STUDY OF THE FLAVONOIDS OF ROUND-LEAVED ANEMONE (*ANEMONE OBTUSILOBA*) ON ALPINE MEADOW AND THE MECHANISM OF ITS ADAPTATION TO THE ENVIRONMENT BASED ON METABOLIC PATHWAYS

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Abstract. The article aimed to investigate the main pigment composition of the A. obtusiloba, focusing on the changes in absorbance spectra in relation to environmental factors of the alpine meadows of Maqu County, China, using UV-vis spectrophotometry, High Performance Liquid Chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS). The results showed that there were two kinds of pigments in A. obtusiloba, carotenoids, and flavonoids. The absorbance spectra regularly changed with the depth of flower colour. Based on HPLC and LC-MS analyses, seven kinds of flavonoids were inferred, including luteolin-3-7-O-glucoside, quercetin-3-O-rutoside, quercetin-3-O-galactoside, quercetin-3-O-glucoside, quercetin-3-O-rhamnoside, kaempferol-3-O-sophoroside, and myricetin-3-O-rhamnose, and the derivative of quercetin was identified as the main component in A. obtusiloba. Additionally, according to the analysis of the flavonoid metabolism pathway, it could be determined that flavonoid 3'-hydroxylase (F3′H) was the key enzyme in increasing quercetin content. This paper speculates that A. obtusiloba increases the content of quercetin by regulating flavonoid F3′H, so that it can deepen the flower colour to adapt to the environment.
Keywords: *Qinghai Tibet Plateau, flower colour, adaptability, HPLC, LC-MS*

Introduction

Anemone obtusiloba, commonly known as Padar, Rattanjog, or Kawashud, is a flowering plant of the family Ranunculaceae and a densely tufted perennial herb (Thu et al., 2018). It is native to the Himalayan and mountainous regions of Myanmar occurring in the Alpine Himalaya from Kashmir to Sikkim at 2100-4200 m altitude and in the Nilgiri hills at an altitude above 1800 m (Tan, G. L. et al., 2002). It bears buttercup hermaphrodite plural and the stem is short and tufted with a single terminal flower, generally producing one to three individual stems with yellow, light-yellow, or white colour. At present, research mainly focuses on the medicinal value of *A. obtusiloba*, whose ethanolic extract contains saponins (Masood et al., 1979). It also contains protoanemonin, which is an irritating acrid oil and is an enzymatic breakdown product of the glycoside ranunculin (Savita, R. et al., 2011). *A. obtusiloba* is used as a purgative and in the treatment of rheumatic joints, jaundice, spleen disorders, and anxiety neurosis. Besides, it is also used as an antidote to snakebite, while its seed oil is used to cure arthritis (Gupta et al., 2005). Studies have shown that the petal colour of *A. obtusiloba* is related to reproductive characteristics and reproductive distribution. During the flowering period, with the deepening of flower colour, the reproductive characteristics and reproductive distribution of *A. obtusiloba* have been adjusted to

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improve its own nutritional growth and male function. However, in the fruit stage, with the deepening of flower colour, the related phenotypic characteristics and resource allocation of *A. obtusiloba* made adjustments to improve reproductive function and female function. From flowering to fruiting, dark flowers are more competitive and more adaptable to the environment (Li, 2012). However, there are few studies on the mechanism of petal colour adaptation to the environment.

In the Tibetan Plateau, *A. obtusiloba* has its own adaptive mechanism to the local environment. During the past years, affected by global warming, there have been significant environmental changes in the plateau area—The temperature rises and the precipitation increases (Zheng, 2015). After years of observation by our research team, we find an obvious decrease of white flowers and an increase of yellow and light-yellow flowers of *A. obtusiloba*. It is speculated that this phenomenon may be related to its adaptability to the environment and biological evolution. Flower colour is one of the most important traits of plants and is attributed to various pigments that are composed of three major classes of compounds including flavonoids, carotenoids, and anthocyanin (Grotewold, 2006). Among these compounds, flavonoids are responsible for the yellow colour. At the same time, the number and position of phenolic groups in the chemical structure have a certain influence on the flower colour. The colour of the flower will change from yellow to orange and to red by the hydroxylation at C_3 (Yu et al., 2002). However, increasing the hydroxylation of the B ring will change the colour to blue, and the methylation of the B ring will make it red (Harborne 1993). Carotenoids are responsible for colours ranging from yellow to red (Tanaka et al., 2008). Cyanidin, which controls red, blue, purple, and other colours, can be divided into three types: pelargonidin, delphinidin, and anthocyanins (Hall, 1897).

