Anthocyanin essence of the purple pigment and positive correlation of the anthocyanin content and the total ginsenoside content of the root tuber of *Panax notoginseng*

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Abstract: *Panax notoginseng* is"the first medicinal material"in Yunnan of China and *P. notoginseng* produced in the Wenshan eparchy of Yunnan is the Genuine Medicinal Material of *P. notoginseng*. The transverse sections of the root tubers of *P. notoginseng* range from yellowish white to purple. The root tubers with pure purple occupy about 28.21% of the root tubers researched, and their pericycles, endodermises, cortexes or epidermises are purple. Specific color reactions and UV-vis spectra indicated that the purple pigment of the root tuber of *P. notoginseng* belongs to flavonoids, probably holding phenolic *o*-dihydroxyls, excluding carotenoids, chalcones, aurones, isoflavones and catechins. Anthocyanins and/or their aglycones, namely anthocyanidins, underlay the pigmentation of the purple root tuber and other non-red flavonoids function as co-pigments. The average anthocyanin content and the average total ginsenoside content of the root tubers with pure purple are all the highest, next are those of the root tubers with farraginous color of yellow and purple, and the lowest are those of the root tubers with pure yellow, which is consistent with the changing trend of the color hues of the different root tubers. The difference of anthocyanin contents of the root tubers with different colors reaches the most significant level, but the difference of anthocyanin contents does not reach the significant level. Every root tuber contains anthocyanins of different quantity, and along with increase of the anthocyanin content, the purple of the root color basically becomes more and more obvious gradually. The anthocyanin content of the root tuber is positively related with the total ginsenoside content at the significant level and the correlation coefficient(r)is 0.355. This paper can provide a reference for the exploration on the mechanism of the tuber coloration and the identification of the molecular structures of the tuber pigments of *P. notoginseng*.

Key words: *Panax notoginseng*; purple pigment of root tuber; anthocyanin essence; total ginsenoside content; positive correlation

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Belonging to the herb plant of *Panax* genus of Araliaceae family, *Panax notoginseng* is one of the rare Chinese medicinal materials. It is usually named"the supernatural herb of south China", and is the main in-

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gredient of the world-famous "Yunnan Baiyao". Modern iatrical practice has proved that *P. notoginseng* is provided with the remedial functions of different degrees to the diseases of the cordis and cerebral vascular, neural and immune systems, etc, and with the pharmacological activities of anti-inflammation, anti-senescence and anti-tumour (Zheng & Yang, 1999; Hu, 2000; He, 2004). The usage history of *P. notoginseng* in China and abroad has been more than 600 years, but its cultivating history is only about 400 years (Hu, 2000; He, 2004; Cui, *et al.*, 2005).

Belonging to the sub-tropic and alpine shade plant with a limited ecological scope, *P. notoginseng* distributes mostly in the regions with the altitudes from 1 200 to 2 000 m which are located between Yunnan and Guangxi Province of China, and in the neighborhood of the Tropic of cancer. The most concentrative producing region is the Wenshan County, Yanshan County and Maguan County of the Wenshan Eparchy of Yunnan Province. The annual output of the root tuber of *P. notoginseng* in the Wenshan eparchy is approximate 4 000 000 kg, occupying 98% of the total output of China. Furthermore, the quality of *P. notoginseng* in this eparchy is also the best in China, resulting in the fact that *P. notoginseng* is "the first medicinal material" in Yunnan and *P. notoginseng* produced in the Wenshan eparchy is the Genuine Medicinal Material of *P. notoginseng* (Cui, *et al.*, 2005). In Yunnan province, *P. notoginseng* in the Wenshan eparchy is the first Chinese medicinal material which has been authenticated by the Good Agricultural Practice (GAP) of China (Zhang, 2003). In 1995, the Wenshan eparchy was officially denominated "the village of *P. notoginseng* of China". The base-constructing of *P. notoginseng* in the Wenshan eparchy is being carried out according to the Standard Operation Procedure (SOP) of GAP (Wang, *et al.*, 2002; Jin, *et al.*, 2006), and the demonstrating base of *P. notoginseng* has been established more than 2 000 hm^2, presumedly occupying the 30% of the total planting area in the eparchy (Yu, 2005). It is forecasted that, in 2015, the income produced by *P. notoginseng* in the Wenshan eparchy will reach 10 000 millions yuan (Huang, 2003).

