Autofluorescence-decrease phenomenon of woody cell in Lophira alata

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Abstract

Background: Fluorescence is an intrinsic property of lignin. However, the autofluorescence of *Lophira alata* (*L. alata*) was found to be almost invisible during an occasional fluorescence observation experiment. The purpose of this study was to investigate the reason why lignin autofluorescence is invisible in *L. alata*.

Results: Herein, the autofluorescence microscopy, diffuse reflection spectra and UV-Vis absorption spectra of *L. alata* have been performed. In order to recognize the relationship between autofluorescence phenomenon and anatomical structure, themacroscopic, microscopic and ultramicroscopic characteristics of *L. alata* are also examined. Results show that both the longitudinal parenchyma and the rays are rich in extractives. Moreover, these extractives have infiltrated into the vessels and fibers. The autofluorescence of the wood becomes increasingly clear after the benzene–alcohol extraction treatment. Meanwhile, UV-Vis absorption spectrashow that the extractives from *L. alata* have a strong absorption to light at a wavelength range of 200-500 nm.

Conclusions: The complex compounds like polyphenols or terpenoids contained in the rich extractives of *L. alata* are likely to affect the autofluorescence of lignin.

Background

Autofluorescence is a general phenomenon due to a contribution of fluorescent compounds located in different cellular compartments [1, 2]. Lignin in the woody cell wall of plants has been reported to discover the picturesque autofluorescence as early as the sixteenth century [3]. It is well known that the autofluorescence phenomenon of lignin has been attributed to the presence of conjugated bonds in its aromatic backbone [4, 5]. It could help to differentiate these lignified cells from non-fluorescent hemicellulose to pectin cell wall components due to autofluorescence of lignin [6]. Fluorescence microscopy is an effective tool for viewing plant internal anatomy [7]. Compared to the cell staining, the autofluoroscope is a specific approach to lignin localization [8]. It has the potential to be used to assess cell wall modifications resulting from a range of biological, chemical and physical treatments. It is interesting to improve the growth-ring visualization and false ring identification in several species by the utilization of fluorescence [9].

Lophira alata Banks ex Gaertn.f. is one of the most common imported timbers from Africa in recent years. It is frequently used as marine material and railway sleepers due to their fantastic durability [10]. The microstructure of *L. alata* is occasionally performed by a fluorescence observation, which is shown in Fig. 1. It is explored that the lignin autofluorescence of *L. alata* is almost invisible. In this study, the heartwood and sapwood of *L. alata* were examined by epifluorescence microscopy firstly. Next, the macrostructure, microstructure and the characteristics in fiber morphology of *L. alata* were determined by light microscopy and scanning electron microscopy (SEM). The diffuse reflectance spectra and UV-Vis absorption spectra was applied to investigate the absorption and reflection to light by wood and its

extractives. The results would demonstrate the possible reason for the decreased autofluorescence in *L. alata.* it could also verify the theoretical guidance for purpose of the scientific processing and efficient utilization of *L. alata*.

Materials And Methods

Materials

The *L. alata* was grown in the tree farm of Yihua Lifestyle Technology Co., Ltd. in Gabon. One tree with a straight trunk and ~110 cm in diameter at breast height was sampled for this study. Wood discs about 5 cm thickness was collected at the height of 1.2 m. Then it was stored for more than 6 months to adjust the moisture content to ~12%. Wood samples were collected after air-drying and processed into test samples with different dimensions. Wood powders with ~200 mesh sizes were prepared for determination of diffuse reflection and UV-Vis absorption spectrum. As a comparison of fluorescence observation, a poplar tree (*Populus deltoides*) was sampled in the Jiaozuo experimental field in Henan, China.

Autofluorescence experiment

Heartwood and sapwood samples of 10 × 5 × 5 mm^3 (Longitudinal × Radial × Tangential) were prepared for fluorescence observation, respectively. Meanwhile, wood blacks of the poplar wood with the same size was prepared for comparative observation. The samples were softened until it could be easily sliced off with a small blade [11]. 15-μm-thick slices of cross sections were cut with a sledge microtome (G.S.L.1, Switzerland), and placed between microscope glass slide and coverslip [3]. The properties of wood autofluorescence were observed by fluorescence microscopy (Nikon i55) under the excitation of blue, green and ultraviolet (UV) light, respectively [12].

Diffuse reflection spectra and UV-Vis absorption spectra

The diffuse reflection spectra were recorded on a UV-Vis-NIR spectrometer (Lambda 750, PerkinElmer) within range of 200 to 800 nm by a diffuse reflectance regime. The sample for this test was wood powder of 200 mesh sizes.

The UV-Vis absorption spectra were measured using a UV-Vis spectrophotometer (N5000, PerkinElmer) in the range 200 to 800 nm. 0.1 g and 1 g of air-dried wood powders (200 mesh) of *L. alata* were extracted in a benzene-ethanol mixture solution for 8 hours, respectively. Then they were placed in a 100 mL hydrothermal reactor by a hydrothermal carbonization treatment at 140°C for 24 hours, respectively [13]. The solution may contain some lignin after hydrothermal treatment, so 0.1 g of commercial lignin solution was prepared as a comparison. The supernatant was taken from the filtrated solution to obtain the UV-Vis absorption spectra.

Macrostructural observation

Wood samples of 50 × 50 × 30 mm^3 (L × R × T) were cut for macrostructure observation. A sharp blade was used to trim their plane to ensure the flatness and smooth surface. The macrostructure was observed by a stereo microscope (MZS0745/MZS0745T). The main macroscopic structural features were examined including color, smell, the size and distribution of vessels, rays and grains.

