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EFFECTS OF ECOLOGICAL FACTORS ON CONTENT OF FLAVONOIDS IN *ROSA STERILIS* FROM DIFFERENT KARST AREAS OF GUIZHOU, SW CHINA

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Abstract

The samples collected from 11 areas of Guizhou Province, China, were investigated to illustrate the effects of soil indicators and environmental factors on flavonoids in *R. sterilis*. A facile method for the simultaneous determination of the main flavonoids in *R. sterilis* fruits was established with high performance liquid chromatography. The major flavonoids, accounting for 1.13-5.98% of the total flavonoids, consist of rutin (1.05-5.58%), quercetin (0.02-0.09%), and kaempferol (0.06-0.31%). Moreover, four local environmental factors and eleven soil indicators were determined from each planting area. The results of variance analysis showed significant differences in soil properties from different planting areas. Multivariate analysis (redundancy analysis and partial correlation analysis) indicated that sunshine intensity and annual precipitation were the major environmental factors for the content of flavonoids in the fruits, while soil organic matter (SOM), pH, available nitrogen (AN), total phosphorus (TP) and available potassium (AK) are basic and essential nutrients for plants growth that influence the content of flavonoids in *R. sterilis*. The results revealed that sunlight intensity was the main factor for the content of flavonoids in the fruits by affecting the synthesis rate. The soil indicators, such as SOM, pH, AN, TP and AK, should be adequate and balanced, which supply essential nutrients to contribute to maintaining health and the content of active substances in *R. sterilis*.

Key words: *Rosa sterilis*; The content of flavonoids; Environmental factors; Soil characteristics.

Introduction

Rosa sterilis S. D. Shi is a deciduous shrub with perennial rootstock, which is a shallow rooted tree of the Rose family. It has been widely cultivated in Guizhou province. This species of Rose is an attractive plant in orchards and gardens and also as hedge plant. *Rosa. Sterilis* is a climbing and branching tree, which grow up to a height of 10 meters and a crown of 4-5 meters in the natural environment. It is a wild fruit tree, which grows at the altitude of 500 to 2500 meters above the sea level on sunny slopes, in valleys, along roads and with bushes. It is apparent that *R. sterilis* can be readily rooted in any buried root material through sexual propagation. Soil and weather conditions are also ideal for the growth of *R. sterilis* in Guizhou province. And it is reproduced through stem cutting propagation or tissue culture. It was at first transplanted to other regions by cutting in the 1980s. Due to its fast-growth and high economic values, it is always planted for soil improvement, soil and water conservation and rocky desertification control. The main planting locations are in Anshun City, Guiyang City, southwest and south of Guizhou province.

During 1990s, *R. sterilis* as a new species was discovered by S.D. Shi, a researcher focused on the collection of germplasm resources, molecular identification, natural products and ecological management (Liang *et al*., 1989; Wen *et al*., 2004; Yang *et al*., 2014; Li *et al*., 2016). According to the flower morphology, genetic system,*R. sterilis*is closely related to *R. roxbunghii* Tratt. In recent years, the biological active substances are found in the fruits, such as flavonoids and superoxide dismutase (SOD). The effects of antioxidant, anti-aging and others can be found in the flavonoids and SOD. Its fruit edible and is rich in vitamin C, SOD, flavonoids and other antioxidant bioactive substances; it can also be used as the medicine. Its lateral root system was well-developed, which is one of the best suitable tree species for rocky desertification control. It has been widely used in the restoration of local rocky desertification. The China State Forestry Administration declared *R. sterilis* as new plant variety in 2015. As a medicinal plant in the southwest of china, the values of ecological, social and economic need to be investigated. This development trend promotes *R. sterilis* from the scientific research field to the production practice. In the recent years, it has been used in the production of pharmaceuticals, cosmetics, health care products.

Flavonoids are commonly found in fruits, vegetables, which play an important role in medical care (Toledo *et al*., 2013). Flavonoids have a curative effect on angiocardiopathy and cancers and can be used for the inhibition of heavy metal toxicity (Li *et al*., 2011; Shagirtha & Pari, 2011; Toledo *et al*., 2013; Jagetia *et al*., 2014). The content of total flavonoids in the *R. sterilis* fruit is at a high level, which is related to local climate and management, especially environmental and soil factors (Passioura, 2002; Li *et al*., 2006).

Guizhou province is located in the southwest China where the carbonate rocks and Karst landforms are widely distributed. As a relatively distinctive region in the world, the complicated geographic feature forms a various climate and diversity of soil properties (e.g. physical and chemical properties). For these reasons, the variation may be found in fruit quality and content of effective components. To our knowledge, only several studies have been conducted on the biological morphology and genetic characteristics of *R. sterilis* where as reports are available on the flavonoids and the local ecological systems. This is the first? Study investigating the type of flavonoids, and the relationship between flavonoids and ecological factors of *R. sterilis*.

