Advancement in Medicinal Plant Research
Vol. 2(2), pp. 34-40, June 2014
ISSN: 2354-2152
Full Length Research Paper

Authentication of morphological and microscopic features of stem and leaf of *Panax quinquefolius* L. grown in Ontario, Canada

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Accepted 26 May, 2014

ABSTRACT

The present paper highlights an authentication of microscopic features of stem and leaf of *Panax quinquefolius* L. grown in Ontario, Canada, including microscopy of stem, leaf, leaf stalk and surface view of leaf epidermis, powder and dissociation of seed, stem and leaf of *P. quinquefolius* L. The methods could be adopted to distinguish Panacis quinquefolii Radix imported from Canada, which is available or presented as mixtures in the raw herbs marketed in China at present. The number of compound leaf, palisade ratio, vein islet numbers, starch grain, calcium oxalate, cluster crystals and secretory canal characteristics were presented. So it was suggested that the stem and leaves could be further researched on biological active effects.

Keywords: *Panax quinquefolius* L., morphological and microscopic features, authentication, Ontario, Canada.

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INTRODUCTION

Canada is currently the largest producer of Panacis Quinquefolii Radix recognized by the Canadian regulatory agency as a natural health product for use as an adaptogen which is a biological response modifier (Azike et al., 2011). The dried root of *P. quinquefolius* Linn., which belongs to Genus *Panax* of Family Araliaceae (Chinese Academy of Sciences,1978), is called Panacis Quinquefolii Radix, also named as American Ginseng (Shao, 2010). It was recorded in the *Supplement to Compendium of Material Media* (1765), and *People's Republic of China Pharmacopoeia*. Panacis Quinquefolii Radix has played a pivotal role in traditional Chinese medicine for thousands of years; its use has become increasingly popular, in part due to the many claims of its immune-enhancing properties (Lemmon et al., 2012).

Now, the species is cultivated widely in Ontario Province of Canada, Wisconsin of America, and Jilin, Liaoning and Heilongjiang Provinces of China. As a traditional and precious Chinese herbal medicine, it is accepted that the price and medicinal quality of the Panacis Quinquefolii Radix are higher when imported from Canada and America. However, it is quite difficult to distinguish between imported and domestic variety (China) in the raw herbs market, which is introduced and cultivated from Canada and America.

Researchers of Canada and America usually pursue research on the efficacy and pharmacology of the monomers and extracts, e.g. ginsenoside of Panacis Quinquefolii Radix of the roots, stems and leaves (Wang et al., 2013; Zhang et al., 2010), but less attention is paid to the pharmacognosy of Panacis Quinquefolii Radix. Because of the limitations of the geographical conditions and long sampling period, the pharmacognosy of *P. quinquefolius* Linn., which is originated from Canada and America, is even less studied by the researchers of China

(Wang et al., 2007). Herein, for the sake of offering a scientific database for further research to obtain the biological characteristics (Santos and Rivera, 2013; Lee et al., 2010; Salem et al., 2013; Raman et al., 2013; Wong et al., 2012; Lee and Jernstedt, 2013), systematic research on pharmacognosy including the microscopy of stem, leaf, leaf stalk and surface view of leaf epidermis, powder and dissociation of seed, stem and leaf of five-year-old *P. quinquefolius* L. was studied to establish a method to facilitate the authentication of *P. quinquefolius* Linn.