Microstructural and SEM observation

Test samples of 10 × 5 × 5 mm^3 (L × R × T) were prepared for microstructural observation. 15-μm-thick slices were cut from softened samples including cross, tangential and radial sections. After stained with 1% safranine O solution and dehydrated, sections were fixed on the slide with neutral quick-drying glue and observed under a biological optical microscope (Nikon ECLIPSE Ci-L). Thirty groups of fibers were randomly selected in cross-section and their double wall thickness and cell lumen diameter were measured using image analysis software (ImageJ 1.50i). Meanwhile, 12 cross sections were observed and photographed to measure the proportion of each major cell types.

Small blocks with dimension of 5 × 5 × 5 mm^3 (L × R × T) were trimmed by hand with a new single-edged razor blade for each surface [12]. Before testing, the blocks were coated with a thin layer of gold under vacuum. Then, samples were observed with scanning electron microscope (SEM, ZEISS Sigma 300) under an accelerating voltage of 15 KV.

Results

Autofluorescence experiment

The images of fluorescence observation of heartwood and sapwood of *L. alata* are shown in Fig. 1. Both the sapwood and heartwood of *L. alata* show slightly fluorescence signal as observed by fluorescence microscopy (Fig. 1). In other words, its autofluorescence signal is so weak that it is almost invisible. Only faint fluorescence signals can be vaguely observed in a few fibers cell walls. It was also observed that both the heartwood and sapwood of *L. alata* had distinct ribbon cells of longitudinal parenchyma that appeared black under fluorescence observation. As a comparison, Figure S1 demonstrated notable fluorescence of cell walls of poplar wood. The cell walls show green, red and blue fluorescence under excitation with blue, green and UV light, respectively. Fluorescence image of poplar wood shows clear fiber cells outlines. Therefore, the reason why the autofluorescence of *L. alata* is almost invisible has been further investigated.

Diffuse reflection spectra and UV-Vis absorption spectra

To clarify the properties of wood in terms of spectral absorption and reflection, diffuse reflection spectra and UV-Vis absorption spectra has been carried out. Results of diffuse reflection spectra of the wood powder are shown in Fig. 2. The spectral show that the *L. alata* has weak reflection to light at a wavelength range of 200–500 nm. This indicates that it has strong absorption to light in this range. In the

region of wavelength above 500 nm, the reflection of light increases significantly. Based on the Kubelka-Munk formula [14]:

$$k/s = (1 - R)^2/2R$$

1

where *R* represents the diffuse reflectance of the layer relative to the standard, *k* is the absorption coefficient and *s* is the scattering coefficient. This theory is created for poorly absorbing materials. Since wood is a good light absorber, the K-M theory is widely applied to determine its light absorption [15, 16]. As shown in the inset of Fig. 2, the diffuse reflectance spectra have been transformed to an absorption spectrum. The measured *k/s* values are usually higher than five units at the 200–400 nm region. This means that less than 10% of the incident light can be collected from the surface of the wood by the detector [16]. This confirms that the material has an extremely high absorption capacity to light.

UV-Vis absorption spectra of the extracted solutions and pure lignin are shown in Fig. 3. The spectrum of pure lignin shows a distinct absorption peak at 280 nm, which is the characteristic absorption peak for lignin [17]. Compared with the UV absorption spectra of commercial pure lignin, the extractives of *L. alata* have strong absorption of light at a wavelength range of 200–500 nm. The UV-Vis absorption spectra of the extractives obtained from 1 g of wood powder is significantly higher than that of 0.1 g. Meanwhile, extractives obtained by 1 g of wood powder have a strong absorption peak at 235 nm, and a slightly weaker peak at about 360 nm and 391 nm. Under the conditions of the same extraction time, extractives of *L. alata* have a significant strong absorption in the position of these peaks. Absorption bands within 200–500 nm decrease in intensity when the wood powder used for extraction reduced from 1 g to 0.1 g. Meanwhile, the absorption peaks at 360 nm and 391 nm may be related to the content of lignin-polyphenols and ketones in the wood [18, 19]. Comparing to the absorption spectra of commercial pure lignin, there is almost no lignin in the extracted solutions. This demonstrates that the extractives from *L. alata* have a significant absorption to light range of 200–500 nm.

Macroscopic observation

As shown in Fig. 4, the heartwood of *L. alata* presents dark brown color. It has indistinct growth rings and diffuse porous. Most of vessel lumens are filled with rich extractives (Fig. 4A-c, B-c), which become more obvious from the view of radial (Fig. 4C) and tangential sections (Fig. 4D). Under the stereo microscope, both the longitudinal parenchyma (Fig. 4A-b, B-b) and wood rays are visible. The wood rays are narrow and densely distributed. The longitudinal parenchyma tissue looks like a thin-white line perpendicular to the wood ray (Fig. 4B). *L. alata* has a dense and hard texture. The dark color also allows the wood to absorb light easily.

Microscopic and SEM observation

The microstructure images of *L. alata* are shown in Fig. 4. From the observation on cross section (Fig. 5A), the shape of vessels is oval with radial multiple pores (mostly two) and occasionally solitary pores. A few vessels also contain extractives (Fig. 5e) inside. The perforation plate between vessels is inclined

simple perforation, and the spiral thickening is absent. An abundance of longitudinal parenchyma is observed in cross-sections of the wood, appearing as a string-like apotracheal banded pattern with 3 to 4 cells width (Fig. 5A-c, 5B). The fibers of *L. alata* are elliptical in cross section and the cell walls are thick (Fig. 5A, 5B).

According to the measurement results of cross section in Table 1, the fiber cell wall to lumen ratio is 6.1 and the cavity to diameter ratio is only 0.14. The cavities of the fibers are very small and the cell walls are extremely thick. Rays are homogeneous, uniseriate and multiseriate (usually 2 to 3 cells width), which are composed of procumbent cells (Fig. 5D, 5E). Most of the cells in wood rays are filled with extractives and gum (Fig. 5D-b). Microscopic observation clearly reveals an abundance of dark extractives in the longitudinal parenchyma and rays.