Flavonoids represent a large subgroup of plant secondary metabolites including flavones, isoflavones, anthocyanins, flavanols, flavonols, and derivatives (Gao et al., 2019) with various biological activities such as anti-inflammation, anti-cancer, etc. (Arora et al., 2015). Over the past decade, studies have revealed the core biosynthesis pathways of flavonoids in various plants such as *Arabidopsis* (Tohge et al., 2017), *Lactuca sativa* (Zhang et al., 2017), *Camellia sinensis* (Liu et al., 2018), *Salvia miltiorrhiza* (Deng et al., 2018), *Oroxylum indicum* (Deshmukh et al., 2018), *Chrysanthemum morifolium* (Yue et al., 2018), *Chrysanthemum indicum* (Jiang et al., 2019), and *Scutellaria baicalensis* (Zhao et al., 2016). Flavones are synthesized via the flavonoid pathway, which is part of phenylpropanoid metabolism (Ferreyra et al., 2012). Naringenin is a central intermediate in the biosynthesis of normal 4'-hydroxyflavones (Martens and Mithöfer, 2005). The enzymatic properties related to flavonoid accumulation in vitro have been confirmed (Zhu, et al., 2020). Flavonoid biosynthetic genes can be classified into two categories: (i) early biosynthetic genes, such as genes encoding phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), and chalcone isomerase (CHI); (ii) late biosynthetic genes, such as those encoding flavanone 3-hydroxylase (F3′H), dihydroflavonol reductase (DFR), and anthocyanidin synthase (ANS) (Patra et al., 2013). CHS, the first key enzyme in the anthocyanin synthesis pathway, is affected by multiple factors, such as environmental stress, light, inducer, etc. (Takeuchi et al., 1995). There are six genes involved in anthocyanin synthesis in different tissues of grape, CHS, CHI, F3′H, DFR, leucoanthocyanidin reductase (LAR), and flavonoids glycosyltransferase (UFGT), while the accumulation of anthocyanidin is absent in white grape. Therefore, the accumulation of anthocyanidin is not only related to tissue, but also related to the alternative gene expression. The study aimed to

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qualitatively and quantitatively identify the chemical composition using different analytical methods including ultraviolet-visible (UV-vis) spectroscopy, HPLC, and LC-MS, and to further analyse the key enzyme synthesizing this pigment.

Materials and methods

Materials

The experimental materials light-yellow and yellow *A. obtusiloba* fresh petals about 10 gwere collected in early July at the peak of flowering from Maqu County, Gansu Province of China in 2018 (101.52E, 33.40N, altitude 3500 m). The flowers were rinsed, and the stamens were removed. After fixating at 80 °C for 30 min, they were dried at 60 °C and crushed into powder with a pulveriser (Tianjin Taisite Instrument Co. Ltd., Tianjin, China). Then the powder sample was stored in a sealed bag and kept in a dark place before using.

Spectral analysis by UV-vis spectroscopy

Spectral analysis of flavonoids

The flavonoid extraction was identified according to the method of Deepika et al. (2018) with some modifications. To extract flavonoids, 0.1 g of flower powder was treated with methanol/formic acid (98:2, v/v) for 24 h in a Soxhlet apparatus under dark conditions. The filter residue was repeatedly extracted by the solvent. After the filtrate was combined and diluted to 10 mL, the spectral scanning was carried out by a UV spectrophotometer (UV-3000, Shanghai JiaPeng technology Co., Ltd., Shanghai, China) with a range of 200–700 nm. The measurements was repeated three times for each sample.

Content calculation (Cai et al., 2010):

$$\text{Total flavonoids content (mg/g)} = A_{max} \times 320 \times V_1/(V_2 \times 1000 \times m) \quad \text{(Eq.1)}$$

A_{max}: Maximum absorbance; V_1: Total sample volume; V_2: Sample volume; m: Weight of flower powder.

Spectral analysis of carotenoids

The carotenoids of *A. obtusiloba* were extracted based on the method reported by Cai et al. (2010) with necessary modifications. Briefly, each sample of flower powder (0.1 g) was mixed with acetone/ethanol (1:1, v/v), and the mixture was repeatedly extracted 24 h in a Soxhlet apparatus under dark conditions. After the filtrate was combined and diluted to 10 mL, the spectral scanning was carried out with a range of 200–700 nm. The measurements was repeated three times for each sample.

