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REGULAR ARTICLE

# Influence of altitudinal and latitudinal variation on the composition and antioxidant activity of polyphenols in *Nicotiana tabacum* L. leaf

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## ABSTRACT

This present comparative study was to investigate the influence of different altitude and latitude on the polyphenols, composition and its antioxidant activity in *Nicotiana tabacum* L. leaf. Polyphenol content and its antioxidant activities of methanol extracts of *Nicotiana tabacum* L. leaf from different altitude (70 m to 2100 m) and latitude (23.26° N to 34.61° N) were studied. The compositions of polyphenol were detected by UPLC-MS, and the total polyphenols content (TPC) and total flavonoids content (TFC) were measured by spectroscopic methods. The antioxidant activity assays were measured by the bleaching of the DPPH radical, reducing power ability, and ferric reducing antioxidant power. Results showed that chlorogenic acids (CQAs, including 3-CQA, 4-CQA and 5-CQA), rutin, and amount phenolic compounds (CQAs and rutin) showed high significant positive correlation with altitude; besides, TPC and TFC were significant positive correlation with altitude. Otherwise, the results of them with latitude were almost no correlation, while TPC was significant negative correlation with latitude. The higher altitude of extract of tobacco sample the stronger antioxidant capacity was, but there was little relationship with the latitude. The compositions of polyphenol were various from different altitude, and there was a significant positive correlation between altitude and rutin proportion. Based on the molecular structures, it's probably because the UV absorbing ability and antioxidant activity of rutin are better than these of CQAs. These preliminary findings demonstrated that influence of altitude on the content of individual polyphenol, TPC, TFC and antioxidant activities was significant, while the relationship with latitude was not significant overall.

**Keywords:** *Nicotiana tabacum* L.; Altitude; Latitude; Polyphenols; Chemical composition; Antioxidant activity

## INTRODUCTION

*Nicotiana tabacum* L. is a model plant of scientific research as well as an important economic crop. Large amounts of resources (including leaves and stems) are discarded as processing waste in China every year. In fact, they are economically valuable because of lots of potentially useful compounds in them, such as phenolic compounds, proteins, and polysaccharides (Lizcano et al., 2012; Ru et al., 2012; Wang et al., 2008; Zhang et al., 2012). Several polyphenols were identified in tobacco leaves and the antioxidant activities of flavonoids and polysaccharides were investigated (Ru et al., 2012; Wang et al., 2008; Wang et al., 2010). Polyphenols are very important multifunctional secondary metabolites, defending against insects, birds, mammals, fungi, and bacteria, color pigmentation, antioxidant, regulate seed longevity and dormancy, and UV protection (Brown et al., 2003; Izhaki, 2002; Nour-Eldin et al., 2008). Especially, polyphenols can protect plants and animals against DNA damage induced by ultraviolet radiation (Charles and Cockell, 1999; Kootstra, 1994), contribute directly to antioxidant activity because of the scavenging activity provided by the phenol hydroxyl groups (Margraf et al., 2016; Morais et al., 2015). Interest in polyphenols is increasing in the agriculture and food industry not only because of the capacity to delay oxidant degradation of lipids, in order to improve the quality and nutrition of foods (Kuczmannova et al., 2015; Nowak et al., 2016; Stevanato et al., 2014), but also the defensive or other role in scientific researches (Martín et al., 2015; Morales et al., 2010; Spitaler et al., 2008; Zhang et al., 2014).

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In fact, it is well documented that, when plants are exposed to oxidative stress, the antioxidant systems can be potentiated in order to protect plants from free radical damages. As important anti-ultraviolet secondary metabolites, the higher the altitude and lower the latitude, the more the ultraviolet rays, does this means that the more polyphenols in plants could be accumulated? The conclusions are almost proved by many experiments, but there are some different research findings (Carrillo et al., 2014; Kishore et al., 2010; Nchabeleng et al., 2012; Alonso-Amelot et al., 2004). For example, Kishore et al. (2010) found that a good positive correlation with total polyphenols and antioxidant potential in fifteen seed samples of Tartar Buckwheat between altitude in Western Himalaya. While, Carrillo et al. (2014) found that the lower the altitude, the more polyphenols in eighteen cocoa bean samples from eleven different cocoa-growing areas of Colombia.