In the cross section of *L. alata*, the main cell tissues include vessels, fibers, rays and longitudinal parenchyma. The results from measuring the proportions of the four tissues are shown in Table 2. Longitudinal parenchyma and wood rays containing abundant extractives account for 14.4% and 17.2%, respectively. 58.2% of fibers is the predominant cell tissue in *L. alata*, which has a dense and hard texture. It may be suspected that the rich extractives and extremely thick cell wall of fibers could explain the wood properties of *L. alata*.

As shown in Fig. 6, the near-solid state fibers of *L. alata* can be seen more clearly under SEM observations. The cell walls of fiber are very thick, and their cell lumens are almost absent or very narrow (Fig. 6A). The vessels are the shape of drum and cylindrical in radial and tangential section, with part of the cell lumen containing abundant extractives (Fig. 6B, 6C). The intervascular pits are alternate, approximately 2–3 μm in diameter, with elliptic and slit type (Fig. 6D). Vestured pits can also be found occasionally (Fig. 6D-d). Abundant extractives have been observed in the lumen of longitudinal parenchyma (Fig. 6E) [20]. Besides, a large amount of extractives is filled in the wood ray cells (Fig. 6F). In addition, many pits in the cell walls of the longitudinal parenchyma could be observed (Fig. 6F-e). Microscopic and SEM observations of *L. alata* revealed the presence of large amounts of extractives in longitudinal parenchyma and wood rays, which basically filled the cell lumen. Microscopic and SEM observations confirm that the wood has abundant extractives and the fibers are near solid. This may contribute to the wood absorbing light more readily and make it more difficult for light to transmit.

Discussion

Two necessary conditions are universally acknowledged to a substance to produce fluorescence as following: (1) the relatively weak energy absorbed by a photon undergoing multiple invariant transitions, (2) a fluorophore containing an unsaturated bond in the structure of substance [21]. Based on the aforementioned conditions, it is hypothesized that there are two main reasons for the decrease of fluorescence in *L. alata*. The first reason is possible to be the lack of fluorescent luminescence factors. Secondly, it may be affected by certain components of the wood, and the occurring fluorescence is absorbed or blocked.

In this work, in order to explain the reasons for the decrease autofluorescence in *L. alata*, anatomical features including fiber morphology and tissue ratios were investigated. Table 1 shows that the fiber cell wall to lumen ratio is 6.1 and the cavity to diameter ratio is only 0.14. The *L. alata* has very thick fiber cell walls and the fibers are almost solid cells under microscopic and SEM observations. Meanwhile, from the measurement results in Table 2, fibers with the proportion of 58.2% are the predominant cell tissues in *L. alata*. It is well known that wood cell walls are made up of cellulose, hemicellulose and lignin [22]. As the main component of the cell wall of wood, lignin has a broad fluorescence emission range and can be excited with both UV and visible light [23]. In general, the presence of thick-walled fibers, which account for nearly 60% of wood, rather increases the autofluorescence of the wood. However, the observation by fluorescence microscopy has revealed that the autofluorescence of *L. alata* is almost invisible (Fig. 1). At the same time, the presence of lignin in the fiber cell walls suggests that the before-mentioned (1)-reason is not valid.

In Fig. 5, microscopic observations present that most of the longitudinal parenchyma and wood rays are filled with dark extractives. SEM observations further confirmed the presence of abundant extractives in the longitudinal parenchyma (Fig. 6E) and ray cells (Fig. 6F). And Table 2 shows the longitudinal parenchyma and rays account for a total of 31.6% of the tissue ratios. Besides, Fig. 6D also reveals that some pits in the vessels wall are filled with extractives. It can be demonstrated that through the perennial growth and accumulation of trees, these rich extractives may penetrate from the parenchyma tissue and ray cells into part of the vessels through the pits in the cells wall. The abundant pits (Fig. 6D, F) in the cell walls of the vessels and rays provide a pathway for the penetration of extractives. The presence of extractives in the vessels in Fig. 6B and 6C gives support to this interpretation. The high dense and hard texture of *L. alata* may be related to the rich extractives and extremely thick cell wall of fibers. The rich extractives in the wood cell are also responsible for the dark brown color under macrostructural observation (Fig. 4). Therefore, it can be assumed that the large amounts of extractives in the wood affects the autofluorescence of the wood.

To confirm the effect of extractives on the autofluorescence, benzene-ethanol extraction tests were carried out on wood slices. Some 15-μm-thick slices of cross sections were cut by a microtome. These slices were placed in a soxhlet extractor for benzene-alcohol extraction (benzene: 95% alcohol = 2:1) [24]. During the extraction, two slices were removed every 2h in order to examine a fluorescence observation. In Fig. 7, the fluorescence of the wood gradually appears after benzene-ethanol extraction. It is interesting that a relatively clear outline of the fiber cells as the time emerges up to 8h. Meanwhile, the longitudinal parenchyma and wood rays still remain invisible in a fluorescence observation. It could be hypothesized that the rich extractives accumulated in the longitudinal parenchyma and ray cells is so amazing. Furthermore, it is possible that the method of benzene-alcohol extraction could not subtract the a few decades deposition in the parenchyma tissues. Whereas the small quantities of extractives in the fiber cells may be relatively easy for extraction. Therefore, the extractives should continually be worth to be explored and analyzed in the subsequent investigations as following: Liquid Chromatography-Mass Spectrometry (LC-MS), Nuclear Magnetic Resonance (NMR), Matrix-Assisted Laser Desorption / Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS), and so on.

