Variation Analysis of the Difference in Anatomical Properties and Chemical Composition of the Bark and Branch Wood of *Pteroceltis tatarinowii* at Different Annual Rings

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The bark of *Pteroceltis tatarinowii* is one of the main raw materials for the manufacturing of Xuan paper. In order to guide the production of Xuan paper, the anatomical properties and chemical compositions of the bark and branch wood from *P. tatarinowii* at different years of age were analyzed in this study. The results from a variation analysis of the anatomical properties indicated that the ratio of the length to width of the bark and branch wood was greater than 30, while the ratio of the lumen diameter to the wall thickness was less than 1. Furthermore, there were significant differences in the length and the ratio of length to width of the bark and branch wood. The results from a variation analysis of the chemical composition indicated that the lignin content of branch wood at various ages (years) was greater than the lignin content of bark. Additionally, the cellulose extractive and pentosan contents of the branch wood was less than the contents in the bark. Based on the analysis of the anatomical and chemical composition, barks that were 2 to 3 years old were the most suitable raw materials for the manufacturing of Xuan paper.

Keywords: Pteroceltis tatarinowii; Bark; Branch wood; Anatomical properties; Chemical compositions; Variation analysis

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INTRODUCTION

The Chinese paper industry has grown rapidly since the 1990s, and China is now at the top of the world in terms of the production and consumption of paper and cardboard (Ma *et al*. 2015). Over the past decades, in order to meet the growing demands for raw materials, considerable efforts have been made to improve the quality of trees, particularly in *P. tatarinowii* (Fang *et al*. 2004, 2006; Gao *et al*. 2007). The utilization of *P. tatarinowii* by the pulp industry has developed during recent years, and its usage will continue to increase in the future. The reason for this phenomenon is that the bark of *P. tatarinowii* is the main raw material in the manufacturing of traditional Xuan paper. Xuan paper is one of the most famous traditional inventions in China (Fang *et al*. 2004). Furthermore, as one of the Four Treasures. It is called the “King of Papers”, due to its thin, dense, durable, non-absorbent, and tough characteristics, as well as its pure white appearance (Gao *et al*. 2016). In addition, Xuan paper is made *via* an 18-step process and it is said to maintain its texture for over 1000 y. As the primary raw material of Xuan paper, *P. tatarinowii* has a long history and a special purpose in Chinese culture.

In the past few decades, scholars (both domestic and overseas) have become interested in handmade Xuan paper, which is peculiar to China. This interest is for the purpose of conservation (Shi and Tao 2013; Wang *et al.* 2014; Brown *et al.* 2017), dating (He *et al.* 2010; Helman-Ważny 2016) or assessment of authenticity (Li *et al.* 2017), and the exploration of applicable and reliable new, analytical methods (Yang *et al*. 2011).

P. tatarinowii is the only deciduous leaf arbor of the Ulmaceae, which is one of the tertiary residual plants in China. It is an endangered tertiary relic tree endemic to China with a high ecological and economic value (Fang *et al*. 2004, 2006). As such, *P. tatarinowii* has been designated as a national third class protected plant in China. It is distributed sporadically in Anhui, Shandong, Henan, Hubei, and 19 other provinces in China (Li *et al.* 2015). Due to the widespread vegetation destruction and excessive utilization of resources, the *P. tatarinowii* was decreased rapidly in recent years (Zhang and Liu 2019). Presently, there are multiple studies on the seed source selection, chemical components, productivity and bark quality, and rheological models of Xuan paper (Zuo *et al*. 1998; Wang *et al*. 2000; Fang *et al*. 2001, 2004; Gao *et al*. 2007; Gao *et al*. 2016). Since the properties of wood are affected by the environment, there are drastic variations in the structure and function of the wood. The same phenomenon is also present in the bark and branch wood. Therefore, the anatomical properties and chemical components of the bark and branch wood should be studied. Liu and Hu (1985) and Liu and Qu (1986) reported that the bark quality has a direct influence on the quality of the Xuan paper. The toughness of the *P. tatarinowii* branch wood has the advantages of a thin cell wall, which leads to high performance in terms of adsorbed ink (Chai *et al*. 2010). The chemical components of the raw material have an influence on the process, economic benefit, and pollution of the chemical pulping. At this point, a study on the variation of wood chemical components could provide a theoretical basis for the early selection and prediction of the wood properties of pulp forests and for reasonable rotation periods.

Based upon previous literature, multiple studies were focused on the anatomical properties and chemical components of *P. tatarinowii* that were 2 years to 3 years old (Gao *et al*. 2007; Li *et al*. 2012). Thus, in this paper, the anatomical properties and chemical components of the bark and branch wood from *P. tatarinowii* with an age range of 2 to 6 were studied. Moreover, the variation features in the axial direction were identified, in order to provide a basis for the utilization of *P. tatarinowii*, as well as to increase the value derived from the bark and branch wood. Therefore, this study could be a theoretical foundation for the high value utilization of *P. tatarinowii*.

EXPERIMENTAL

Materials

The *P. tatarinowii* samples, with an age range of 2 years to 6 years were collected on 27th July 2015 from Jing county, Anhui province, China. The collection site was located at approximately 117°57′ to 118°41′E longitude and 30°21′ to 30°50′N latitude, which is a humid subtropical monsoon climate zone. The area has a climate with an annual average temperature of approximately 15 °C, a mean annual precipitation of 1300 to 1600 mm, and an annual evaporation of 1400 to 1600 mm during the growing seasons. The frost-free period is approximately 230 d every year, and the average relative humidity is 79%. The total solar radiation is 484.68 kJ/cm^2 to 501.48 kJ/cm^2, the sunshine time is greater than 2000 h, and the relative sunshine duration is 42% to 49% (Fang *et al.* 2002). The collected samples were grown in the limestone highland and in calcareous soils.

Bark Preparation

Different aged branch wood of *P. tatarinowii* were used in this study. The *P. tatarinowii* branch was cut and then placed in water for two days. Then the bark and the wood were easily separated from the branch (as shown in Fig. 1).

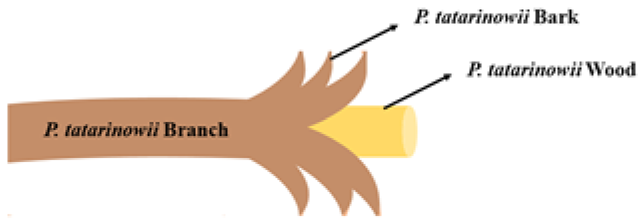

Fig. 1. Schematic diagram of sampling

Wood Anatomical Properties

Measurement of the fiber length

The *P. tatarinowii* bark and branch wood were cut into small pieces. The samples were placed in the test tube with acetic acid and hydrogen peroxide (at a 1 to 1 ratio by v/v) for segregation. Then, the test tube was plugged and placed in an oven at 80 °C for 8 h. Finally, the segregated fibers were prepared for the slices and observed under an electron microscope (DS RI-2, Nikon, Tokyo, Japan) to measure the length of the fibers. Approximately 500 fibers from different aged barks and branch wood were measured under a 100-fold microscope.

Preparation of the samples

The slices samples of the bark and branch wood were prepared according to the previous method with a minor modification (Barbosa *et al*. 2010). In brief, the samples were softened with a hot-cold cycle and then embedded in polyethylene glycol. After drying, the sample was dehydrated with 50%, 75%, 85%, 95%, or 100% alcohol in advance. Next, the samples were transparent treated and sealed; xylene was used for the transparent treatment of dehydrated slices, in order to make the slices clean and transparent. Afterwards, a neutral gum solution was added to the slices. Finally, the slices were placed in an oven at 40 °C. The fiber length, width, lumen diameter, and wall thickness of the bark and branch wood of *P. tatarinowii* were measured *via* an electron microscope (Nikon DS RI-2). The morphologies of samples were investigated by field emission scanning electron microscopy using a Hitachi S-3400N II (Hitachi, Japan) instrument at 15 kV. Before SEM observation. Transmission electron microscopy (TEM) images were obtained using transmission electron microscope (TEM, HT7700, Hitachi, Japan) under an acceleration voltage.