Content calculation (Cai et al., 2010):

$$\text{Total carotenoid content (mg/g)} = 4 \times A_{max} + 3.28 \times A_{665} - 11.64 \times A_{649} \quad \text{(Eq.2)}$$

Qualitative and quantitative analysis of petal pigments

Flavonoids in petals of *A. obtusiloba* were extracted with methanol/formic acid (98:2, v/v) in darkness at 4 °C for 24 h. Then the mixture was centrifuged at 12,000 rpm

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for 5 min at 4 °C to remove the precipitants. The supernatant was collected and filtered through a 0.45 μm micropore membrane, and the as-prepared sample was stored for qualitative and quantitative analysis (Wang et al., 2018).

The quantitative analysis of flavonoids was performed on HPLC-PAD (Waters 2685, USA) and HPLC-MS (Agilent 6460, USA). A C18 column of Inertsil Zorbax SB (4.6 mm × 250 mm, 5 μm, Shimadzu GL, Shanghai, China) was used to separate the individual flavonoids with mobile phase A (double distilled water containing 0.1% phosphoric acid) and mobile phase B (acetonitrile). The elution gradient was set as follows: 0 min, 1% B; 15 min, 8% B; 35 min, 22% B; 45 min, 42% B; 55 min, 75% B; 65 min, 100% B. Then, 2 μL of the sample was injected for HPLC analysis. The chromatogram showed horizontal coordinate and vertical coordinate, corresponding to the retention time (min) and response value (m AU), respectively. The flow rate was 0.2 mL/min, and the column temperature was 30 ℃. Since flavonoids are expected to be observed at a 300 nm wavelength by UV detector, the spectra were scanned in the range of 190–700 nm.

The MS conditions were set as follows: High purity nitrogen (99.999%) was used as a nebulizing (60 psi) and drying gas at a flow rate of 9.0 L/min. The vaporizer temperature was set at 350 °C. Other parameters were rationally set including an Ion spray voltage of 70–205 V and a scanning range of 100–1000 m/z.

Statistical analysis

The data were processed and presented as mean ± standard deviation (SD). Measurements were performed in triplicates and the statistical analysis was executed by one-way analysis of variance (ANOVA). Significant differences between groups were discerned at $p \leq 0.05$. Statistic software Graph Pad Prism 6.0 (Graph Pad Software Inc., San Diego, USA) was used for all the graphical and statistical evaluations.

Results

Spectral analysisusing UV-vis spectrometer

Spectral analysis of flavonoids

As shown in the UV-vis spectrum of the flavonoid fraction of *A. obtusiloba* (*Fig. 1A*), the maximum absorption peaks were observed at 268 nm (band II) and 330 nm (band I), respectively. There are two typical absorbance bands of flavonoids, band B (310–350 nm) for flavones and band A (250–290 nm) for flavonols (Arora and Itankar, 2018). Generally speaking, band II absorption could be caused by ring A-cyclobenzoic acid system, while band I was caused by ring B-cyclocinnamic acid system (Hu et al., 2008). As shown in *Figure 1A,* the absorption peak at 268 nm indicated the presence of the A-cyclobenzoyl system, while the absorption peak at 300 nm suggested that there was a B-cyclocinnamoyl system in the flavonoid fraction of the flower extract. Based on the observation that band II was the main peak while band I was weak, we speculated that the cinnamoyl system might be destroyed (Liu et al., 2007). This result showed that the extract from *A. obtusiloba* had the basic structure of flavonoids C6-C3-C6.

Spectral analysis of carotenoids

According to the UV-vis spectrum of the carotenoids sample from *A. obtusiloba* (*Fig. 1B*), the maximum absorption peak was at 268 nm, which was consistent with the

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characteristic absorption peak of the carotenoid. With the deeper colour, the absorption peak of the carotenoid sample gradually increased. The total content changed regularly with the shading of the colour (*Fig. 1B*). The maximum light absorption value of the pale-yellow sample was 18.36%less than that of the yellow sample (4.112 and 3.357 of the optical density (OD), respectively).