Previous studies have confirmed that the fluorescence emission of poplar fibers seems to correspond to the known location of syringyl-rich lignin in the fiber secondary wall [5, 25]. Other studies have also demonstrated the fluorescence intensity is not directly proportional to the amount of lignin but is also affected by molecular environment and chemical structure [4, 5]. The extractives in wood are the products of physiological activity or metabolism during the growth of trees and include polyphenols, terpenoids, and others [26]. The mechanisms of autofluorescence in wood are extremely complicated and readily influenced by these compounds. According to the results of UV-Vis absorption spectra, the extractives from *L. alata* have a strong absorption to light at a wavelength range of 200–500 nm. The presence of rich extractives and the strong light absorbing capacity of *L. alata* are the main factors which are almost responsible for its invisible autofluorescence. It is presumed that other tree species with abundant extractives would likely have a similarly almost invisible fluorescence observation.

Conclusions

In this interesting study, the autofluorescence of *L. alata* was explored to be extremely faint and almost invisible by a fluorescence microscopy. The fluorescence was gradually apparent in the fiber cell wall region after being performed a benzene-alcohol extraction. However, it was worth subsequently investigating the autofluorescence-decrease phenomenon in the parenchymatous tissue and ray cells, where these rich extractives could relatively complexly. Meanwhile, UV-Vis absorption spectra of the extracted solutions demonstrated that the extractives in *L. alata* has an extremely high absorption capacity to light. Presumably, the complicated compounds have tightly packaged affect the autofluorescence of lignin.

Declarations

Ethics approval and consent to participate Acknowledgement

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data generated or analyzed during this study were included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no conflicts of interest to report regarding the present study.

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Authors' contributions

ZY Y and DN X: Experiment and Writing-Original Draft. JB H and SS C: Supervision, Writing- Reviewing and Editing. GG L and XD W: Data Curation. QT H and T L: Resources. J H: Experiments of UV-Vis absorption spectrum. Y L: Investigation. All authors contributed to and approved the final manuscript.

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Tables

Tables 1 to 2 are available in the Supplementary Files section

Figures

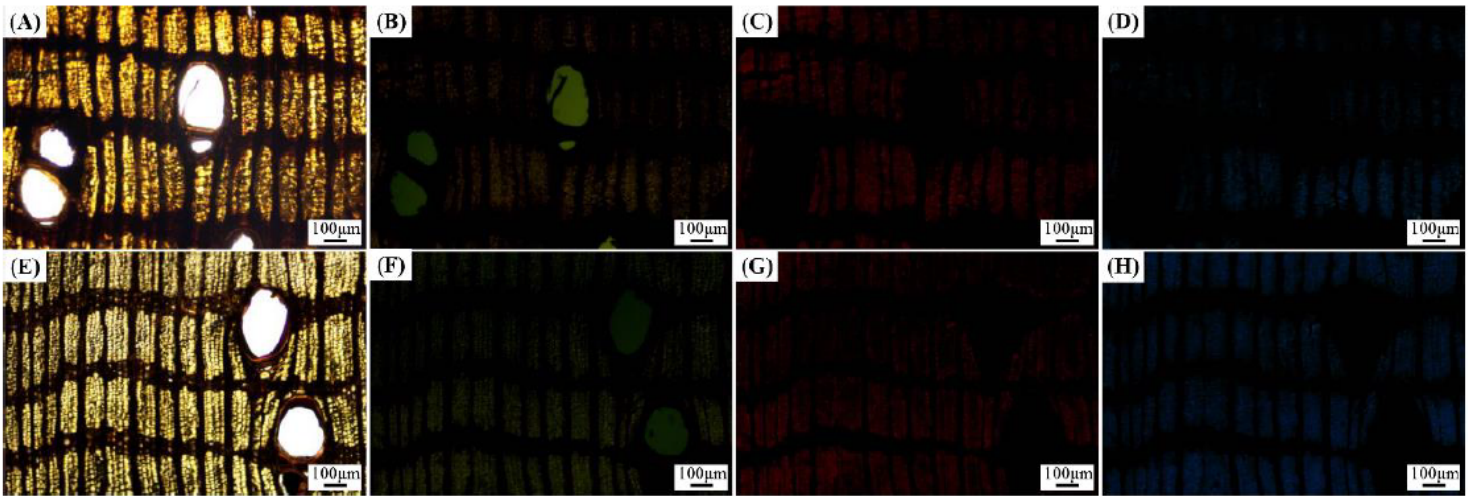

Figure 1

Fluorescence images on cross section of the heartwood (A, B, C and D) and sapwood (E, F, G and H) of *L. alata*. Observed in natural light (a), blue (b), green (c) and UV (d), respectively. Scale bars = 100μm