The root tuber is the primary medicinal part of *P. notoginseng*. Although the root tuber is usually yellow or yellowish white, a minority of root tubers have been found to be purple. Chen *et al.* (2001) and Sun *et al.* (2003) classified the root tubers of *P. notoginseng* into two categories, namely the green and the purple. The section of the former is green or yellowish green when the root tuber is fresh, and changes to dark green when the root tuber is dried. The section and the peel of the later are purple when the root tuber is fresh, when the root tuber is dried, the peel becomes faint purple, and the section becomes dark purple. Moreover, Chen *et al.* (2001) found that the purple root tuber is provided with a plentiful of red purple substance which the green root tuber does not almost hold, and the medicinal quality of the purple root tuber is better than that of the green root tuber. It is because ginsenoside has been thought to be the main pharmacological incomponent of *P. notoginseng* (Dong *et al.*, 2003; Zhang, *et al.*, 2003), and the total ginsenoside content of the purple root tuber is 48.52% higher than that of the green root tuber (Chen *et al.*, 2001).

However, heretofore, the phytochemical essence of the purple pigment of the root tuber of *P. notoginseng* has not been reported. The relationship between the purple pigment and the total ginsenoside content has not been exploited either. It is well known that the weak ecological adaptability of *P. notoginseng* results directly in the cabined geographical distribution. Researches on the purple pigment of the root tuber can not only reveal profoundly and completely the pharmacological activities of *P. notoginseng*, but also produce probably new insight into the ecological adaptability of *P. notoginseng*, which is of great significance to the industrialization development of *P. notoginseng*.

For the first time, this paper dealt with the phytochemical essence of the purple pigment of the root tuber and the probable correlation of the pigment content and the total ginsenoside content of the root tuber of *P. notoginseng*, providing a reference for the exploration on the mechanism of the root tuber pigmentation and on the integrated pharmacological activities of *P. notoginseng*.

1 Materials and methods

1.1 General

All solvents used were of analytical grade made in China. All color reactions were carried out in capped test tubes and repeated three times. Determinations of the anthocyanin and the total ginsenoside contents of the root tubers were repeated three times too. UV-Vis spectrum was measured at 22 ℃ in a 1 cm pathlength quartz cell in the 200—700 nm range using a Shimadzu-2450 UV-Vis spectrophotometer, and then was photographed by a digital camera (Nikon COOLPIX P5100).

1.2 Plant material

On Oct 14 of 2005, 40 root tubers of *P. notoginseng* were randomly selected and collected from the Huazhuang village of the Matang Town of the Wenshan Eparchy. The hypsography of the fields selected is comparatively complanate, the average altitude is 1 580 m, and the soil belongs to loamy clay. Every root tuber was quickly cleaned with tap water, and the exterior water of the tubers was absorbed entirely by filter papers at once.

1.3 Investigation on the morphological and statistical characteristics of the root tuber coloration

For the 40 root tubers, every one was cut breadthwise into almost two even parts with a unilateral stainless steel blade. The coloration of the transverse section was observed, and photographed by the digital camera.

1.4 Systematic preparatory tests on the pigments of the root tubers with purely purple, farraginous color of yellow and purple, and purely yellow

The two parts of a root tuber prepared in 1.3 were cut lengthways again, respectively, producing two pairs of lengthways homologous parts which were frozen at -20—-22 ℃, and hereinafter used to study pigment and the total ginsenoside content respectively.

2.0 g of the parts of the root tubers with pure purple, farraginous color of yellow and purple and pure yellow were cut up, and ground in a white porcelain pestle quickly and completely at about 15℃ after mixing with about 5 mL of petroleum ether, 10% hydrochloric acid and 25% ammonia solution, respectively. Extracts were filtered immediately (Antian, 1989; Cheng, 2000).

2.0 g of the parts of the root tubers with above color difference were ground quickly and completely at about 15 ℃ after mixing with approximate 15 mL methanol containing 1% concentrated HCL (v/v) (Markham, 1982). Extracts were filtered and the residues were washed till they became full white. The final extracts were diluted to 50 mL with the above acidic methanol. The extracts were refrigerated under 3 ℃ in darkness, and tested with follow reactions respectively (Lin, 1977; An, 1996).