As the ultraviolet radiation is not only related to altitude, but also related to latitude, therefore, the present work was to investigate polyphenol composition, antioxidant activity, and polyphenol content of tobacco leaf from forty-four counties throughout China with different altitudes and latitudes.

## MATERIAL AND METHODS

### Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl), TPTZ (2, 4, 6-tripyridyl-S-triazine), BHT (butylated hydroxytoluene), chlorogenic acids (5-O-Caffeoylquinic acid, 5-CQA; 4-O-Caffeoylquinic acid, 4-CQA; 3-O-Caffeoylquinic acid, 3-CQA) and rutin (purity > 98%) were purchased from Solarbio Science & Technology Co, Ltd (Beijing, China). The other chemical reagents used were of analytical grade and purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China).

### Plant materials and sample preparation

The samples of tobacco from forty-four counties (70-2100 m altitude, 23.26-34.61° N latitude) were provided by Zhengzhou Tobacco Research Institute. These counties are located in the main tobacco-producing provinces throughout China. Only healthy samples, without any infection or physical damage, were processed. Random sampling was carried out. The samples were brushed clean, dried at 70 °C, grounded into powder and sieved over a 40-mesh screen, and then stored at -18 °C until sample preparation. One hundred milligrams of the powders were mixed with 25 mL of 50% (v/v) methanol aqueous solution in the ultrasonic extraction tank for 20 min at at room temperature with a frequency of 40 kHz. The extracts were centrifuged (3,000 × g) for 10 min and the upper layer was filtered (0.45 μm) and then the extract of tobacco sample (ETS) was obtained.

### LC-MS/MS Analysis

UPLC-MS was conducted on a LC-30A UPLC (Shimadzu Corporation, Kyoto, Japan), coupled with hybrid quadrupole time-of-flight tandem mass spectrometry LC-MS-Q-TOF (LC-MS-Triple TOF 5600, AB SCIEX, Foster City, CA), which equipped with an LC-30AD binary pump, an SIL-30AC auto sampler, and a CTO- 30A column oven. Chromatographic separation was performed on C18 reversed phase LC column (Shimadzu Corporation, 1.6 μm particles, 2.0 mm×50 mm). The analysis sequence of the different samples was randomized. Mobile phase A: Ultrapure water with formic acid (1%); mobile phase B: Acetonitrile with formic acid (1%). A gradient elution system was used: 98% A from 0 to 0.5 min, 98-85% A for 0.5 - 5.0 min, 85 - 65% A for 5.0 - 8.0 min, 65 - 5% A for 8.0 - 11.0 min, 5% A for 11.0 -13.0 min, and 5.0 - 98% A for 13.0 -13.1 min, and finally, the column was holding the composition of 98% A until 15.0 min for column balance; this system was used as a rinse, and the column flow rate was 500 μL/min.

The samples were analyzed in negative ionization mode by hybrid triple quadrupole time-of-flight mass spectrometer equipped with Turbo V sources. The data acquisition using information-dependent analysis (IDA) method, and 8 MS/MS scans were obtained for each cycle. The TOF MS scan was operated with the mass range of 100-1000, and the TOF MS/MS was an m/z scan range of 50-1000. The following MS conditions were used: Ion-spray voltage (ISVF), -4500V; Collision energy (CE), 40 ± 20V; the turbo spray temperature, 550 °C; declustering potential (DP), -100 V; nebulizer gas(GS1), 55 psi; heater gas (GS2), 55 psi; curtain gas(CUR), 35 psi. Nitrogen was kept as nebulizer and auxiliary gas. Calibration was auto-carried by CDS after every five samples analysis.