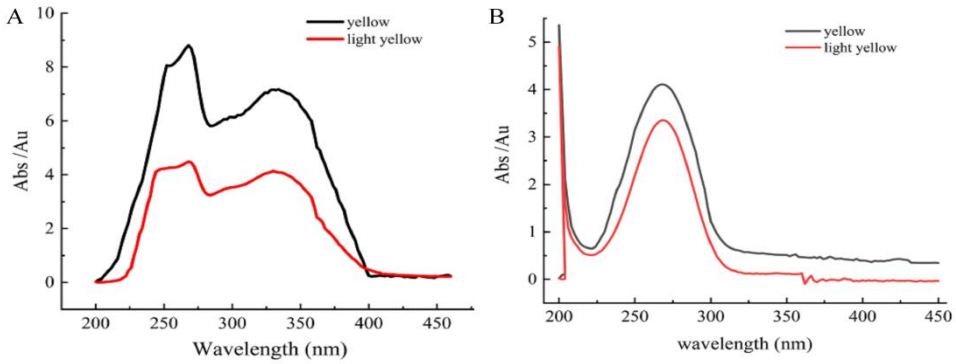

Figure 1. *The UV-visible spectra of petal pigments in Anemone obtusiloba, Flavonoid (A), Carotenoid (B)*

According to the Total flavonoid content and total carotenoid content ANOVA tables (*Table 1*), significant differences between groups were discerned at $p \leq 0.05$. However, differences of total flavonoid content between groups was more significant, and the content of total flavonoids is higher than that of total carotenoids. Flavonoids were identified as the main components of flower colour, and the specific components were analysed and determined.

Table 1. *Total flavonoid content and total carotenoid content ANOVA tables*

Total flavonoid content ANOVA table					
Source	**SS**	**df**	**MS**	**F**	**P-value**
Group	127.1716882	1	127.1716882	11137.17493	4.83E-08
Error	0.045674667	4	0.011418667		
Total	127.2173628	5			
Total carotenoid content ANOVA table					
Source	**SS**	**df**	**MS**	**F**	**P-value**
Group	0.02535	1	0.02535	13.82727273	0.020501259
Error	0.007333333	4	0.001833333		
Total	0.032683333	5			

Qualitative and quantitative analysis of petal pigments

Qualitative analysis of petal pigments from Maqu

Based on the HPLC-PDA analysis of the flavonoid extract of yellow flowers of *A. obtusiloba* (*Fig. 2*), 7 kinds of compounds were detected at 300 nm with a retention time ranging from 30 to 50 min. Meanwhile, there were 8 kinds of compounds detected in light-yellow of flavonoid fraction of *A. obtusiloba*. Zhao et al. (2005) reported that

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the different colour of flowers were not determined by the structure of the compound, but by the change in the content of some pigment molecules. Studies have also found that the red colour of cotton is mainly caused by flavonoid accumulation. Except for the flavonoid-related enzyme genes in red cotton such as CHS and F3'H, other genes that regulate flavonoid biosynthesis have higher transcription levels than those in white cotton (Long et al., 2019). In the HPLC chromatogram (*Fig. 2A*), peaks 3 and 8 were close to each other, suggesting that they might be isomers. Therefore, the flavonoid components of the two coloured flowers are supposed to be the same. Mostly, the peak intensity (response value) of yellow flowers (B) was higher than that of the light yellow (B) ones. Therefore, it could be suggested that the colour intensity of *A. obtusiloba* was caused by the content of pigment molecules. The components (*Fig. 2A*) at peaks 3 (1.775 mg/g), 6 (1.56 mg/g), and 7 (2.687 mg/g) were more dominant in light-yellow flowers, whereas peaks 3 (4.124 mg/g), 4 (2.754 mg/g), and 7 (2.651 mg/g) were more dominant in yellow flowers (*Fig. 2B*).

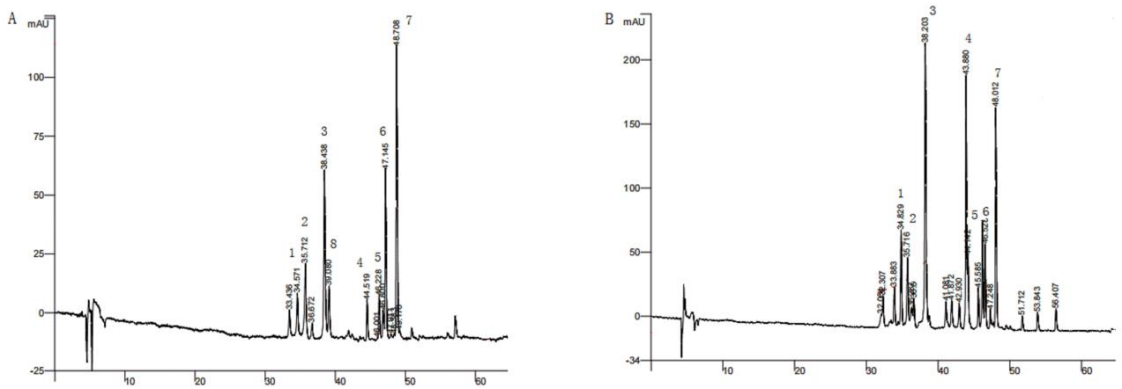

Figure 2. *HPLC chromatogram of flavonids from Anemone obtusiloba, Light yellow (A), Yellow (B)*