Wood Chemical Composition

Alcohol-benzene extractives

Standard GB/T 2677.6 (1994) was referenced to measure the content of the extractive, and the dried *P. tatarinowii* specimens were ground into 40 to 60 mesh particles for further experimentation. The *P. tatarinowii* powder was extracted with ethanol/toluene (at a 1 to 2 ratio by v/v) in a Soxhlet apparatus for 6 h and dried in a cabinet oven with air circulation at 60 °C for 16 h.

Holocellulose extraction

Standard GB/T2677.10 (1995) was referenced to extract the holocellulose from the *P. tatarinowii* samples. First, 2 g of the extracted sample, 65 mL of distilled water, 0.5 mL of glacial acetic acid, and 0.75 g of sodium chlorite ($NaClO_2$) were mixed and heated in a 75 °C water bath for 1 h. Then, 0.5 mL of glacial acetic acid and 0.75 g of sodium chlorite ($NaClO_2$) were continuously added to the reaction. This procedure was repeated three times until the sample became white. Finally, the sample was washed with distilled water to remove the acid, and then washed with acetone three times. The sample was dried at 105 °C overnight to obtain the holocellulose content.

Alpha-cellulose extraction

Using standard GB/T2677.10 (1995), the alpha cellulose content of the samples was obtained. The hemicellulose content was removed using sodium hydroxide. Then, 2 g of holocellulose was transferred to a small breaker, 30 mL of 17.5% NaOH was added, and the sample was mercerize-treated for 45 min in a 20 °C water bath. Afterwards, 30 mL of distilled water was added, and the sample was stirred for 1 to 2 min. The reaction solution was carefully removed into a pre-weighed glass filter. All retentate was filtered through the pre-weighed glass filter by washing it with 9.5% NaOH (3 mL x 25 mL). After washing with distilled water (400 mL), the solution was neutralized *via* soaking in 2 mol/L of acetic acid for 5 min. Finally, the sample was dried at 105 °C overnight to obtain the alpha cellulose content.

Acid-insoluble lignin extraction and acid-soluble lignin extraction

Using standard GB/T10337 (1989) and standard GB/T2677.8 (1994) the acid-insoluble and acid-soluble lignin content of the samples were obtained, respectively. The extracted sample (1 g) and 15 mL of 72% H_2SO_4 (10 °C to 15 °C) were added to a 250 mL Erlenmeyer flask. The mixture was heated in a 20 °C water bath for 2 h and was shaken during the water bath. After that, the reaction was transferred to a 1000 mL Erlenmeyer flask and then diluted with water until the concentration of the acid was 3% (approximately 560 mL of distilled water). The Erlenmeyer flask was placed on an electric furnace for 4 h and the total volume of the solution was maintained at 575 mL *via* the addition of hot water. Then, the sample was filtered and washed until a neutral pH was reached, in order to obtain the acid-insoluble lignin content of the samples.

The filtrate of the acid-insoluble lignin contained a small amount of acid-soluble lignin, which showed characteristic absorption in the ultraviolet region, and its absorption intensity was related to the content of the acid-soluble lignin. The content of acid-soluble lignin was obtained *via* measuring the absorbance of the filtrate at 205 nm.

Pentosan extraction

Using standard GB/T2677.9 (1994), the pentosan content of the samples was obtained. First, 0.5 g of the *P. tatarinowii* sample, 10 g of NaCl, and 100 mL of 12% HCl were mixed in a round bottom flask. The temperature of the furnace was adjusted to keep the distillation rate at 30 mL of distillate per 10 min. Next, 30 mL of 12% HCl was added after 30 mL of the distillate was distilled off. This procedure was repeated until the total volume of the distillate reached 360 mL. The distillate was transferred to a volumetric flask and fixed to 500 mL with 12% HCl. Then, 200 mL of the distillate, 150 g of crushed ice, and 25 mL of a mixed solution of potassium bromide and potassium bromate were added to an iodine flask. The mixtures were placed in the dark for 5 min. Finally, 10 mL of a 10%

potassium iodide solution was added to the mixture and allowed to stand for 5 min. The pentosan content was calculated *via* the titration of 0.1 mol/L sodium thiosulfate.

Data Analysis

SPSS 20.0 software IBM (USA) and Microsoft Excel 2010 were used to perform statistical analysis, *i.e.*, T-test, variance, and experimental data. In the analysis of variance, it was assumed that the treatment variances were equal. That was known as homogeneity of variance (HOV). As one of the most commonly used was HOV test, Levene 's test was selected for testing whether the variance was equal. The output in the difference T-test for equality of means by SPSS included two rows: equal variances assumed and equal variances not assumed. Levene's test indicated that the variances were equal (*i.e*., p-value large), the first row of output was reliable in the results from the actual independent samples T-test. If Levene's test indicated that the variances were not equal, the second row of output was reliable. The difference between these two rows of output above rely on the way the T-test statistic was calculated.

RESULTS AND DISCUSSION

Analysis of the Anatomical Properties of the Bark and Branch wood of *P. tatarinowii* at Different Years of Age

The anatomical properties of the bark and branch wood of *P. tatarinowii* at different years of age are listed in Table 1 and Fig. 2. As depicted in Table 1 and Fig. 2a, the fiber lengths of the bark and branch wood at 3 years were longer than the rest, while the largest ratio of length to width from the bark was also the longest at 3 years from 1 to 6 years. Generally, the fiber length determines the most suitable raw material for the paper industry. The paper properties, such as the tensile strength, optical property, and surface quality are affected by the fiber length (Zhang *et al*. 2006; Gao *et al*. 2007). The contact site and binding force of the pulp fibers were directly influenced by the fiber length. In recent work by Gao *et al*. (2007), it was shown that the quality of Xuan paper can be directly influenced by the fiber length. The fiber aspect ratio is one of the most important factors that affects the quality of the paper. As the fiber aspect ratio is increased, the probability of hydrogen bonds occurring between the fibers is also increased, and the mechanical properties of the paper would be increased. In general, larger fiber lengths and narrower fiber widths (with a length to width ratio greater than 30) were the first choice for pulp and paper production; this led to higher density paper, smoother paper, more uniform formation, *etc*. (Ma *et al*. 2015). All the data presented in Table 1 had a ratio of length to width (for both bark and branch wood) greater than 30, which met the prerequisites for paper-making raw materials.

The fiber wall thickness, lumen diameter, and ratio of lumen diameter to wall thickness are the standards for measuring the merit of a papermaking material. Usually, the thinner the cell wall and the larger the lumen diameter, the easier it is for the fiber to collapse during the papermaking process. This leads to the binding ability of the fibers being drastically enhanced. For wood fibers, the ratio of lumen diameter to wall thickness is less than 1, which is classified as the finest papermaking material (Niu *et al*. 2010). Based on the anatomical analysis (Table 1), it was found that 2- to 3-year-old bark was the most suitable raw materials for manufacturing Xuan paper.