(1) Concentrated HCL-Mg or Zn powder reaction: 2 mL extract was added a little Mg or Zn powder, and shaken to make the powders be submerged completely, then added 5 drops of concentrated HCl, placed quietly for 5 min. (2) $NaBH_4$ reaction: 2 mL extract was added 8 mg $NaBH_4$, added 1 mL of 1.0% HCL again, shaken adequately, and finally placed quietly for 1 h. (3) Paper spot reaction of $AlCl_3$: 5 drops of the extract were dripped on a piece of filter paper, quickly dripped 5 drops of 1.0% $AlCl_3 \cdot 6H_2O$-C_2H_5OH solution (w/v), and churned with a slender glass stick. After being placed quietly for 15—20 min, the spots dried entirely, and were observed under a UV light. (4) Ammoniac $SrCl_2$ reaction: 10 mL CH_3OH was diluted to 25 mL with 25% ammonia solution, producing CH_3OH solution saturated by ammonia. 2 mL extracts were added 10 drops of 0.01 mol/L $SrCl_2 \cdot 6H_2O$-CH_3OH solution (w/v), added 10 drops of the CH_3OH solution saturated by ammonia, shaken adequately, and finally placed quietly for 1 h. (5) $FeCl_3$ reaction: 2 mL extract was added 2 mL of 5.0% $FeCl_3 \cdot 6H_2O$, shaken adequately, and finally placed quietly for 5 min. (6) $Pb(CH_3COO)_2$ reaction: 2 mL extract was added 2 mL of 1.0% $Pb(CH_3COO)_2 \cdot 3H_2O$, shaken adequately, and finally placed quietly for 5 min. (7) H_3BO_3 reaction: 2 mL extract was added 10 drops of 1.0% $H_2C_2O_4 \cdot 2H_2O$, added again 3 mL of 2.0% H_3BO_3, shaken adequately, and finally placed quietly for 5 min. (8) Na_2CO_3 reaction: 2 mL extract was added 2 mL of

5% Na_2CO_3, shaken adequately, and finally placed quietly for 30 min, then ventilated for 10 min. (9) Concentrated H_2SO_4 reaction: 2 mL extract was added 1.5 mL of concentrated H_2SO_4, shaken adequately, and placed in boiling bath for 5 min.

1.5 UV-vis spectra of the root tuber pigment

The pigments of the root tubers with pure purple, farraginous color of yellow and purple and pure yellow were respectively extracted with the methanol containing 1% concentrated HCl(v/v) according to the operation procedure in 1.4(Markham, 1982). Extracts were diluted properly and the UV-Vis spectra were performed as soon as possible using the spectrophotometer.

1.6 Determination of the anthocyanin contents of the root tuber

2.000 g of the root tubers with pure purple, farraginous color of yellow and purple and pure yellow were respectively extracted with the methanol containing 1% concentrated HCL(v/v)(Markham, 1982). The final extracts were diluted to 100 mL with the same acidic methanol. Anthocyanin contents were determined by the method proposed by Rabino & Mancinelli (1986), and worked out by the formula (A_{530}-0.25A_{657})/g(FW).

1.7 Determination of the total ginsenoside content of the root tuber

The root tubers with pure purple, farraginous color of yellow and purple and pure yellow were treated at 105 ℃ for 10 min, and roasted at 80 ℃ till their constant weights, then ground into fine powders which could pass the sieve with the mesh of 80. In a centrifugation tube of 10 mL, being treated by an ultrasonic of 40 000 Hz and 100 W(Ma, *et al.*, 2005), 0.100 g powder was extracted with 2 mL methanol for 40 min. After being centrifugated at 7 000 g for 15 min at 22 ℃, the supernatant was transferred into another centrifugation tube, added the solution of vanillin-acetic acid and perchloric acid, and heated in 60 ℃ water bath for 15 min. Cooled to 22 ℃, the final solution was used to determined A_{560} in the same spectrophotometer. The total ginsenoside content was figure out by the standard curve equation proposed by(Chen *et al.*, 2002).

1.8 Analysis on the correlation of the anthocyanin content and the total ginsenoside content of the root tuber

On the bases of 1.6 and 1.7, the correlation of the anthocyanin content and the total ginsenoside content of the root tuber was analyzed with the software SPSS 11.5.

2 Results and analyses

2.1 Morphological and statistical characteristics of the root tuber coloration of *P. notoginseng*

The transverse sections of the root tubers are yellow, yellowish green, dark yellow or purple(Fig. 1). Thus, the coloration of the transverse section can be thought to range from yellowish white to purple piece by piece. It seems that, if the transverse section becomes from yellow to purple, the pericycle becomes purple first(Fig. 1). As to the purple root tuber, the pericycle, endodermis, cortex or epidermis, sometimes including the primary phloem, are purple(Fig. 1 F).

Being analyzed from the color differences of the transverse sections of the 40 root tubers, 11 can be regarded as purely purple, 9 as purely yellow and other 20 as farraginous color which is mixed by yellow and purple diversely. They occupy 28.21%、48.72% and 23.77% of the 40 root tubers respectively(Fig. 2).