In this study, it is aimed to determine the relationship between soil environmental factors and flavonoid compounds and the content of flavonoids in *R. sterilis* fruits in SW China. This study is important for quality evaluation and management of *R. sterilis* in different planting areas.

Materials and Methods

The *R. sterilis* fruits and soil samples were collected from 11 towns in the Karst mountain areas from Guizhou province, SW China from mid-October 2015.The geographical coordinates are 25º 21´-26° 57´N latitudes, 105º 06´-106° 59´E longitudes, 898-1541m above sea level (Table 1). The study areas are in a subtropical climate, and the soil zonality is yellow. The planting areas of *R. sterilis* are Xiaba, Hefeng, Huilong, Yuzhang, Shichang, Ninggu, Jichang, Longgong, Qiyanqiao, Shuangpu and Xiayun, (these planting areas are put in numerical order: 1-XB, 2-HF, 3-HL, 4-YZ, 5-SC, 6-NG, 7-JC, 8-LG, 9-QYQ, 10-SP, 11-XY) (Fig. 1). Age of each *R. sterilis* planting area is 5-6 years old. Management activities are absent in these study areas except for weeding. Four environmental factors (altitude, mean annual air temperature, annual precipitation and sunshine intensity) and eleven soil indicators (total phosphorus (P), potassium (K), nitrogen (N), pH and soil organic matter (SOM), available phosphorus (AP), available potassium (AK) and available nitrogen (AN)) were selected as main impact indicators for the content of total flavonoids and three major of flavonoids.

Research approach, soil samples and analysis. Soil samples were collected at a depth of 0–20cm (top layer) of each planting area in October 2012 (just after the fruits of *R. sterilis* were ripe). Five plots were selected in each planting area, and 1 kg mixed soil sample was collected using five-point sampling method in each plot. Then, soil samples were immediately transported to the laboratory, and air-dried at room temperature. Visible plant residues and stones were removed and then soil particles were sieved (Gartzia-Bengoetxea *et al*., 2009; Guo *et al*., 2015). All soil samples were screened through 1 mm and 0.15 mm sieve for the determination of total P, K, N, pH, SOM and AN, AP, AK, respectively. The analytical protocols used are listed in Table 2.

Table 1. Background information of study areas in Guizhou province, SW China.

Study area	Coordinate	Altitude/ m	Stratum	Lithology	Annual mean temperature/ºC	Annual precipitation/mm	Sunshine intensity/Lux
XB	26º45.609´N 106º58.675´E	1104	Shizipu Fm	Limestone	15.2	1315	896.00
HF	26º56.630´N 106º55.214´E	898	Emeishanxuanwuyan Fm	Limestone	15.3	1120	862.00
HL	25º31.477´N 105º30.376´E	1426	Longtan Fm	Limestone	15	1178	912.00
YZ	25º21.199´N 105º06.240´E	1541	Emeishanxuanwuyan Fm	Limestone	15	1450	637.00
SC	26º14.586´N 105º58.774´E	1362	Anshun Fm	Limestone	14.8	1356	790.00
NG	26º12.334´N 105º58.807´E	1319	Anshun Fm	Limestone	14.5	1350	613.00
JC	26º05.882´N 106º03.768´E	1275	Maokou Fm	Limestone	14	1300	788.00
LG	26º05.803´N 105º53.218´E	1146	Longtan Fm	Limestone	14.5	1365	619.00
QYQ	26º15.102´N 106º03.711´E	1371	Anshun Fm	Limestone	14.2	1330	701.00
SP	26º07.663´N 106º07.757´E	1248	Daihua Fm	Limestone	14.8	1380	720.00
XY	26º26.881´N 106º19.181´E	1274	Erqiao Fm	Limestone	14.1	1181	717.00

