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AUTHENTICATION OF MORPHOLOGICAL AND MICROSCOPIC FEATURES OF ROOT OF THE ONE TO FIVE-YEAR-OLD *PANAX QUINQUEFOLIUS* L. GROWN IN ONTARIO, CANADA

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Abstract

Morphological and microscopic features of root of one to five-year-old *Panax quinquefolius* L. grown on Canada has been authenticated.The study includes also fibrous root, powder and maceration of root. The methods used in this research could be adopted to distinguish one to five-year-old Radix Panacis Quinquefolii imported from Canada, which is available or presented as mixtures in the raw root selling markets in China. The peduncle of terminal umbel, starch grain, calcium oxalate cluster crystals and secretory canal and the number of "Lo Bowl" characteristics have been presented. It is therefore suggested that the roots could be used to authenticate the one to five-year-old *P. quinquefolius* grown in Canada.

Introduction

Dried root of *Panax quinquefolius* L. (Fam.: Araliaceae) is called '*Radix Panacis Quinquefolii*' and is also named American ginseng (Chinese Pharmacopoeia Commission 2010). It was not only recorded in the *Supplement to Compendium of Materia Medica* from AD 1765, also in every version of *the People's Republic of China Pharmacopoeia*. Modern researches have shown that the chemical compositions of Radix Panacis Quinquefolii are quite complex, including ginsenosides, polysaccharides, volatile oils, amino acids and a lot of extracts from it (Chinese Pharmacopoeia Commission 2010, Tung and Shoyama 2012). It has been shown that these chemical compositions have the biological activity of anticancer, antioxidant, enhancing memory, improving immune ability, decreasing blood sugar, blood pressure and blood lipids, etc. (Li *et al.* 2012, Poudyal *et al.* 2012, Sen *et al.* 2012, Tárrega *et al.* 2012, Wilson *et al.* 2012). Radix Panacis Quinquefolii has played a pivotal role in traditional Chinese medicine for thousands of years. Moreover, its use has become increasingly popular, in part due to the many claims of its immune-enhancing properties. The immune-pharmacology of the North American variety of Radix Panacis Quinquefolii and its extracts is needed to substantiate these claims (Lemmon *et al.* 2012). Canada is currently the largest producer of Radix Panacis Quinquefolii recognized by the Canadian regulatory agency as a natural health product for use as an adaptogen, i.e., biological response modifier (Azike *et al.* 2011).

P. quinquefolius in wild has already been in danger and because of high demand of Radix Panacis Quinquefolii product in the herbal market, it is one of the top ten famous herbs in terms of sale-volume in north America. *P. quinquefolius* is cultivated widely in Ontario of Canada, Wisconsin of the USA and Jilin, Liaoning and Heilongjiang Provinces of the People's Republic of

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China. As a traditional and precious Chinese herbal medicine, it is accepted that the price and medicinal quality of the Radix Panacis Quinquefolii are higher when it is imported from Canada and the USA. However, it is quite difficult to distinguish between the imported and domestic Radix Panacis Quinquefolii in the raw herbs market of China.

Researches in the efficacy and pharmacology of the monomers and extracts, e.g. ginsenoside of Radix Panacis Quinquefolii from the roots, stems and leaves are limited in Canda and the USA (Wang *et al.* 2013, Zhang *et al.* 2010) and less attention has been paid to the pharmacognosy of Radix Panacis Quinquefolii. Because of the limitation of the geographical conditions and long sample collecting time, the pharmacognosy of *P. quinquefolius*, which is originated from Canada and the USA, is even less studied by the researchers of China (Wang *et al.* 2007). So, it is necessary to create a scientific database for further research to obtain the biological characteristics of the product (Santos and Rivera 2013, Lee *et al.* 2010, Salem *et al.* 2013, Sen *et al.* 2012, Raman *et al.* 2013, Wong *et al.* 2012, Lee and Jernstedt 2013). In the present research, pharmacognosy including the morphological and microscopical authentication of the roots of one to five-year-old *P. quinquefolius* grown in the Great Mountain Farm, Scotland, Ontario, Canada was undertaken. In addition, fibrous root, powder and maceration of root of five-year-old *P. quinquefolius* were also authenticated. Subsequently, representative and unique morphological and microscopic features were illustrated, characterized and described. Detailed key of the authenticated parameters based on these anatomical characteristics were presented for the *P. quinquefolius*.

