Pharmacognostic Identification of the Green Peel of Juglans mandshurica Maxim. from Different Regions and Harvest Times

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Research

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Abstract

Background: The green peel of *Juglans mandshurica* Maxim. (QLY) can be used as medicine, also known as Qinglongyi[1]. The aim of this study was to identify QLY from the different regions and harvest times by pharmacognostic identification.

Methods: In this study, the morphological character, microscopic character and juglone's content of QLY from different regions and harvest times were compared by morphological identification, paraffin section and powder section, physico-chemical identification and High Performance Liquid Chromatography (HPLC).

Results: Morphological identification results that the colour of outer surfaces of peels which harvested from Tangyuan county of Heilongjiang Province in July, August and September are respectively yellow brown, tan and black brown , and the color of the solution graded gradually from light brown yellow to brownish black by water-based test (WBT). The colour of outer surfaces of peels which harvested from Changbai county of Jilin Province in July, August and September are respectively yellowish white, brownish yellow and pale brown, and the color of the solution gradually changed from light brown to dark brown by WBT. As well as the colour of outer surfaces of peels which harvested from Qingyuan county of Liaoning Province in July, August and respectively September are Light yellowish green, light brownish green and brown green, and the color of the solution gradually changed from light green to dark brown by WBT. With the increase of growth period, the number of microscopic characteristics of different Qinglongyi increased. Through physico-chemical identification, it was found that the yield of juglone in QLY picked on September 21, 2016 was the highest, and the content of juglone, whose order was Heilongjiang > Jilin > Liaoning, was as high as 203.476 μg/g.

Conclusions: Through comparison, it is concluded that Heilongjiang Province is one of the high-quality producing areas of QLY, and its best harvest time is in the middle and first ten-day period of September to harvest QLY without softening, yellowing and decay. This experiment provides a theoretical basis for the determination of the best harvest conditions in QLY and the establishment of identification standards for medicinal materials.

Background

Juglans mandshurica Maxim.(JMM) is one of the deciduous trees belongs to Juglans, Juglandaceae[2]. In China, the tree is commonly known as Hutaoqiu, Qiushu, Qiuzishu, Shanhetao and Sanqiuzi, and it is mainly distributed in Heilongjiang, Jilin and Liaoning Provinces of China, North Korea, Russia, etc[3–10]. JMM likes a sunny environment and is commonly found in broad-leaved forest and forest margins. It grows in deep humus, moist and fertile soil, well-drained valley, river alluvial plain or hillsides[11]. It is recorded that QLY is used as medicine, tastes bitter, and is cold. It has the effect of eliminating heat, detoxification, detumescence, check dysentery and improving eyesight[12, 13]. QLY is widely used in folk[14]. It can be used for to treat gastritis, stomachache, digestive tract cancers, bacterial dysentery and

enteritis with remarkable effect, which are made into tea and wine[15–17]. If its fresh peel is mashed into juice for external coating, it also has good curative effect in the treatment of headache and neurodermatitis[18].

In recent years, although some scholars have made a preliminary pharmacognotic identification about it, there are no comparative study on pharmacognosy characteristics and quality evaluation for QLY in different regions and harvest times[19, 20]. Traditional pharmacognosy studies identify medicinal materials from shape, size, surface character, color, texture, fraction, odour, taste, WBT and fire-based test (FBT). Both physical-chemical identification and microscopic identification have the characteristics of good reproducibility and high stability, but it is relatively complicated to identify the same species in different regions and harvest times in daily life[21]. In Chinese herbal medicine market, the most common method for people to identify medicinal materials is macroscopic identification. However, with the variability of region and harvest time, macroscopical identification has been unable to meet the needs. At this time, it is necessary to identify the microstructure and physico-chemical properties of medicinal materials. The combination of these three methods can effectively solve the problem of pharmacognostic identification. Therefore, in this study, pharmacognostic identification will be made for QLY from different origins and harvest times, which provides a scientific theoretical basis for the further development and utilization of QLY and the establishment of identification standard about medicinal materials.

Methods

Plant materials

The materials were collected from Tangyuan County, Heilongjiang Province on July 7, 2016, August 28, 2016 and September 21, 2016 respectively; collected from Changbai County, Jilin Province on July 9, 2016, August 19, 2016 and September 15, 2016; collected on July 15, 2016, August 21, 2016 and September 16, 2016 in Qingyuan County, Liaoning Province. All of them were identified as the fruits of JMM which belongs to Juglans, Juglandaceae by Professor Wang Lihong of Jiamusi University. The peel was peeled and dried at 35 ℃ for 48 h. And after being crushed by pulverizer, QLY passes through a mesh screen with an aperture of 0.25 mm for reserve.

Apparatus

FA2004 analytical balance (Shanghai Hengping Science and Technology Co. Ltd); BP211D precision electronic balance (Sartorius Stedim Biotech GmbH); Chinese herbal medicine pulverizer (Tianjin Texter Instrument Co. Ltd); biological microscope, ruler of microscope, anatomical microscope (Jiamusi University Teaching Equipment); thermostat water bath (Zhengzhou Great Wall Science, Industry and Trace Co. Ltd); Agilent 1100 SeriesHPLC, EclipseXDB-C18 column, Diode array detector and HPLC Autosampler Syringes (Agilent Technologies Co. Ltd).

Reagents

Except methanol (chromatographic grade, Tianjin Kermeo Chemical Reagent Co., Ltd, Tianjin, China), all chemicals used in this study were of analytical grade. All the water used in the study was distilled, obtained from Jiamusi University. Anhydrous ethanol, sodium hydroxide, ether, methanol, acetic acid, isopropanol and phosphoric acid were purchased from Tianjin Kaitong Chemical Reagent Co., Ltd (Tianjin, China). Chloral hydrate was purchased from Shanghai Chemical Reagent Purchase and Supply Wulian Chemical Plant (Shanghai, China). Dilute hydrochloric acid was purchased from Harbin Xinda Chemical Factory (Harbin, China). Medical paraffin was purchased from Shanghai Specimen Model Factory (Shanghai, China). The formaldehyde acetate alcohol (FAA) fixative is used to fix plant tissue. Tissue sections were stained with Safranin and Fast Green. Juglone reference substance (≥ 98%) was purchased from Henan Standard Biological Technology Co., Ltd (Henan, China).

Macroscopical identification of medicinal materials

Visual observation method was used to observe QLY in July, August and September in Tangyuan County, Heilongjiang Province, Changbai County, Jilin Province, and Qingyuan County, Liaoning Province with its shape, color, surface character and fraction[22]. Its odour was smelt. And its size was measured by ruler. After separating the medicinal materials with hands, its texture was identified. WBT and FBT were used to observe other morphological characteristics, and the data were recorded and compared[23].

Microscopical identification of medicinal materials

Fabrication of paraffin sections of QLY

Fixation: After washing the fresh QLY collected in Tangyuan County, Heilongjiang Province in mid-to-late September, it was immersed in the newly prepared FAA fixative. Then put it in refrigerator at 4 ℃ overnight[24].

Dehydration: The peel tissues of JMM were taken out and dehydrated in 50%, 60%, 75%, 80%, 95% ethanol and anhydrous ethanol for 90 min respectively[25].

Transparent: The peel tissues of JMM after dehydration were put in the solution of anhydrous ethanol: xylene (1:1) for 2 h, and then impregnated to xylene[26]. If xylene does not appear turbidity, the validation of dehydration is successful.

Wax impregnation: The transparent tissues was immersed in a covered crucible with xylene: paraffin (3:1, 31 ℃~32 ℃), xylene: paraffin (1:1, 36 ℃), xylene: paraffin (1:3, 44 ℃~45 ℃) and pure paraffin Ⅰ (52 ℃), pure paraffin Ⅱ (54 ℃) and pure paraffin Ⅲ (56 ℃) for 4 h[27].

Embedding: The pre-melted paraffin (58 ℃) was filtered through filter paper to remove impurities, and poured into a folded kraft paper box. The tissue was quickly put into the paraffin in the box with preheated tweezers, and the bubbles around the material in the paraffin were removed. After the paraffin

surface was solidified, it was quickly placed in a container with cold water, so that it was completely submerged in water to cool for 24 h[28].

Section: Took out the embedded paraffin block, removed the surrounding debris, used the blade to form a neat trapezoid, and stuck to a small rectangular block. The block was fixed on the paraffin slicing machine, and the embedded tissue was sectioned after adjusting the angle[29].

Adhesive and baking sheet: The better paraffin sections were screened and put on the glass slide with 1 ~ 2 drops of distilled water. After they were unfolded, used filter paper to absorb excessive moisture. And put the glass slides into the constant temperature drying oven to dry at 38 ℃ for 6 ~ 8 h.

Staining: Put dried paraffin sections respectively in 100 % xylene (10 min)→ 100% xylene Ⅰ (5 min)→ xylene:anhydrous ethanol (2:1, 5 min)→ xylene:anhydrous ethanol (1:1, 5 min)→ xylene: anhydrous ethanol (1:2, 5 min)→ anhydrous ethanol Ⅰ (5 min)→ anhydrous ethanol Ⅱ (5 min)→ 95% ethanol (3 min)→ 90% ethanol (3 min)→ 80% ethanol→ 70% ethanol (3 min)→ 60% ethanol (3 min)→ safranin in 50% ethanol solution (50 min)→ 60% ethanol (3 min)→ 70% ethanol (3 min)→ 90% ethanol (3 min)→ fast green in 95% ethanol solution (50 s)→ 95% ethanol (3 min)→ anhydrous ethanol Ⅰ (3 min)→ anhydrous ethanol Ⅱ (3 min)→ xylene: anhydrous ethanol (1:2, 5 min)→ xylene Ⅰ (5 min) → xylene Ⅱ (10 min), and set aside[30, 31].