2.2 Specific color reactions of the root tuber pigments of *P. notoginseng*

In the petroleum ether, hydrochloric acid and ammonia solution tests, the root tubers with pure purple, farraginous color of yellow and purple and pure yellow expressed colorless, red of different degrees and greenish yellow of different degrees respectively(Table 1). The yellowish green emerged in the ammonia solution tests should be the result which the blue produced by the reaction of anthocyanins with the ammonia solution mixes with the yellow produced by the reaction of other flavonoids with the ammonia solution. So, the pigments of the root tubers with pure purple, farraginous color of yellow and purple and pure yellow of *P. notoginseng* may belong to flavonoids, excluding carotenoids(Fig. 3 A). The purple may result radically from anthocyanins, and the hue differences of the root tuber

purple may only because of the content differences of the anthocyanins (Fig. 3 B) (Antian, 1989; Cheng, 2000). On the other hand, no red or orange red arose in the ammonia solution reactions of the root tubers with pure purple, farraginous color and pure yellow, suggesting that the root tuber pigments may not contain aurones(Yimidafu, 1985).

The extracts of the root tubers with pure purple, farraginous color and pure yellow prepared by using the methanol containing 1% concentrated HCL(v/v)as solvent are dark mauve, light mauve and faint mauve respectively.

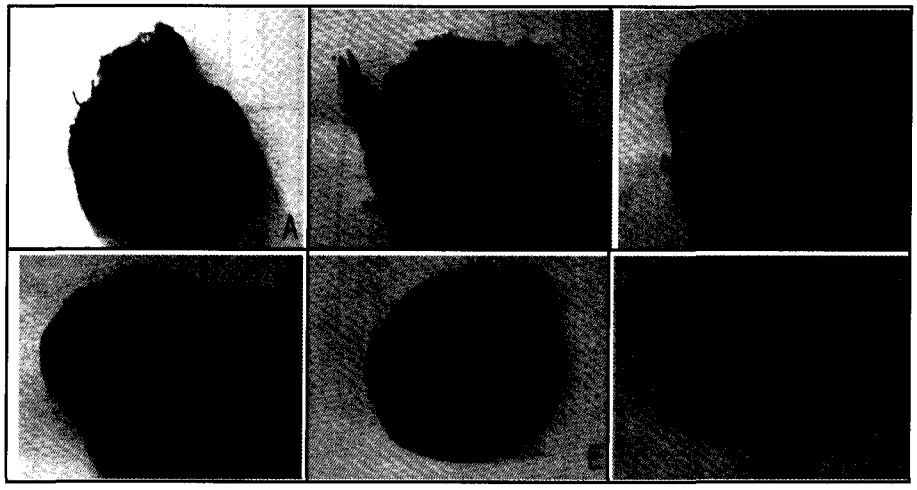

Fig. 1　Coloration of the transverse sections of the root tubers of *P. notoginseng*

From A to F, the transverse section colors of the root tubers are thought to range from yellowish white to purple piece by piece

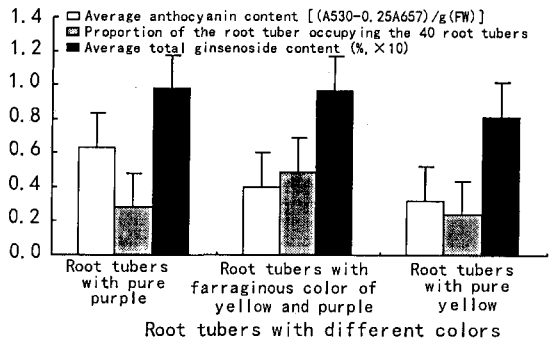

Fig. 2　Average anthocyanin contents, proportions and average total ginsenoside contents of the root tubers of *P. notoginseng* with different colors

In the concentrated HCl-Mg powder reactions, the extracts of the root tubers with pure purple, farraginous color and pure yellow produced spumes which are red of different degree (Table 1). Therefore, the root tuber pigments may contain anthocyanins(and/or their aglycones, namely anthocyanidins) (Fig. 3 B), flavonols, flavanonols and flavanones, excluding isoflavones, chalcones, aurones and catechins. Moreover, the fact that the solutions are almost colorless after the spumes disappear implies that the 3-hydroxyls of the pigment molecules are glycosylation(Lin, 1977; Xiao, 1987).