Quantitative analysis of petal pigments from Maqu

To further determine the specific type of pigment molecules, HPLC-MS analysis was carried out on yellow flowers with high total contents. The MS peaks of four standards (Quercetin, Kaempherol, Luteolin, and Rutin) were shown in *Figure 3*. According to the total ion chromatogram and positive ion mode of flavonoid from the yellow flower (*Figs. 4* and *5*), the analysis results in *Table 2* were obtained.

Table 2. *HPLC-MS analysis results of flavonid from Anemone obtusiloba yellow flower*

Peak No.	Retention time (min)	Molecular ion (m/z)	Fragment ions (m/z)	Identification
7	31.88	645.18($[M + H]^+$)	465, 287, 248	luteolin-3-7-O-glucoside
6	33.24	625.15($[M]^+$)	465, 303, 274	quercetin-3-O-rutoside
5	36.19	611.2($[M + H]^+$)	445, 287, 263	kaempferol-3-O-sophoroside
4	42.58	483.1($[M + 3H]^+$)	318, 146	myricetin-3-O-rhamnose
3	45.31	465.1($[M + H]^+$)	303, 287, 274	quercetin-3-O-galactoside
2	45.74	465.1($[M + H]^+$)	303, 287, 274	quercetin-3-O-glucoside
1	47.40	449.2($[M + H]^+$)	303, 274, 179	quercetin-3-O-rhamnoside

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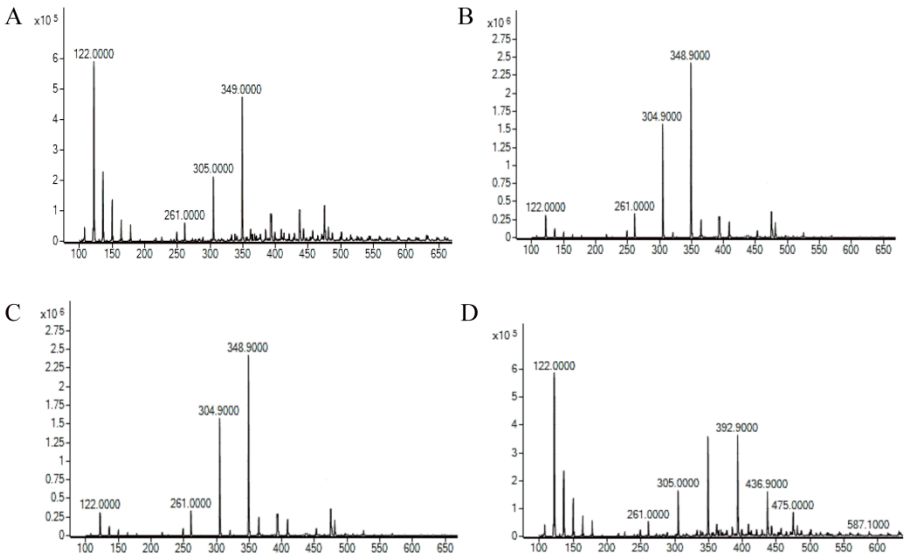

Figure 3. *Total ion chromatogram of standards Lquercetin (A), Kaempherol (B), Luteolin (C), and quercetin-3-O-rutinoside (D)*

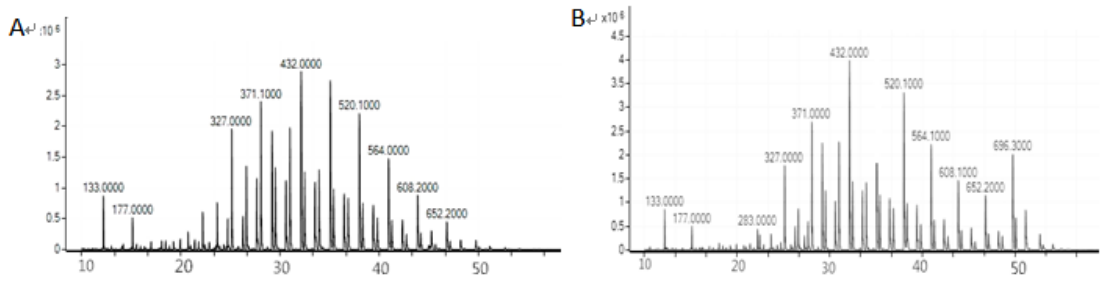

Figure 4. *Total ion chromatogram of flavonoid from Anemone obtusiloba Light yellow (A), yellow (B)*

The molecular ion peak was observed at m/z 645.18 [M + H]+ of peak 7. The fragment at m/z 465 represented the loss of one glucose molecule. Then one glucose molecule was removed to obtain a fragment m/z 287, which was luteolin aglycone. It was speculated to be luteolin-3-7-O-glucoside since it was consistent with the result reported by Susan (1985).