Sealing: Quickly added 1 ~ 2 drops of Canadian gum to the glass slide with the paraffin section, covered the coverslip and let it stand horizontally for 24 h to ensure that the tissue sections in the paraffin will not slide. Then placed under a microscope and photographed with an imaging system.

Preparation of temporary slide of QLY's powder

After the proper amount of powder for each period was sifted with a 0.25 mm sieve, the proper amount of powder was placed on a clean microscope slide with a small spoon. Then added 1 drop of chloral hydrate (chloral hydrate was prepared by glycerol as solvent for observation of crystal and transparent tissue packing), the powder was stirred and mixed continuously with tweezers to make the observed cells more clear, and the solution no longer swam to facilitate observation[32]. Finally, the evenly mixed powder and chloral hydrate were covered with cover glass, the peripheral residual liquid was dried with absorbent paper, and then the powder was placed under the microscope to observe the powder characteristics and the size of each characteristic was measured with the ruler of microscope. In each period, 30 pieces of powder temporary slide were made, 8 pieces of slide were taken for specific analysis, and the frequency of each characteristic was observed and recorded under microscope.

Physico-chemical identification of medicinal materials

Took 5.000 g QLY powder of JMM from three regions with 30 mL ethanol. After heating reflux extraction for 40 min, rinsed with cold water until cooled and then performed extraction filtration. 15 mL filtrate were taken and evaporated, added an appropriate amount of 4 % sodium hydroxide to the residue, heated to

dissolve and then filtered. The filtrate was acidified with dilute hydrochloric acid and extracted with ether. Take 1 mL ether extract, add appropriate amount of sodium hydroxide solution, and evaporate the ether. The residue was dissolved with 1.5 mL ethanol, and 5 drops of 9 % magnesium acetate methanol solution were added to observe the color of the solution[20]. (Preparation of magnesium acetate methanol solution: A small amount of magnesium powder was added with appropriate acetic acid. After sufficient reaction, the filtrate of filtration was constant volume with methanol solution).

Preparation of reference solution: The standard juglone was accurately weighed and dissolved with methanol, and the standard solutions with concentrations of 5.09, 10.18 μg/mL, 25.45 μg/mL, 50.9 μg/mL, 101.8 μg/mL and 203.6 μg/mL were prepared[33–35]. The sample was filtered with 0.45 μm millipore filter before injection.

Preparation of sample solution: 1.00 g QLY powder was accurately weighed and extracted by ultrasonic for 40 min at 30.5 ℃, 300W, solid-liquid ratio of 1:14.30 and ethanol volume fraction of 89.80 %. After filtration, the filtrate was centrifuged with 3500 r/min for 15 min, and the supernatant was collected and put into 10 mL volumetric flask. The sample was filtered with 0.45 μm millipore filter before injection[36].

Chromatographic conditions: The chromatographic column was EclipseXDB-C18 column (4.6 mm × 150 mm, 5 μm). Methanol: water (55:45) was used as mobile phase, and pH was adjusted to 3.60 with phosphoric acid (about 0.1%)[37]. The flow rate was 1 mL/min, the detection wavelength was 248 nm, the column temperature was room temperature, and the injection volume was 10 μL[38].

Results

Result of morphological identification of medicinal materials

Tangyuan County, Heilongjiang Province:

 Fresh product: Fresh fruit is oval, its apex is cuspate, and the color of exocarp changes from light green to turquoise to yellow-green in July, August and September. The fresh product is often put into 2 ~ 3 pieces, connected by ridge lines and about 2 ~ 5 mm thick with densely claybank glandular hairs and nonglandular hairs.

 Dried product: Dried product is fleshy peel with rough surface, curled and crinkled inward, and plicated lump or hemispherical shape with broken scar of carpopodium at one end. The diameters in July, August and September are 2.4 ~ 3.5, 4.0 ~ 5.3, 4.2 ~ 6.3 cm long, about 1 ~ 3 cm thick. The outer surface is yellowish-brown, tan and black brown respectively, with a dense layer of claybank glandular hairs whose length are uneven and nonglandular hairs; the inner surface is black gray or black brown and uneven. Dried product is hard but brittle, not easily broken and flat or spiny in section; The odour is fragrant and

the taste is spicy. It floats in water, settles later, is non-viscous, and forms transparent solution of light claybank, dark brown and brownish black respectively; Burning it has a slight aroma with black smoke, and leaving black ash after burning.

Changbai County, Jilin Province:

 Fresh product: Fresh fruit is oval, its apex is slightly cuspate, and the color of exocarp changes from light green to turquoise to yellow-green in July, August and September. The fresh product is often put into 2 ~ 3 pieces, connected by ridge lines and about 2 ~ 5 mm thick with densely brown glandular hairs and nonglandular hairs.

 Dried product: Dried product is fleshy peel with rough surface, curled and crinkled inward, and plicated lump or hemispherical shape with broken scar of carpopodium at one end. The diameters in July, August and September are 3.0 ~ 4.4, 3.5 ~ 4.6, 3.8 ~ 4.9 cm long, about 1 ~ 3 cm thick. The outer surface is yellowish white, claybank and sandy beige respectively, with a dense layer of deep yellow glandular hairs which are shorter and nonglandular hairs; the inner surface is black yellow or furvous and uneven, and the ridge edges are visible intermittently. Dried product is tenacious, not easily broken and flat or spiny in section; The odour is fragrant and the taste is astringent. It floats in water, settles later, is non-viscous, and forms transparent solution of light brown, brown and dark brown respectively; Burning it has a slight aroma with black smoke, and leaving black ash after burning.

Qingyuan County, Liaoning Province:

 Fresh product: Fresh fruit is pyriform or oval, and the color of exocarp changes from light green to turquoise to yellow-green in July, August and September with small white spots. The fresh product is often put into 2 ~ 3 pieces, connected by ridge lines and about 2 ~ 5 mm thick with densely greenyellow glandular hairs and nonglandular hairs.

 Dried product: Dried product is fleshy peel with rough surface, curled and crinkled inward, and plicated lump or hemispherical shape with broken scar of carpopodium at one end. The diameters in July, August and September are 3.0 ~ 4.4, 3.5 ~ 4.6, 3.8 ~ 4.9 cm long, about 1 ~ 3 cm thick. The outer surface is light greenyellow, light breen and breen respectively, with a dense layer of yellow glandular hairs and nonglandular hairs; the inner surface is black yellow or furvous and uneven with yellow nearly powdery substance, and the ridge edges are visible intermittently. Dried product is hard, not easily broken and flat or spiny in section; It is odourless and the taste is astringent. It floats in water, settles later, is non-viscous, and forms transparent solution of light green, breen and dark brown respectively; Burning it has a slight aroma with black smoke, and leaving black ash after burning.

Microscopic identification of medicinal materials

Histological structure of QLY

The exocarp is a row of fine and roughly circular epidermis cells which is 9 ~ 15 μm thick, and covered with a thickness of 6 ~ 9 μm cuticle, densely glandular hairs and nonglandular hairs. The head of glandular hair is composed of 4 ~ 5 cells, and glandular stalk is multicellular; Nonglandular hair is branched. The stoma protrude in the epidermis.The mesocarp is relatively thick, whose thickness reaches 1200 ~ 2300 μm. A few columns of cells on the outside of the mesocarp are roughly circular and closely arranged with thickened cell walls, and there are usually the girdle of sclereids which are composed by 2 ~ 3 columns sclereids in the interior. Sclereids are different, such as roughly circular, oval, polygonal and so on. Most of the other mesocarp cells are roughly circular parenchyma cells. The parenchyma cells are assimilation parenchyma, and contain granular chloroplasts with occasionally small calcium oxalate solitary crystal. In the parenchyma of the mesocarp, there are collateral bundles and scattered secretory cells.

The tissue characteristics of QLY are shown in Fig. 1-1, 1-2, 1-3 and 1-4

Powder characteristics

The powder characteristics of QLY are shown in Fig. 1–5.

The powder characteristics of QLY are nonglandular hair, glandular hair, stone cell, parenchyma cell debris, visible secretory tissue and vessel. There are two or three nonglandular hairs, but most of them exist alone. The adenophore of glandular hair is long and straight, but individually curved. Stone cells are polygonal, irregular, roughly circular and round, and of varying sizes. There are obvious yellowish-brown secretions in the secretory tissues. The vessels are mostly spiral vessels and a few are juxtaposed with annular vessels.

The microscopic characteristics of QLY from each origin and harvest time were as follows:

 July, Heilongjiang: Most of stone cells are oval, but there are also branched stone cells of different sizes, which are divided into two types: wall thinness and wall thickness. There are small pits in the stone cells, and most stone cells accumulate or clumped together. The stone cell is 48 ~ 120 μm long. Parenchyma cells have thin walls, loose arrangement and large vacuole. Most of them are generally assimilation parenchyma and exist in slices. The glandular hair has a head and a petiole, its head consists of 1 ~ 2 cells, and its adenophore is composed of 7 cells. The adenophore is thicker but thinner near the head and 36 ~ 192 μm long. Nonglandular hair is slender, branched and 48 ~ 240 μm in length. The vessels are spiral vessels and 16 ~ 43 μm in diameter. Secretory tissues are mostly oval or branched with secretion function. They become dead and stored cells after being filled with secretions. The quantity statistics of powder characteristics in this period are shown in Table 1-1.