In the concentrated HCL-Zn powder reactions, the extracts produced the similar results as in the concentrated HCL-Mg powder reactions, showing that the pigments may also contain flavanonols and flavonol-3-O-glycosides, but do not contain flavonols and flavanonol-3-O-glycosides(Lin, 1977; An, 1996).

In the $NaBH_4$ reactions, the extracts produced white turbidity(Table 1), suggesting that the pigments do not contain flavanonols and flavanones (Lin, 1977; Xiao, 1987; An, 1996).

In the paper spot reactions of $AlCl_3$, the spots produced by the extracts with $AlCl_3$ all produced yellow fluorescence of different intensities under UV light(Table 1), suggesting again that the pigments belong to flavonoids(Fig. 3 A), and exclude 4′-hydroxyl flavonols or 7, 4′-two hydroxyls flavonols(Lin, 1977; Xiao, 1987; An, 1996).

In the ammoniac $SrCl_2$ reactions, the extracts produced green precipitate (Table 1), suggesting that the pigment molecules may be provided with o-dihydroxy-

ls, namely 3′, 4′-two hydroxyls (Lin, 1977; Xiao, 1987; An, 1996).

In the $FeCl_3$ reactions, the extracts produced brown of different degrees (Table 1), suggesting that the pigment molecules are consequentially provided with phenolic hydroxyls and the 3 positions do not possess of any dissociative hydroxyls (Lin, 1977; An, 1996).

Table 1 Specific color reactions of the pigments of the root tubers with pure purple, farraginous color and pure yellow of *P. notoginseng*[a]

Reaction reagent	Root tuber		
	Pure purple	Farraginous color[b]	Pure yellow
Petroleum ether	Colorless	Colorless	Colorless
HCL	Pink	Light pink	Faint red
Ammonia solution	Greenish yellow	Light greenish yellow	Faint greenish yellow
Concentrated HCl-Mg powder	Red spume	Pink spume	Faint red spume
Concentrated HCl-Zn powder	Red spume	Pink spume	Faint red spume
$NaBH_4$	White turbidity	White turbidity	White turbidity
$AlCl_3$[c]	Yellow fluorescence	Light yellow fluorescence	Faint yellow fluorescence
Ammoniac $SrCl_2$	Green precipitate	Green precipitate	Green precipitate
$FeCl_3$	Light brown	Light brown	Faint brown
$Pb(CH_3COO)_2$	White precipitate	White turbidity	White turbidity
H_3BO_3	Colorless	Colorless	Colorless
Na_2CO_3	Light yellowish green precipitate	Light yellowish green turbidity	Faint yellowish green turbidity
Concentrated H_2SO_4	Orange red	Light orange red	Faint orange red

a. All color reactions were repeated three times. b. "Farraginous color" indicates that the root tuber color is mixed by yellow and purple diversely. c. Reaction was carried out on a piece of filter paper.

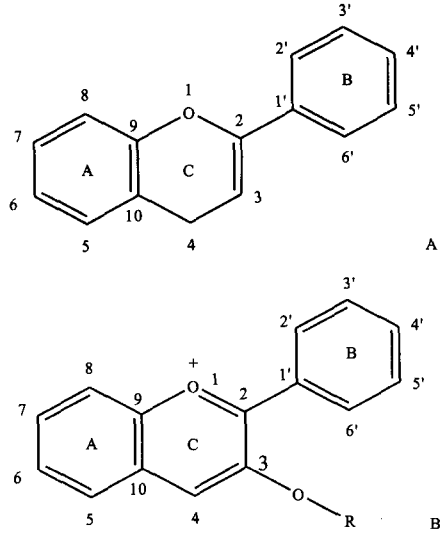

Fig. 3 Structure of the basic framework of flavonoids

A. General framework of flavonoids. B. Framework structure of anthocyanins which belong to flavonoids. "R" stands for the saccharide residue.

In the $Pb(CH_3COO)_2$ reactions, the extracts produced white precipitate or turbidity (Table 1), suggesting that the pigments may hold phenolic *o*-dihydroxyls, and may also hold the structure of "4-C=O, 3-OH" or "4-C=O, 5-OH", excluding chalcones and aurones (Lin, 1977; An, 1996).

In the H_3BO_3 reactions, the extracts expressed colorless (Table 1), suggesting that the pigments may not hold 5-hydroxyl (Lin, 1977; An, 1996).

In the Na_2CO_3 reactions, the extracts produced light yellowish green precipitate or turbidity (Table 1), and after being ventilated, no color changes were observed except a little precipitate emerged, suggesting again that the pigments belong to flavonoids and may hold phenolic or other *o*-dihydroxyls, possibly including flavones and excluding chalcones, aurones, flavonols and flavanones (Lin, 1977; Xiao, 1987; An, 1996).