Table 1. Descriptive Statistics of the Anatomical Properties of *P. tatarinowii* Bark and Branch wood at Different Years of Age

Parameters	Years (µm)						Mean (%)	Standard Deviation (SD)	Coefficient of Variation (CV)
	1	2	3	4	5	6			
Length (Bark)	2193	2416	2432	2392	2129	2167	2289	139	0.060
Length (Branch wood)	624	840	903	842	665	613	748	128	0.171
Width (Bark)	11.47	12.29	11.12	11.06	11.75	11.56	11.54	0.450	0.039
Width (Branch wood)	9.74	13.77	12.33	11.31	15.13	14.94	12.87	2.136	0.166
Lumen Diameter (Bark)	8.24	9.57	8.16	8.12	7.47	7.23	8.13	0.813	0.100
Lumen Diameter (Branch wood)	6.24	10.10	8.62	7.57	10.96	9.78	8.88	1.749	0.197
Ratio of Length to Width (Bark)	191	196	218	216	181	187	199	15.522	0.078
Ratio of Length to Width (Branch wood)	64.09	61.02	73.31	74.50	43.99	41.03	59.66	14.318	0.240
Wall Thickness (Bark)	5.74	2.72	2.96	2.93	3.28	3.79	3.57	1.131	0.317
Wall Thickness (Branch wood)	3.50	3.67	3.70	3.74	4.17	4.34	3.87	0.348	0.090
Ratio of Lumen Diameter to Wall Thickness (Bark)	0.70	0.29	0.36	0.36	0.44	0.52	0.44	0.150	0.341
Ratio of Lumen Diameter to Wall Thickness (Branch wood)	0.56	0.36	0.43	0.49	0.38	0.44	0.45	0.070	0.156

Statistical analysis of the anatomical properties indicated that the coefficient of variation (CV) of the factors were ranked as follows: the ratio of lumen diameter to wall thickness of the bark (0.341) > wall thickness of the bark (0.317) > ratio of length to width of the branch (0.240) > length of the branch (0.171) > width of the branch (0.166) > ratio of the lumen diameter to the wall thickness of the branch (0.156). The CVs of the other parameters were relatively small (CV was less than 0.1).

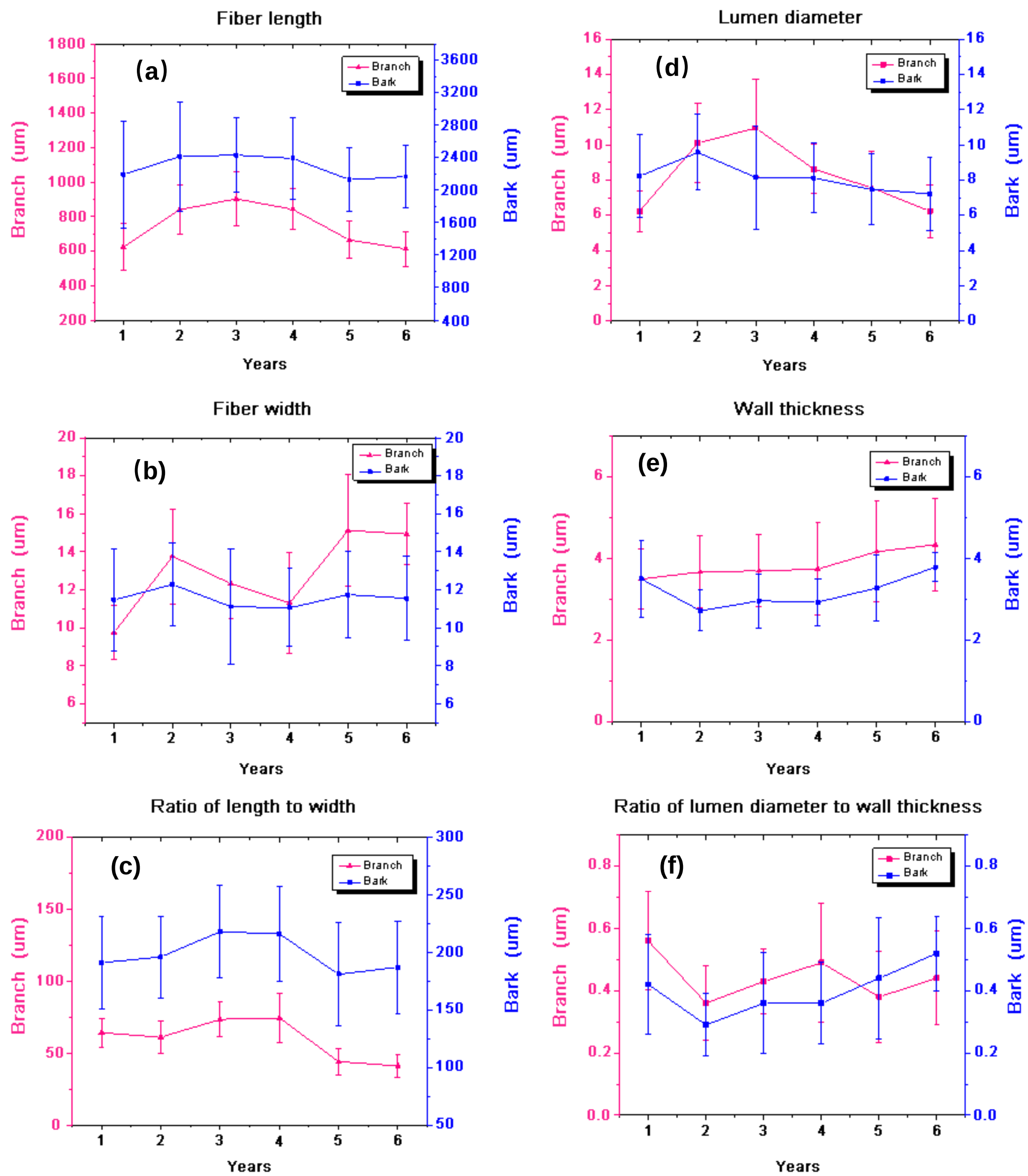

Fig. 2. Variation in the anatomical properties of *P. tatarinowii* at different years of age (a: fiber length; b: fiber width; c: ratio of length to width; d: lumen diameter; e: wall thickness; and f: ratio of lumen diameter to wall thickness)

Illustration of the Bark Anatomy

The anatomical properties of 2-year-old to 3-year-old *P. tatarinowii* bark were reported in this study (as shown in Figs. 3 through 8). Illustrated in the cross-section pictures, the bark of a *P. tatarinowii* branch was composed of the epidermis, periderms, cortex, and secondary phloem (Fig. 4a). The optical and electron microscopic observations showed that the secondary phloem of the bark primarily consisted of bast fiber cells, sieve tubes, companion cells, axial parenchyma, and ray parenchyma cells. The bast fibers were agglomerated or slightly broken. The individual bast fibers were spindle-shaped cells with sharp ends, and the fiber width was irregular with an uneven thickness. There were multiple forms of fibers, which included the abnormal fibers. The ray parenchyma cells were primarily rectangular, with both single and double rows. Most of the parenchyma cells contained starch granules and red inclusions, which were probably the extractive of the wood such as tannins (Fig. 5b) (Helama *et al.* 2010). The sieves and parenchyma cells became small clusters scattered among the fibers.