Table 1-1
Statistics on the powder characteristics of QLY (July, Heilongjiang)

Quantity/Name	Stone cell	Parenchyma cell debris	Secretory cavity	Glandular hair	Nonglandular hair	Spiral vessel
Group 1	112	64	6	11	8	19
Group 2	105	46	6	1	8	8
Group 3	112	59	20	9	13	6
Group 4	147	46	13	10	15	9
Group 5	103	71	15	4	6	10
Group 6	153	33	7	11	3	10
Group 7	107	25	12	12	15	21
Group 8	133	43	13	5	4	16

 August, Heilongjiang: Most of the stone cells are oval and globose, and a few are branched. Its cell cavity is dark brown, its laminae is not obvious, and its size is 60 ~ 144 μm. The glandular hairs are mostly composed of 3 ~ 8 cells of different sizes in its adenophore, and the distribution of cells which is 120 ~ 228 μm long are obvious. Glandular hair' s head is slightly cuspidal, and the cell wall has thickening of the phenomenon. Nonglandular hair is more curved and consists of a single cell with darker color and a length of 98 ~ 204 μm. And there is little difference in the size of nonglandular hair in the metaphase. Spiral vessels are mostly distributed within secretory tissue or parenchyma, and a few of them exist individually. The vessel is slightly wider and has a diameter of 24 ~ 52 μm. The secretory tissue is disorganized and dendritic, and the incrassate secretory canal can be observed by rotating fine adujstment. The parenchyma cell walls are thickened and the cells are slightly larger to fill and connect with other tissues. The quantity statistics of powder characteristics in this period are shown in Table 1–2.

Table 1-2
Statistics on the powder characteristics of QLY (August, Heilongjiang)

Quantity/Name	Stone cell	Parenchyma cell debris	Secretory cavity	Glandular hair	Nonglandular hair	Spiral vessel
Group 1	174	17	7	7	6	4
Group 2	105	12	4	18	2	17
Group 3	222	39	11	5	5	20
Group 4	74	20	7	4	0	13
Group 5	137	19	4	12	1	12
Group 6	168	16	7	10	10	6
Group 7	121	43	6	6	6	8
Group 8	136	23	12	12	6	7

 September, Heilongjiang: Most of the stone cells which is 102 ~ 211 μm long are oval or globose, the cell wall is thickened, the cell cavity is light brown, the pit of cell cavity is branched, and the laminated striation is not obvious. Most of the secretory tissues are scattered and obvious thickened. The glandular hairs are relatively long and straight, the color is dark, the head and petiole are composed of 5 ~ 8 cells, the adenophore is narrow, the outer wall is thickened, and the length is 194 ~ 350 μm. The number of nonglandular hairs decreased compared with July and August, but the shape and size did not change, and the length is 148 ~ 258 μm. Parenchyma cells are arranged closely and vacuoles become smaller. Most of the spiral vessels in September are in secretory tissues or in parenchyma cells, and a very few are alone, whose diameter is 29 ~ 72 μm. The quantity statistics of powder characteristics in this period are shown in Table 1–3.

Table 1-3
Statistics on the powder characteristics of QLY (September, Heilongjiang)

Quantity/Name	Stone cell	Parenchyma cell debris	Secretory cavity	Glandular hair	Nonglandular hair	Spiral vessel
Group 1	95	19	2	2	1	2
Group 2	126	22	5	12	0	3
Group 3	184	8	8	12	1	14
Group 4	79	22	5	12	0	4
Group 5	99	9	5	10	1	9
Group 6	94	11	1	18	1	2
Group 7	193	28	8	11	0	21
Group 8	137	17	2	18	2	17

 July, Jilin: The number of stone cells is large, they are oval, dozens of cells accumulate together with small cell cavity, the laminated striation is not obvious, stone cells are small, and it is 25 ~ 53 μm long. Large parenchyma cells constitute assimilation parenchyma, containing a large number of chloroplasts because they are green. The lacuna of secretory cavity is small, and some stone cells and parenchyma cells are also mixed with secretory cavity. The number of nonglandular hairs and glandular hairs is less. The length of glandular hair is 19 ~ 96 μm, and the length of the nonglandular hair is 48 ~ 132 μm, but the nonglandular hair is longer. Most of the vessels in July are combined with secretory cavities and are spiral vessels, with a diameter of 25 ~ 39 μm. The quantity statistics of powder characteristics in this period are shown in Table 1–4.

Table 1-4
Statistics on the powder characteristics of QLY (July, Jilin)

Quantity/Name	Stone cell	Parenchyma cell debris	Secretory cavity	Glandular hair	Nonglandular hair	Spiral vessel
Group 1	283	30	10	2	0	17
Group 2	175	17	6	2	0	42
Group 3	145	11	6	0	1	17
Group 4	172	14	8	0	1	13
Group 5	275	24	6	1	1	18
Group 6	214	22	13	1	0	16
Group 7	177	10	8	1	0	22
Group 8	342	23	7	1	0	20

 August, Jilin: A number of stone cells are oval, small, and mostly in clusters. Many stone cells accumulate together, and the cell cavities are mostly light brown. The number of stone cell is large with 29 ~ 62 μm in length and 19 ~ 36 μm in width. Parenchyma cells are generally assimilation parenchyma. The shapes of the cells are spherical and cylindrical. Usually when viewed under a microscope, the dark brown and light-colored secretory cavities are flickering by rotating fine adujstment. The number of glandular hairs and nonglandular hairs is less. The adenophore of glandular hair is composed ~ 3 ~ 6 cells, and the head of it is usually composed of 6 ~ 8 cells. The head of glandular hair is smaller, the adenophore of glandular hair is curved, and glandular hair is 48 ~ 120 μm long. The nonglandular hairs consist of a single cell, 84 ~ 144 μm in length. The vessels are curved, mostly spiral vessels and occasionally reticulated vessel with a diameter of 34 ~ 60μm. A few crystal fibers appear in the powder. The quantity statistics of powder characteristics in this period are shown in Table 1–5.

Table 1-5
Statistics on the powder characteristics of QLY (August, Jilin)

Quantity/Name	Stone cell	Parenchyma cell debris	Secretory cavity	Glandular hair	Nonglandular hair	Spiral vessel
Group 1	162	21	3	7	1	6
Group 2	92	18	3	2	5	1
Group 3	168	28	8	3	9	19
Group 4	82	5	0	1	8	5
Group 5	193	5	1	3	9	17
Group 6	104	26	2	1	11	7
Group 7	90	4	1	2	6	1
Group 8	145	2	0	2	4	11

 September, Jilin: The powder is black brown, the stone cells are oval and polygonal, the annular striation is more obvious, the pit is bigger, the stone cell wall is thinner, and the length is 36 ~ 72 μm. Parenchyma cells are smaller and the cell wall is thinner. The secretory tissue does not have obvious cell cavity, but the thickened part is obviously visible. Glandular hair is less and 216 ~ 360 μm long. The head of glandular hair is slightly narrowed, and the adenophore of glandular hair consists of 5 ~ 8 cells. Nonglandular hairs are slightly curved and branched, whose cell wall are not thickened and branches are 120 ~ 288 μm long. Spiral vessels are mostly alone with a diameter of 42 ~ 72μm. The quantity statistics of powder characteristics in this period are shown in Table 1–6.

Table 1-6
Statistics on the powder characteristics of QLY (September, Jilin)

Quantity/Name	Stone cell	Parenchyma cell debris	Secretory cavity	Glandular hair	Nonglandular hair	Spiral vessel
Group 1	197	9	4	0	2	14
Group 2	183	6	5	7	1	8
Group 3	144	4	3	5	0	11
Group 4	276	7	8	3	6	13
Group 5	124	4	3	3	6	16
Group 6	204	10	2	4	3	22
Group 7	217	4	6	4	2	21
Group 8	159	9	4	6	4	13

 July, Liaoning: Stone cells are smaller than those form the origins in Heilongjiang Province and Jilin Province. There is a brown pit in the cell cavity. The cell laminated striation is branched. The cell is 24 ~ 62 μm long. Parenchyma cells are large and polygonal. The cell wall of secretory cavity is obviously thickened and the color is darker. Glandular hair is long. The adenophore of glandular hair consists of 5 ~ 8 cells and is slightly thickened, whose cell distribution is obvious. The head of glandular hair is small with 69 ~ 204 μm in length. The number of nonglandular hairs in July is less, mostly curved, composed of single cell, and 37 ~ 168 μm long. The vessels are mostly spiral vessels, the diameter is 24 ~ 47 μm and cluster crystals are seen occasionally in the vessel. The quantity statistics of powder characteristics in this period are shown in Table 1–7.

Table 1-7
Statistics on the powder characteristics of QLY (July, Liaoning)

Quantity/Name	Stone cell	Parenchyma cell debris	Secretory cavity	Glandular hair	Nonglandular hair	Spiral vessel
Group 1	69	25	8	4	0	4
Group 2	60	12	17	4	0	5
Group 3	70	21	27	6	0	6
Group 4	154	2	1	7	3	0
Group 5	93	1	4	2	0	7
Group 6	104	4	5	1	0	13
Group 7	67	7	6	1	4	3
Group 8	68	7	5	2	6	2

 August, Liaoning: Stone cells are of different shapes and 31 ~ 96 μm long, with large cells, darkened cell cavity, unconspicuous annular striation, and cell wall of uneven thickening. Parenchyma cells are larger with slightly thickened cell walls. The secretory cavity is yellowish-brown. The nonglandular hairs are relatively wide, the walls are thickened and the cells are 55 ~ 329 μm long. The length and size of glandular hair are basically the same as that in July, but the color of glandular hair becomes lighter in color, and the cell cavity of adenophore is black brown. Most of adenophore are composed of 3 ~ 5 cells. The length of glandular hair is 84 ~ 254 μm. Spiral vessel is slightly curved, mostly 3 ~ 5 vessels side by side, and 17 ~ 55μm in diameter. The quantity statistics of powder characteristics in this period are shown in Table 1–8.