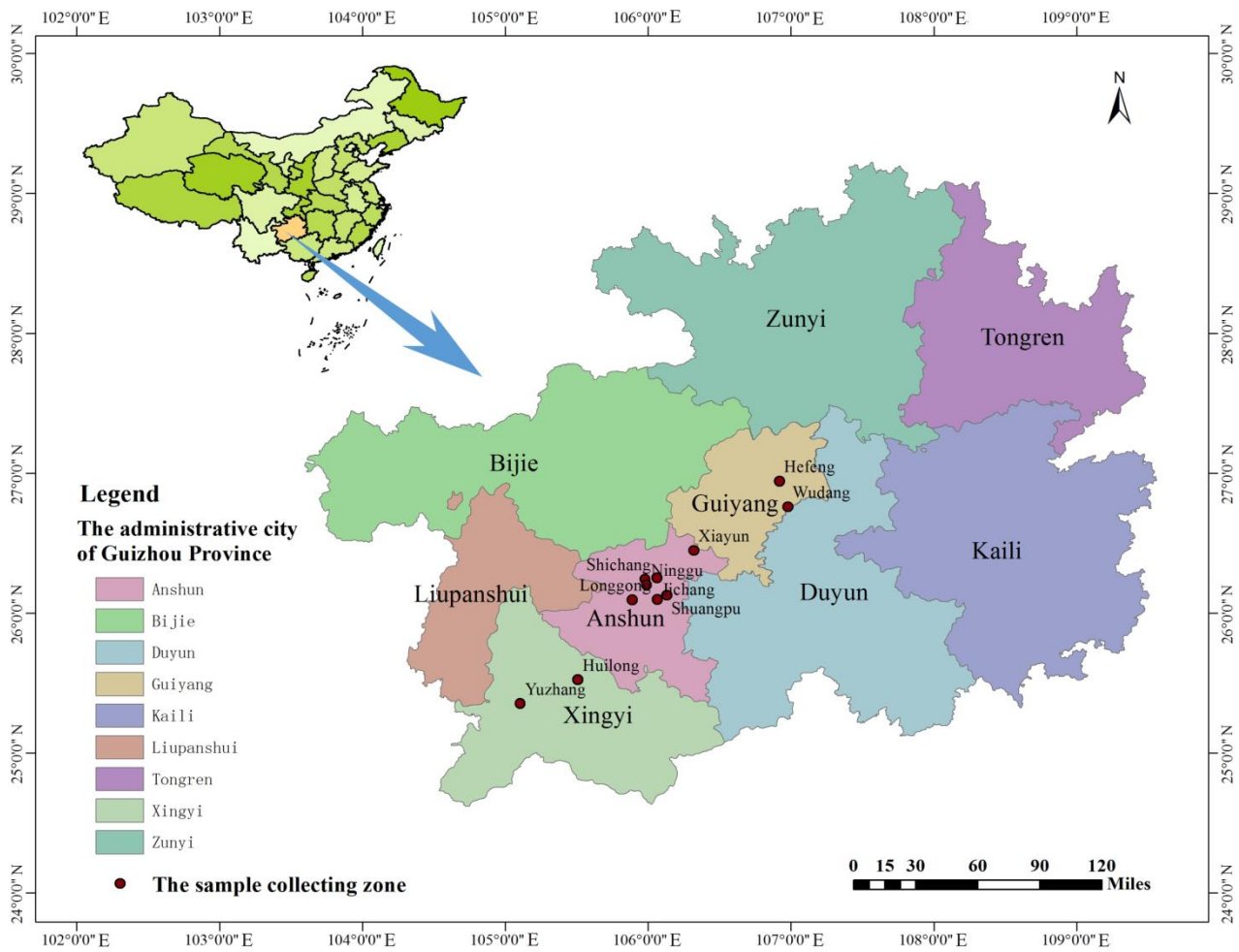

Fig. 1. Location of study areas in Guizhou province, SW China.

The Analysis of flavonoids in *R. sterilis* fruits: All fully ripe fruits of *R. sterilis* were collected from the healthy plants of 5a-6a. In order to ensure the representation of the experiment, the fruits were selected from more than 4 fruit trees around each random point of soil, and more than 20 fruit trees were selected in each plot. Over 50 fruits were collected from the east, west, north and south of the tree crown, respectively. The fruits were brought back to the laboratory and preserved at low temperature (30ºC±2) for analysis.

Total flavonoids in the fruit was determined by spectrophotometric method using UV-2550 (SHIMADZU). A new facile method was established for measuring major flavonoids of *R. sterilis* fruits, which was extracted with methanol and measured by high performance liquid chromatography (HPLC).

Extraction and isolation of major flavonoids: The fruits were extracted using 100% methanol, 100% ethanol, ethanol-water and methanol-water. The HPLC chromatograms obtained showed that methanol is better than ethanol in term of the extraction effect. And there may be sugar, acid and other polar components in these fruits, which are water-soluble components. The water-soluble components influenced the base line of the chromatograms. Flavonoids were extracted twice from 1 g dried powder of *R. sterilis* fruits using 50 ml 100% methanol (25 ml 100% methanol each time). The extracts of fruits were filtered and the methanol phases were collected. Methanol was evaporated to 1-2 ml and volume to 5 ml with deionized water. Then, flavonoids were extracted twice with30 ml ethyl acetate (15 ml ethyl acetate each time) and 30 ml water saturation n-butyl alcohol (15 ml water saturation n-butyl alcohol), respectively. The ethyl acetate and water saturation n-butyl alcohol phases were collected and evaporated.5ml 100% methanol was used for voluming the sample. The quantitative analysis of flavonoids compounds was carried out in a SHIMADZU LC-20A high-performance liquid chromatography (HPLC) system, equipped with ultraviolet detector (UV) (wavelength is 360 nm.), binary pump, online degasser, auto sampler. The data were processed using the SHIMADZU® Workstation software. The injection volume was 10 µl. The baseline resolution was obtained at setting temperature (30±2ºC) using an Agilent ODS-C-18 HPLC column(4.6 mm×250 mm, 5µm) and a gradient combining solvent A (acetonitrile) solvent B (orthophosphoric acid 0.1%, adjusted to pH 3.0) as shown by the elution program in Table 3. The mobile phase was prepared daily and degassed by sonication before use. The flow rate was kept constant at 0.7 ml min^{-1} and the chromatograms were recorded at 360 nm while the UV spectra were monitored over a range of 450 to 200 nm using UV-2550 (SHIMADZU). The peaks were