### Total polyphenols content

Total polyphenols content (TPC) was analyzed by the Folin-Ciocalteu reagent based on Mishra et al. (2015) and gallic acid was used as standard, with little modifications. In brief, 5.0 mL of freshly diluted 10-fold Folin-Ciocalteu reagent and 0.5 mL of ETS were mixed well together. The solution was placed for 3 min and 4.0 mL of sodium carbonate aqueous solution (7%, w/v) was added. After 60 min the result of reaction system was read at 760 nm by an ultraviolet visible spectrophotometer against a blank sample. A calibration curve using gallic acid as standard was produced. The TPC was expressed as milligram equivalent of gallic acid (mg GAE) per gram of dried sample (mg GAE/g dried sample).

### Total flavonoid content

A colorimetric aluminum chloride method was used for determination the total flavonoid contents (TFC) by Islam et al. (2014) with some modifications. One milliliter ETS were mixed with 1 mL $NaNO_2$ aqueous solution (5%, w/v) and kept at room temperature for 6 min. Thereafter, 0.3 mL aluminium chloride aqueous solution (10%, w/v) was added and kept to react for another 6 min. Then, 2.0 mL NaOH (4%, w/v) solution was added to each solution and reacted for 10 min at room temperature. The result of the reaction mixture was recorded at 510 nm using an ultraviolet visible spectrophotometer. The calibration curve was established by preparing rutin methanol solutions. The yield of the flavonoids was expressed as mg of rutin equivalents per gram (mg RE/g).

### Reducing power ability (RP)

The reducing power ability was performed according to the literature (Sharma et al., 2015) with slight modifications. Three hundred microliter ETS, aqueous ethanol solution (2.2 mL, 80%), 2.5 mL potassium ferricyanide solution (1%, w/v) and 2.5 mL sodium phosphate buffer (0.2 M, pH 6.6) were mixed vigorously, and placed at 50 °C for twenty minutes. And then, 2.5 mL trichloroacetic acid (10%, w/v) was added to end this reaction, and the mixed solution was centrifuged (3,000 × g) for 10 min. A 2.5 mL upper layer fraction was mixed with 2.0 mL pure water and 0.5 mL $FeCl_3$ solution (0.1%, w/v) and mixed vigorously. The record was read at 700 nm. Then, a calibration curve using BHT ethanol solution was established. The RP assay results were showed as mg of BHT equivalents per gram (mg BE/g).

### Ferric reducing antioxidant power (FRAP)

The FRAP method was performed according to the method described by literatures (Grąbkowska et al. 2016; Pushparaj and Urooj 2014) with slight modifications. The FRAP reagent was made freshly by 300 mM acetate buffer (pH 3.6), a 10 mM TPTZ solution in 40 mM hydrochloric acid and 20 mM ferric chloride solution in proportions of 10:1:1 (v/v), sequentially. A 2.5 mL FRAP reagent and 0.6 mL of ETS were mixed well together, and the reaction solution was warmed at 37 °C for 10 min, after that, the result was read at 593 nm. And then, $FeSO_4$ solution was used to establish a calibration curve. The FRAP assay record was expressed as μg of $FeSO_4$ equivalents per gram (μg FeE/g).

### Scavenging activity on DPPH radicals

DPPH radical scavenging capacity was performed referring to Santiago-López et al. (2016). Two hundred microliters ETS and 1.5 mL DPPH methanol solution (0.3 mM) were mixed together, then shaken vigorously and kept at 30 °C for thirty minutes in the darkness. The result of the reaction mixture was read at 517 nm. The scavenging capacity on DPPH radicals was used the formula: Scavenging capacity (%) = $(1 - A_s/A_c) \times 100\%$, where $A_c$ is the absorbance of control without the tobacco extract, and $A_s$ is the absorbance include the tobacco extract. The tobacco extract concentration offering 50% inhibition (EC50) was evaluated from the curve of scavenging effect percentage against tested sample concentration. BHT was implied for comparison, and the DPPH assay results were presented as μg of BHT equivalents per gram (μg BE/g).

### Statistical Analysis

Every one of the experiment was performed three times and the results were presented as average ± standard deviation (SD). SPSS 19.0 was used to analyze the data.