The fragment at m/z 303 corresponded to quercetin aglytin and its cleaved m/z 274 fragments were found the MS of peaks 6, 3, 2, and 1, which were presumed to be derivatives of quercetin. The [M + H]+ of peak 6 was m/z 625.15, which contained fragment ions m/z 465 and 303, indicating that two molecules of six-carbon sugar have been removed. As a result, it could be quercetin-3-O-rutoside (Susan et al., 1985). Besides, a molecular ion peak was observed at m/z 465.1 [M + H]+ of peaks 3 and 2, and the removal of one six-carbon sugar molecule obtained m/z 303. This result could indicate that peak 3 was quercetin-3-O-galactoside (Harborne 1986) and peak 2 was quercetin-3-O-glucoside (Yu et al., 2007). Fragments at m/z 303 showed that the removal of rhamnose and fragments at m/z 179 could be the loss of one water molecule representing the [M + H]+ peak 1. The result indicated that it might be quercetin-3-O-

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rhamnoside (Li et al., 2008). The fragment ion m/z 287 matched the molecular weight of kaempferol. Therefore, it could be speculated that kaempferol was contained in its structure. The fragment m/z 445 indicated the loss of two molecules of glucose, which was consistent with the peak of kaempferol-3-O-sophoroside reported previously (Li et al., 2008). The molecular ion peak 4 was observed at m/z 483.1 [M + H]+. Based on the characteristic ion of myricetin at m/z 318, and the glycosyl ion at m/z 146, the data were found to be consistent with the result of myricetin-3-O-rhamnose (Chosson et al., 1998). The content of the flavonoids was measured (*Table 3*). The content of quercetin-3-O-galactoside (4.124 mg/g) was found to be relatively higher than the other components. As a result, quercetin derivatives were dominant in flavonoids of *A. obtusiloba* flowers.

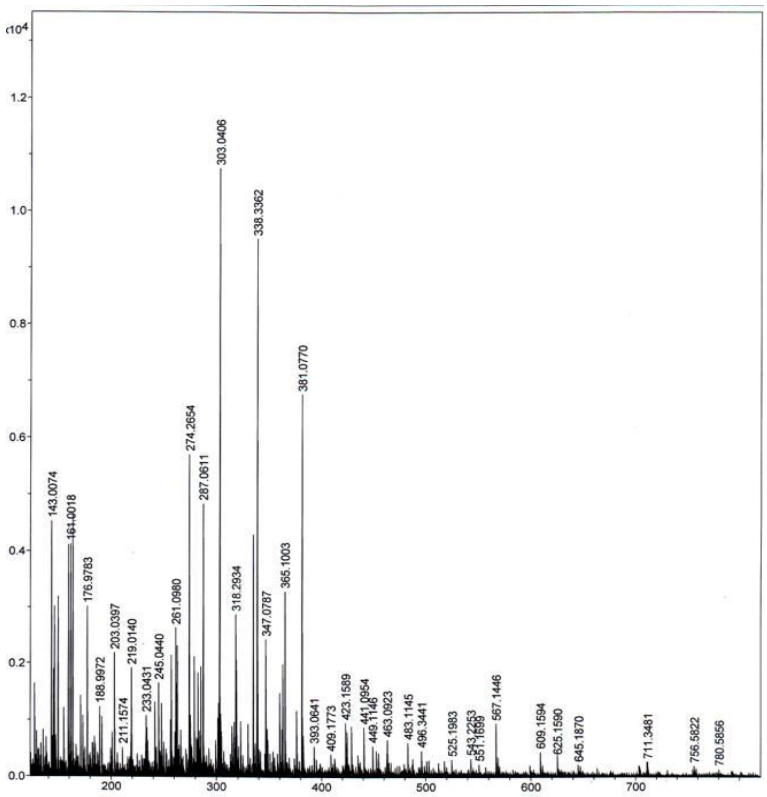

Figure 5. *Cationic mass spectrograms of flavonoid from yellow Anemone obtusiloba*