characterized by comparing the retention time and UV spectra with the reference standards, and by the co-injection of the sample and authentic samples. Three flavonoids, including rutin, quercetin and kaempferol were confirmed from fruits of *R. sterilis* (Fig. 2), and the recoveries of the targets were 93.40%, 99.98%, and 100.58%, respectively. The RSD of precision was 1.102%, 0.991%, 1.110%, respectively. The RSD of repeatability was 1.51%, 2.01%, 1.78%, respectively and the RSD of stability was 1.102%, 0.991%, 1.110%, respectively.

Statistical analysis: A general linear model of variance was used to describe the relation between the content of flavonoids in *R. sterilis* fruit and environmental factors. The least significant difference (LSD) test was used for each environmental factor, indicating that their interactions were significant. Difference at $p<0.05$ level was considered to be significant. All these statistical analyses were performed with SPSS 19.0 (IBM Corp., Armonk, NY, USA). Differences in major flavonoids were evaluated by principal component analysis. Partial correlation analysis and redundancy analysis were performed to gain insights into the relationships between flavonoids composition and soil indicators with SPSS 19.0 and CANOCO software (version 4.5, Microcomputer Power, Inc., Ithaca, NY, USA), respectively.

Table 2. Measurements protocols for indicators selected in this study.

Soil parameters	Reference
soil bulk density	Blake & Hartge, 1986
Soil moisture content	Sivakumar, 2014
Soil field capacity	Franz *et al*., 2010
pH	Wei *et al*., 2008; Cheesman*et al*., 2012
Soil organic matter	Walkley & Black, 1934
Total N	Anderson & Ingram, 1993; Szulc *et al*., 2016
Total P	Cheesman*et al*., 2012
Total K	Dimr *et al*., 2006
Available nitrogen	Comfield, 1960
Available phosphorus	Olsen & Sommers, 1982 Sui *et al*., 1999
Available potassium	Kim, 2005

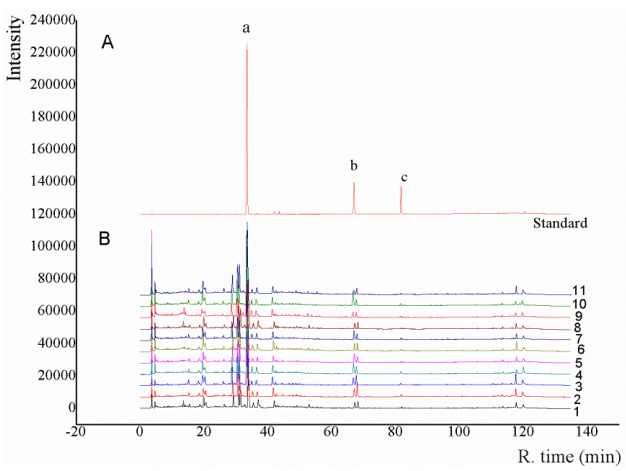

Fig. 2. HPLC chromatogram for major flavonoids with detection at 360 nm: (A) standard, (B) *R. sterilis* samples. Peak identification: a, rutin; b, kaempferol; c, quercetin. 1-11 were the *R. sterilis* furit samples from Xiaba, Hefeng, Huilong, Yuzhang, Shichang, Ninggu, Jichang, Longlong, Qiyanqiao, Shuangpu and Xiayun, respectively.

Results

Physical characteristics of soils: Table 4 shows significant differences in the physical characteristics of soils based on different environmental factors, such as sunlight intensity, altitude, mean annual air temperature and annual precipitation. Analysis of variance for four environmental factors shows that there is only one interaction between annual precipitation and soil moisture density ($p<0.05$), while there are no significant interaction effects of four factors on field moisture capacity and soil bulk density.

Table 3. The elution program established for major flavonoids of *R. sterilis* fruits.

Time	A(%)	Time	A(%)
0-5min	10%-13%	70-80min	30%-40%
5-10min	13%	80-90min	40%-65%
10-15min	13%-15%	90-95min	65%-80%
15-20min	15%-16%	95-105min	80%-90%
20-32min	16%-18%	105-110min	90%-95%
32-35min	18%-20%	110-115min	95%-90%
35-40min	20%-22%	115-120min	90%-80%
40-45min	22%-25%	120-130min	80%-10%
55-70min	25%-30%	130-150min	10%

Chemical characteristics of soil: Table 5 shows significant differences in concentrations of soil chemical attributes from different areas with the same soil management system. Compared with sunlight intensity, altitude, mean annual air temperature and annual precipitation, there was the only interaction between altitude and available nitrogen ($p<0.05$). There were no significant interaction effects of four environmental factors on other soil chemical properties.