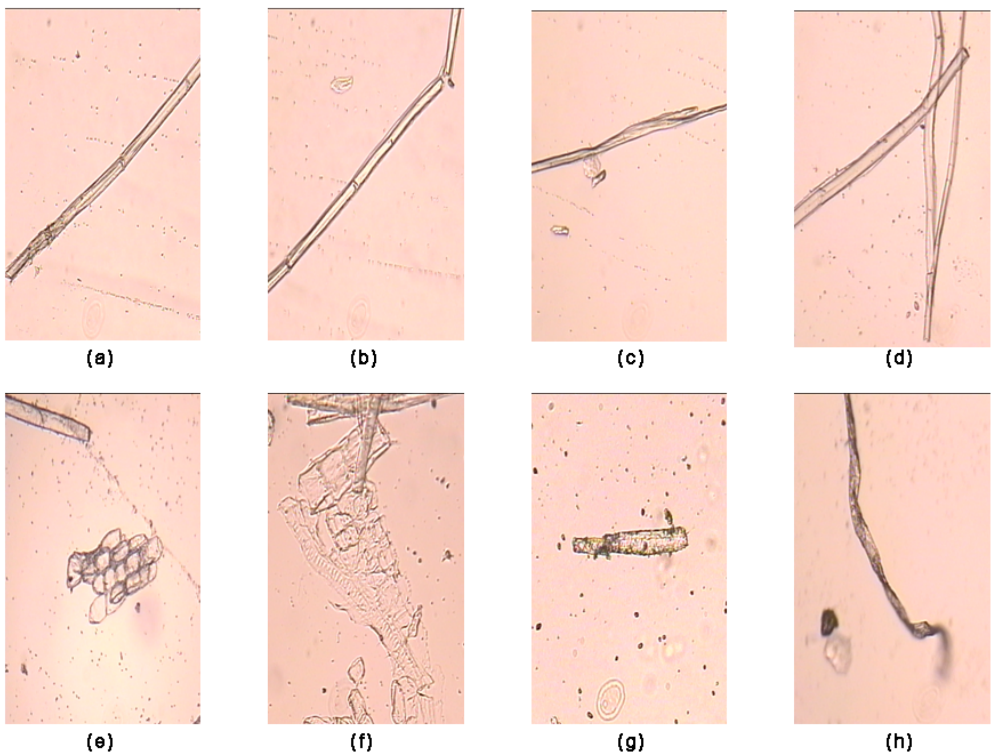

Fig. 3. The anatomy of the 2-year-old to 3-year-old *P. tatarinowii* bark (LM x 100) (a, b, c, and d: fiber; e: axial parenchyma; f: sieve tube; g: pit; and h: abnormal fiber)

As shown in Fig. 6, the cross-section of the *P. tatarinowii* bark fiber was elliptical, polygonal, and irregular. However, the cell wall of the fiber was thin with small lumen. This was due to the fact that the cambium cell was continuously proceeding of periclinal division and anticlinal division during the growth of trees, resulting in the formation of secondary phloem outward and xylem inward (Luostarinen and Hakkarainen 2019). Furthermore, the cells of xylem were usually more than those of phloem in the wood. Therefore, phloem fiber was shoved to the bark, and the fiber cell was thin with small lumen. The cell was mostly oval, polygonal and irregular. The capillary tube had stronger liquid absorption with more gaps among microfibrils and microfibrils, resulting in the Xuan paper having good ink absorption (Fig. 6). There were bordered pits on the cell walls, however, there was a low number of total pits.

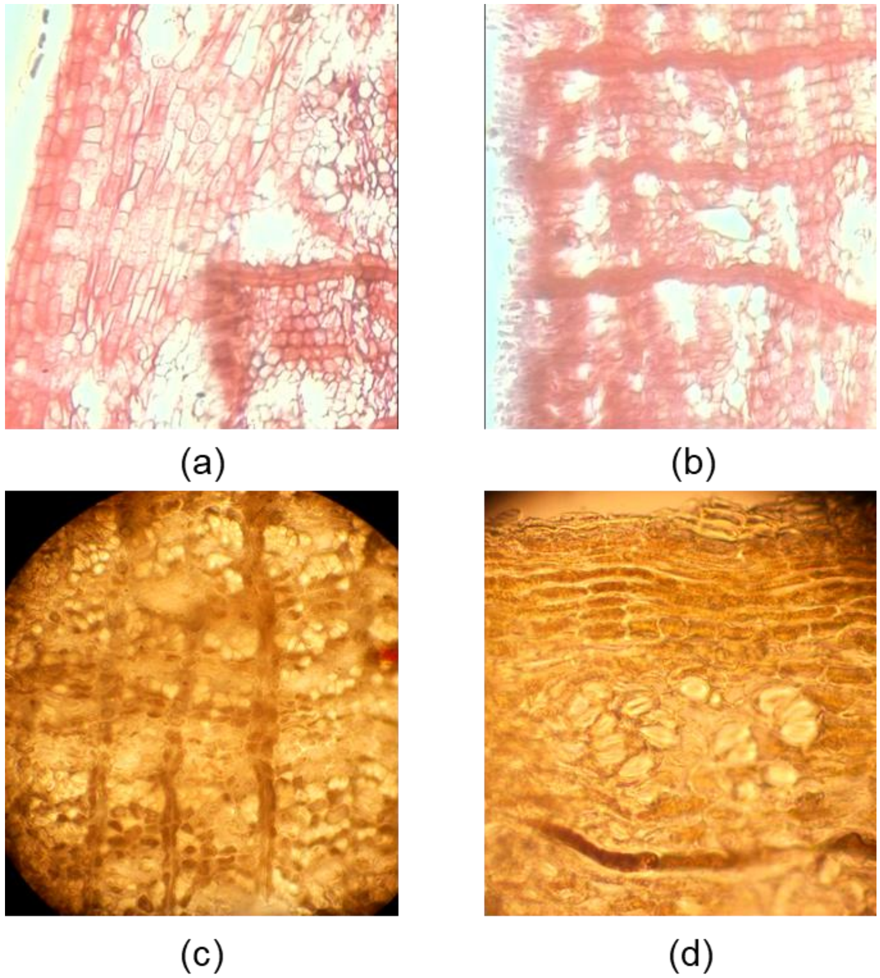

Fig. 4. Cross section of the 2-year-old to 3-year-old *P. tatarinowii* bark (LM x 100)

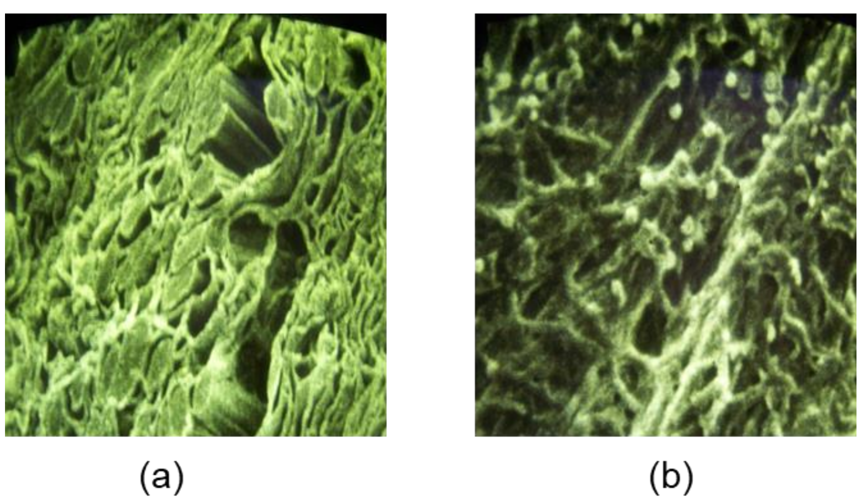

Fig. 5. Scanning electron microscopy of the 2-year-old to 3-year-old *P. tatarinowii* bark (a) fiber bundle (SEM x 885) and (b) starch particles (SEM x 1490)).