## RESULTS AND DISCUSSION

### Polyphenols and flavonoids in tobacco leaves

HPLC, with reverse phase column technology, is usually used to analyze the separation and characterization of polyphenols (Zhang et al., 2015a). In the present study, the major components in ETS were identified by UPLC-Q-TOF MS system. According to the literatures published in the past, there are dozens of phenolic compounds, and mainly of them are chlorogenic acids (3-CQA, 4-CQA and 5-CQA) and rutin, and the content of other polyphenols are minimal (Wang et al., 2008 and 2010). So, in this present study, these four phenolic compounds were detected. By comparing with the standards and MS data, these four phenolic compounds were confirmed. The contents of forty-four samples of 3-CQA, 4-CQA, 5-CQA, chlorogenic acids (CQAs, including 3-CQA, 4-CQA and 5-CQA), rutin and amount phenolic compounds (APC, CQAs and rutin) were 1.94± 0.45 mg/g, 2.43±0.57 mg/g, 11.52±1.52 mg/g, 15.89±2.02 mg/g, 8.06±2.24 mg/g and 23.95±3.82 mg/g, respectively.

The method of Folin-Ciocalteu reagent to evaluate the TPC and colorimetric aluminum chloride to determine the TFC, using gallic acid and rutin as standard respectively, were used commonly to evaluate the antioxidant activities (Mishra et al., 2015; Islam et al., 2014). In this work, the contents of forty-four samples of TPC and TFC were 22.96±2.45 mg GAE/g and 31.09 ± 3.70 mg RE/g, respectively.

Generally speaking, the higher the altitude and lower the latitude, the stronger ultraviolet will be. So a wide range samples were employed to analyse their relationship. The Pearson correlation coefficients for the altitude and latitude of the growing site and 3-CQA, 4-CQA, 5-CQA, CQAs, rutin, APC, TPC and TFC were summarized in Table 1. The contents of 5-CQA, CQAs, rutin, and APC showed highly significant positive correlation with altitude. The contents

**Table 1: Pearson correlation coefficients (*r*) and corresponding p values for the observed correlation between the contents of tobacco leaf phenolics and the altitude and latitude of the growing site**

| | 3-CQA | 4-CQA | 5-CQA | CQAs | Rutin | APC | TFC | TPC |
|---|---|---|---|---|---|---|---|---|
| Altitude | | | | | | | | |
| r | 0.321* | 0.257 | 0.417** | 0.458** | 0.579** | 0.583** | 0.307* | 0.376* |
| p | 0.034 | 0.092 | 0.005 | 0.002 | 0.000 | 0.000 | 0.043 | 0.012 |
| Latitude | | | | | | | | |
| r | –0.447** | –0.253 | –0.055 | –0.213 | –0.013 | –0.120 | –0.121 | –0.412** |
| p | 0.002 | 0.097 | 0.724 | 0.166 | 0.932 | 0.436 | 0.433 | 0.005 |

*$p \leq 0.05$, **$p \leq 0.01$, the same as follows

of 3-CQA, TFC, and TPC showed significant positive correlation with altitude. While, the content of 4-CQA showed no significant positive correlation with altitude.

Compared to the Pearson correlation coefficients for the altitude and polyphenols, the results of latitude and polyphenols were different. The content of 3-CQA and TPC showed highly significant negative and significant negative correlation with latitude, respectively. But there was no significant correlation between the content of 4-CQA, 5-CQA, rutin, APC, TFC and latitude. Overall, the polyphenols content increased with increasing altitude, but the relationship between polyphenols content and latitude was not significant. This may be a greater impact on the elevation of UV radiation, but less impact on the latitude of UV radiation.

Radiation ultraviolet could increase the synthesis of polyphenols (Al-Rashed et al., 2016; Zhang et al., 2014), and strong ultraviolet radiation at high altitude area, which was assumed to be the main factor responsible for these differences in previous studies (Alonso-Amelot et al., 2007; Spitaler et al., 2006; Zidorn et al., 2010), in addition, high altitude area not only has strong ultraviolet radiation and low temperature, which might also be the cause of the high content of polyphenols (Bilger et al., 2007). Ultraviolet radiation and temperature can cause the plant polyphenol accumulation, but which factors play a leading role is rarely reported in the literature.