Composition of *R. sterilis* flavonoids: Significant effects of altitude on rutin of *R. sterilis* fruits, and mean annual air temperature on total flavonoids are shown in Table 6 ($p<0.05$). There is no significant interaction between other environmental factors and flavonoids of the fruits.

The three flavonoids were identified in *R. sterilis* fruits from different planting areas with the same extraction and determination method. The rutin, quercetin and kaempferol account for about 1.05-5.58%, 0.02-0.09%, 0.06-0.31% of total flavonoids, respectively. Principal component analysis extracted two principal components, PC1 and PC2, accounting for 47.706% and 34.074% of the overall variances, respectively (Eigen value >1). The dominant flavonoids of PC1 were quercetin and kaempferol, and PC2 were quercetin and rutin (loading value >0.7) (Fig. 3a). Principal component analysis for 4 environmental factors indicated that the contribution rate ranking of each factor to the principal component was sunshine intensity, annual precipitation, altitude and annual mean temperature, respectively (Fig. 3b).

Table 4. Changes in physical characteristics of soils from study areas.

	SMC	SBD	FMC
1.	23.57 ± 1.36cd (BCD)	1.47 ± 0.04ab (AB)	30.42 ± 3.03abcd (ABC)
2.	30.78 ± 1.95 ab (AB)	1.22 ± 0.04de (DE)	44.78 ± 4.89ab (AB)
3.	21.71 ± 3.28de (CD)	1.29 ± 0.16 cd (BCDE)	32.57 ± 4.04abcd (ABC)
4.	26.06 ± 1.75 cde (BCD)	1.14 ± 0.05 de (DE)	44.40 ± 6.49 abc (ABC)
5.	26.30 ± 1.78 bc (ABC)	1.29 ± 0.1cde (DE)	30.85 ± 3.63d (C)
6.	17.78 ± 1.18e (D)	1.11 ± 0.07de (DE)	41.23 ± 4.37 bcd (ABC)
7.	24.52 ± 0.74bcd (BCD)	1.19 ± 0.06cd (CDE)	37.06 ± 1.96a (A)
8.	34.45 ± 1.75a (A)	1.14 ± 0.06e (E)	40.20 ± 2.58cd (ABC)
9.	27.60 ± 2.03 ab (AB)	1.40 ± 0.08bc (ABCD)	30.75 ± 1.88cd (BC)
10.	24.35 ± 1.13bcd (BCD)	1.44 ± 0.1 ab (ABC)	36.69 ± 3.94d (C)
11.	21.73 ± 1.44 cde (CD)	1.51 ± 0.09a (A)	30.16 ± 3.61d (C)
Analysis of variance			
Sunlight intensity	Ns	Ns	ns
Altitude	Ns	Ns	ns
Mean annual air temperature	Ns	Ns	ns
Annual precipitation	*	Ns	ns

Different letters in a column indicate significant differences at the level of 5% or 1%. Values are mean ± standard errors. ns no significance, *p<0.05

Table 5. Changes in concentrations of chemical characteristics of soils from the study areas.