MATERIALS AND METHODS

Samples of *P. quinquefolius* Linn. (Five-year-old) were collected from the Great Mountain Farm, Scotland, Ontario, Canada (43°2'N, 80°20'W) from May to October of 2009, about 251 m above sea level. Mean annual temperature is 8.1°C. Annual rainfall is 769.6mm. Mean humidex is 30.83. The samples of collected specimens were powdered by High speed pulverizer (YiKang YK-1000A, China) and cleared in 200% (w/v)chloral hydrate, then mounted in 33% glycerin. Samples were studied for the key morpho-anatomy and microscopy to authenticate the salient features. Late, samples (stem, leaf, leafs talk and surface view of leaf epidermis, powder and dissociation of stem, leaf and seed of five-year-old specimen) were used for microscopy study for authentication, to be authenticated by Prof. Yuan Liu (Ethnic Medicine Institute, Southwest University for Nationalities, Chengdu, P. R. China). Safranine T in 50% alcohol, and safranine-fast green solution were prepared for specimen staining. 95%, 100% alcohol and Canadian balsam used in this study were analytically pure grade. Water used in the study was all double distilled water. Samples were embedded in Embedding matrix for frozen sections (Triangle Biomedical Sciences 72592, America). All the transverse sections of the materials were prepared using microtome (Leica CM3050, Germany). An imaging system consisting of an optical microscope (Olympus BX41, Japan) equipped with a micrometer and a digital camera for acquisition of photographs was used for photography. All the fresh and dried samples were weighted by Electronic balance (Mettler MS105, Switzerland).

The five-year-old leaves were prepared following the practical peeling technique and clearing method (Berlyn and Miksche, 1976; Leng et al., 2007); stem and leaves of *P. quinquefolius* Linn. were isolated by nitric acid chromate method (Shao, 2010). Fresh stem and leaf of *P. quinquefolius* Linn. were divided into appropriate sizes (3 to 5 mm), and 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 with microtome; next sliced into 10 mm when specimen was the same density as frozen tissue; then 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.

RESULTS

Plant morphology

P. quinquefolius Linn.: Perennial herb, about 9.75-90 cm tall, hermaphroditic. Roots 1.5 to 30 cm length × 0.12 to 3.6 cm diameter. Stems erect and longitudinal wrinkles, 5.1 to 39.6 cm length × 0.15 to 1.74 cm diameter, Short rhizome, and spindle-shaped and fleshy root. Leaves palmately compound, leaflets 3 to 5; usually one leaf of leaflets 3 or 4 or 5 for one-year-old; usually two or one leaf of leaflets 5 for two-year-old; usually three or two leaf of leaflets 5 for three-year-old; usually four or three leaf of leaflets 5 for four-year-old; usually five or four leaf of leaflets 5 for five-year-old; verticillate at the apex of stem; leaflets oblong-obovate, 1.89 to 14 × 0.69 to 8 cm, membranous, sparsely setose on veins or glabrous adaxially, margin coarsely serrate or dentate, apex abruptly or boldly acuminate; Inflorescence a solitary, terminal umbel 6 to 20-flowered; peduncle much shorter than petioles; calyx 5, green, mitriform; petal 5, white green, quadrature circle; stamen 5; pistil 1, ovary hypogynous, 2 carpellate,1 anatropous ovule for per locule; styles 2; disc chuck fleshy, circulared. Berried drupe; immature fruit green, mature fruit red, 1 to 2 cm; seed 1 to 4, usually 2, length 0.5 to 0.8 cm, width 0.4 to 0.6 cm, thickness 0.2 to 0.3 cm.

Pictures of the whole plant of one to five-year-old are shown in Figure1.

Microscopic characteristics

P. quinquefolius L. is a perennial herb, the tissue structure of stem, leaf and leafstalk is the same in every year. The dissociation and powders of five- year-old of *P. quinquefolius* L. were as an example for showing the microscopy features in authentication. So, they were just focused on the five-year-old sample of that.

The microscopy in authentication of stem, leaf, leafstalk and surface view of leaf epidermis, powder and dissociation of stem and leaf of five-year-old of *P. quinquefolius* L. were shown in Figures 2 to 4.

Microscopic characteristics of transverse sections (TS)

TS of stem

The epidermal cells were square and single-layered, covered with thin cuticle and prickles. Cortex was narrow and 7 to 9 layers with 5 to 6 angular collenchyma under the epidermal cells and 2 to 3 layers parenchymaous cell near to vascular bundle sheath cell. Vascular bundle 16 to18 covered 5 to 6 fiber bundle cell dyed red-purple color and outside for per vascular bundle. Phloem was narrow. Secretory canal was found. 5 to 6 vessels cell were radially arranged in xylem. Detailed features were shown in Figure 2.