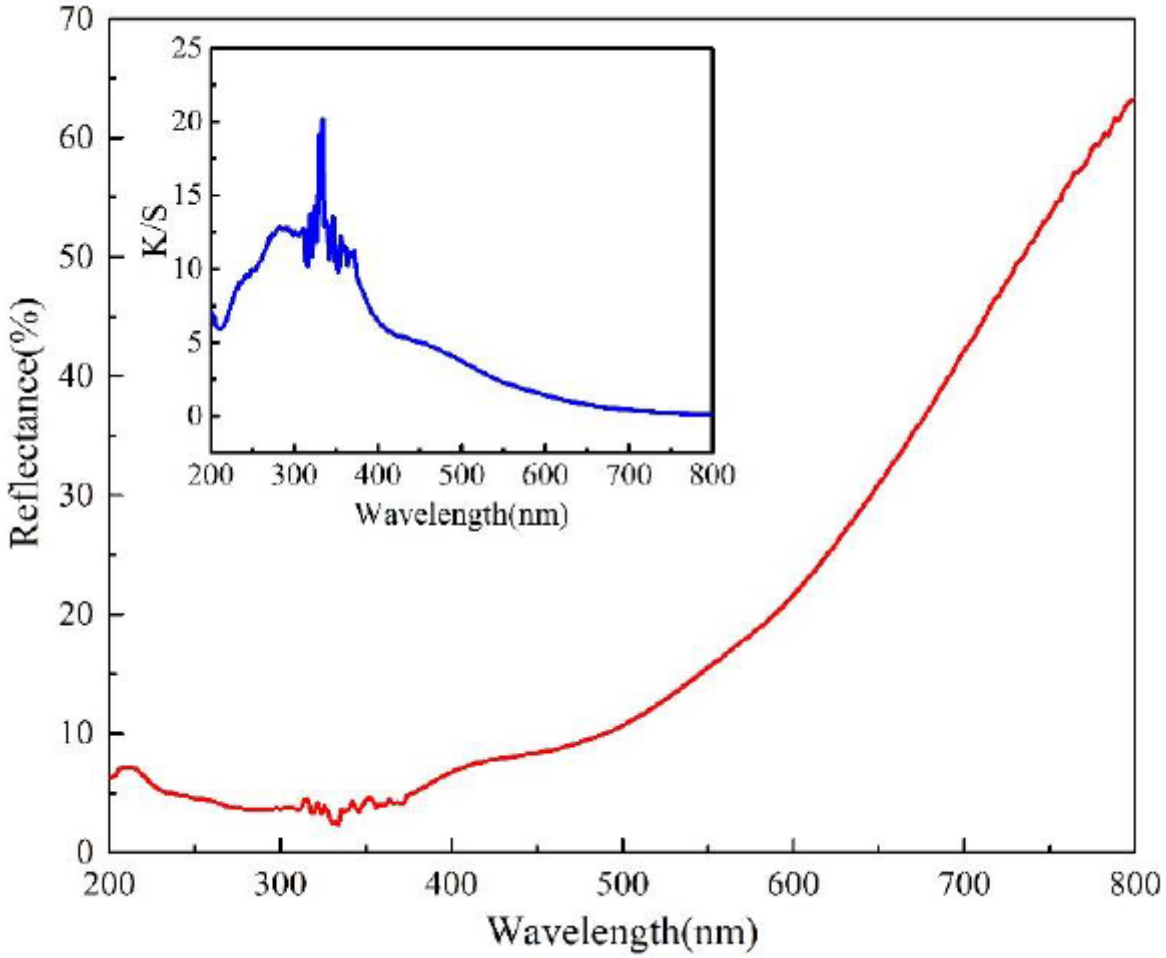

Figure 2

The diffuse reflection spectra and its absorption spectrum (the inset) which was converted according to the diffuse reflection spectra of *L. alata*