	pH	TN	TP	TK
1.	6.93 ± 0.32b (B)	1.70 ± 0.28a (A)	0.37 ± 0.07h (H)	10.01 ± 1.34f (F)
2.	6.49 ± 0.17c (C)	3.20 ± 1.67b (B)	0.53 ± 0.16f (F)	13.86 ± 5.47d (D)
3.	6.91 ± 0.38b (B)	2.58 ± 0.67c (C)	0.78 ± 0.09d (D)	8.70 ± 2.46g (F)
4.	6.36 ± 0.12d (D)	0.58 ± 0.42d (D)	0.75 ± 0.03e (E)	0.89 ± 0.12j (J)
5.	6.88 ± 0.21b (B)	3.11 ± 1.64e (E)	0.78 ± 0.52d (DE)	13.50 ± 1.66de (D)
6.	6.87 ± 0.05b (B)	4.40 ± 0.85f (F)	0.84 ± 0.44c (C)	23.87 ± 6.88b (B)
7.	6.04 ± 0.45e (E)	4.16 ± 3.28g (G)	1.08 ± 0.47a (A)	27.77 ± 14.94a (A)
8.	7.12 ± 0.06a (A)	1.12 ± 0.81h (H)	0.39 ± 0.18gh (GH)	4.23 ± 1.44i (H)
9.	5.99 ± 0.23e (E)	6.79 ± 3.57i (I)	1.06 ± 0.08b (B)	15.02 ± 2.46c (C)
10.	5.49 ± 0.18f (F)	1.89 ± 1.47j (J)	0.42 ± 0.29g (G)	5.34 ± 3.23h (G)
11.	5.96 ± 0.4e (E)	2.86 ± 1.39k (K)	0.11 ± 0.003i (l)	13.52 ± 1.08e (D)
Analysis of variance				
Sunshine intensity	ns	ns	ns	ns
Altitude	ns	ns	ns	ns
Mean annual air temperature	ns	ns	ns	ns
Annual precipitation	ns	ns	ns	ns
	SOM	**AN**	**AP**	**AK**
1.	29.87 ± 0.88g (F)	83.80 ± 23.05i (H)	5.65 ± 0.08a (A)	8.93 ± 1.52j (H)
2.	31.71 ± 2.57e (DE)	114.05 ± 55.19h (G)	33.15 ± 0.16b (B)	13.02 ± 3.29e (E)
3.	36.49 ± 4.39b (B)	165.88 ± 30.01d (C)	2.35 ± 0.09c (C)	11.83 ± 0.95g (F)
4.	28.50 ± 2.01g (F)	173.35 ± 26.04b (B)	3.99 ± 0.03d (D)	28.42 ± 0.35a (A)
5.	35.34 ± 1.74c (BC)	183.22 ± 64.95a (A)	26.61 ± 0.52e (E)	20.41 ± 3.79d (D)
6.	34.97 ± 5.52cd (C)	170.41 ± 35.43c (C)	6.85 ± 0.44f (F)	26.54 ± 4.58b (B)
7.	36.48 ± 1.55b (B)	137.33 ± 45.56e (D)	11.26 ± 0.47g (G)	21.06 ± 4.12c (CD)
8.	31.75 ± 1.02f (E)	68.03 ± 32.10j (I)	4.96 ± 0.18h (H)	12.47 ± 2.71f (EF)
9.	41.36 ± 0.38a (A)	129.05 ± 29.09f (E)	26.81 ± 1.08i (I)	21.01 ± 2.30c (C)
10.	33.33 ± 0.78e (D)	183.72 ± 28.76a (A)	3.17 ± 0.29j (J)	9.78 ± 2.67i (H)
Analysis of variance				
Sunshine intensity	ns	ns	ns	ns
Altitude	ns	*	ns	ns
Mean annual air temperature	ns	ns	ns	ns
Annual precipitation	ns	ns	ns	ns

Different letters in a column indicate significant differences at the level of 5% or 1%. Values are mean ± standard errors. no significance, *p<0.05

Table 6. Changes in composition of major flavonoids of *R. sterilis* from study areas.

	Total flavonoids	Rutin	Quercetion	Kaempferol
1.	0.089 h (G)	4.8866a (A)	0.0515f (F)	0.202a (A)
2.	0.07 j (I)	3.9034b (B)	0.0535e (E)	0.2168b (B)
3.	0.204 a (A)	3.0521e (E)	0.0713b (B)	0.1275c (C)
4.	0.078 i (H)	2.6805f (F)	0.0714b (B)	0.1527d (D)
5.	0.105 g (F)	2.5974g (F)	0.0556e (E)	0.1422e (E)
6.	0.193 b (B)	3.5536c (C)	0.0494g (F)	0.1985a (A)
7.	0.146 f (E)	3.2752d (D)	0.0619c (C)	0.1679f (F)
8.	0.16 d (D)	3.2016d (D)	0.0603d (D)	0.1692f (F)
9.	0.187 b (B)	1.9610h (G)	0.037h (G)	0.1758f (F)
10.	0.151 e (E)	3.5649c (C)	0.0939a (A)	0.1139g (G)
11.	0.176 c (C)	3.5295c (C)	0.0295i (H)	0.2372h (H)
Analysis of variance				
Sunshine intensity	ns	ns	ns	ns
Altitude	ns	*	ns	ns
Mean annual air temperature	*	ns	ns	ns
Annual precipitation	ns	ns	ns	ns

Different letters in a column indicate significant differences at the level of 5% or 1%. Values are mean ± standard errors. *ns* no significance, *p<0.05; **p<0.01