Table 1-8
Statistics on the powder characteristics of QLY (August, Liaoning)

Quantity/Name	Stone cell	Parenchyma cell debris	Secretory cavity	Glandular hair	Nonglandular hair	Spiral vessel
Group 1	253	2	1	4	4	4
Group 2	438	5	1	15	14	14
Group 3	687	4	1	15	12	24
Group 4	330	1	1	5	3	3
Group 5	293	5	7	5	3	2
Group 6	175	4	5	2	1	1
Group 7	271	9	4	3	2	5
Group 8	291	7	4	3	2	5

 September, Liaoning: Stone cells become smaller, the cell cavity is black brown, the cell pit is obvious, and the length is 49 ~ 114 μm. Parenchyma cells are smaller. The secretory cavity changed from dark brown in August to grayish brown in September, and the thickened part of the cell wall was observed by rotating the fine adujstment. The head of glandular hair is obvious in September, which is composed of many small cells. The adenophore of glandular hair is composed of 5 ~ 10 cells, the cell wall of adenophore is thickened obviously, and the length of glandular hair is 96 ~ 336 μm. The cells of nonglandular hairs are blackened, the cell walls are thickened, and the length is 65 ~ 336 μm. In this period, the wall of spiral vessel is thickened obviously, and there are scattered secretory cells around the vessel with darker color, some of which are scattered in the secretory tissue with a diameter of 24 ~ 64 μm. The quantity statistics of powder characteristics in this period are shown in Table 1–9.

Table 1-9
Statistics on the powder characteristics of QLY (September, Liaoning)

Quantity/Name	Stone cell	Parenchyma cell debris	Secretory cavity	Glandular hair	Nonglandular hair	Spiral vessel
Group 1	43	8	6	1	0	10
Group 2	63	12	3	5	0	9
Group 3	102	22	7	6	0	9
Group 4	58	22	3	2	0	5
Group 5	70	13	2	9	1	8
Group 6	43	38	6	2	0	9
Group 7	38	6	2	3	2	8
Group 8	155	4	4	7	2	11

The average quantity statistics of the powder characteristics of QLY in July, August and September in Heilongjiang, Jilin and Liaoning provinces are shown in Table 1–10, and the statistics on the powder characteristics size of QLY in different origins and harvest times are shown in Table 1–11.

Tab. 1-10

Quantity statistics of the powder characteristics of QLY in different origins and harvest times

Quantity/Name	Stone cell	Parenchyma cell debris	Secretory cavity	Glandular hair	Nonglandular hair	Spiral vessel
July, Heilongjiang	122	48	12	8	9	12
August, Heilongjiang	142	24	7	9	5	11
September, Heilongjiang	126	17	5	12	1	9
July, Jilin	223	18	8	1	1	21
August, Jilin	130	14	3	3	7	8
September, Jilin	188	7	4	4	3	15
July, Liaoning	86	10	9	3	2	5
August, Liaoning	342	5	3	7	5	7
September, Liaoning	72	16	4	4	1	9

Table. 1-11

Statistics on the powder characteristics size of QLY in different origins and harvest times

Quantity/Name	Stone cell/ μm	Glandular hair/ μm	Nonglandular hair/ μm	Spiral vessel/ μm
July, Heilongjiang	48~120	36~192	48~240	16~43
August, Heilongjiang	60~144	120~228	98~204	24~52
September, Heilongjiang	102~211	194~350	148~258	29~72
July, Jilin	24~53	19~96	48~132	25~39
August, Jilin	29~62	48~120	84~144	34~60
September, Jilin	36~72	216~360	120~288	42~72
July, Liaoning	24~62	65~204	37~168	24~47
August, Liaoning	31~96	84~254	55~329	17~55
September, Liaoning	49~114	96~336	65~336	24~64

The statistics on the powder characteristics size of QLY in different origins and harvest times are shown in Figure 1-6.

Physico-chemical identification of medicinal materials

The reaction between the QLY's powder extract from three places of origin and magnesium ion was red, which proved that the powder from all producing areas contained quinones.

Taking the mass concentration of juglone (μg/mL) as the abscissa and the chromatographic peak area (mAu) as the ordinate, and the standard curve of juglone was drawn. The standard curve data of juglone were shown in Table 3 − 2, the standard curve of juglone was shown in Fig. 3 − 1, and Fig. 3 − 2 showed the high performance liquid chromatography of the reference substance of jujube. The regression equation was Y = 38.10X-172.6, r^2 = 0.9991, which showed that there was a good linear relationship between the injection concentration of juglone and the peak area in the range of 5.09 ~ 203.6 μg/mL. The comparison of the yield of juglone in different periods was shown in Table 3–3. The results showed that the content of juglone in QLY from the same area was in September > August > July; and the content of juglone in QLY at the same harvest time was produced in Heilongjiang > Jilin > Liaoning. It is speculated that with the growth and development of the fruit of JMM, the content of juglone continues to accumulate, and the accumulation of juglone will reach the maximum from the middle of September to the end of September.

Table 1-12
Juglone Standard Curve Data

C μg/mL	5.09	10.18	25.45	50.9	101.8	203.6
mAu	21.329	215.258	797.045	1766.69	3705.98	7584.56

Table 1-13
Determination of the content of juglone in QLY in different producing areas and harvesting

Origin	Harvest Times	Peak Area/mAu	Juglone Yield/μg.g^{-1}
Qingyuan, Liaoning	2016.7.15	59.479	6.090
Qingyuan, Liaoning	2016.8.21	173.184	9.075
Qingyuan, Liaoning	2016.9.16	253.676	11.188
Changbai, Jilin	2016.7.9	47.270	5.771
Changbai, Jilin	2016.8.19	146.041	8.363
Changbai, Jilin	2016.9.15	321.427	12.967
Tangyuan, Heilongjiang	2016.7.7	296.472	12.312
Tangyuan, Heilongjiang	2016.8.28	549.256	18.946
Tangyuan, Heilongjiang	2016.9.21	7579.836	203.476

Discussion

In the process of macroscopical identification, the respective characteristics of its endocarp and exocarp softened by water should be observed under an anatomical microscope because the dried QLY will curl and corrugate. At the same time, the rational use of the magnification of the anatomical microscope is helpful to the observation of the characteristics.

In the process of making paraffin slides of QLY, fixation and dehydration should be adjusted according to the degree of softness and hardness of QLY after soaking in FAA stationary liquid. After dehydration, it was impregnated with xylene. If xylene becomes turbid, it will prove that dehydration is incomplete and we need to redehydration. When soaking the paraffin, it is necessary to adjust its time properly, so that QLY's tissue is soaked in completely. After safranin dyed it takes about 20 minutes for luminous red to be observed under the microscope. The dyeing time of fast green is suitable for 15 ~ 60 s, and insufficient dyeing time will lead to uneven dyeing of QLY so that the dyeing time should be sufficient.

Conclusion

In macroscopical identification, the fresh fruit of JMM is ellipse. The tip of QLY in Heilongjiang is the most cuspidal. Glandular hairs of QLY form Heilongjiang are claybank, dense and long. Glandular hairs from Jilin are brown and moderate density. Glandular hairs from Liaoning are greenyellow, short and sparse. Dried QLY is the largest in Heilongjiang, the least in Liaoning, and medium in Jilin. QLY picked in July, August and September in Tangyuan County, Heilongjiang Province were all floating in water, later settling, non-viscous, and forming light claybank, dark brown and brownish black transparent solutions respectively. The WBT's results of QLY collected in July, August and September in Changbai County, Jilin Province show that they are floating in water, later settling, non-viscous, and forming light brown, brown and dark brown transparent solutions respectively. The results of WBT of QLY collected in July, August and September in Qingyuan County, Liaoning Province show that they are floating in water, later settling, non-viscous, and forming light green, breen and dark brown transparent solutions respectively. In addition, the solution color of QLY, after WBT, in September, Heilongjiang Province is the deepest, which is brownish black; Burning them all has a slight aroma with black smoke, and leaving black ash after burning.

The characteristics of histological structure: Exocarp had dense glandular hair and nonglandular hair, mesocarp had girdle composed of 2 ~ 3 columns stone cells, visible secretory cavity and scattered vascular bundle.

In powder characteristics, among QLY of three origins at different harvest times, the number of nonglandular hair and secretory cavity of the powder was the most in July, Heilongjiang Province. In September, Heilongjiang Province, the number of stone cells and secretory cells of the powder was the

largest. In each harvest time of Jilin Province, the stone cells were smaller, and the glandular hairs of QLY in September were the longest, but the number was less. In August, Liaoning Province, the number of stone cells of QLY was the largest but it was smaller; In September, Liaoning Province, the nonglandular hairs were longer than those in other origins, but the number of non-glandular hairs was the least. The quantity of microscopic characteristics of the powder in each origin and harvest time was different. With the growth and development of the fruit, the organizational characteristics of three origins increased with the growth period.