### Antioxidant activity

The antioxidant activities of tobacco leaf were measured by RP, FRAP and DPPH methods. In order to describe the antioxidant activity of the sample more clearly, the antioxidant activity index was introduced according to the method described by Zhang et al. (2015b). For each of the antioxidant assay, the antioxidant index score was based on the formula: Antioxidant index score = [(sample score/highest score) × 100%], and total antioxidant activity composite (TAAC) index was according to the mean value of the antioxidant index score of each assay.

RP values of the samples from different areas varied from 0.94 to 1.33 mg BE/g and the mean value was 1.14 ± 0.12 mg BE/g. In this assay, BHT as a current antioxidant in food industry was used for comparison, and the larger the value indicated the stronger the antioxidant capacity. High RP values of ETS meant the extraordinary potential to donate electrons to reactive free radicals, which would turn them into more stable non-reactive objects and finally stop the chain reaction of free radical (Plazonic et al., 2013; Pushparaj and Urooj 2014). As to the RP index, the minimum value was 70.75, indicating that the activity was various among the samples.

FRAP values of ETS were from 71.95 to 139.31 μg FeE/g and the mean value was 107.34± 14.92μg FeE/g. In this assay, $FeSO_4$ was used for comparison, and the larger the value indicated the stronger the antioxidant capacity. As to the FRAP index, the minimum value was 51.65, which showed that the antioxidant activity of ETS in different regions was relatively large.

DPPH values of the samples from different areas varied from 3.05 to 5.96 μg BE/g and the mean value was 4.34 ± 0.73 μg BE/g. In this assay, BHT was used for comparison, and EC50 was used to evaluate the activity, so the smaller the value indicated the stronger the antioxidant activity. In order to keep the consistency of the results, index DPPH was also introduced in, and the greater the DPPH index the stronger the antioxidant activity was. In order to keep the consistent of the results, DPPH index was also introduced in this assay, and the greater the DPPH index indicated the higher the antioxidant activity. As to the DPPH index, the minimum value is 51.17, which showed that the antioxidant activity of ETS in different regions was relatively large.

There were many researches on the content of polyphenols and antioxidant capacity (Carrillo et al., 2014; Kishore et al., 2010; Nchabeleng et al., 2012; Alonso-Amelot et al., 2004). Some literatures considered that there was a positive correlation between them, and some did not think that there was a certain correlation. From the results summarized in Table 2, there were very good positive correlation between polyphenols and antioxidant activity overall. Since 3-CQA, 4-CQA, 5-CQA have very similar structure, with the same location and number of phenolic hydroxyl group, so the

**Table 2: Pearson correlation coefficients (*r*) and corresponding p values for the observed correlation between the antioxidant activity assays and polyphenols, altitude and latitude of the growing site of tobacco leaf**

| | 3-CQA | 4-CQA | 5-CQA | CQAs | Rutin | APC | TFC | TPC | Altitude | Latitude |
|---|---|---|---|---|---|---|---|---|---|---|
| RP index | | | | | | | | | | |
| r | 0.294 | 0.287 | 0.392** | 0.442** | 0.690** | 0.640** | 0.189 | 0.317* | 0.577** | –0.056 |
| p | 0.053 | 0.059 | 0.008 | 0.003 | 0.000 | 0.000 | 0.219 | 0.036 | 0.000 | 0.719 |
| FRAP index | | | | | | | | | | |
| r | 0.190 | 0.193 | 0.601** | 0.550** | 0.490** | 0.579** | 0.427** | 0.400** | 0.515** | –0.283 |
| p | 0.216 | 0.209 | 0.000 | 0.000 | 0.001 | 0.000 | 0.004 | 0.007 | 0.000 | 0.062 |
| DPPH index | | | | | | | | | | |
| r | 0.347* | 0.343* | 0.512** | 0.560** | 0.542** | 0.615** | 0.248 | 0.241 | 0.445** | –0.263 |
| p | 0.021 | 0.023 | 0.000 | 0.000 | 0.000 | 0.000 | 0.105 | 0.114 | 0.002 | 0.085 |
| TAAC index | | | | | | | | | | |
| r | 0.368* | 0.365* | 0.668** | 0.688** | 0.742** | 0.801** | 0.383** | 0.413** | 0.663** | –0.280 |
| p | 0.014 | 0.015 | 0.000 | 0.000 | 0.000 | 0.000 | 0.010 | 0.005 | 0.000 | 0.066 |