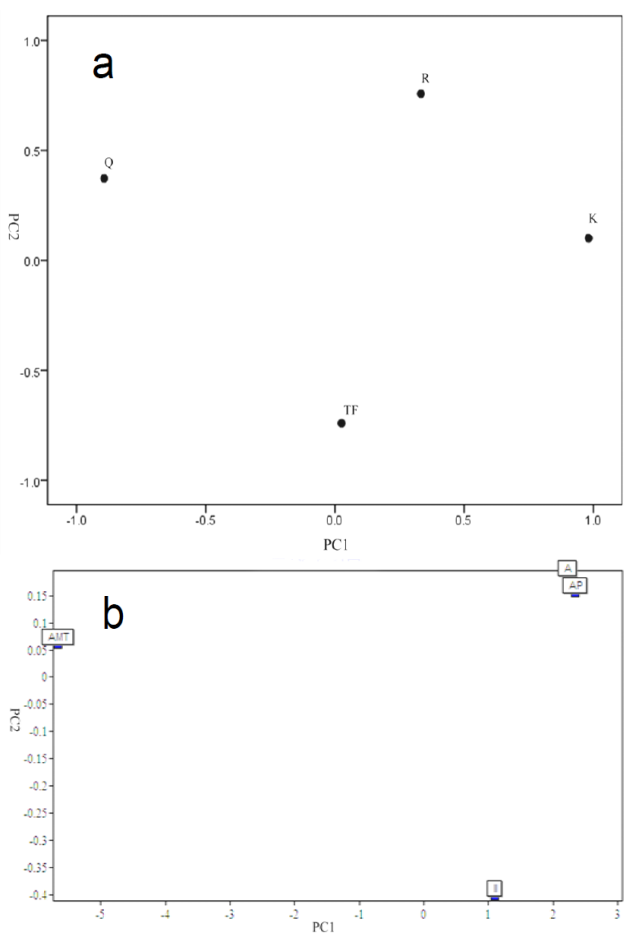

Fig. 3. a. Loading values for individual flavonoid from principal component analysis; b. Principal component analysis of the environment factors from different *R. sterilis* planting areas. Two components (PC1 and PC2) were extracted by principal component analysis, and PC1 accounted for 47.706% and PC2 for 34.074% of the original variance. SI, sunshine intensity; AP, annual precipitation; A, altitude; AMT, annual mean temperature.

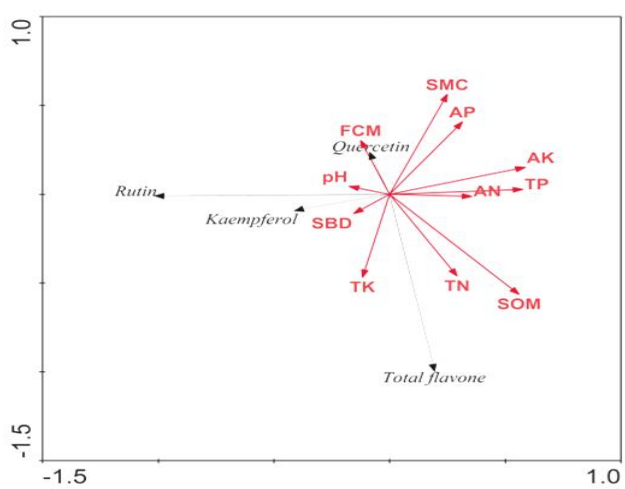

Fig. 4. Redundancy analysis of *R. sterilis* flavonoids and soil indicators from different planting bases. The amount of variability explained by all canonical axes was 96.7%.pH Potential of Hydrogen, FMC field moisture capacity, SMC soil moisture content, SBD soil bulk density, SOM soil organic matter, SMC soil moisture content, AP available phosphorus, AK available potassium, AN available nitrogen, TP total phosphorus, TK total potassium, TN total nitrogen.

Redundancy analysis of *R. sterilis* flavonoids showed that the coordinate from the first two ordination axes explained 96.7 % (the first axis 91.7 % and the second 5.0 %) of the variances (Fig. 4). The flavonoids were distinctly separated by the first two principal components. The AN, TP and AK content of the soil and pH along the first axis were the most influential factors for flavonoids in the fruits. The AN, TP and AK content of soil were positively related to the first axis, and the pH of soil was negatively correlated with the first axis. In addition, among all soil factors, FMC had the highest degree of correlation with quercetin. Partial correlation analysis indicated that SOM was significantly and positively correlated with total flavonoids (Table 7). In general, there were no significant relationships between the other soil factors and flavonoids.

Table 7. Partial correlation analysis between *Rose sterilis* flavones parameters and soil characteristics.

	SMC	SBD	FMC	pH	TN	TP	TK	SOM	AN	AP	AK
Total flavone	-0.508	0.071	-0.308	-0.084	0.478	0.095	0.407	0.649*	0.112	-0.371	-0.047
Rutin	-0.256	0.156	0.122	0.175	-0.291	-0.574	0.118	-0.556	-0.351	-0.324	-0.591
Quercetin	0.064	-0.18	0.399	-0.142	-0.51	0.11	-0.501	-0.332	0.373	-0.366	-0.1
Kaempferol	-0.076	0.093	-0.018	0.094	0.249	-0.373	0.396	-0.068	-0.512	0.21	-0.087

* and ** indicated 0.05 and 0.01 significant levels, respectively

Discussion

Effect of environmental factors on soil characteristic: In our study, there are significant differences in physical and chemical characteristics of soils (Tables 4 and 5). The soil conditions directly affect the growth of plants. The main soil forming factors are the climate (principally water balance), parent material (texture, porosity, and chemical composition), vegetation (especially on avalanche slopes), topography (aspect and slope angle), and time (Alvarez & Lavado, 1998; Bockheim *et al*., 2014). The diversity in soil characteristics (the physical, chemical and other aspects) was due to the complex underlying surface of the Karst area in Guizhou and the differences in geographical environment and ecological system.

