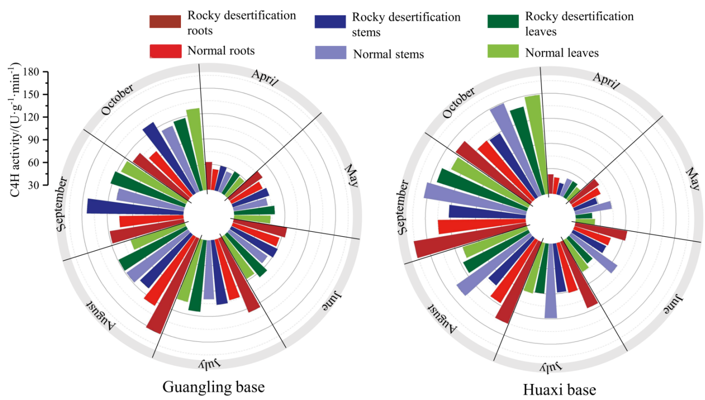

Rocky desertification
Normal
A
B
C
D
E
F
Tannic content/(mg•g-1)
Time/m

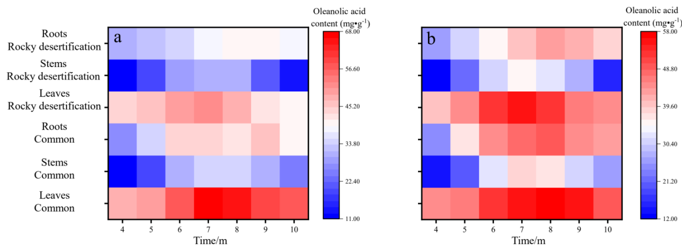

Rocky desertification
Normal
A
B
C
D
E
F
Flavonoids content/(mg•g-1)
Time/m

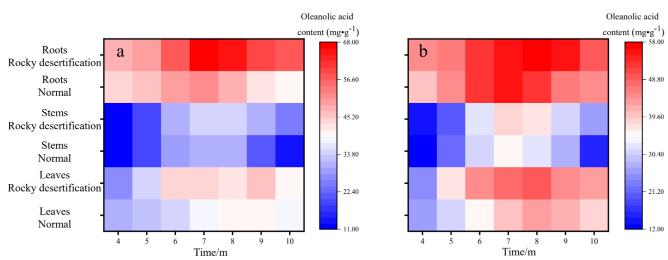

Rocky desertification
Normal
A
B
C
D
E
F
Total phenol content /(mg•g-1)
Time/m

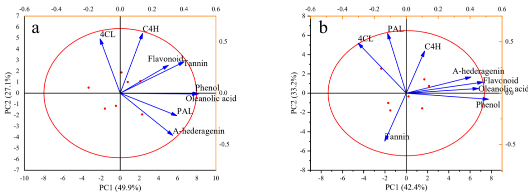

Rocky desertification roots
Normal roots
Rocky desertification stems
Normal stems
Rocky desertification leaves
Normal leaves
PAL activity/(U·g-1·min-1)
April
May
June
July
August
September
October
Guangling base
Huaxi base


Rocky desertification roots
Normal roots
Rocky desertification stems
Normal stems
Rocky desertification leaves
Normal leaves
4CL activity/(U·g⁻¹·min⁻¹)
350
300
250
200
150
100
50
April
May
June
July
August
September
October
Guangling base
Huaxi base


Rocky desertification roots
Normal roots
Rocky desertification stems
Normal stems
Rocky desertification leaves
Normal leaves
C4H activity/(U·g⁻¹·min⁻¹)
180
150
120
90
60
30
April
May
June
July
August
September
October
Guangling base
Huaxi base


a
b
Roots
Rocky desertification
Stems
Rocky desertification
Leaves
Rocky desertification
Roots
Common
Stems
Common
Leaves
Common
4
5
6
7
8
9
10
Time/m
Oleanolic acid
content (mg•g-1)
68.00
56.60
45.20
33.80
22.40
11.00
58.00
48.80
39.60
30.40
21.20
12.00


a
b
Roots
Rocky desertification
Roots
Normal
Stems
Rocky desertification
Stems
Normal
Leaves
Rocky desertification
Leaves
Normal
4
5
6
7
8
9
10
Time/m
Oleanolic acid
content (mg•g-1)
68.00
56.60
45.20
33.80
22.40
11.00
58.00
48.80
39.60
30.40
21.20
12.00


a
4CL
C4H
Flavonoid
Tannin
Phenol
Oleanolic acid
PAL
A-hederagenin
PC2 (27.1%)
PC1 (49.9%)
b
4CL
PAL
C4H
A-hederagenin
Flavonoid
Oleanolic acid
Phenol
Tannin
PC2 (33.2%)
PC1 (42.4%)