TS of leaf

Leaf bifacial or isobilateral; both upper and lower epidermis was composed of oblong or sub-rounded and

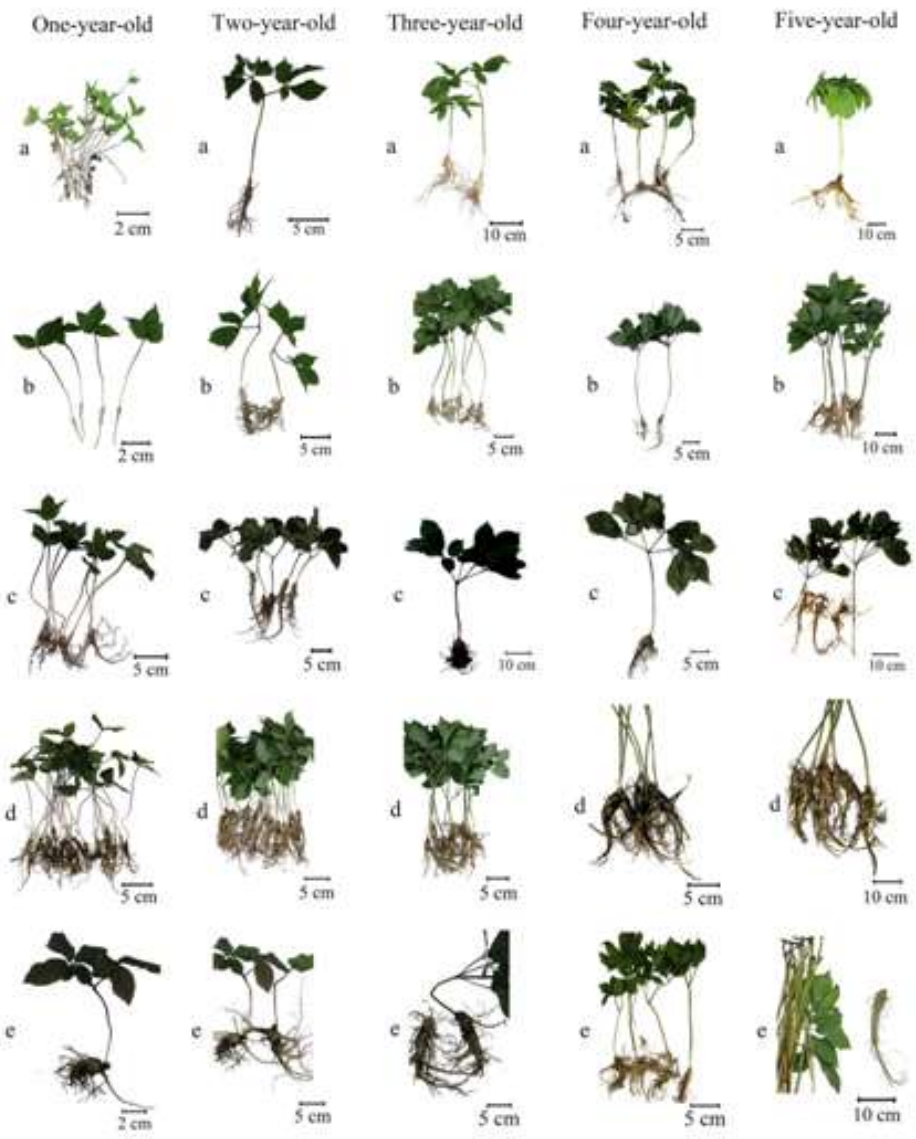

Figure 1. Pictures of the whole plant of *P. quinquefolius* L. of one to five-year-old. a. May 24, 2009; b. June 22, 2009; c. July 27, 2009; d. August 23, 2009; e. September 20, 2009.

single-layered cells that were arranged closely and covered with cuticle. Stomata could be observed in lower epidermis. The palisade tissues and spongy tissues could not be obvious distinguished; mesophyll tissue cells were oval, arranged loosely with broad intercellular space. Vascular bundles of the midrib were of the ectophloic type, circular-shaped, and enclosed by 2 to 3 layer collenchyma under upper epidermis and 1 to 2 layer collenchyma under lower epidermis. The cambium was not conspicuous. Secretory canal was not found. Detailed features were shown in Figure 2.