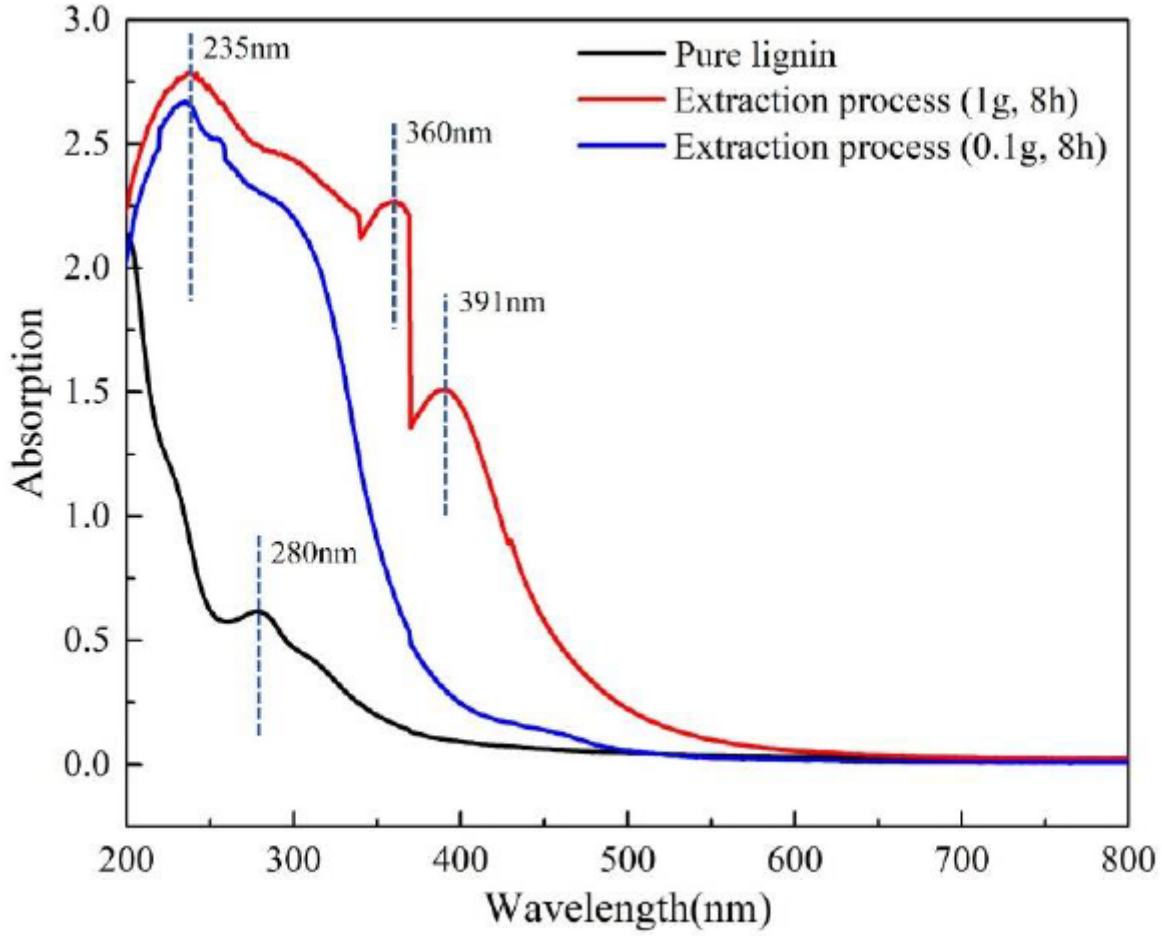

Figure 3

UV-Vis absorption spectra of extracted solutions and commercial pure lignin

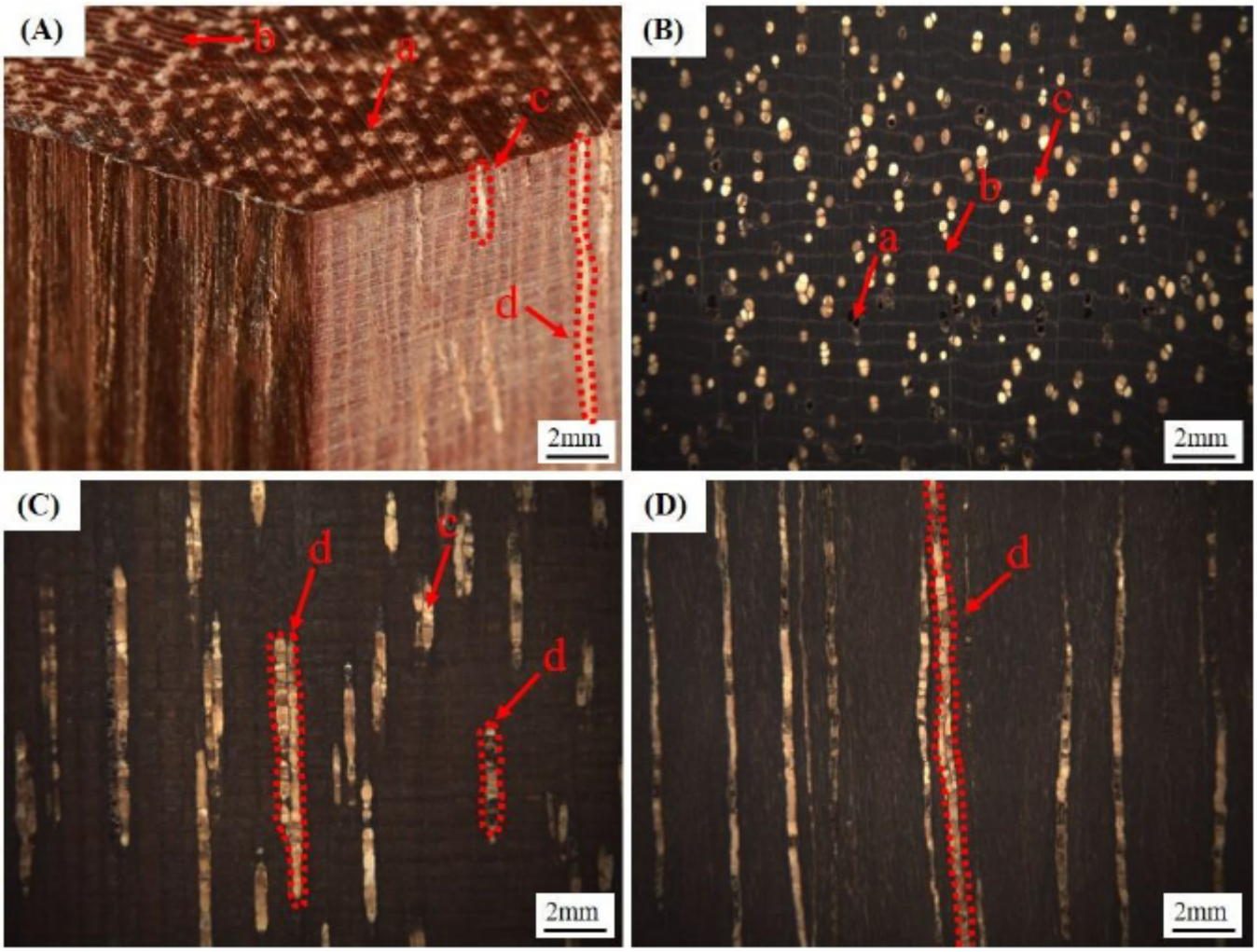

Figure 4

The macrostructure photos of *L. alata* in stereo image (A), cross section (B), radial section (C) and tangential section(D). Vessel pore (a), longitudinal parenchyma (b), extractives(c), vessel channel (d). Scale bars = 2 mm