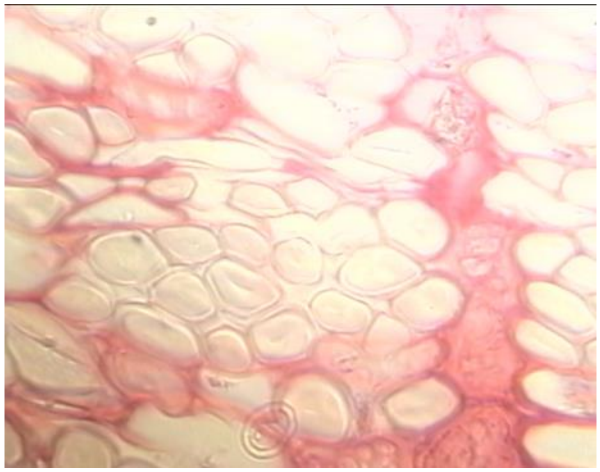

Fig. 6. Fiber of the 2-year-old to 3-year-old *P. tatarinowii* bark (LM x 400)

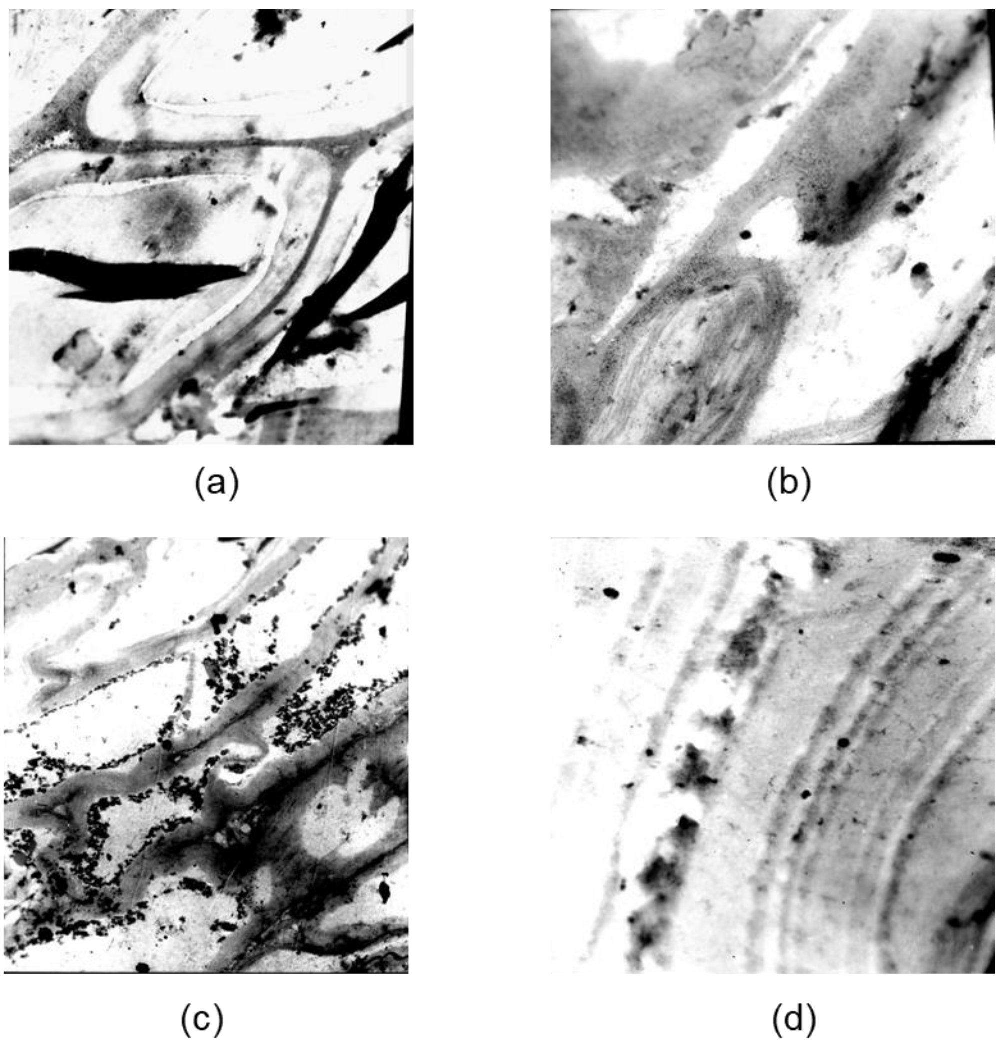

Fig. 7. Transmission electron microscopy of the 2-year-old to 3-year-old *P. tatarinowii* bark (a: cross section (TEM x 10000); b: bordered pit (TEM x 15000); c: parenchyma (TEM x 6000); and d: lamellar structure of cell wall (TEM × 15000))

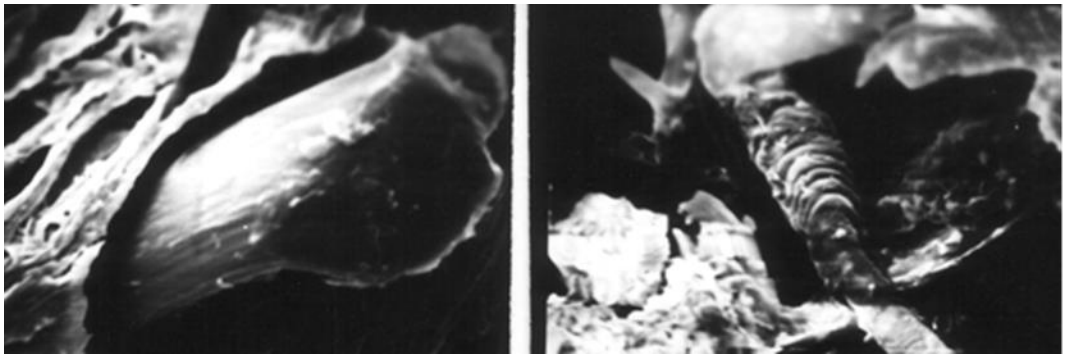

Fig. 8. SEM of Giant fibril of the 2-year-old to 3-year-old *P. tatarinowii* bark (SEM×2500)

The scanning electron microscopy pictures showed that the giant fibrils of the *P. tatarinowii* bark cell walls were substantially cylindrical (Fig. 8). When the giant fibril surface crust was completely removed, a helical fiber was found to be wrapped around the fibrils. A single fiber could possibly have formed *via* multiple intertwined microfibers. The outer wall of the primary wall and secondary wall would be broken during the beating of the pulp. Multiple pores remained between the intertwined fibers, which could accommodate and retain small calcium carbonate particles to increase the adsorption of ink (Hoeger *et al.* 2014).

Differences in the Anatomical Properties of the Bark and Branch wood of *P. tatarinowii*

The T-test is mainly used for the normal distribution with small sample size and unknown population standard deviation. The analysis of the T-test results on differences in the anatomical properties of the bark and branch wood of *P. tatarinowii* are listed in Table 2. The data revealed that there were significantly differences in the length and the ratio of length to width of the bark and branch wood (P-value was less than 0.05). In contrast, the lumen diameter, width, wall thickness, and ratio of lumen diameter to wall thickness showed no significant difference (P-value was greater than 0.05). When compared to the other indexes, the ratio of length to width and the length of the fibers were significantly different at different years of age. Therefore, this result suggested that the differences in the anatomical properties between the bark and branch wood of *P. tatarinowii* were mainly the ratio of length to width and the length of the fibers. And both parameters were the main factor in the pulping processing.

Analysis of Variance on Anatomical Properties of *P. tatarinowii*

Table 3 shows the variability of the anatomical properties at different years of age. The lumen diameter, wall thickness, and ratio of the lumen diameter to wall thickness at different years of age showed the largest variability (the F-value was greater than $F_{0.01}$ and the P-value was less than 0.01). Variation of the width were also significantly different (the F-value was greater than $F_{0.05}$ and the P-value was less than 0.05). However, variations in the length and the ratio of length to width were relatively small, with no significant differences at different years of age (the F-value was less than 1 and the P-value was greater than 0.05). The variability results suggested that the anatomical properties were different among different years. Therefore, the age was one of the main factor for the material properties.