Table 3. *The content of main components of anthocyanin in Maqu Anemone obtusiloba (mg/g)*

Peak No.	Component	Yellow flowers	Light yellow flower
7	luteolin-3-7-O-glucoside	2.754	2.687
6	quercetin-3-O-rutoside	0.982	1.56
5	kaempferol-3-O-sophoroside	1.2	0.394
4	myricetin-3-O-rhamnose	2.651	0. 34
3	quercetin-3-O-galactoside	4.124	1.775
2	quercetin-3-O-glucoside	0.867	0.815
1	quercetin-3-O-rhamnoside	1.05	0.461

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The keyenzyme analysis in the regulation of anthocyanin in A. obtusiloba

Although the anthocyanins of *A. obtusiloba* are mainly composed of four sugar derivatives of luteolin, kaempferol, myricetin and quercetin. In these major flavonoids, quercetin with the highest relative abundance may be one of the main active ingredients. According to the metabolic pathway of flavonoids (*Fig. 6*) (He et al., 2020), quercetin could only be synthesized from dihydroquercetin and catalysed by flavonol synthetase (FLS). There were two pathways of dihydroquercetin synthesis: one was eriodictyol catalysed by flavanone-3-hydroxylase (F3H), and the other was dihydrokaempferol catalysed by flavonoid-3'-hydroxylase (F3'H). However, eriodictyol could be also catalysed by flavone synthase II (FNSII) to form luteolin. In the study, the derivatives of luteolin (2.754 mg/g) were observed. Therefore, luteolin pathway was not prohibited, and the activity of FNSII might be reduced or the gene expression of FNSII was down regulated to reduce the decomposition of luteolin. F3'H catalysed the synthesis of dihydrokaempferol and dihydroquercetin from senkyolin and naringenin, respectively. According to the reactions in *Figure 6*, F3'H showed a higher affinity with naringenin. So it was speculated that the affinity of FLS with dihydroquercetin was enhanced in the pathway of the synthesis of luteolin, which competed with dihydrokaempferol and dihydromyricetin to increase the content of quercetin. According to the content of flavonoids in *A. obtusiloba*, it was speculated that the dihydrokaempferol pathway improved the translation of F3'H to increase the content of dihydroquercetin. And the affinity between FLS and dihydroquercetin was enhanced, so quercetin content could be eventually increased.

Therefore, enhancing the translation of F3'H gene could not only increase the content of dihydroquercetin, but also increase the content of eriodictyol, which could indirectly increase the content of dihydroquercetin and finally achieve the purpose of increasing the content of quercetin.

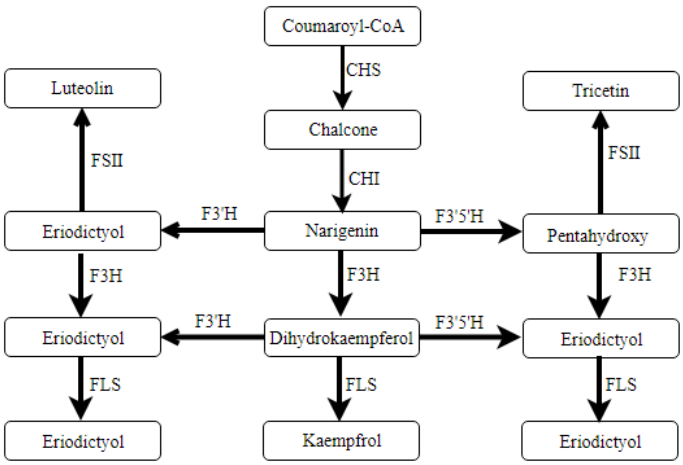

Figure 6. *The metabolic pathway of flavonoid*

Discussion

In recent years, with changes in the environment in the alpine region—The temperature rises and the precipitation increases (Zheng, 2015), the pigment of the unique plant *A. obtusiloba* has also been changed. The pigment of *A. obtusiloba* has

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undergone significant changes to adapt to the environment. In the Hezuo plot with lower altitudes (altitude 3000 m), the pigment of the *A. obtusiloba* is obviously darkened, white flowers are not easily observed. Compared with low altitude Hezuo plot, the higher the altitude, the deeper the colour, in the Maqu plot with higher altitudes, and even no white flowers are observed (*Fig. 7*) (Lan, 2016). The same experiment was carried out in the HeZuo sample plot, and the results were consistent with this study. Seven kinds of components in flavonoids were speculated by HPLC-MS, increases the content of quercetin by regulating the expression or activity of F3'H to deepen the flower colour so that it could adapt to the environment (Lv et al., 2019). The side shows that, the evolutionary direction and adaptive mechanism of *A. obtusiloba* at different altitudes are the same.