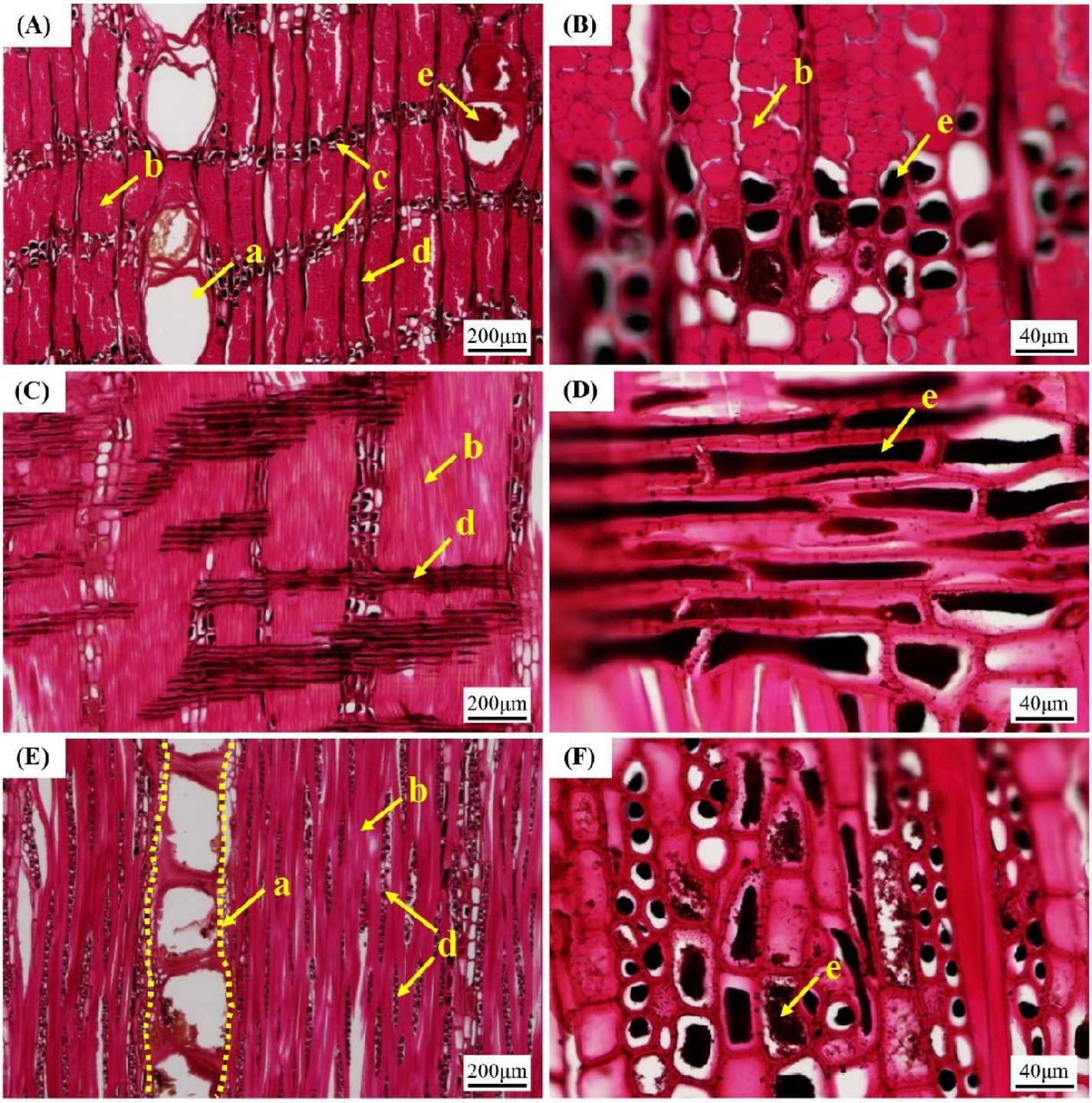

Figure 5

The microstructure of *L. alata* in cross section (A, B), radial section (C, D) and tangential section (E, F). B, D and F: the magnified view of fiber, longitudinal parenchyma and wood ray. Vessel (a), fiber (b), longitudinal parenchyma (c), wood ray (d), extractives (e). Scale bars: A, C, E = 200μm; B, D, F = 40μm