Table 2. Analysis of T-test on the Differences in the Anatomical Properties of the Bark and Branch wood of *P. tatarinowii*

Levene's Test for Equality of Variances				Difference T-test for Equality of Means			
		Significance (Sig)	F-statistic	T-value	Degrees of freedom (df)	P-value	Mean Difference
Length	Equal Variances Assumed	0.474	0.553	19.933	10	0.000	1540
	Equal Variances Not Assumed	-	-	18.416	9.929	0.000	1540
Width	Equal Variances Assumed	12.412	0.006	1.494	10	0.166	1.328
	Equal Variances Not Assumed	-	-	1.494	5.449	0.191	1.328
Lumen Diameter	Equal Variances Assumed	4.389	0.063	0.949	10	0.365	0.749
	Equal Variances Not Assumed	-	-	0.949	7.062	0.374	0.749
Ratio of Length to Width	Equal Variances Assumed	0.094	0.765	-16.110	10	0.000	-138
	Equal Variances Not Assumed	-	-	-16.110	9.927	0.000	-138
Wall Thickness	Equal Variances Assumed	2.992	0.118	0.619	10	0.550	0.298
	Equal Variances Not Assumed	-	-	0.619	5.997	0.559	0.298
Ratio of Lumen Diameter to Wall Thickness	Equal Variances Assumed	2.047	0.183	0.031	10	0.976	0.002
	Equal Variances Not Assumed	-	-	0.031	7.313	0.976	0.002
Note: The statistical significance was at the 95% confidence level							

Table 3. Analysis of Variance of the Anatomical Properties of *P. tatarinowii*

	Length	Width	Lumen Diameter	Ratio of Length to Width	Wall Thickness	Ratio of Lumen Diameter to Wall Thickness
Sample ID	210	210	210	210	210	210
Mean (%)	1519	12.063	8.514	128	3.520	0.421
F-statistic	0.151	3.710	4.374	0.346	3.968	8.456
P-value	0.978	0.010	0.004	0.881	0.007	0.000
Std error	132	0.306	0.219	11.718	0.079	0.013
Note: $F_{0.05}$ (6, 29) = 2.43; $F_{0.01}$ (6, 29) = 3.70; Sample ID represents the total test sample numbers						

Analysis of the Chemical Composition of the Bark and Branch wood of *P. tatarinowii* at Different Years of Age

The chemical components have a major influence on the pulping and papermaking process. The cellulose, hemicellulose, and lignin are the three main components of a plant cell wall, and the minor components are the extractives and ash. The yield of the pulp can be directly affected by the chemical composition and proportions of the cellulose, hemicellulose, and lignin content. However, when the lignin is removed and accompanied by the degradation of the cellulose, the lignin content can have adverse effects on the pulping process (Ma *et al*. 2015). According to Hart *et al*. (2013), higher pulp yields and quality are associated with lower lignin and higher cellulose contents. Lignin has a drastic adverse effect on the paper strength and increases the ease in which the paper oxidizes back to yellow. It is necessary to remove most of the lignin content during the cooking process. It was also necessary to degrade the residual lignin in the subsequent bleaching process. As shown in Table 4 and Fig. 9d, the acid-insoluble lignin content in the bark was less than the content in the branch wood at different years of age. Furthermore, with an increasing age, the acid-insoluble lignin content in the bark increased gradually; while the acid-insoluble lignin content in the branch wood increased first, and then declined, as shown in Fig. 9d. Moreover, the acid-insoluble lignin content increased from 6.05% to 7.89% from 2 years to 6 years. The average acid-insoluble lignin content was 6.77% with a standard deviation of 0.009 and a coefficient of variation of 0.129. Variation in the percentage of lignin was associated with the differences in age.

In addition to the two major components of the wood chemical composition, the resin, ash, and other trace elements had an impact on the pulp and paper properties (Xu 2004). Extractives have an impact on the properties of the wood, as well as impacting the processing and utilization. Generally, the physical strength of paper decreases when the raw material contains a higher amount of alcohol extractive. The extractives content showed an increasing trend (Fig. 9a). The analysis of the variance of the different chemical components in *P. tatarinowii* is shown in Table 4. It could be seen that the extractives content of the branch wood ranged from 1.62% to 2.67%. However, the drug consumption increased when the extractives content was low. Based on the results shown in Fig. 9, the α-cellulose, extractives, and pentosan content of the branch wood were less than the content of the bark. The α-cellulose content in the branch wood gradually increased, while it decreased in the bark (Fig. 9b). This is relevant when considering that a high cellulose contents would be favorable in terms of a higher pulp yield production. The α-cellulose content decreased from 45.82% to 42.3% in 2-year-old to 6-year-old samples.

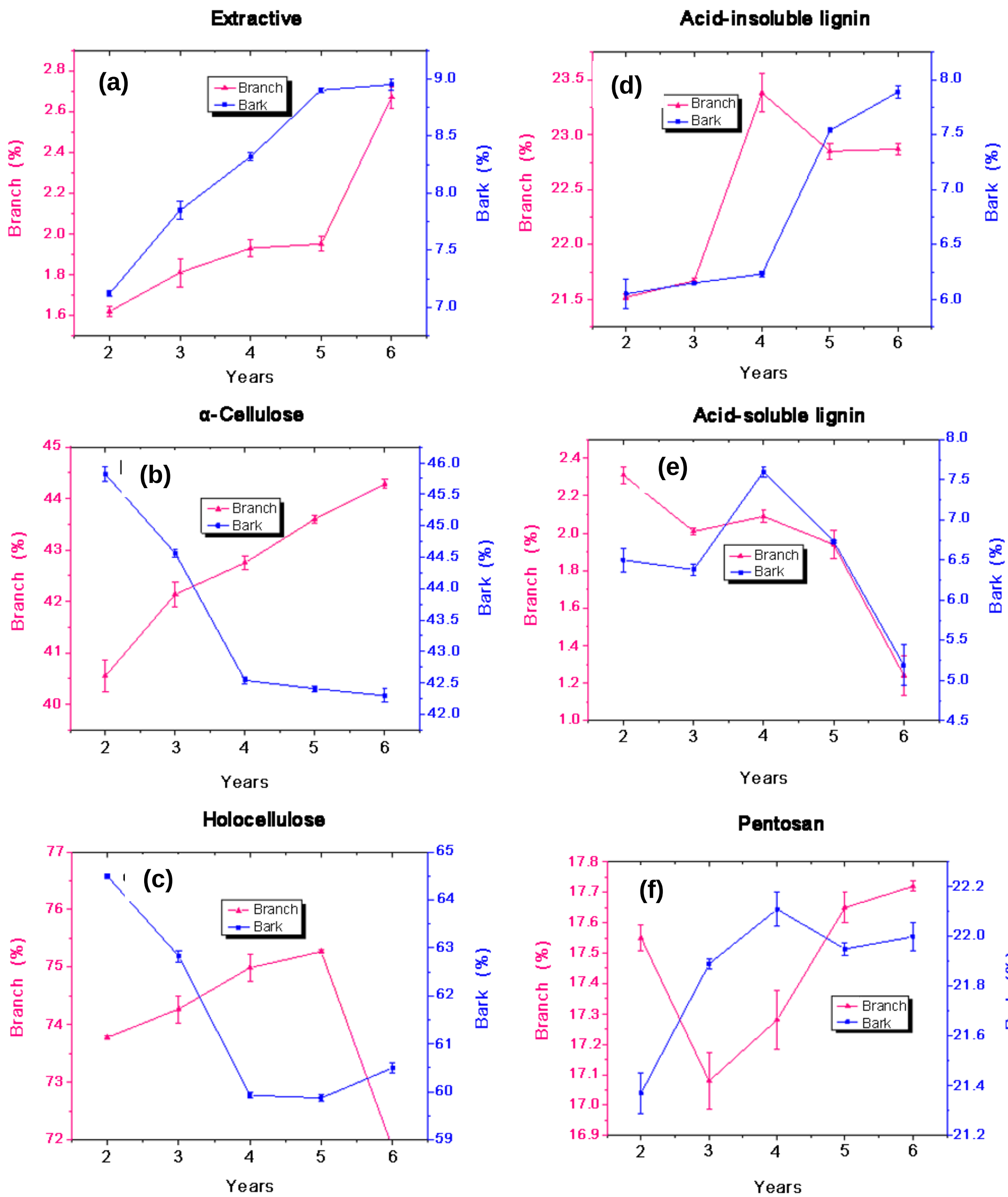

Fig. 9. Variation in the chemical composition of *P. tatarinowii* at different years of age (a: extractive; b: α-cellulose; c: holocellulose; d: acid-insoluble lignin; e: acid-soluble lignin; and f: pentosan)