Studies have shown that the petal colour of *A. obtusiloba* is related to reproductive characteristics and reproductive distribution, moreover, with the increase of reproductive pressure (altitude), yellow flowers are more competitive than other colours (Li, 2012). Therefore, the number of yellow flowers increased with the increase of altitude, this is consistent with our observations. There is also a certain relationship between petal colour and genetic diversity. The research shows that there are more genetic diversity polymorphic loci in anemone with deeper flower colour in different regions (Liu, 2017). Moreover, the number of seeds per fruit and seed setting rate of yellow plants in the same population were significantly higher than those of white plants (Hu, 2013). It can be inferred that the yellow flower of *A. obtusiloba* is the direction of its adaptive evolution.

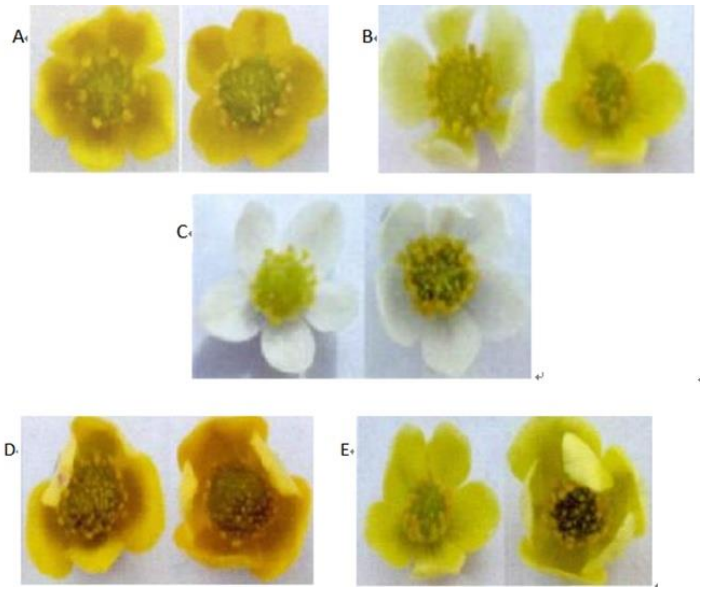

Figure 7. *Three Pigment of Anemone obtusiloba. (A) Hezuo Yellow, (B) Hezuo Pale Yellow, (C) Hezuo White, (D) Maqu Yellow and (E) Maqu Pale Yellow*

Conclusion

In this study, the main components and their key enzymes in different colourful flowers of *A. obtusiloba* were analysed by UV-vis spectroscopy, HPLC-PAD, and HPLC-MS. Seven kinds of components in flavonoids were speculated by HPLC-MS, including luteolin-3-7-O-glucoside, quercetin-3-O-rutoside, quercetin-3-O-galactoside,

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quercetin-3-O-glucoside, quercetin-3-O-rhamnoside, kaempferol-3-O-sophoroside, and myricetin-3-O-rhamnose. According to the results of qualitative and quantitative analysis, quercetin showed the largest proportion of sugar derivatives and the most obvious change of flower colour, it is the main chromogenic pigment that causes the difference in flower colour of *A. obtusiloba*. According to the flavonoid metabolic pathways, there are three main ways to increase the content of quercetin: (1) reducing the activity of FNSII or attenuating the translation of FNSII gene to reduce the path of eriodictyol; (2) enhancing the affinity of FLS binding with dihydroquercetin to make dihydroquercetin superior in substrate competition; (3) enhancing the translation of F3'H genes, such as increasing the transcription factor in the promoter region to increase the content or activity of F3'H to increase the contents of dihydroquercetin and saugenin. This paper speculates that *A. obtusiloba* increases the content of quercetin by regulating the expression or activity of F3'H to deepen the flower colour so that it could adapt to the environment.

Through this study, the evolution of *A. obtusiloba* response to the environment was analysed. This conjecture is also applicable to the adaptation to altitude changes. It provides a theoretical basis for the study of the evolution mechanism of plateau plants responding to the environment and provides a reference for the relationship between plateau environmental change and vegetation, it also provides a research direction for studying the adaptive evolution of plateau plants. In the future, the adaptability of *A. obtusiloba* to the environment can be studied from the perspective of molecular biology, especially F3'H related genes, and further study its evolutionary direction, genetic diversity and the impact of environment on its evolution.

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