In the concentrated H_2SO_4 reactions, the extracts produced orange red of different degrees, and after being treated in boiling water bath, the expressed colors did not change (Table 1), suggesting again that the pigments may hold flavones, flavonols and flavanones (Lin, 1977; Xiao, 1987; An, 1996), but excluding isoflavones and isoflavanones which are the characteristic of Leguminosae (Harborne, 1973; Harborne, 1984).

2.3 UV-vis spectra of the root tuber pigments of *P. notoginseng*

For the solutions of the root tubers prepared by using the methanol containing 1% concentrated HCL

(v/v) as solvent, three main absorption peaks, namely at 240—285 nm, 290—330 nm and 530 nm, were observed in the UV-visible spectra of the root tubers with pure purple and farraginous color (Fig. 4 A and B), and two main absorption peaks, namely at 240—285 nm and 290—330 nm, were observed in the spectra of the root tubers with pure yellow (Fig. 4 C).

The basic structures of the purple pigment can be reflected by the UV-vis spectra. The peaks at 240—285 nm and 300—350 nm have proved to be the typical absorptions of Band Ⅱ and Band Ⅰ of flavonoids (Markham, 1982; Tan, 2002). The peaks at 530 nm are the characteristic absorption peak of anthocyanins (Zhang, 1990; Meng *et al.*, 2001). The high intensity of the peaks at 252 or 251 nm show once more that the pigments do not contain chalcones and aurones, the weak shoulder peaks at 320 nm imply the pigments may by acylated by cinnamonic acids, and the second peak of Band Ⅱ between 240 and 285 nm indicates the existence of 3′, 4′-dihydroxyl system (Markham, 1982; Tan, 2002). Thus, the purple of the root tubers of *P. notoginseng* with pure purple and farraginous color should result from anthocyanins and/or their aglycones, namely anthocyanidins, and the light yellow of the root tubers with pure yellow should result from other non-red flavonoids (Zhao, *et al.*, 2005).

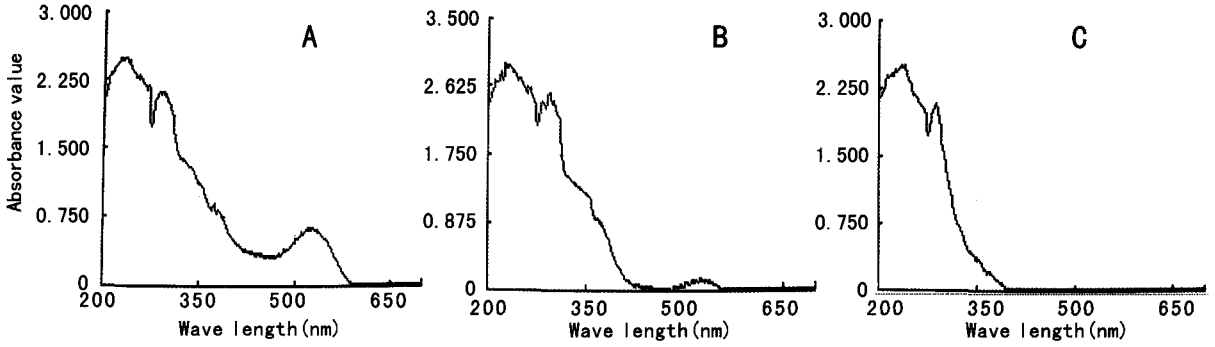

Fig. 4　UV-visible spectra of the root tubers with pure purple, farraginous color and pure yellow of *P. notoginseng*

A is the spectra of "the root tubers with pure purple", B is that of "the root tubers with farraginous color of yellow and purple", and C is that of "the root tubers with pure yellow". The solvent is methanol containing 1% HCL (v/v).

Furthermore, the hue difference of the root tubers of *P. notoginseng* with pure purple, farraginous color of yellow and purple and pure yellow can be reflected by their UV-vis spectra (Fig. 4). The spectrum of the root tubers with pure purple is very similar to that of the root tubers with farraginous color, and the obvious difference is that the absorption peak of the extract of the root tubers with pure purple at 530 nm is much higher than that of the extract of the root tubers with farraginous color (Fig. 4, A and B), which is consistent with the hue differences of the three kinds of root tubers (Fig. 1, 2). The above specific reactions indicated that the root tubers with pure yellow still hold anthocyanins and/or their aglycones, namely anthocyanidins (Table 1), but no peak was found at 530 nm in the spectrum of their extract (Fig. 4, C), which should due to the lower anthocyanins content in the root tubers with pure yellow (Fig. 2), being directly evidenced by the yellow of the root tubers (Fig. 1).

