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Fluorescence Properties of *Pterocarpus* Wood Extract

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Abstract: The water immersion of *Pterocarpus* wood produces strong blue fluorescence, which comes from the extract. The fluorescence contained in the extract is of interest for the identification of *Pterocarpus* wood. We conducted an investigation into the extraction solution of *Pterocarpus* wood and analyzed the mechanism of fluorescence in this species. Possible species of the fluorescent molecules are discussed based on the mixture. Liquid chromatography mass spectrometry (LC-MS) is used for an analysis of the extract, the obtained substances that may be fluorescent in *Pterocarpus* wood. In addition, the change in the fluorescence intensity with changes in the pH and concentration in the extract is also studied. The results show that the fluorescent molecule is quenched by aggregation (Aggregation-Caused Quenching; ACQ) and is unstable in over-acidic and over-alkaline conditions (especially acidic).

Keywords: *Pterocarpus* wood; extract solution; fluorescence intensity; conjugate structure

1. Introduction

Pterocarpus wood is a kind of rare wood [1]. It is often used as a raw material for furniture and wood-based panels because of its unique fragrance, high density, and beautiful patterns [2,3]. Extracts of *Pterocarpus* wood also have medicinal value [4]. The phenomenon of counterfeiting red *Pterocarpus* wood in the market for profit often appears [5,6]. There is a need to find a method for the identification of Pterocarpus wood [7]. When people identify *Pterocarpus* wood, they often soak it in water and observe whether it has the fluorescence phenomenon [8]. The fluorescence intensity of *Pterocarpus* wood in a water immersion is high. The fluorescence may come from the extract of *Pterocarpus* wood. This study on the fluorescence of *Pterocarpus* wood extract is helpful for the identification of this valuable wood. Fluorescence is a photoluminescence phenomenon. When a substance at ambient temperature is irradiated by a certain wavelength of incident light (UV, etc.), photons of the incident light transfer energy to the molecules being radiated [9]. If the molecule has fluorescent properties (conjugated electrons), the valence electrons of the substance undergo energy-level transitions with the increase in energy and then be in an excited state. Due to the electron displacement effect in a π–π conjugated system or a p–π conjugated system, these electrons can have relatively free movement after generating an energy-level transition. Then, they emit light in the form of light energy released during the movement, which is called fluorescence [10–12]. In addition, when two molecules are in close contact (5–10 nm), energy can be transferred from one excited molecule to another in a non-radiative manner, which is called FRET [13,14]. It is worth mentioning that this principle has been known for some time as fluorescence energy transfer. In fact, the F in FRET stands for Forster, not fluorescence. The principle can be used as a complement to the causes of formation. Fluorescence has many advantages, such as its sensitivity to excitation light. Fluorescence usually has extremely short response times, on the order of 10^{-8} s [15,16]. The extremely sensitive generation efficiency allows it to be used as a probe in detection systems. Moreover, fluorescence has a certain lifetime. This is due to the delay

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Citation: Li, R.; Li, J.; Shi, J.; Zhang, Y.; Sun, Y.; Chen, Y.; Liu, Z. Fluorescence Properties of *Pterocarpus* Wood Extract. *Forests* **2023**, *14*, 1094. https://doi.org/10.3390/f14061094

Academic Editor: Joana Patrícia Araújo Ferreira

Received: 29 March 2023
Revised: 17 May 2023
Accepted: 23 May 2023
Published: 25 May 2023

between the fluorescent molecular transition and emission. This characteristic can be used to observe processes that cannot be observed by ultraviolet–visible light, which makes it an important complement to detection methods. Meanwhile, the emission light path of a fluorescent molecule has an angle with the excitation light path, and the fluorescence can be detected in the vertical direction of the excitation light. The detection process is not disturbed by the excitation light during the generation process, which makes its wavelength have a relatively stable value [17]. Fluorescence is widely used in biology, detection, and other fields. Studying the fluorescence of *Pterocarpus* wood extract is helpful for the further application of fluorescence. This study on the fluorescence of *Pterocarpus* wood is of practical significance.