As an important chemical component in QLY, juglone has analgesic, anti-tumor, antibacterial and other effects, especially significant anti-tumor effects, and has great potential for development. Therefore, the content of juglone can be used as a main index to screen the high quality region and harvest time of QLY. The content of this experiment showed that the yield of juglone was the highest QLY picked in Heilongjiang on September 21, 2016. Therefore, Heilongjiang Province is one of the high-quality producing areas of QLY, and the best harvest time is in the middle and first ten days of September every year to harvest QLY which is not softened, yellowed and rotten.

To sum up, the authenticity of QLY collected from three origins and harvest times in Heilongjiang, Jilin, Liaoning can be identified by the identification of crude drug, and QLY can be identified by its trait, powder and physico-chemical characteristics in different origins and harvest times.

Abbreviations

QLY: The green peel of *Juglans mandshurica* Maxim.; HPLC: High Performance Liquid Chromatography; WBT: Water-based test; FBT: Fire-based test; FMM: *Juglans mandshurica* Maxim.; FAA: Formaldehyde acetate alcohol.

Declarations

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Authors’ contributions

YY and LW designed the study. CW and JC collected the herbs. CW carried out partial physico-chemical identification. JC carried out the macroscopic, microscopic and physico-chemical identification. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

We declare that the Publisher has the Author’s permission to publish the relevant Contribution.

References

1. Cao LJ, Xv Z, Chen ZY, Li J, Zhao SL. Research on the Chemical Components and Pharmaceutical Actions of Walnut Green Husk. Hubei Agricultural Sciences. 2016;55(18):4629–33 + 4663.

2. Editorial Committee of Chinese Flora. Chinese Academy of Sciences. Flora of China. Beijing Science Publishing House. 1979;21:32–3.
3. Son JK. Isolation and structure determination of a new tetralone glucoside from the roots of Juglans mandshurica. Arch Pharm Res (Seoul). 1995;18:203–5.
4. Machida K, Matsuoka E, Kasahara T, Kikuchi M. Studies on the constituents of Juglans species. I. Structural determination of (4S)- and (4R)-4- hydroxy-alpha-tetralone derivatives from the fruit of Juglans mandshurica MAXIM. var. sieboldiana. MAKINO Chem Pharm Bull. 2005;53:934–7.
5. Bai WN, Liao WJ, Zhang DY. Nuclear and chloroplast DNA phylogeography reveal two refuge areas with asymmetrical gene flow in a temperate walnut tree from East Asia. New Phytol. 2010;188:892–901.
6. Wang RJ, Wang S, Xia YJ, Tu M, Zhang LJ, Wang YM. Antitumor effects and immune regulation activities of a purified polysaccharide extracted from Juglan regia. Int J Biol Macromol. 2015;72:771–5.
7. Hu Z, Zhang T, Gao XX, Wang Y, Zhang Q, Zhou HJ, et al. De novo assembly and characterization of the leaf, bud, and fruit transcriptome from the vulnerable tree Juglans mandshurica for the development of 20 new microsatellite markers using Illumina sequencing. 2016;291:849–862.
8. Hu Z, Zhang T, Gao XX, Wang Y, Zhang Q, Zhou HJ, et al. De novo assembly and characterization of the leaf, bud, and fruit transcriptome from the vulnerable tree Juglans mandshurica for the development of 20 new microsatellite markers using Illumina sequencing. 2016;291:849–862.
9. Zhao P, Zhou HJ, Potter D, Hu YH, Feng XJ, Dang M, et al. Population genetics, phylogenomics and hybrid speciation of Juglans in China determined from whole chloroplast genomes, transcriptomes, and genotyping-bysequencing (GBS). Mol Phylogenet Evol. 2018;126:250–65.
10. Zhao Y, Zhou W, Diao SB, Jiang Z, Jin M, Li G. Phytochemical investigation on the roots of Juglans mandshurica and their chemotaxonomic significance. Biochem Systemat Ecol. 2019;87:103957.
11. Institute of Forestry and soil, Chinese Academy of Sciences. Primary medical herbs in the northeast of China. Heilongjiang science and technology press. 1989:207–209.
12. Zhu FQ, Liang YM, Xv L, Wang YF, Ding JL, Kang TG. Study on Manchu Medicine Juglans mandshurica Herbal Textual Research and DNA Barcoding Identification. Journal of Chinese Medicinal Materials. 2018;41(9):2073–8.
13. Li J, Xu KP, Zou ZX, Zou H, Long HP, Tan LH, et al. Two new compounds from the green peel of Juglans mandshurica. J Asian Nat Prod Res. 2017;19(11):1087–92.
14. Chen G, Pi XM, Yu CY. A new naphthalenone isolated from the green walnut husks of Juglans mandshurica Maxim. Formerly Natural Product Letters. 2015;29(2):174–9.
15. Jin M, Diao SB, Zhang CH, Cao S, Sun JF, Zhe RL. Two new diarylheptanoids isolated from the roots of Juglans mandshurica. Natural Product Research: Formerly Natural Product Letters. 2015;29(19):1839–44.
16. Luan F, Wang Z, Yang Y, Ji Y, Lv H, Han K, et al. Juglans mandshurica Maxim.: A Review of Its Traditional Usages, Phytochemical Constituents, and Pharmacological Properties. Front Pharmacol.

2021;21(11):1–31.
17. Jin M, Sun J, Li R, Diao S, Zhang C, Cui J, et al. Two new quinones from the roots of Juglans mandshurica. Arch Pharm Res. 2016;39(9):1237–41.
18. Jiangsu New Medical College. Chinese Medicine Dictionary (I). Shanghai Science and Technical Publishers. 1744.
19. Wang SL, Wang SD, Liu JF. The study on dentification of pharmacognosy and anti-neoplasm of Juglans mandshurica. Journal of Pharmaceutical Practice. 1995;13(1):40–2.
20. Zhao YQ, Jin Y. Pharmaceutical Identification of Green pericarp of Juglans mandshurica. LIAONING PHARMACY CLINICAL REMEDIES. 2002;5(129):101.
21. Chen KL, Huang LF, Liu YM. Development history of methodology of Chinese medicines' authentication. Zhongguo Zhong Yao Za Zhi. 2014;39(07):1203–8.
22. Wang TM, Xv SZ, Yv WJ, Di X, Zhang H, Zhai YJ, et al. Morphological study on Juglans mandshurica. Chinese Archives of Traditional Chinese Medicine. 2018;36(03):675–7.
23. Li YF. Study on Pharmacognosy Identification, Composition Analysis and Anti-inflammatory and Bacteriostatic Effects of Raccoon Dog Bile. Jilin Agricultural University; 2019.
24. Qin GH, Liu CY, Xv YL, Li YL, Qi YJ, Pan HF, et al. Microstructure Observation of Seed Coats and Its Development of Pomegranate (Punica granatum L.). Chinese Journal of Tropical Crops. 2018;39(3):489–93.
25. Gao DJ, Bao WM, Chen Y, Pan JS, Zhang WW. Paraffin Section Technology of Fruit Tumor Tissues in Cucumber (Cucumissativus L.). Molecular Plant Breeding. 2020;18(21):7143–8.
26. Wang YF, Ye Z, Liu H, Liu QL, Zhang B, Hao YY. Studies of paraffin section manufaction for apple fruits in different development stages. Journal of Fruit Science. 2014;31(5):973–6.
27. Wang YH, Liu HM, Liu X, Yang H, Li R, Huang P. Study on Preparation and Structural Observation of Paraffin Setions of Three Kinds of Rhodiola. Seed. 2018;37(7):28–31.
28. Sadeghipour A, Babaheidarian P. Making Formalin-Fixed, Paraffin Embedded Blocks. Methods Mol Biol. 2019;1897:253–68.
29. Sy J, Ang LC. Microtomy. Cutting Formalin-Fixed, Paraffin-Embedded Sections. Methods Mol Biol. 2019;1897:269–78.
30. Zaqout S, Becker LL, Kaindl AM. Immunofluorescence Staining of Paraffin Sections Step by Step. Front Neuroanat. 2020;14:582218.
31. Li XL, Shao AJ, Zhan XR, Wei Y, Huang LQ. Study on microscopic identification of Astragalus complanatus and A. adsurgens seeds. Zhongguo Zhong Yao Za Zhi. 2015;40(7):1271–3.
32. Li MM. Study on the Macroscopic and Microscopic Characteristics of Centellae Herba. Asia-Pacific Traditional Medicine. 2020;16(07):65–7.
33. Huo JH, Zhang HY, Wang WM. Simultaneous Determination of Contents of Juglone and Regiolone in Juglans mandshurica by Quantitative Analysis of Multi-components by Single-maker. China Pharmacy. 2017;28(3):380–3.

34. Li WJ, Wang CZ, Liu CJ. Extraction and Antimicrobial Activity of Juglone from Pericarpium Juglandis. Chem Indus For Prod. 2019;39(5):95–100.
35. Pan WD, Hu YT, Hu X, Wang QM. Smimultaneous UFLC - MS/MS Quantitation of Chemical Component in Different Part of Juglans Mandshurica Maxim. Chinese Archives of Traditional Chinese Medicine. 2020;38(9):195–8.
36. Li FR, Shi WF, Cheng GL, Zhang JY. Determination of Juglone in the green peel of Juglans Mandshurica Maxim. by HPLC. Journal of Pharmaceutical Research. 2007;4(12):164–5.
37. Wang WZ, Liu HZ, Liu SY, Cai MY. Study on Extraction Process of Juglone in Green Husks of Juglans mandshurica. Northern Horticulture. 2013;(15):153–157.
38. Wang WZ. Study on four kinds of chemical compositions in Juglans mandshurica Green husks. Jilin Agricultural University; 2013.