TS of leaf petiole

Almost circular with irregularly waved curving-shaped. Epidermis was composed of oblong or subrounded and single-layered cells. Cortical layer were arranged closely and enclosed by 5 to 6 layer collenchyma and 5 to 6 layer parenchymaous cell arranged loosely. Vascular bundle was 10 to 12 ringed and ectophloic type. Secretory canal was observed and composed of 6 to 7 secretory cells in phloem and cortical layer. Parenchymaous cell was sporadically contained clusters of calcium oxalate. The cambium was not conspicuous. Detailed features were shown in Figure 2.

Microscopic characteristics of surface view of leaf epidermis

The anticlinal walls of the leaf epidermal cells were wavy.

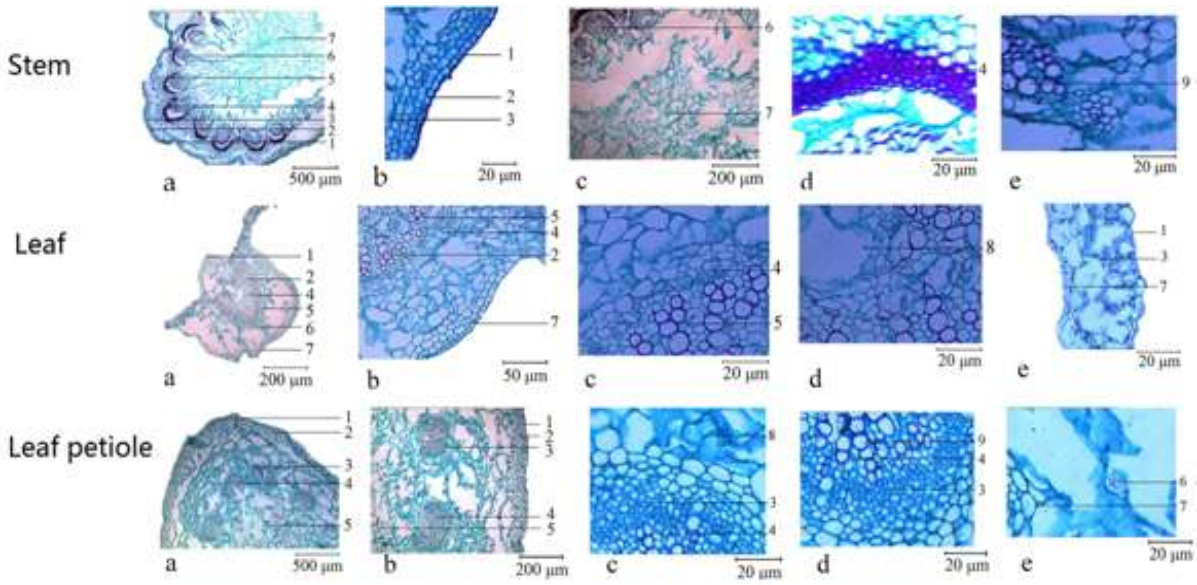

Figure 2. TS of the five-year-old *P. quinquefolius* L. **Stem:** a. Showing outline; b. Showing epidermis and cortex; c. Showing pith; d. Showing pericycle fiber; e. Showing xylem. 1. Cuticle; 2. Epidermis; 3. Cortex; 4. Pericycle fiber; 5. Phloem; 6. Xylem; 7. Pith; 8.Secretory canal; 9. Vessel. **Leaf:** a. Showing outline; b. Showing vascular bundles of the midrib; c. Showing phloem and xylem; d. Showing calcium oxalate cluster crystal; e. Showing mesophyll. 1. Upper epidermis; 2. Vascular bundles of the midrib; 3. Mesophyll; 4. Phloem; 5. Xylem; 6.Collenchyma; 7. Lower epidermis; 8. Calcium oxalate cluster crystal. **Leaf petiole:** a. Showing outline; b. Showing epidermis and cortex; c. Showing phloem; d. Showing xylem; e. Showing calcium oxalate cluster crystal. 1. Epidermis; 2. Cortex; 3. Phloem; 4. Xylem; 5. Pith; 6. Calcium oxalate cluster crystal; 7. Parenchymatous cell; 8. Secretory canal; 9. Vessel.