2.4 Anthocyanin and total ginsenoside contents of the root tuber of *P. notoginseng*

The average anthocyanin content and the average total ginsenoside content of the root tubers of *P. notoginseng* with pure purple are all the highest, next are those of the root tubers with farraginous color of yellow and purple, and the lowest are those of the root tubers with pure yellow (Fig. 2), which is consistent with the changing trend of the color hues of the different root tubers (Fig. 1 and 2).

On one hand, the average anthocyanin content of the root tubers with farraginous color or pure yellow is only 63.17% or 50.85% of that of the root tubers with pure purple respectively, and the difference of anthocyanin contents of the root tubers with different colors reaches the most significant level because variance analysis reflected that: F value=117.32>30.82

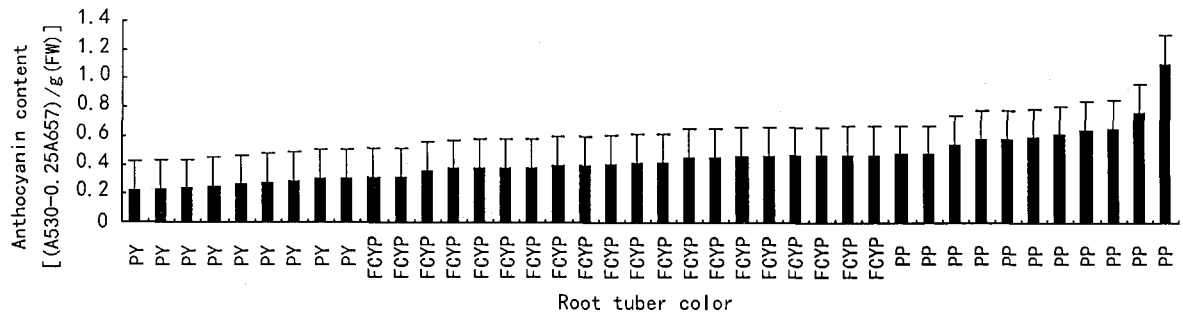

Fig. 5 Aanthocyanin content of the individual root tuber of *P. notoginseng* with different colors
"PY"stands for"pure yellow","FCYP"stands for"farraginous color of yellow and purple"and"PP"stands for "pure purple".

$=F_{0.99(2,3)}$.

In fact, every root tuber of *P. notoginseng* was determined to contain anthocyanins of different quantity. If the aithocyanin contents of the 40 root tubers were arranged from the smallest to the biggest, it was found that, along with increase of the anthocyanin content, the purple of the root color basically becomes more and more obvious gradually, namely from pure yellow to pure purple(Fig. 5). As a result, it is impossible and insignificant that the category-compartmentalizing of the root tubers of *P. notoginseng* is only based on the anthocyanin content value of the individual root tuber. Recognition of human eyes to the hue differences of the root tubers can not exactly reflect the anthocyanin content of the individual root tuber either. On the side, so far, no evidence shows whether the purple-increasing of the root tuber is one of the obvious evolutionary trends of *P. notoginseng*.

On the other hand, the average total ginsenoside content of the root tubers with farraginous color or pure yellow is 98.61% or 83.38% of that of the root tubers with pure purple respectively, and the difference of anthocyanin contents of the root tubers with different colors does not reach the significant level because variance analysis reflected that: F value $=8.42<9.55=F_{0.95(2,3)}$.

2.5 Correlation of the anthocyanin content and the total ginsenoside content of the root tuber of *P. notoginseng*

Analysis of the software SPSS 11.5 indicated that the anthocyanin content of the root tuber of *P. notoginseng* is positively related with its total ginsenoside content at the significant level and the correlation coefficient(r)is 0.355(Table 2).