Figures

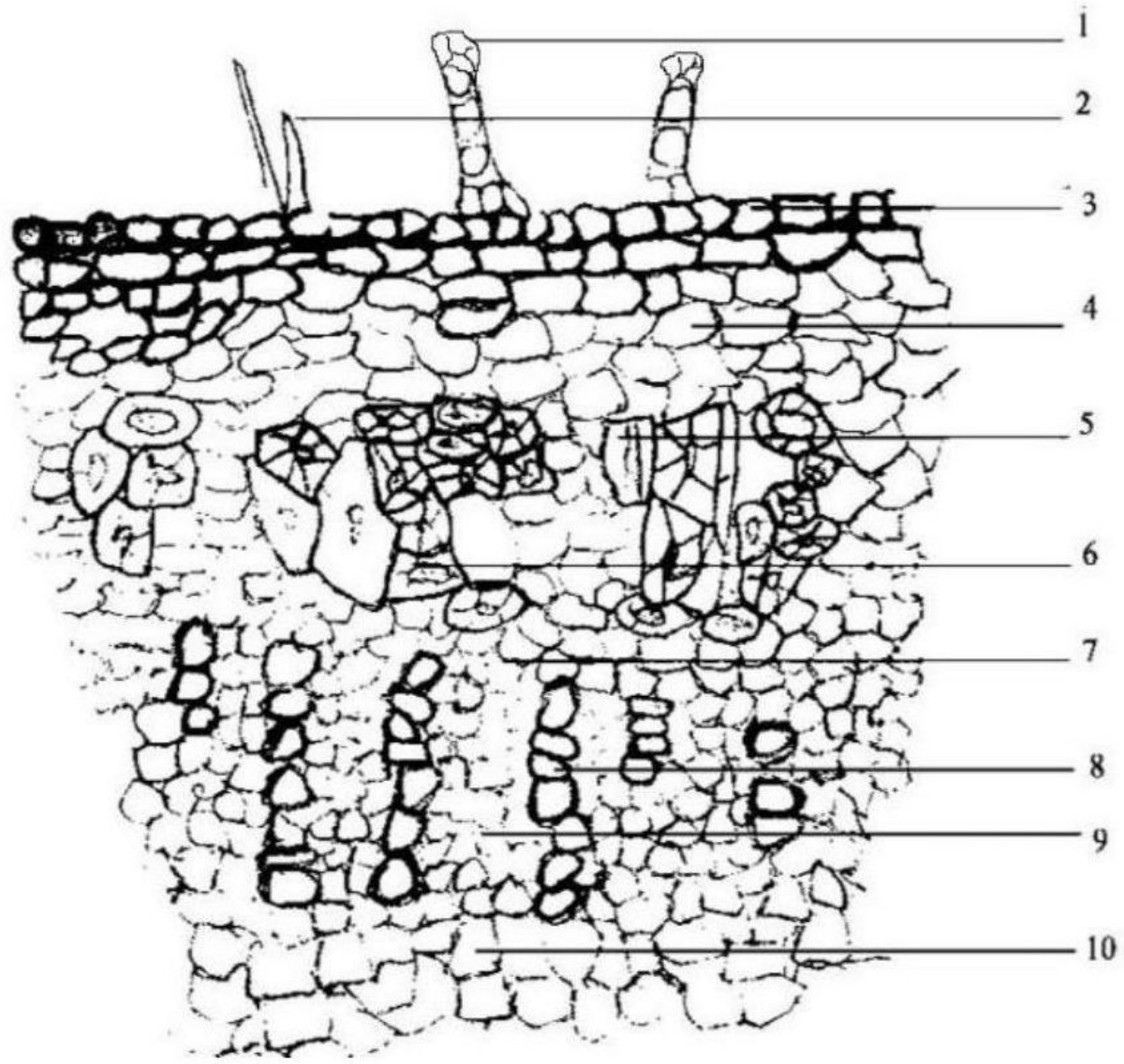

Figure 1

1-1 Partial detail drawing of transverse section of QLY (10×40) 1 glandular hair; 2 nonglandular hair; 3 epicarp; 4 mesocarp; 5 secretory cells; 6 stone cell; 7 phloem; 8,9 xylem; 10 parenchyma cells

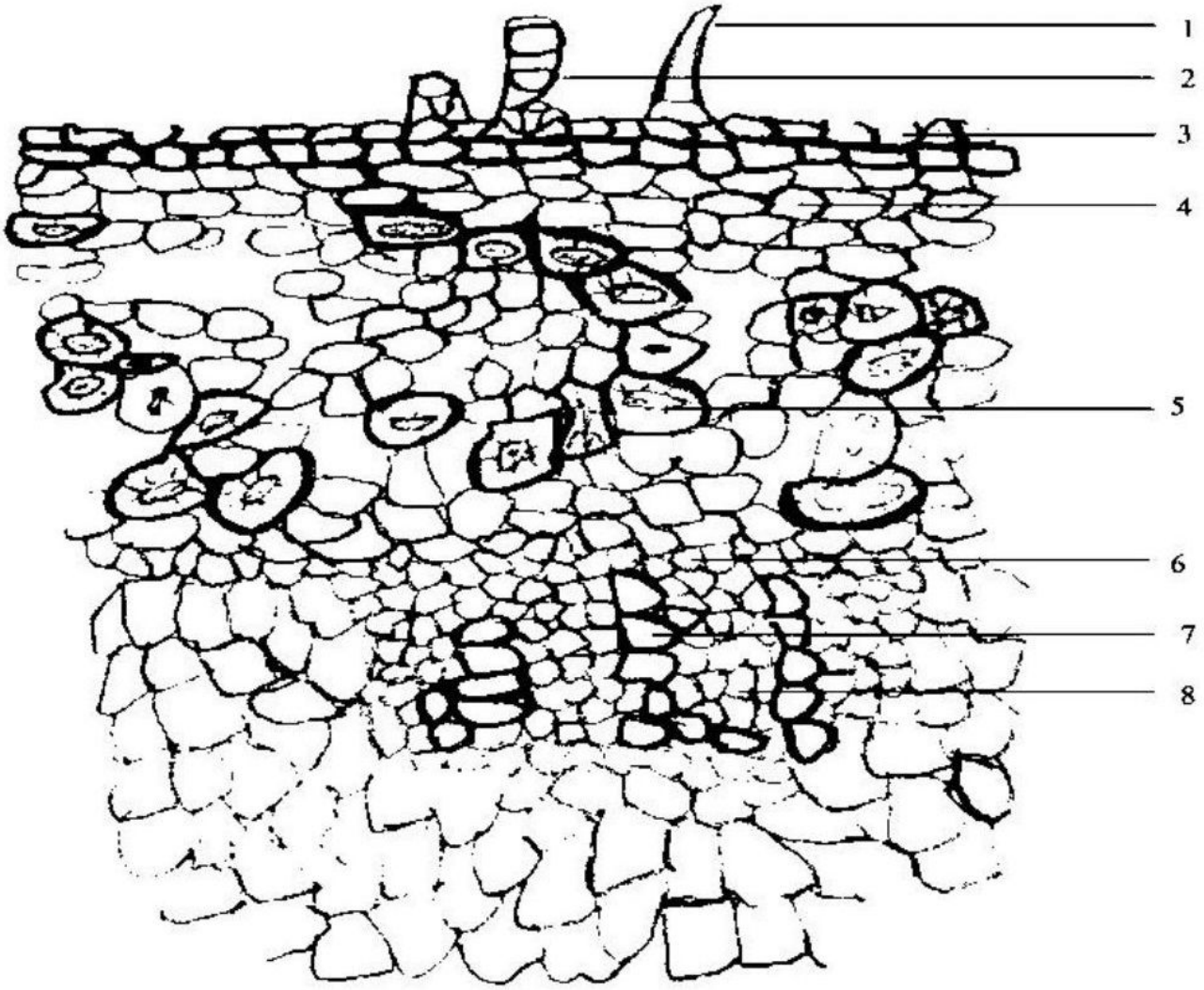

Figure 2

1-2 Partial detail drawing of longitudinal section of QLY (10×40) 1 nonglandular hair; 2 glandular hair; 3 epicarp; 4 mesocarp; 5 stone cell; 6 phloem; 7,8 xylem