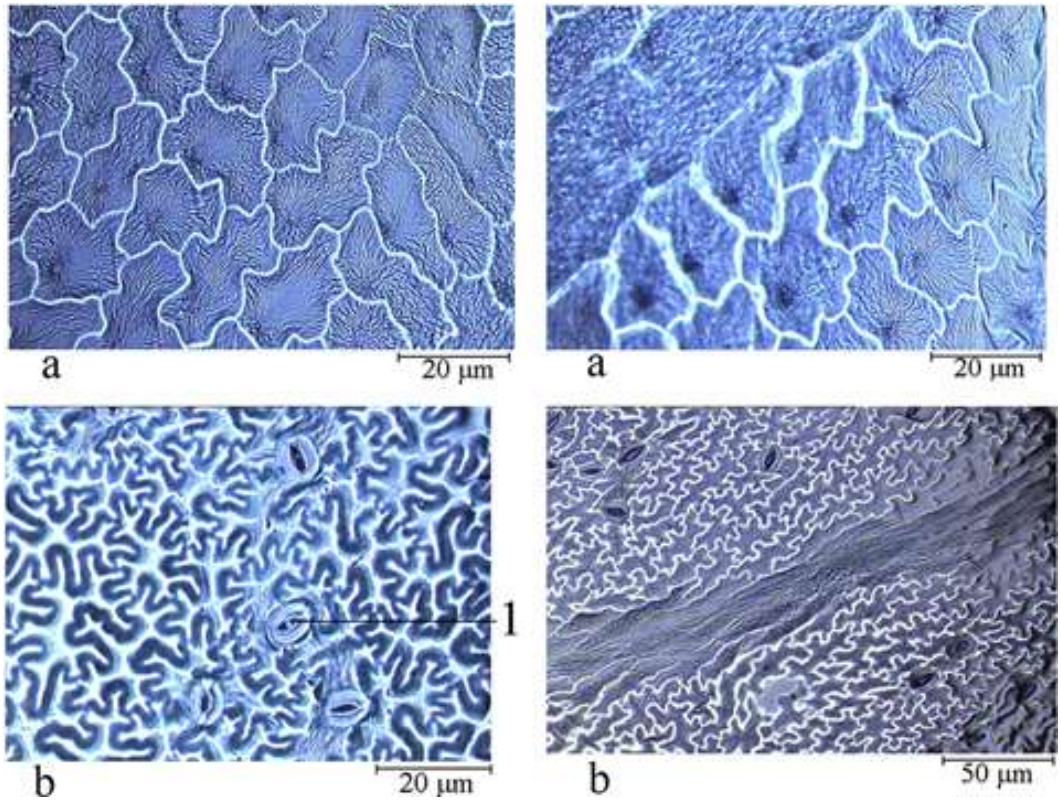

Figure 3. Surface view of leaf of the five-year-old *P. quinquefolius* L. a. Showing upper epidermis; b. Showing lower epidermis. 1. Stomata.