antioxidant activity should be little difference. The following analyses were carried out with the total of them (CQAs) instead of 3-CQA, 4-CQA and 5-CQA.

The contents of CQAs, rutin, and APC showed highly significant positive correlation with RP index, FRAP index, DPPH index and TAAC index. The contents of TFC showed highly significant positive correlation with FRAP index and TAAC index, but not significant positive correlation with RP index and DPPH index, and the *p* values were 0.219 and 0.105, respectively. The contents of TPC showed highly significant positive correlation with TAAC index and FRAP index, and significant positive correlation with RP index, but no significant positive correlation with DPPH index, with the *p* value of 0.114.

All of the antioxidant activity of ETS including RP index, FRAP index, DPPH index and TAAC index showed highly significant positive correlation with altitude. The results of them with latitude showed not significant correlation. These results were similar to the regularities of polyphenol content and altitude and latitude.

Through the above results could be seen, the more the total polyphenols content and the higher altitude of ETS the stronger antioxidant capacity were, but there were little relationship with the latitude. Moreover, the correlations between the contents of CQAs, rutin and APC with antioxidant capacity were much higher than these of TPC and TFC and antioxidant capacity. This might be because CQAs, rutin and APC were detected with LC, and their accuracy was higher than these of TPC and TFC. After all, the detection principle of TPC and TFC's just use some of the chromomeric properties of polyphenols, while some impurities might cause interference.

### The composition of polyphenols in tobacco leaf

From the previous results and discussion, it could be seen that the higher the altitude, the higher the polyphenols content, but not with the latitude. What is the relationship between the composition of polyphenols and the altitude and latitude? As can be seen from Table 3, the higher the altitude, the greater the rutin proportion (content of rutin/content of APC), but there was no significant relationship with latitude. Overall, rutin proportion with the RP index, TAAC index and DPPH index were highly significant positive correlation or significant positive correlation, and with FRAP index was close to significant positive correlation ($p$=0.057). Based on the previous studies, the effects of polyphenols to protect plants against ultraviolet radiation might be two main aspects (Markham et al., 1998; Kootstra, 1994; Paul and Gwynn-Jones, 2003; Stevanato et al., 2014), first, because it has the ability to absorb ultraviolet light, and second, because of its antioxidant activity can reduce the free radicals caused by UV.

**Table 3: Pearson correlation coefficients (*r*) and corresponding p values for the observed correlation between the polyphenols composition and antioxidant activity assays and the altitude and latitude of the growing site**

| | RP index | FRAP index | DPPH index | AAC index | Altitude | Latitude |
|---|---|---|---|---|---|---|
| Rutin proportion | | | | | | |
| r | 0.578** | 0.290 | 0.357* | 0.518** | 0.427** | 0.145 |
| p | 0.000 | 0.057 | 0.018 | 0.000 | 0.004 | 0.349 |

For the ultraviolet absorption ability, from the view of the structure of polyphenols, chlorogenic acid belong to phenolic acid, the molecular structure contains a benzene ring, a C = C bond and a C = O bond in the conjugated system. Otherwise, rutin, belong to flavonoid, has a cross conjugated system composed of benzoyl conjugated system and cinnamyl conjugated system. Overall, the larger conjugation system, the stronger UV absorbing ability is, moreover, OH groups in the ring B of rutin, the abortion of UV spectra would be systematically red-shifted, and the absorption band would be wider (Anouar et al., 2012; Severino et al., 2009).