Soil moisture conditions are crucial for conserving soil water in forests and playing important roles in increasing the vegetation for maintaining a favorable hydrological balance (Zheng *et al*., 2015). The SMC of soil increased following the increase in precipitation (Gao *et al*., 2011; Liu *et al*., 2016). Previous research showed that the changes in altitude affected the temperature of the soil, directly affecting the rate of soil organic carbon and nitrogen mineralization (Fang *et al*., 2005). In our study, the annual precipitation and altitude has significantly positive correlation with soil moisture content and available N, respectively (Tables 2 and 3). Similarly, Alvarez & Lavado (1998) and Hassan *et al*. (2015) reported that favorable water content and soil temperature of top soil layer caused fast residue decomposition.

In the present study, annual precipitation and altitude significantly influenced the soil characteristics instead of other environmental factors ($p<0.01$, Tables 4 and 5), possibly because soil moisture and AN content are more sensitive than other properties of soil in different environments (Hupet *et al*., 2002). Soil moisture is a key variable of the climate system (Seneviratne *et al*., 2010).

Effects of environmental factors on major flavonoids: Table 6 shows that the annual mean temperature and altitude were correlated with total flavonoids and rutin, respectively. Therefore, suitable temperature and altitude can provide a better environment for flavonoids to influence the accumulation of *R. sterilis* fruit. However, we did not observe other correlation with environmental factors and flavoniods, and environmental sunshine intensity and precipitation were essential conditions for plant growth. Similar results were reported by Anderson *et al*. (1969) and Körner (2015). Therefore, we confirmed the contribution rate by principal component analysis. The results showed that sunshine intensity and annual precipitation were the main factors influencing the content of flavonoids (Fig. 3). Temperature, sunlight intensity and precipitation are not only the basic condition of plant growth (Haraguchi & Yamada, 2011), but also the key factors for the effective components of the fruits and plants. Previous studies have already established the environmental and related factors which could affect the rutin content in the buckwheat leaves (Wijngaard & Arendt, 2006; Drazic *et al*., 2016). These conditions constantly changed with altitude. Given the dependence of the relationship between altitude and rutin, precipitation and sunlight have an influence on the synthesis rate and content of flavonoids with a change in temperature (Wang *et al*., 2014). Additionally, Brevik *et al.* (2004) suggested that the content of flavonoids in the fruit might be related to seasonal changes.

Relationships between soil characteristics and major flavonoids: Redundancy analysis of *R. sterilis* flavonoids and soil indicators showed that eleven soil indicators were related to flavonoids parameters (Fig. 4). The main indicators consisted of AN, TP, AK and pH, respectively. Moreover, partial correlation analysis also showed that the SOM content was significantly related to total parameters, and the partial correlation coefficient of the SOM was significantly higher than other indicators (Table 7). The soil content of SOM, AN, TP, AK and pH could change the soil quality of planting areas. Thus, these indicators impact the content of major flavonoids and nutrient contents in the plants (Reimberg *et al*., 2009; Arjumend & Abbasi, 2016).

Guizhou is located in typical Karst mountain areas with heavy soil erosion and desertification, and the soil environment is relatively weak. The pH value, as an inactive indicator, reduced the soil quality by affecting soil nitrification, microbial communities (Sarathjith *et al*., 2014; Siciliano *et al*., 2014). Then, the pH value further influenced plant uptake and vegetation process (Petersen *et al*., 2012; Thomaes *et al*., 2012).

SOM is the major pool of organic carbon that is sensitive to a change in the local climate or environment (Parry *et al*., 2007; Schmidt *et al*., 2011). Soil with rich organic matter has influences on vegetative growths, which increases the total fruit yields as well as improve the fruit quality (Suge *et al*., 2011). The content of N, P and K in soils can effect on establishment, survival and development of plant species and are considered to be the most important limiting factor in Karst areas, which directly affect the yield of the fruits and its product quality (Pérez-Álvarez *et al*., 2013). In this study, the flavonoids and the major soil indicators in different planting areas are significantly correlated (Fig. 4). However, excessive use of these chemical inputs N, P and K has affected the soil health leading to a reduced yield (Verma *et al*., 2016). Mahdy (2011) pointed out that the proper nutrient ratio of soil should not only be beneficial

to plant growth but also sustainable for the development of soil ecology. Furthermore, the local environmental factors and soil characteristics could mutually affect the content of flavonoids by seasonal variations, water conditions and soil quality.

Conclusions

The results show that there exists a significant correlation between the content of flavonoids and local environmental factors. Sunshine intensity and annual precipitation have a positive effect on the content and synthesis rate of flavonoids in *R. sterilis*. Similarly, soil indicators (SOM, AN, TP and AK) played positive roles in improving the content of flavonoids, except for pH. Therefore, management methods need to adjusted, based on the situation of soil and local environmental factors. The key is to study the optimum ratio of fertilization so as to avoid fertilizer pollution. In addition, the experiments were conducted in a short period, thus a further study is needed to verify the relationships between the content of favonoids in *R. sterilis* and ecological factors from different planting areas in Karst regions.

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