Materials and Methods

Samples of *P. quinquefolius* were collected from about 251 meters above sea level, the Great Mountain Farm, Scotland, Ontario, Canada from May to October, 2009. Samples used for authentication via microscopical work were: one to five-year-old root, fibrous root, powder and maceration of root of five-year-old in September 20, 2009. The products were authenticated by Prof. Yuan Liu (Ethnic Medicine Institute, Southwest University for Nationalities, Chengdu, P. R. China). Voucher specimens were deposited in the Herbarium of Ethno-herbs, Ethnic Medicine Institute, Southwest University for Nationalities (SWUN) (Table 1).

Safranine T in 50% alcohol and safranine-fast green solution were prepared for specimen staining. 95% to 100% alcohol and Canada balsam of chemical grade were used in this study.

Embedding matrix for frozen sections was from Triangle Biomedical Sciences (72592). All the transverse sections of the materials were prepared using microtome (Leica CM 3050, Germany). An imaging system consisting of an optical microscope equipped with a micrometer (Axioskop, Germany) and a digital camera for acquisition of photographs was used for photography. All the fresh and dried samples were weighed by electronic balance (Mettler MS105, Switzerland).

The fresh roots of *P. quinquefolius* were divided into appropriate sizes, embedded in embedding matrix for frozen sections. The specimen was placed on a metal tissue disc which was then secured in a chuck and frozen rapidly to about −16 to −18°C. When the frozen was the same density as frozen tissue, then the specimen was sliced to 10 μm.The section was picked up on a glass slide and stained with safranine-fast green (Berlyn and Miksche 1976), and finally mounted in Canada balsam for observation. The root of *P. quinquefolius* was isolated by Nitric acid Chromate method (Chinese Pharmacopoeia Commission 2010) and was observed. The samples of roots were powdered and cleared in chloral hydrate, then mounted in glycerin. Samples were studied for the key morpho-anatomy and microscopy in authentication features.

Table 1. Source of materials of the one to five-year-old *P. quinquefolius*.

Sl. No.	Taxon	Batches	Locality	Elevation (m)	Date of collection	Voucher
1	One-year-old	No.1	The Great Mountain Farm, Scotland City, Ontario, Canada	251	May 24, 2009	Y. Liu 090524-1 (SWUN)
		No.2			June 22, 2009	Y. Liu 090622-1 (SWUN)
		No.3			July 27, 2009	Y. Liu 090727-1 (SWUN)
		No.4			August 23, 2009	Y. Liu 090823-1(SWUN)
		No.5			September 20, 2009	Y. Liu 090920-1 (SWUN)
2	Two-year-old	No.1			May 24, 2009	Y. Liu 090524-2(SWUN)
		No.2			June 22, 2009	Y. Liu 090622-2 (SWUN)
		No.3			July 27, 2009	Y. Liu 090727-2 (SWUN)
		No.4			August 23, 2009	Y. Liu 090823-2(SWUN)
		No.5			September 20, 2009	Y. Liu 090920-2 (SWUN)
3	Three-year-old	No.1			May 24, 2009	Y. Liu 090524-3 (SWUN)
		No.2			June 22, 2009	Y. Liu 090622-3 (SWUN)
		No.3			July 27, 2009	Y. Liu 090727-3 (SWUN)
		No.4			August 23, 2009	Y. Liu 090823-3(SWUN)
		No.5			September 20, 2009	Y. Liu 090920-3 (SWUN)
4	Four-year-old	No.1			May 24, 2009	Y. Liu 090524-4(SWUN)
		No.2			June 22, 2009	Y. Liu 090622-4 (SWUN)
		No.3			July 27, 2009	Y. Liu 090727-4 (SWUN)
		No.4			August 23, 2009	Y. Liu 090823-4(SWUN)
		No.5			September 20, 2009	Y. Liu 090920-4 (SWUN)
5	Five-year-old	No.1			May 24, 2009	Y. Liu 090524-5(SWUN)
		No.2			June 22, 2009	Y. Liu 090622-5 (SWUN)
		No.3			July 27, 2009	Y. Liu 090727-5 (SWUN)
		No.4			August 23, 2009	Y. Liu 090823-5(SWUN)
		No.5			September 20, 2009	Y. Liu 090920-5 (SWUN)

Results and Discussion

The length (l) and diameter (dia) of the roots were 1.5 - 30 × 0.12 - 3.6 cm. The number of "Lo Bowl", which refers to a bowl shaped stem scars from roots, is directly related to the age of Radix Panacis Quinquefolii. Images of dried roots of one to five-year-old *P. Quinquefolius* are shown in Fig.1.