For the antioxidant capacity, from the view of the structure of polyphenols, polyphenols have antioxidant activity because of their phenolic hydroxyl groups. Generally speaking, the more phenolic hydroxyl groups, especially the two adjacent hydroxide groups in benzene ring, the stronger the antioxidant activity was (Anouar et al., 2012; Severino et al., 2009; Rzepecka-Stojko et al., 2015), other more, the additional presence of an OH group in ring B at either R3' or R5' could greatly increase the antioxidant capacities of flavonoid (Promden et al., 2014; Severino et al., 2009; Ponomarenko et al., 2014). Chlorogenic acid has two adjacent hydroxide groups and the rutin has four phenolic hydroxyls including two adjacent hydroxide groups. Therefore, the antioxidant activity of rutin is stronger than that of chlorogenic acid.

Therefore, no matter from ultraviolet absorption capacity or antioxidant activity ability, rutin is stronger than that of chlorogenic acid. Plants are sessile organisms and dependent on deployment of secondary metabolites for their response to external stress. A trade-off is envisioned between resources allocated to growth, development, and reproduction and to the biosynthesis, storage, and maintenance of secondary metabolites (Neilson et al., 2013). Plants would synthesize more efficient defensive substances, which might be the cause of the increase of rutin proportion with the increase of altitude (Fig. 1). Similarly, Morales et al. (2010) found that the addition of UV-A and UV-B could induce the increase of polyphenols in Betula pendula leaves, in which the increase of flavonoids was much higher than phenolic acids. Harbaum-Piayda et al. (2010) found that UV-B treat could increase the contents of polyphenols in pak choi (Brassica campestris L. ssp. chinensis var. communis) leaves, the contents increase of flavonoids but not of hydroxycinnamic acids. Martinez-Luscher et al. (2014) found that total and almost all the individual flavonol concentrations in the skins were significantly higher in grapes treated with UV-B, and the molar relative abundance of monosubstituted flavonols and the total accumulated dose of UV-B showed a highly significant correlation. In addition, Liang et al. (2014) found that a high proportion of trihydroxylated flavonols were always found in the higher altitude western region grapes, whereas dihydroxylated flavonols were more prominent in the lower altitude eastern regions.

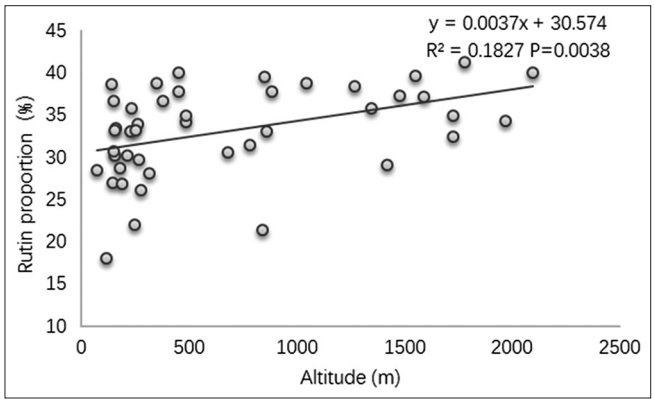


**Fig 1.** Pearson-correlation analysis between rutin proportion and altitude.

## CONCLUSIONS

With the increase of altitude, the content of individual polyphenol, total polyphenols, total flavonoids and their antioxidant activities increased significantly, however, there was no significant corresponding relationship between them and the latitude, over all. Moreover, with the increase of altitude, the rutin proportion of the total polyphenols was increased significantly, that's probably because the UV absorbing ability and antioxidant activity of rutin are better than these of chlorogenic acid.

### Authors' contributions

All authors contributed extensively to the work presented in this article. Zhao M. Q. and Liu P. F. designed experiments; Wang X. L. carried out experiments; Wang F. H., Fu B., and He F. helped to analyze experimental results; Wang X. L. and Liu P.F. wrote the manuscript. All authors read and approved the final manuscript.

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