There was no obvious change in the pentosan content among the 2-year-old to 6-year-old samples (Fig. 9f). The CVs for α-cellulose, extractives, pentosan, and hemicellulose were 0.037, 0.097, 0.011, and 0.033, respectively. Statistical analysis of the chemical composition (for the bark) indicated that the coefficient of variations were as follows: the acid insoluble lignin CV was greater than the alcohol benzene extractive, which was greater than the α-cellulose, which was greater than the acid soluble lignin, which was greater than the holocellulose, which was greater than the pentosan CV. The CVs of branch wood were as follows: acid soluble lignin was greater than the alcohol benzene extractive, which was greater than the acid insoluble lignin, which was greater than the α-cellulose, which was greater than the holocellulose, which was greater than the pentosan CV. The variability in the chemical composition of the bark was larger in relation to the acid insoluble lignin, α-cellulose, and holocellulose contents than in relation to the extractive, acid-soluble lignin, and pentosan contents.

Table 4. Descriptive Statistics of the Chemical Composition of *P. tatarinowii* between Bark and Branch wood at Different Years of Age

Parameters	Years (μm)						Mean (%)	Standard Deviation (SD)	Coefficient of Variation (CV)
	1	2	3	4	5	6			
Extractive (Bark)	7.12	7.85	8.32	8.90	8.95	8.23	0.008	0.097	7.12
Extractive (Branch wood)	1.62	1.81	1.93	1.95	2.67	2.00	0.004	0.200	1.62
Acid-insoluble Lignin (Bark)	6.05	6.15	6.23	7.54	7.89	6.77	0.009	0.129	6.05
Acid-insoluble Lignin (Branch wood)	21.52	21.67	23.38	22.85	22.87	22.45	0.008	0.037	21.52
Acid-soluble Lignin (Bark)	6.50	6.38	7.60	6.73	5.19	6.48	0.009	0.013	6.50
Acid-soluble Lignin (Branch wood)	2.31	2.01	2.09	1.94	1.24	1.92	0.004	0.208	2.31
α-cellulose (Bark)	45.82	44.56	42.54	42.41	42.3	43.53	0.016	0.037	45.82
α-cellulose (Branch wood)	40.55	42.14	42.75	43.6	44.28	42.66	0.014	0.034	40.55
Pentosan (Bark)	21.37	21.89	22.11	21.95	22.00	21.86	0.002	0.011	21.37
Pentosan (Branch wood)	17.55	17.08	17.28	17.65	17.72	17.46	0.003	0.016	17.55
Holo-cellulose (Bark)	64.50	62.83	59.93	59.87	60.50	61.53	0.021	0.033	64.50
Holo-cellulose (Branch wood)	73.78	74.27	74.99	75.27	71.86	74.03	0.013	0.018	73.78

As shown in Table 4, a statistical analysis of the chemical compositions indicated that the major chemical compositions (acid insoluble lignin, α-cellulose, and holocellulose) of *P. tatarinowii* were relatively stable. The minor compositions (extractive) could be influenced by the age and growth status of *P. tatarinowii*.

Table 5. Analysis of the T-test on the Differences in the Chemical Constituents of the *P. tatarinowii* Bark and Branch wood

Levene's Test for Equality of Variances				Difference T-test for Equality of Means			
		Significance (Sig)	F statistic	T-value	Degrees of freedom (df)	P value	Mean Difference
Extractive	Equal Variances Assumed	0.156	2.457	-16.122	8	0.000	-0.062
	Equal Variances Not Assumed	-	-	-16.122	6.018	0.000	-0.062
Acid-insoluble Lignin	Equal Variances Assumed	0.692	0.168	29.342	8	0.000	0.157
	Equal Variances Not Assumed	-	-	29.342	7.967	0.000	0.157
Acid-soluble Lignin	Equal Variances Assumed	0.361	0.938	-10.689	8	0.000	-0.046
	Equal Variances Not Assumed	-	-	-10.689	5.664	0.000	-0.046
α-cellulose	Equal Variances Assumed	0.548	0.394	-0.901	8	0.394	-0.009
	Equal Variances Not Assumed	-	-	-0.901	7.922	0.394	-0.009
Pentosan	Equal Variances Assumed	0.813	0.060	-25.043	8	0.000	-0.044
	Equal Variances Not Assumed	-	-	-25.043	7.962	0.000	-0.044
Holo-cellulose	Equal Variances Assumed	0.170	2.273	11.377	8	0.000	0.125
	Equal Variances Not Assumed	-	-	11.377	6.910	0.000	0.125
Note: The statistical significance was at the 95% confidence level							

Differences in the Chemical Composition of the Bark and Branch wood of *P. tatarinowii*

To determine the difference between the bark and the branch wood of *P. tatarinowii*, the results of the T-test on the differences in the chemical composition are listed in Table 5. For the alcohol benzene extractive, acid-insoluble lignin, acid-insoluble lignin, pentosan, and holocellulose content in the bark and branch wood were significantly different (the P-value was less than 0.05). In contrast, the α-cellulose content showed no significant difference (the P-value was greater than 0.05). The variations in the chemical composition between the bark and branch wood of the *P. tatarinowii* were presented clearly. The results indicated that the chemical compositions were different in the content with the different positon of the plant at different ages.

Analysis of Variance on the Chemical Composition of *P. tatarinowii*

Table 6 shows the variability of the chemical compositions at different years of age. The acid-insoluble lignin, acid-insoluble lignin, α-cellulose, pentosan, and holocellulose content was relatively small, with no significant differences at different years of age (the F-value was less than 1 and the P-value was greater than 0.05). This result indicated that the main chemical compositions of *P. tatarinowii* had variability in different ages. Although the cellulose content was always slight difference, and other main compositions had different patterns.

Table 6. Analysis of Variance on the Chemical Compositions of *P. tatarinowii*

	Extractive	Acid-Insoluble Lignin	Acid-soluble Lignin	*α*-cellulose	Pentosan	Holocellulose
Sample ID	180	180	180	180	180	180
Mean (%)	5.111	14.614	4.162	43.195	19.659	67.629
F-statistic	1.081	0.044	0.431	0.430	0.034	0.176
P-value	0.387	0.996	0.785	0.785	0.998	0.949
Std error	0.006	0.015	0.004	0.003	0.004	0.124
Note: $F_{0.05}$ (4, 25) = 2.76; $F_{0.01}$ (4, 25) = 4.18; Sample ID represents total test sample numbers						

CONCLUSIONS

1. A key point of this study was the significant variation in the wood chemical composition and anatomical properties that occurred in the bark and branch wood of a 2-year-old to a 6-year-old *P. tatarinowii* sample. The fiber length and ratio of length to width of the bark was longer than the branch wood, which would provide the prerequisites necessary for papermaking raw materials. Significant variation was detected for many of the cell wall traits, which included the lumen diameter, wall thickness, and ratio of lumen diameter to wall thickness.
2. The chemical composition showed variation between the bark and branch wood, and the variability was significantly greater for the *α*-cellulose content. The amount of wood extractives increased with age, and for the case studied, the bark had a higher

extractive content and a lower lignin content.

3. Overall, the results of this study showed that the bark from a 2-year-old to 3-year-old *P. tatarinowii* tree was the most suitable raw material for manufacturing Xuan paper.