Fig. 1. Morphological view of one to five-year-old Radix Panacis Quinquefolii

As *P. quinquefolius* is a perennial herb, the fibrous root, the maceration of the root and the powder of the root of five- year-old *P. quinquefolius* were as examples for showing the microscopy features in authentication. So, their authentication were just focused on the five-year-old sample.

The microscopy in authentication of root of one to five-year-old *P. quinquefolius*, and of fibrous root, powder of the root and maceration of the root of five-year-old *P. quinquefolius* are shown in Figs 2-4.

Transverse sections (TS) of root: Five-year-old roots almost circular in outline. Phellem layer 5 - 7, tangentially prolonged brown cork cells arranged closely. Outer-layer sometimes ruptured, exfoliated. Cortex consisted of 5 - 6 layers parenchymaous cells, oval or sub-rounded, arranged loosely, about 26 - 35 μm in dia, contained large number of secretory canals, arrangement oblong, 5 - 6 intermittent ring; elliptical or rotund secretory canals, about 40 - 81 μm in dia, surrounded by 5 - 10 secretory canal. Calcium oxalate cluster crystals present, about 10 - 30 μm in dia. Phloem and xylem rays composed of 1 - 3 layers of cells. Several sieve cells closely packed together, most of them radially arranged in phloem, and many secretory canals around, about 40 - 80 μm. Ten or more vessels closely packed together occasionally, majority of them radially arranged in xylem.

A large quantity of starch grain was found in one to five-year-old roots. The calcium oxalate cluster crystals were found in two to five-year-old (Fig. 2), but it was not found in one-year-old. There was an age-related increase in the deposition of calcium oxalate cluster crystals. The secretory canal was apparent in one-year-old root and its abundance was increased in two to five-year-old roots. Differences in vessel quantity and its degree of lignification were also age related. It was apparent in one-year-old root and its abundance and degree of lignification increased in two to five-year-old roots. Detailed comparison of one to five-year-old roots has been shown in Fig. 2.

TS of fibrous root (five-year-old September): Outline almost circular. Phellem layer 1 - 2, tangentially prolonged brown cork cells arranged closely. Outer-layer sometimes ruptured, exfoliated. Cortex wide, consisted of 12 - 15 layers parenchyma cells, oval or sub-rounded, arranged loosely, about 18 - 25 μm in dia, contained large number of secretory canals; elliptical or rotund secretory canals, about 20-70 μm in dia, surrounded by 5 - 10 secretory cells. Phloem and xylem rays composed of 1 - 3 layers of cells, phloem wide, several sieve cells closely packed together occasionally, most of them radially arranged in phloem, many secretory canals found, about 20 - 37 μm. Xylem narrow, 12 - 15 vessels, radially arranged in xylem. Detailed features are shown in Fig. 3.

Microscopic characteristics of maceration and powders of five-year-old *P. quinquefolius* (September) revealed: white yellow color. Cork cell quadrate, about 60 - 70 μm in length; more than 5 - 6 layers densely with brown-yellow color in powder including brown inclusion, and off-white in maceration mainly reticulate vessel, 50 - 500 μm in length, 10 - 70 μm in width; spiral vessel and scalariform vessel also found. Fibers having wide cell cavity and obvious pit, 390 - 600 μm in length, 12 - 16 μm in width, 3 - 5 μm in thickness; parenchymaous cell in xylem having obvious pit, 50 - 65 μm in length, 15 - 25 μm in width, 2 - 5 μm in thickness and often can be observed. Brown inclusion about 170 - 185 μm in length, 28 - 45 μm in width, 3 - 5 μm in thickness. Clustered crystal about 21 - 35 μm in dia. Many starch grains observed; single particle about 4 - 8 μm in dia; hilum distinct, punctuate, asteroid and crack; striations indistinct; compound starch grain composed of 2 - 5 starch grains. The detailed features are shown in Fig. 4.