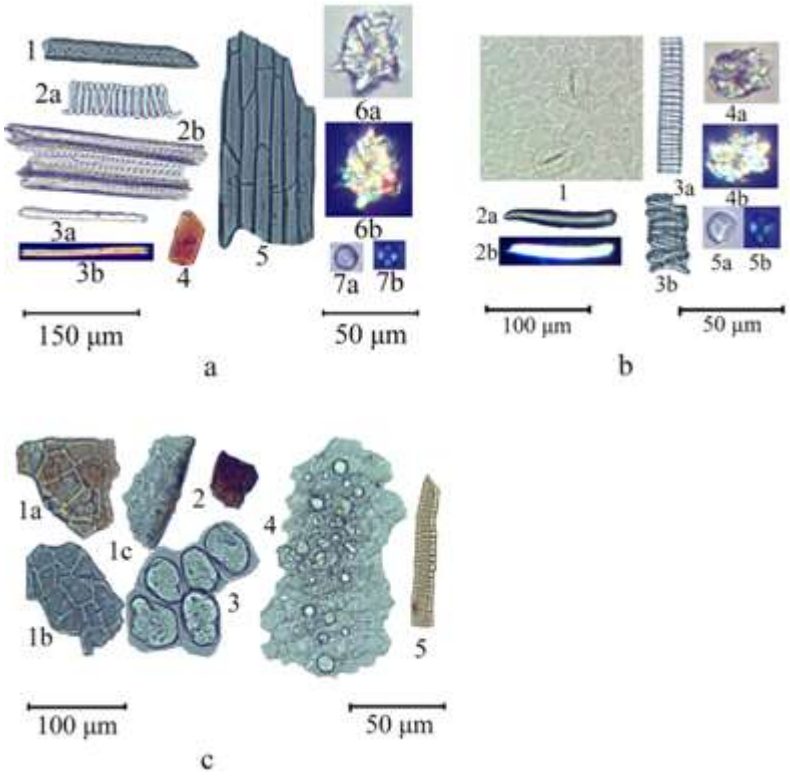

Figure 4. Microscopic characteristics of DAP of *P. quinquefolius* L. **a. Stem:** 1. Xylary parenchyma cells; 2a. Scalariform vessel, 2b. Reticulated vessel; 3a. Fibre, 3b.Fibre in polarized light; 4.Brown inclusion; 5. Cork cell; 6a.Calciumoxalate cluster crystal, 6b. Calcium oxalate cluster crystal in polarized light; 7a. Four starch compound grains, 7b. Four starch compound grains in polarized light. **b. Leaf:** 1. Epidermis cells and stoma; 2a. Fibre, 2b. Fibre in polarized light; 3a. Scalariform vessel, 3b. Spiral vessel; 4a. Calcium oxalate cluster crystal, 4b. Calcium oxalate cluster crystal in polarized light; 5a.Four starch compound grains, 5b. Four starch compound grains in polarized light. **c. Seed:** 1a.Episperm cells (apical view), 1b. Episperm cells (antapical view), 1c. Episperm cells (Side view); 2. Brown inclusion; 3. Stone cells; 4. Endosperm cells; 5. Scalariform vessel.

Stomata type was infinitive or indeterminate, which was 10 to 12.7 × 7.6 to 9 μm dispersedly distributed, and guard cell was kidney-shaped and 2 subsidiary cells with cuticula trace (Figure 3).

The average number of palisade cell under the epidermal cells was called Palisade Ratio (PR). Since palisade ratio was consistent and different from plant to plant, this parameter could be used in authentication, which was also a facile, inexpensive, and objective method. The palisade ratio was 700 to 800 shown in Figure 3.

Stomatal Index (SI) is a basilic parameter in authentication, which can be obtained from the following formula:

Stomatal Index = (number of stomata/ every millimeter-square) × 100 / (number of stomata/ every millimeter-square + number of epidermal cell/ every millimeter-square)

The stomatal index was 24.1% to 25%.

Mesophyll tissue is divided up by slendest vein, which is called vein islet. The numbers of vein islet in every epidermal cell is called Vein Islet Numbers (VIN), which also can be used as a reliable parameter in authentication since it is consistent in a species of plant. The vein islet numbers was 0.85 ± 0.17/mm^2.

Microscopic characteristics of dissociation and powders (DAP)

Microscopic characteristics of DAP of Stem

Green yellow color. Cork cell was quadrate, about 100 to 150 μm in length. Xylary parenchyma cell was about 170 to 220 μm in length, 24 to 30 μm in width, 2 to 4 μm in thickness. Vessel was mainly reticulate vessel and spiral vessel, 40 to 450 μm in length, 10 to 60 μm in width.