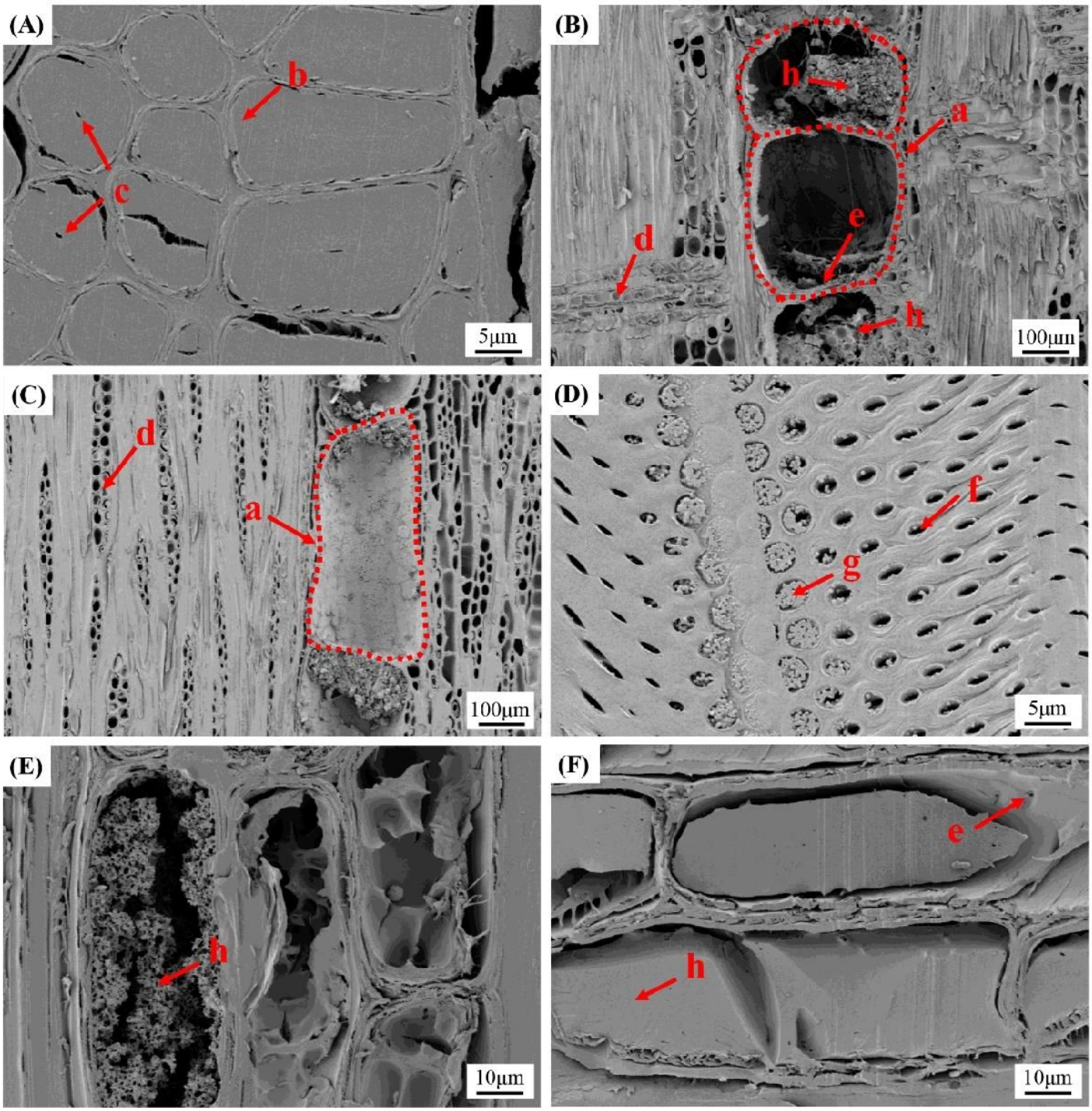

Figure 6

SEM images of *L. alata* in cross section (A), radial section (B) and tangential section (C). Magnified view of vessels wall (D), longitudinal parenchyma (E) and wood ray (F) in tangential section. Vessel (a), fiber (b), cell lumen of fiber (c), wood ray (d), perforation plate (e), pits in the vessel wall (f), vestured pitting (g), extractives (h). Scale bars: A, D = 5μm; B, C = 100μm; E, F = 10μm

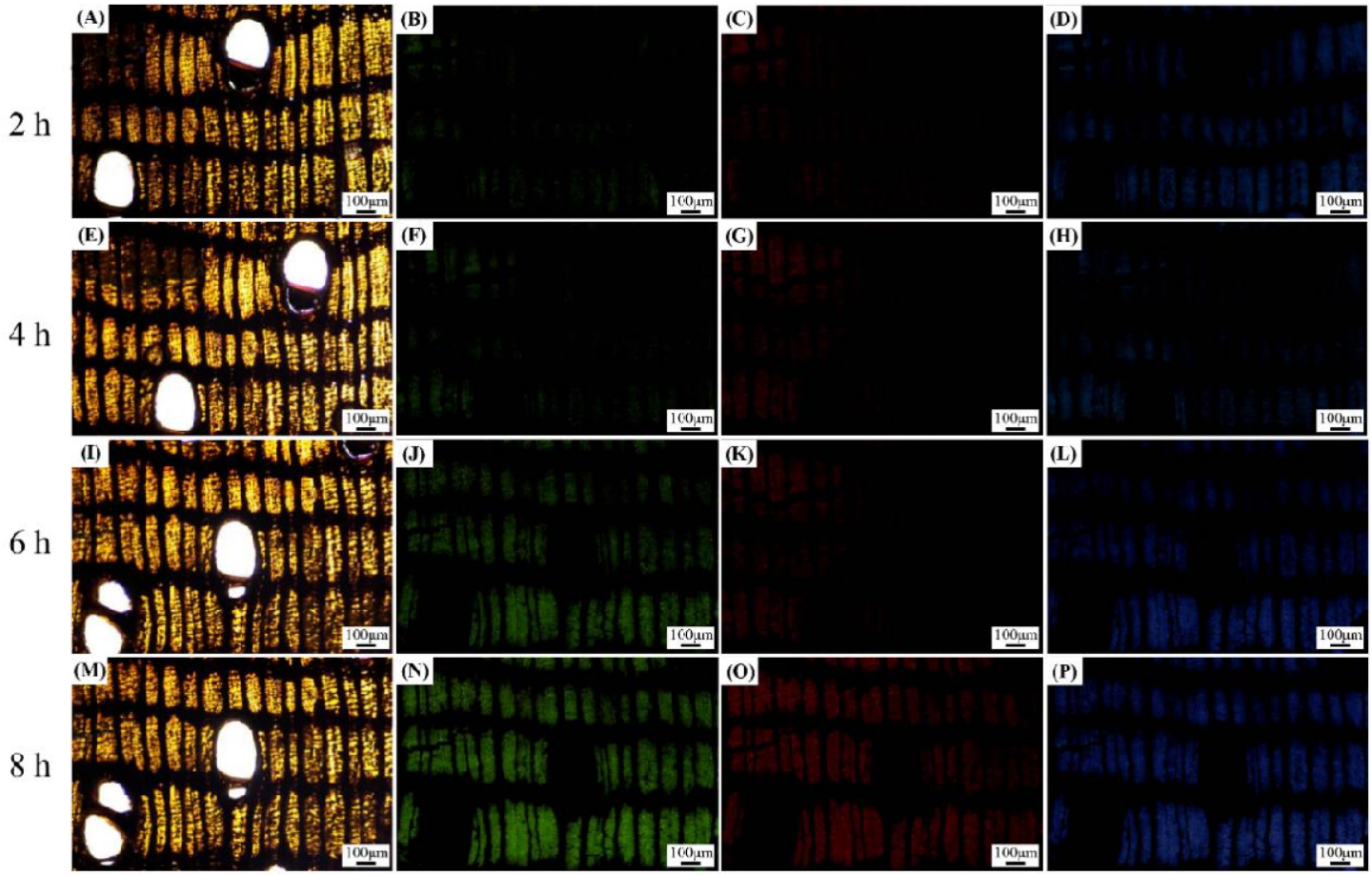

Figure 7

Fluorescence images of *L. alata* after 2, 4, 6 and 8 h benzene-ethanol extractions, respectively. Observe in natural light (a), blue light (b), green light (c), ultraviolet light (d). Scale bars = 100 μm

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Manuscriptelectronicsupplement.xlsx
- SupportingInformation.docx
- Table.1.pdf
- Table.2.pdf