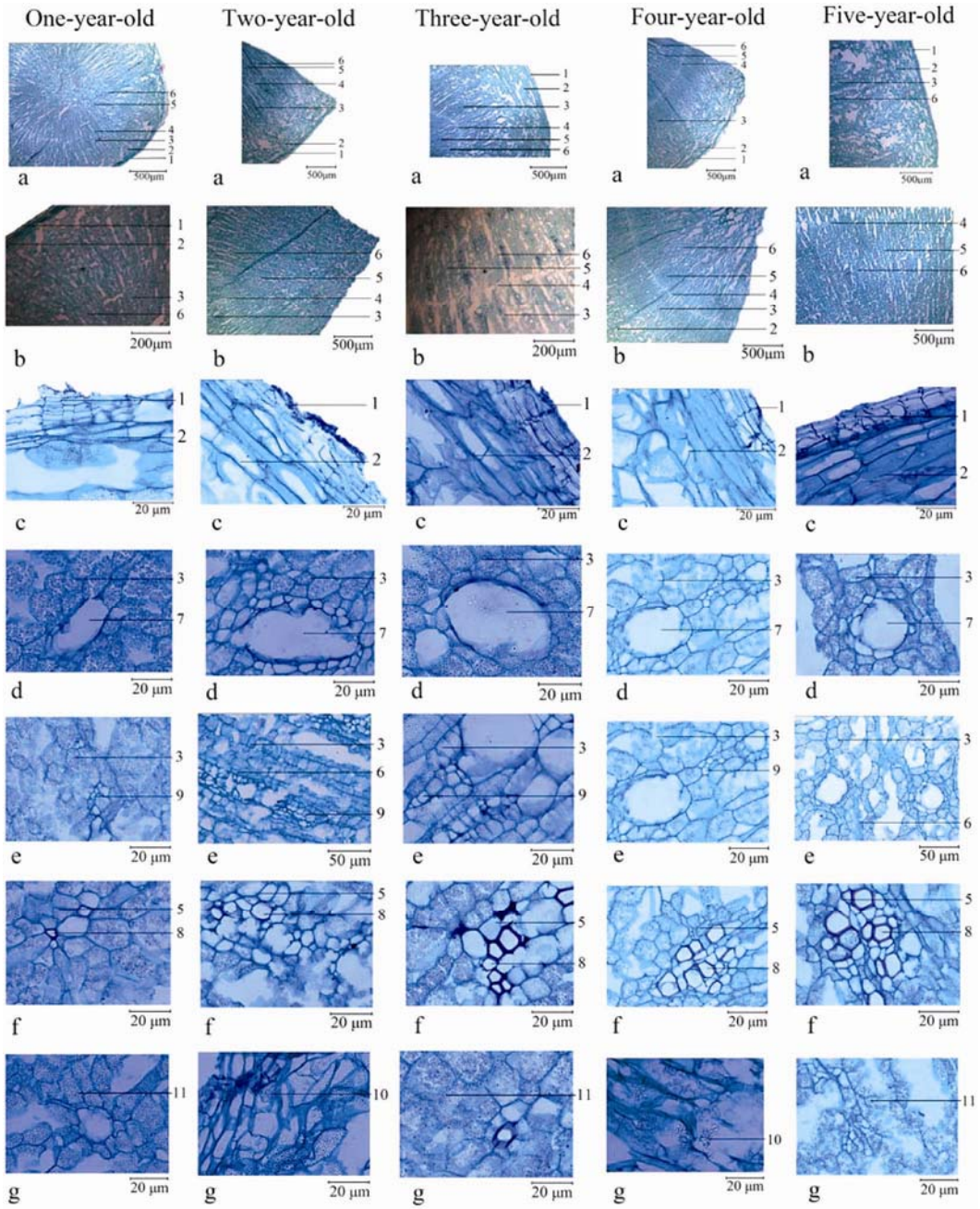

Fig. 2. TS of root of the one to five-year-old *P. quinquefolius* a-g: Outline, cambium, phloem layer and cortex, secretory canal, phloem, xylem and calcium oxalate cluster crystal and starch grain.1-11: Phloem layer, cortex, phloem, cambium, xylem, rays, secretory canal, vessel, sieve cell, calcium oxalate cluster crystal and starch grain.