Fiber was wide cell cavity and obvious pit, 162 to 230 μm in length, 10 to 12 μm in width, 2 to 4 μm in thickness. Brown inclusion was about 18 to 25 μm in length, 11 to 14 μm in width, 3 to 5 μm in thickness. Clustered crystal was about 30 to 40 μm in diameter. Single particle and compound starch grains were observed occasionally, about 9 to 15 μm in diameter. The detailed features were shown in Figure 4a.

Microscopic characteristics of DAP of leaf

Dark green color. Epidermic cell was irregularly small quadrate, about 29 to 35 μm in length. Vessel was reticulate vessel and spiral vessel, 65 to 100 μm in length, 9 to 11 μm in width. Fiber was slender and long cell, 35 to 50 μm in length, 3 to 5 μm in width, 2 to 3 μm in thickness. Clustered crystal was about 18 to 30 μm in diameter. Starch grains were observed occasionally, about 12 to 16 μm; hilum distinct, punctuate, asteroid and crack; striations indistinct. Infinitive stomata were obvious, 13 to 18 in length, 11 to 14 μm in width, 2 to 4 μm in thickness. The detailed features were shown in Figure 4b.

Microscopic characteristics of DAP of seed

Episperm cell was about 36 to 45 in length, 24 to 30 μm in width, 1 to 2 μm in thickness. Brown inclusion was about 42 to 48 in length, 35 to 40 μm in width, 3 to 5 μm in thickness. Stone cell was about 53 to 65 in length, 40 to 50 μm in width, 3 to 5 μm in thickness. Endosperm cell was about 34 to 42 in length, 18 to 24 μm in width, 1 to 2 μm in thickness. Endosperm cell contains a lot of starch grains, aleurone grains and fatty oil. Scalariform vessel was about 70 to 95 in length, 9 to 14 μm in width, 2 to 3 μm in thickness. The detailed features were shown in Figure 4c.

DISCUSSIONS

From the above results knowable, the palmately compound leaves palmate from one to five-year-old generally exhibited the transition phases of three, four, and five stage; the number of compound leaf was excessive, and not exactly be increased the number of five palmately compound leaves year by year. This result was slightly different from the results in literature (Yao, 2007). And the peduncle of terminal umbel was much shorter than the top of the leaves. The result was different from the literature of not exceeding the petioles (Liu et al., 1991; Yao, 2007).

And the results of microscopic structures revealed that: (1) The secretory canal, the inclusion of starch grain and the calcium oxalate cluster crystals were also found in stem and leaf. (2) Isobilateral leaf; the palisade tissues and spongy tissues could not be obvious distinguished; mesophyll tissue cells were oval, arranged loosely with broad intercellular space. This result was consistent with the literature (Liu et al., 1991); the palisade ratio was 700 to 800; the stomatal index was 24.1% to 25%; the vein islet numbers was 0.85 ± 0.17/mm^2.

CONCLUSION

In conclusion, the number of compound leaf, palisade ratio, vein islet numbers, starch grain, calcium oxalate cluster crystals and secretory canal characteristics were presented in this study. Therefore, the methods could be helpful to distinguish Panacis Quinquefolii Radix from Canada. Moreover, it was also suggested that the stem and leaf could be further researched on biological active effects, content of ginsenosides and polysaccharides in order to evaluate the development value of stem and leaf of *Panax quinquefolius* L. grown in Ontario, Canada.

ACKNOWLEDGEMENTS

The authors appreciated the assistance of sample collection by famer Alex and worker Bing Fu of the Great Mountain Farm, Scotland, Ontario, Canada; the assistance of dealing with samples by the Suqi Liu, and Brian Mcgarvey of Southern Crop Protection and Food Research Centre, London, Ontario, N5V 4T3, Canada; China Natural Science Foundation (No. 81173653), the China Ministry of Science and Technology (No. 2012BAI27B07), and the Research Funds of Southwest University for Nationalities (13NLY01).

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