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REFERENCES CITED

Barbosa, A. C. F., Pace, M. R., Witovisk, L., and Angyalossy, V. (2010). "A new method to obtain good anatomical slides of heterogeneous plant parts," *IAWA Journal* 31(4), 373-383. DOI: 10.1163/22941932-90000030

Brown, N., Lichtblau, D., Fearn, T., and Strlič, M. (2017). "Characterisation of 19th and 20th century Chinese paper," *Heritage Science* 5(47), 1-14. DOI: 10.1186/s40494-017-0158-x

Chai, X.-Y., Chen, S.-L., and Xu, W. (2010). "Using inter-simple sequence repeat markers to analyze the genetic structure of natural *Pteroceltis tatarinowii* populations and implications for species conservation," *Plant Systematics and Evolution* 285(1-2), 65-73. DOI: 10.1007/s00606-009-0256-7

Fang, S. Z., Li, G. Y., Li, T. S., and Hui, C. (2001). "The effects of management practice on above-ground biomass production and phloem production of *Pteroceltis tatarinowii* Maxim. Plantations," *Journal of Plant Resources and Environment* 10(1), 21-24.

Fang, S. Z., Li, G. Y., and Fu, X. X. (2002). "Effects of site conditions on mineral element contents in the bark of wingceltis (*Pteroceltis tatarinowii*)," *Scientia Silvae Sinicae* 38(1), 8-14.

Fang, S., Li, G., and Fu, X. (2004). "Biomass production and bark yield in the plantations of *Pteroceltis tatarinowii*," *Biomass and Bioenergy* 26(4), 319-328. DOI: 10.1016/j.biombioe.2003.08.005

Fang, S.-Z., Song, L.-Y., and Fu, X.-X. (2006). "Effects of NaCl stress on seed germination, leaf gas exchange and seedling growth of *Pteroceltis tatarinowii*," *Journal of Forestry Research* 17(3), 185-188. DOI: 10.1007/s11676-006-0043-z

Gao, H., Wang, F., and Shao, Z. (2016). "Study on the rheological model of Xuan paper," *Wood Science and Technology* 50(2), 427-440. DOI: 10.1007/s00226-015-0781-1

Gao, H., Xu, B., and Shao, Z. (2007). "Chemical Compositions and Structure of Cell Wall in Pteroceltis tatarinowii Bark," *Nonwood Forest Research* 4, 008. DOI: 10.1255 /ejms.1226

GB/T 10337. (1989). "Raw material and pulp – Determination of acid-soluble lignin," Standardization Administration of China, Beijing, China.

GB/T 2677.10. (1995). "Fibrous raw material – Determination of holocellulose," Standardization Administration of China, Beijing, China.

GB/T 2677.6. (1994). "Determination of organic solvent extractables content of

papermaking raw materials," Standardization Administration of China, Beijing, China.
GB/T 2677.8. (1994). "Fibrous raw material – Determination of acid-insoluble lignin," Standardization Administration of China, Beijing, China.
GB/T 2677.9. (1994). "Fibrous raw material – Determination of pentosan," Standardization Administration of China, Beijing, China.
He, Q.-J., Li. T., Shi, J.-L, Zhao, R-T., Li, Y-L., and Shao, F. (2010). "Non-destructive in-situ characterization of pigments on a portrait of Chinese Taoism figure," *Sciences of Conservation and Archaeology* 22(3), 61-68.
Helman-Ważny, A. (2016). "More than meets the eye: Fibre and paper analysis of the Chinese manuscripts from the Silk Roads." *Science & Technology of Archaeological Research* 2(2), 127-140. DOI: 10.1080/20548923.2016.1207971
Helama, S., Vartiainen, M., Kolstrom, T., and Merilainen, J. (2010). "Dendrochronological investigation of wood extractives," *Wood Science and Technology* 44(2), 335-351. DOI: 10.1007/s00226-009-0293-y.
Hoeger, I., Gleisner, R., Negron, J., Rojas, O.-J., and Zhu, J.-Y. (2014). "Mountain pine beetle-killed lodgepole pine for the production of submicron lignocellulose fibrils," *Forest Science* 60(3), 502-511. DOI: 10.5849/forsci.13-012.
Li, J.-H., Wang, W., Yao, G.-X., and Wang, D.-P. (2012). "Composition of bark fiber of wild *Pteroceltis tatarinowii* in Daguisi national forest park," *Plant Science Journal* 3-(5), 511-518. DOI: 10.3724/SP.J.1142.2012.50511.
Li, X.-H., Zhang, X.-P., Liu, K., Liu, H.-J., and Shao, J.-W. (2015). "Efficient development of polymorphic microsatellite loci for *Pteroceltis tatarinowii* (Ulmaceae)," *Genetics Molecular Research* 14(4), 16444-16449. DOI: 10.4238/2015.December.9.15.
Li, T., Jin, J., Zhong, Z., and Shi, J. (2017). "A multi-analytical approach to investigate date-unknown paintings of Chinese Taoist priests," *Archaeological and Anthropological Sciences* 9(3), 395-404. DOI: 10.1007/s12520-015-0293-9
Liu, R., and Hu, Y. (1985). "Study on the characteristic of spreading of prepared Chinese ink," *China Pulp & Paper* 3, 23-29.
Liu, R., and Qu, Y. (1986). "A preliminary study on the durability of Xuan paper China pulp and paper," *China Pulp & Paper* 6, 32-36.
Luostarinen, K., and Hakkarainen, K. (2019). "Chemical composition of wood and its connection with wood anatomy in *Betula pubescens*," *Scandinavian Journal of Forest Research* 34(7), 577-584. DOI: 10.1080/02827581.2019.1662939.
Ma, H., Dong, Y., Chen, Z., Liao, W., Lei, B., Gao, K., Li, S., and An, X. (2015). "Variation in the growth traits and wood properties of hybrid white poplar clones," *Forests* 6(4), 1107-1120. DOI: 10.3390/f6041107
Niu, M., Gao, H., and Zhao, G.-J. (2010). "Fiber morphology and chemical composition of tension wood in *Populus* x *euramericana* cv. 'Neva'," *Journal of Beijing Forestry University* 32(2), 141-144.
Shi, J.-L., and Tao, L. (2013). "Technical investigation of 15th and 19th century Chinese paper currencies: Fiber use and pigment identification," *Journal of Raman Spectroscopy* 44(6), 892-898. DOI: 10.1002/jrs.4297
Wang, M., Wang, M., Peng, S., and Ding, L. (2000). "Chemical constituents from the barks of *Pteroceltis tatarinowii*," *Natural Product Research and Devel.* 13(6), 5-8.
Wang, H., Chen, A., Ma, Q., Wang, Z., and Wang, C. (2014). "Identifying the pigments of a Ming dynasty water-and-land painting." *Dunhuang Research* 14(5), 119-124.

Xu, C. –L. (2004). "Using additives for the removal of extractives in kraft pulping and bleaching of hardwood," *Heilongjiang Pulp & Paper* 3, 8-11.

Yang, H.-Y., Guo, J.-L., and Gong, D.-C. (2011). "Study on the relationship between morphological characteristics of the fibers of ancient paper and papermaking technologies," *China Pulp & Paper* 30(12), 32-35.

Zhang, L., Gao, H., Fang, X., and Zhang, Y. (2006). "Preliminary research on pulping property of *Solidago canadensis*," *Nonwood Forest Research* 24(4), 34-37. DOI: 10.14067/j.cnki.1003-8981.2006.04.007

Zhang, L., and Liu, H.-F. (2019). "Fractal properties of spatial pattern of *Pteroceltis tatarinowii* populations in Huangcangyu natural reserve," *Journal of Anhui Normao University (Natural Science)* 42(5), 471-476. DOI: 10.14182/J.cnki.1001-2443.2019. 05. 011.

Zuo, F. S., Mei, Z., Zhong, T. L., Xiang, F. X., and Gen, L. H. (1998). "A preliminary study on seed nutrient ingredient contents and seed vigour for various provenances of *Pteroceltis tatarinowii* Maxim.," *Journal of Plant Resources and Environment* 7(2), 16-24.

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