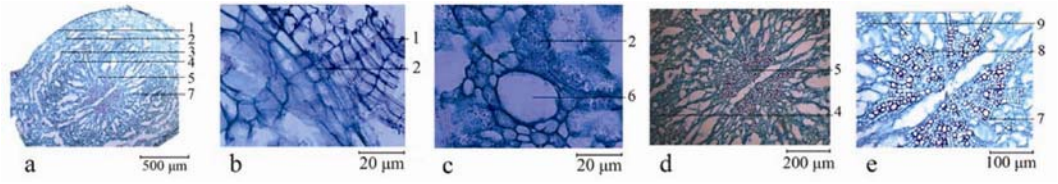

Fig. 3. TS of fibrous root of the five-year-old *P. quinquefolius* L. a-e: Outline, epidermis and cortex, secretory canal, phloem and xylem.1-9: Phellem layer, cortex, fissure, phloem, xylem, secretory canal, rays, vessel and sieve cell.

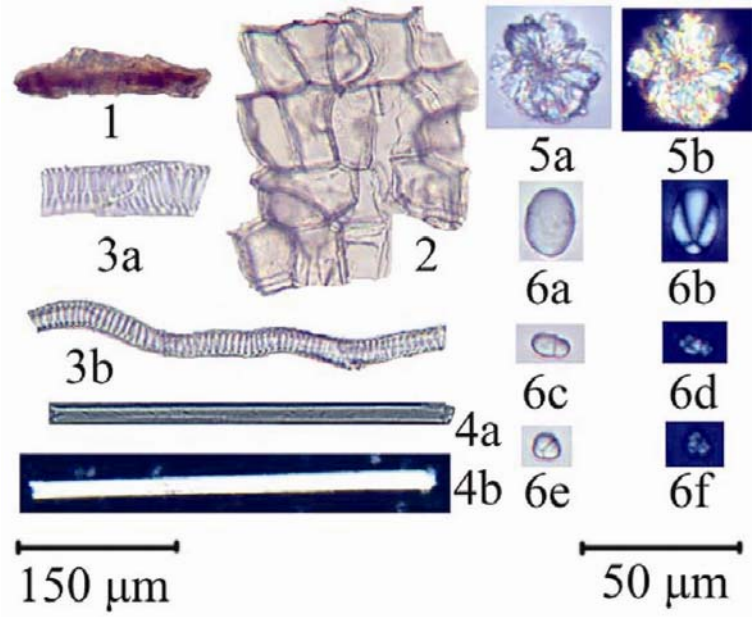

Fig. 4. Microscopic characteristics of maceration and powders of root of *P. quinquefolius* L. 1: Brown inclusion. 2: Cork cells. 3a-b: Reticulated vessel and scalariform vessel. 4a-4b: Fibre and fibre in polarized light. 5a-5b: Calcium oxalate cluster crystal and calcium oxalate cluster crystal in polarized light. 6a-6f: Single starch grain, single starch grain in polarized light, two starch compound grains, two starch compound grains in polarized light, three starch compound grains and three starch compound grains in polarized light.

The results of microscopic structures showed a large quantity of starch grains in one to five-year-old roots. This result was consistent with Su and Hu (1994). The calcium oxalate cluster crystals were found in two-five-year-old, but it was not found in one-year-old; there was an age-related increase in the deposition of calcium oxalate cluster crystals. The secretory canal was apparent in one-year-old root, and its abundance was increased in two to five-year-old roots (Li *et al.*1993). There was an age-related difference in vessel quantity and its degree of lignification which is apparent in one-year-old root, and its abundance and degree of lignification increased in two to five-year-old roots (Guo *et al.*1993).

The "Lo bowl" is often removed before selling of the Radix Panacis Quinquefolii in the raw herbs market of China. As a result, it is difficult to know the accurate age of it in the raw herbs market. Consequently, it becomes hard to estimate the price of Radix Panacis Quinquefolii according to its quality during the purchase. So, it is strongly suggested that they could not get rid

of the “Lo Bowl” of Radix Panacis Quinquefolii when they are sold in the raw herbs market of China. In brief, the one to five-year-old Radix Panacis Quinquefolii are available or presented as mixtures in the raw herbs markets in China. They cannot be distinguished because of being getting rid of “Lo Bowl”, especially when the herbs are in a dried and matted condition. In such cases, discrimination can be very difficult. Herein, morphological and microscopic features should be of great use in identifying these materials.

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