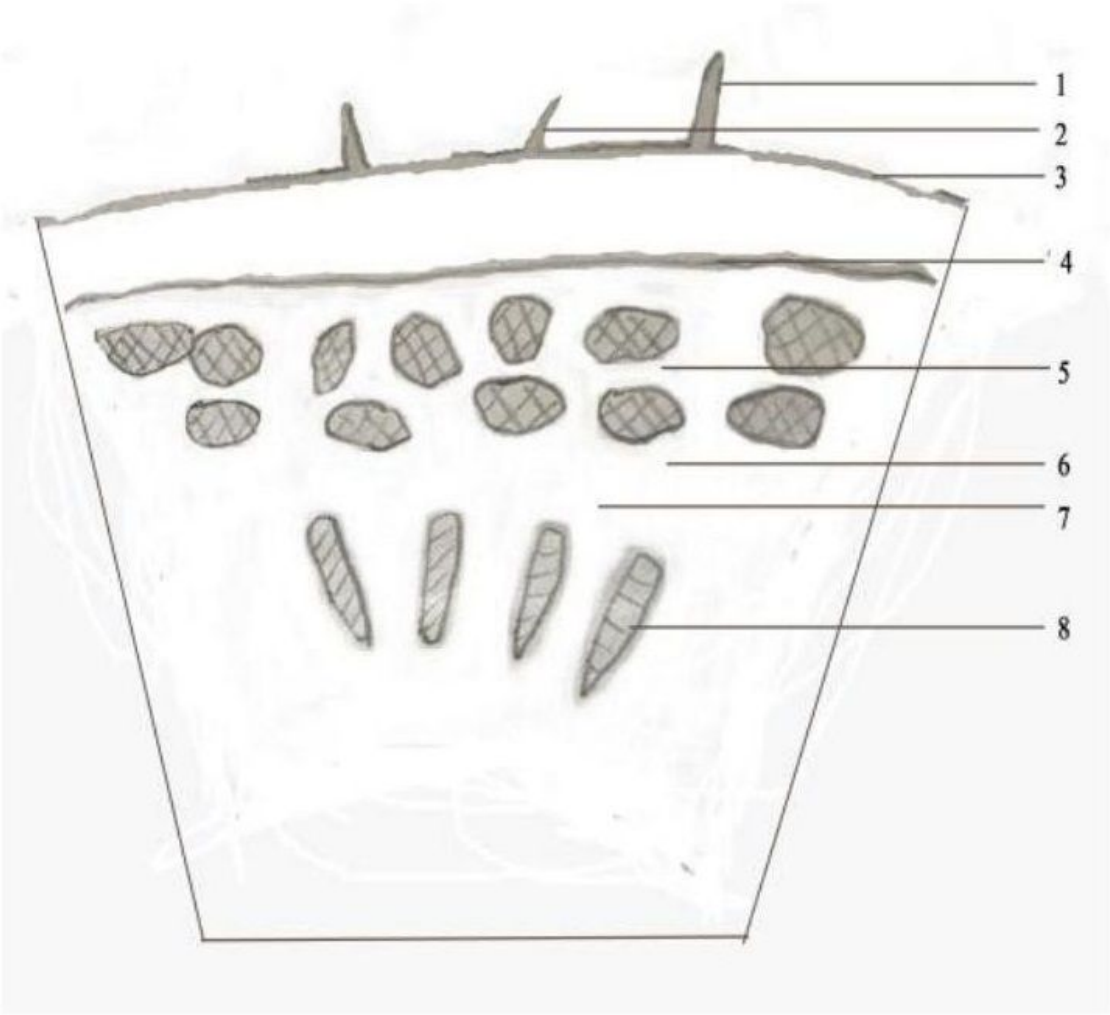

Figure 3

1-3 Partial sketch of transverse section of QLY (10×10) 1 Nonglandular hair; 2 glandular hair; 3 exocarp; 4 mesocarp; 5 stone cells; 6 phloem; 7,8 xylem

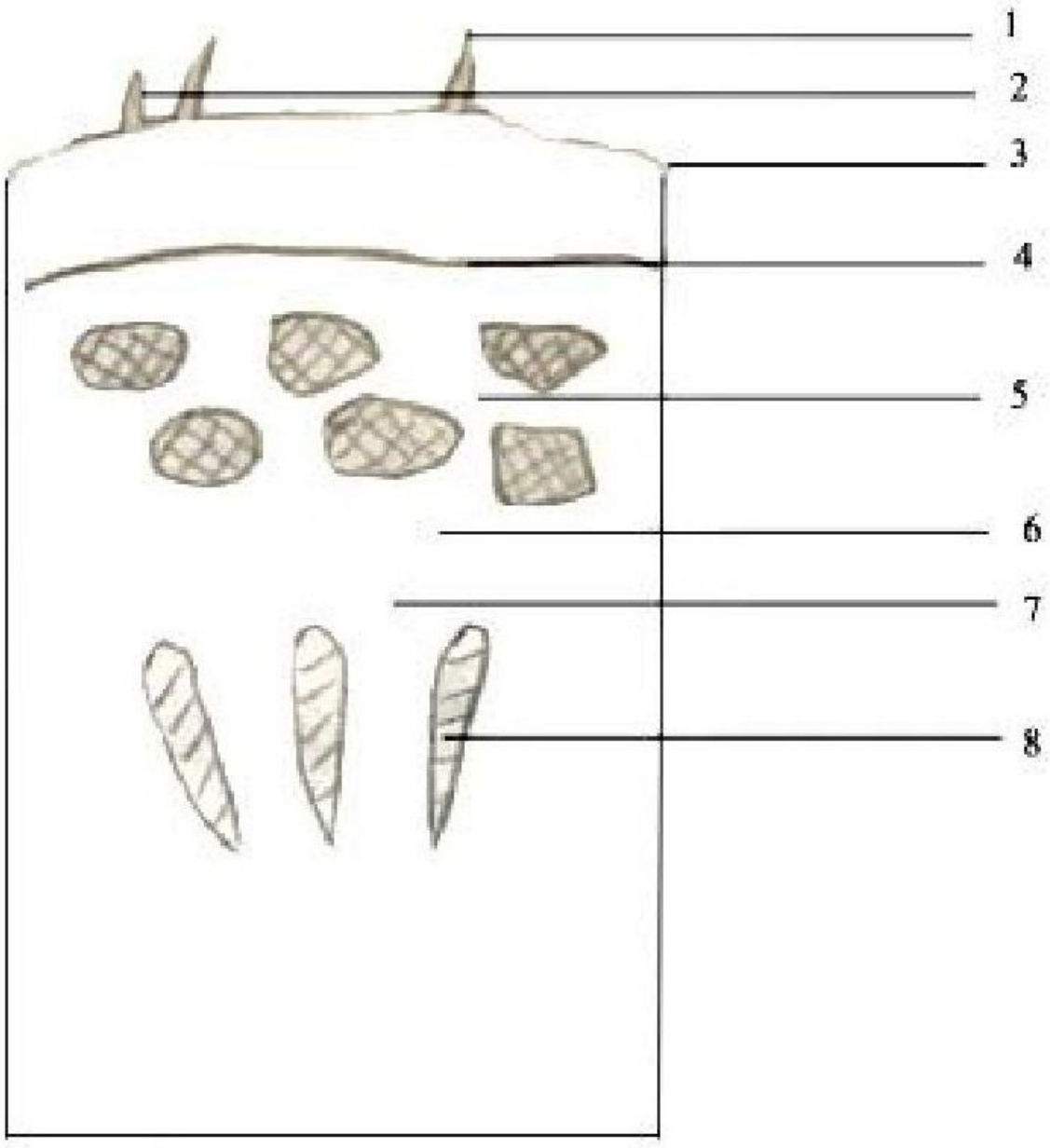

Figure 4

1-4 Partial sketch of longitudinal section of QLY (10×10) 1 glandular hair; 2 nonglandular hair; 3 exocarp; 4 mesocarp; 5 the girdle of sclereid; 6 endocarp; 7 phloem; 8 xylem

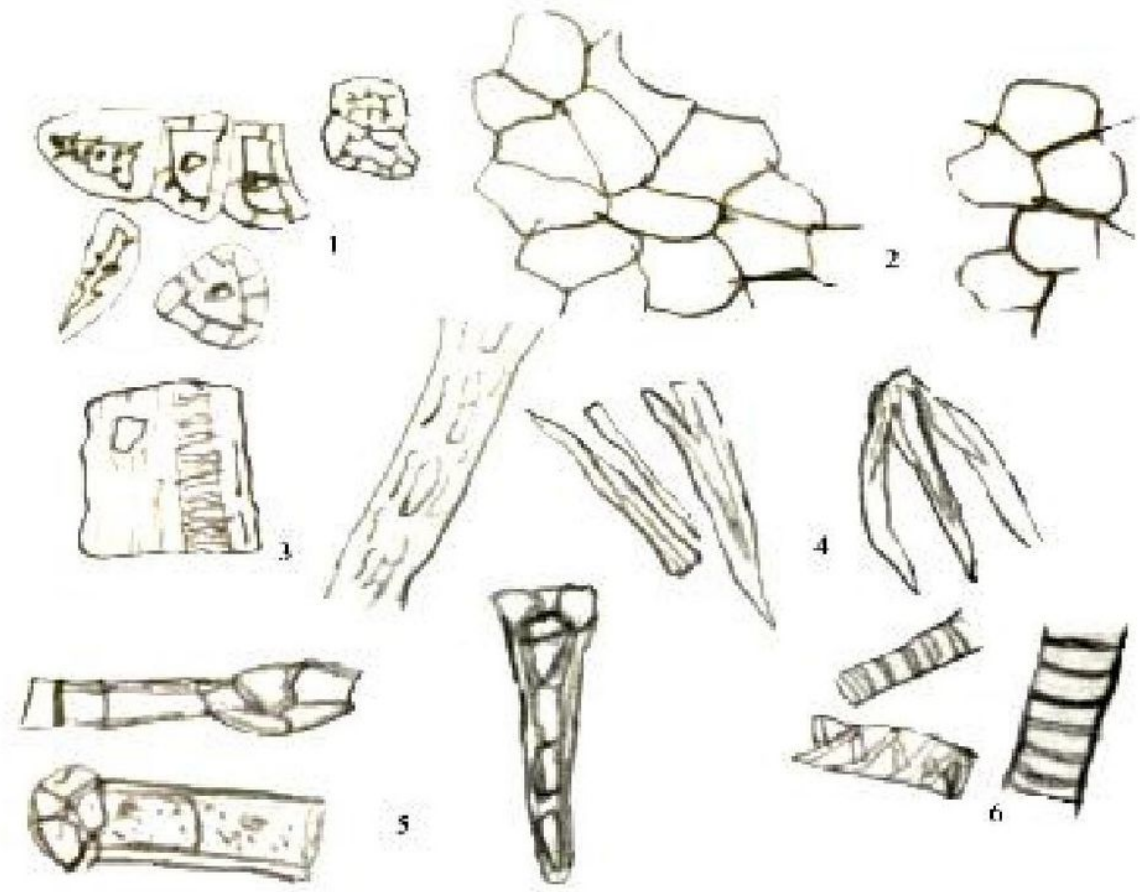

Figure 5

1-5 Powder characteristics of QLY (10×40) 1 stone cell; 2 parenchyma cells; 3 secretory cells; 4 nonglandular hairs; 5 glandular hairs; 6 spiral vessels