Table 2 Correlation of the anthocyanin content and the total ginsenoside content of the root tuber of *P. notoginseng* analyzed by the software SPSS 11.5

		Total ginsenoside	Anthocyanin
	Pearson correlation	1	0.355(*)
Total ginsenoside	Sig. (2-tailed)		0.027
	N	39	39
	Pearson Correlation	0.355(*)	1
Anthocyanin	Sig. (2-tailed)	0.027	
	N	39	39

3 Dicussion

P. notoginseng is "the first medicinal material" in Yunnan of China and *P. notoginseng* produced in the Wenshan eparchy of Yunnan is the Genuine Medicinal Material of *P. notoginseng*. The transverse sections of the root tubers of *P. notoginseng* range from yellowish white to purple. The root tubers with pure purple occupy about 28.21% of the root tubers researched, and their pericycles, endodermises, cortexes or epidermises, sometimes including the primary phloems, are purple. Specific color reactions and UV-vis spectra indicated that the purple pigment of the root tuber of *P. notoginseng* belongs to flavonoids, probably holding phenolic o-dihydroxyls, excluding carotenoids, chalcones, aurones, isoflavones and catechins. Anthocyanins and/or their aglycones, namely anthocyanidins, underlay the pigmentation of the purple root tuber and other non-red flavonoids function as co-pigments. The average anthocyanin content and the average total ginsenoside

content of the root tubers with pure purple are all the highest, next are those of the root tubers with farraginous color of yellow and purple, and the lowest are those of the root tubers with pure yellow, which is consistent with the changing trend of the color hues of the different root tubers. The difference of anthocyanin contents of the root tubers with different colors reaches the most significant level, but the difference of anthocyanin contents does not reach the significant level. Every root tuber contains anthocyanins of different quantity, and along with increase of the anthocyanin content, the purple of the root color basically becomes more and more obvious gradually, namely from pure yellow to pure purple. Analysis of the software SPSS 11.5 indicated that the anthocyanin content of the root tuber of *P. notoginseng* is positively related with the total ginsenoside content at the significant level and the correlation coefficient (r) is 0.355.

Specific color reactions have been thought to be a powerful method to verify the existence of certain kind metabolite in plant cells, however, in our research, different color reactions produced inconsistent results. A series of color reactions used in this study are common and specific reactions to substantiate the presence of the flavonoids, including the concrete types of flavonoids, in plants (Lin, 1977; Yimidafu, 1985; Xiao, 1987; An, 1996). It is a great pity that, as to the flavonoids of the root tuber pigment of *P. notoginseng*, these reactions can not yet prove whether flavonols, flavanonols and flavanones exist together with the anthocyanins. Further researches are urgently needed to solve the problem.

The anthocyanin content of the root tuber of *P. notoginseng* is positively related with its total ginsenoside content, but it is totally unknown what the basis for this correlation is. The biosynthesis pathway of anthocyanin has been found to be nothing to do with that of ginsenoside (Tanaka *et al.*, 1998; Chen & Wu, 2004). Contrarily, the biosynthesis pathway of ginsenoside resembles the primary several reactions of the carotenoids biosynthesis (Zhao *et al.*, 2003; Chen & Wu, 2004). Perhaps, the mathematical correlation of the anthocyanin content and the total ginsenoside content is only propitious to the identification of the root tubers of high quality. In general, because it has been confirmed that light is the most primary factor which influences the biosynthesis and decomposition of anthocyanins (Sweeny *et al.*, 1981; Rabino & Mancinelli, 1986; Beckwith *et al.*, 2004), the root tuber appears to be not the synthesizing organ but the accumulating organ of the anthocyanin pigments. So, the synthesis and accumulation mechanisms of anthocyanins in *P. notoginseng* should be the key to understand the biological and ecological significances of the purple of the root tubers to the survival of *P. notoginseng*.

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三七块根紫色素的花色苷本质及其含量和总皂苷含量的正相关性

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摘 要：三七是中国云南省的“第一药材”，云南文山三七是三七的道地药材。三七块根的横截面为黄白色至紫色。紫色块根约占研究块根总数的28.21%，其中柱鞘、内皮层、皮层或表皮为紫色。特征颜色反应和紫外—可见光谱表明：三七块根紫色素属于黄酮类化合物，可能含有酚性邻位二羟基，不含类胡萝卜素、查耳酮、噢哼、异黄酮、儿茶素。花色苷和/或其苷元花色素奠定了紫色块根着色的基础，其他的非红色的黄酮类化合物起共色素的作用。块根的平均花色苷含量和平均总皂苷含量均以纯紫色块根的为最高，其次是黄紫混合色块根的，纯黄色的最低。块根的花色苷含量差异达到极显著水平，但总皂苷含量差异却没有达到显著水平。每个块根都含有不同量的花色苷，随花色苷量的增加，块根的紫色一般逐渐明显。块根的花色苷含量与其总皂苷含量之间呈显著正相关，相关系数 $r=0.355$。本文可为三七块根颜色呈现的机理探索及其色素的分子结构鉴定提供参考。

关键词：三七；块根紫色素；花色苷本质；总皂苷含量；正相关性