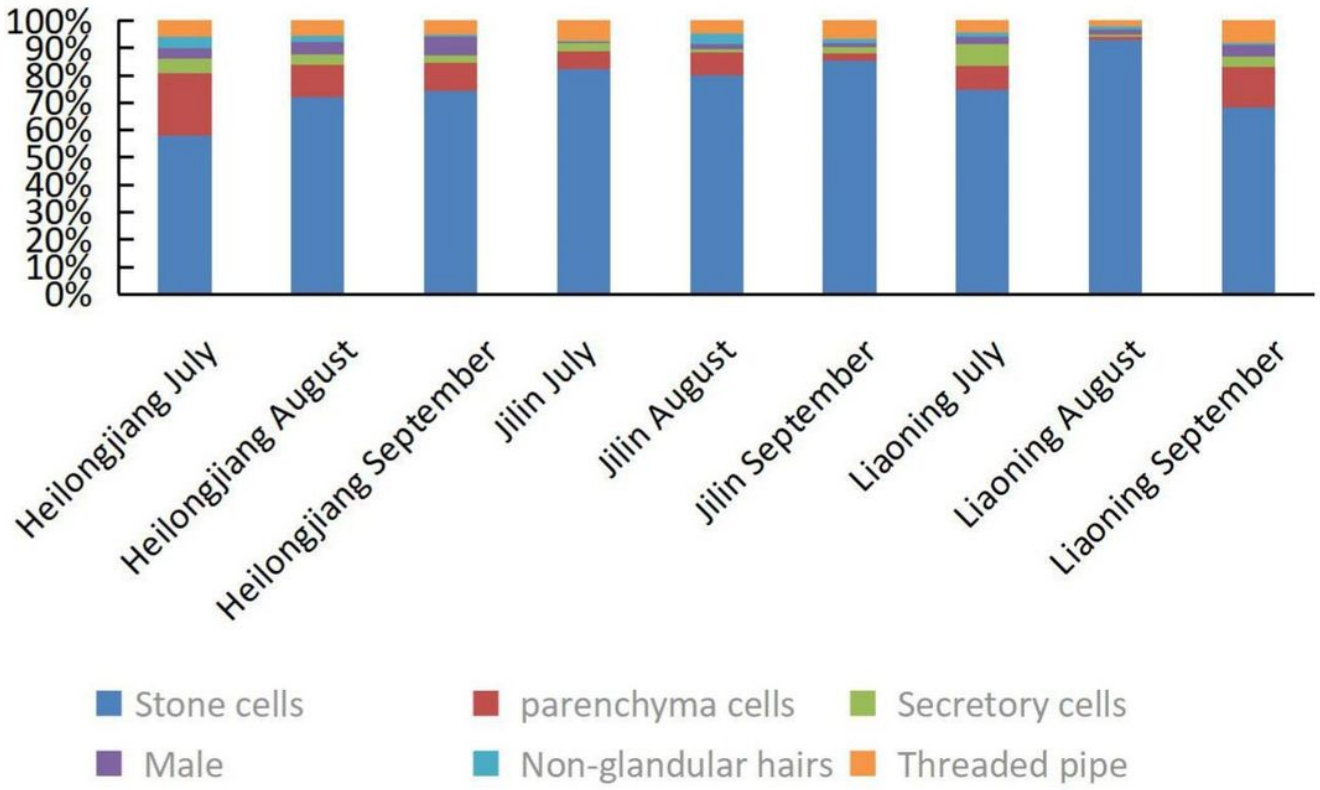

Figure 6

1-6 Statistical chart of the powder characteristics of QLY in each time

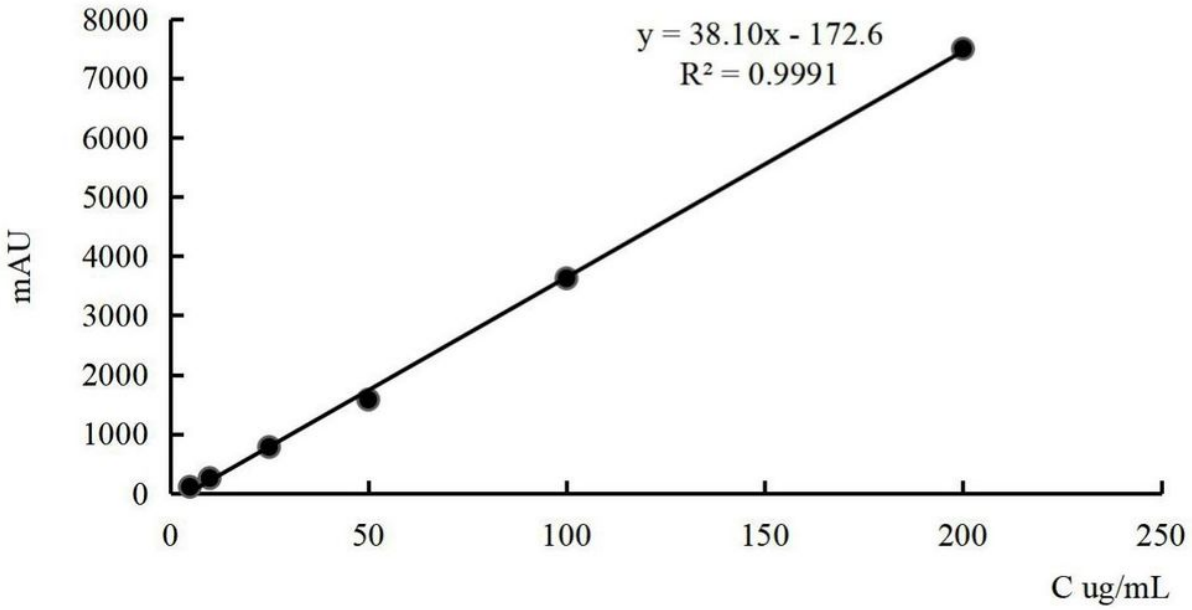

Figure 7

1-7 The standard curve of juglone

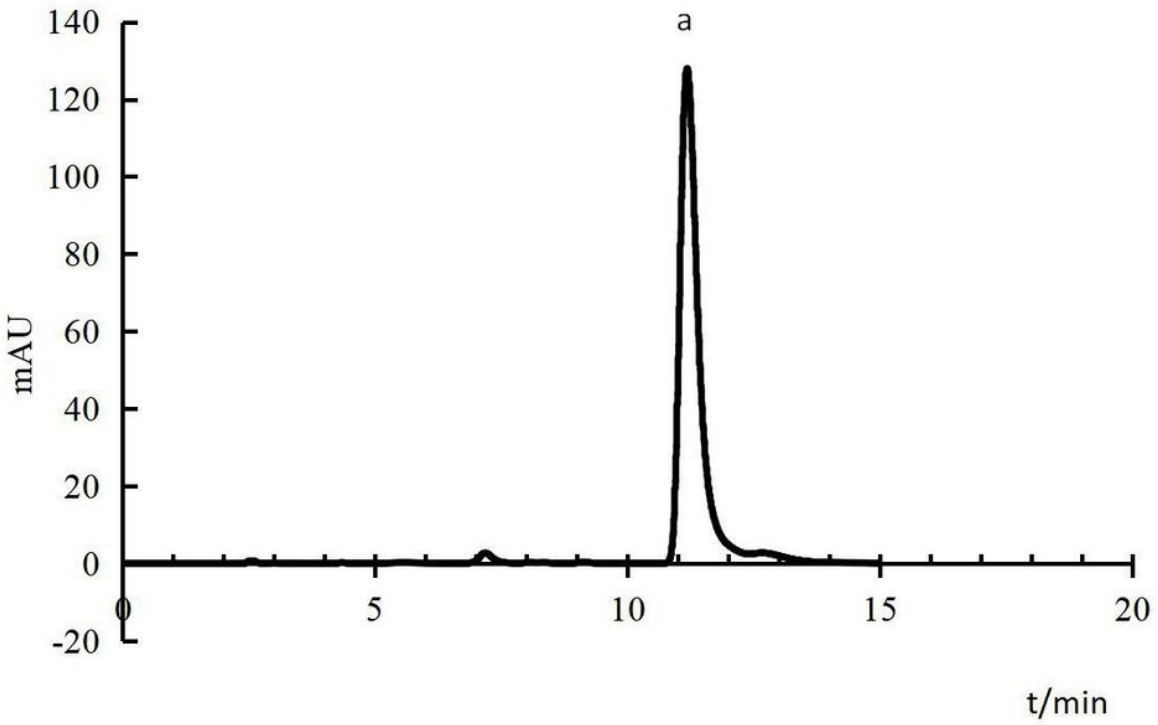

Figure 8

1-8 HPLC chromatogram of the reference substance of juglone (a is the peak of juglone)

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