In previous studies, the response of this fluorescence to external factors was studied based on the water immersion of *Pterocarpus* wood [18].The fluorescence produced by different solvent immersion solutions was studied. Researchers investigated the effect of this fluorescent substance on the external environment (pH, solvent polarity, etc.), and used the special response of red sandalwood species to fluorescence in the identification of red sandalwood species and achieved good results. However, they did not further research the source and production mechanism of fluorescence. Savero [19] used the differences in fluorescence in heartwood extracts from tree species to non-anatomically identify different tree species. They found a large difference in fluorescence in the extractives' solutions from different tree species. This was due to the different species of extractives from different tree species. The fluorescence of *Pterocarpus* wood should be influenced by the kind of extracts. Several studies were undertaken to isolate this fluorescent substance [20]. However, there is no clear method for separating fluorescent substances. In addition, it is worth mentioning that Basudeb [21] performed a fractionation operation on the water infusion of *Pterocarpus* wood, separating the fractions with fluorescence. Then, the functional groups present in it were analyzed by a Nuclear Magnetic Resonance Spectrometer, the structure of a fluorescent molecule was described, and the fluorescent properties of the molecule were proven. However, the substance was inferred, so its authenticity remains to be verified. At present, there are few studies on the categories and possible mechanisms of this fluorescent substance. In fact, the work that has not been done is very meaningful. Only by determining the composition of the fluorescent substances in the species can they be reasonably applied according to their properties. To explore the types of fluorescent molecules in the extracts, the first step is to use a suitable method to separate the molecules in the mixed system. Consequently, further studies on the fluorescence properties of *Pterocarpus* wood extract using different methods are required.

In our experiments, the ideas were combined and improved from previous studies undertaken by others. The change in fluorescence with the change in environment was analyzed by two-dimensional fluorescence spectroscopy, while probing the species of fluorescent molecules in different solvent extracts using LC-MS. The analysis of fluorescent substances by chemical mechanism was performed while using standards for comparison. This helped to explore the application of fluorescence while compensating for the previous methods of fluorescence studies. According to the analysis, it was preliminarily determined that the fluorescent substance comes from the wood extract. In order to increase the concentration of the extracted fluorescent substances, we chose Soxhlet extraction. In addition, the emission wavelengths of the fluorescence in the extract obtained during extraction with different extraction solvents were different. This means that the wood needed to be extracted with different solvents (water, ethanol, chloroform, ethyl acetate, n-hexane, etc.). In addition, the heartwood of this wood had more extracts than the sapwood. The existence of fluorescent molecules only in heartwood extracts can be verified by the extraction and observation of the heartwood and sapwood. Extracts contain a large number of aromatic compounds, mainly tannins and flavonoid [22]. In fact, Jure [23] successfully used liquid chromatography mass spectrometry (LC-MS) to analyze the species of some extracts of Canadian Goldenrod under different solvent extracts. However, they did not explore the types of fluorescent molecules in the extracts. In fact LC-MS is an effective

method for the quantitative and qualitative analysis of small molecules [24]. Moreover, different kinds of extracts were obtained using different extraction solvents. This led to the different fluorescence effects of the extracts in different solvent systems. We can use extracts in different solvents for LC-MS analysis. While obtaining the chemical molecule species in the extracts of different solvents, the solvent effects on the fluorescence intensity and wavelength were explored. This helped to rationalize the selection of solvents in identifying tree species and utilizing fluorescence. A large number of substances in the extract can be screened by LC-MS [25]. The molecules can be screened according to the fluorescence mechanism. Afterward, comparing standard molecules can characterize the type of fluorescent molecules. In addition, the study of this fluorescence needs to serve the utilization. Therefore, the intensity and wavelength of the fluorescence in different extracts with environmental variations were qualitatively analyzed. The response of the fluorescence properties to environmental factors (external forces, temperature, pH, and molecular concentration) was studied by quantitative fluorescence two-dimensional spectroscopy. This helps in the rational application of the fluorescence properties of rosewood.

2. Materials and Methods

2.1. Materials

Indian *Pterocarpus* wood came from the herbarium of Nanjing Forestry University. Methanol, formic acid, acetonitrile, phloridzin, epicatechin, naptalam, and (−)-usnic acid were all purchased from Sigma. Ethanol, ethyl acetate, n-hexane, and chloroform were purchased from Aladdin. The chemical reagents were of analytical grade and were used without further purification. All water used in the experiments was deionized water.

2.2. Methods

2.2.1. Acquisition of Wood Extracts

First, the heartwood and sapwood of Indian lobular rosewood stored for one year were each ground into 80 mesh powder, and 1.5 g of heartwood wood powder was accurately weighed and subjected to Soxhlet extraction with different solvents. Then, the wood powder covered with double-circle filter paper was added to the extraction tube, and 200 mL of extraction solution (deionized water, ethanol, ethyl acetate, n-hexane, and chloroform) were added too. Extraction was carried out at 35 °C above the boiling point of the solvent using an oil bath for 8 h. The obtained extraction was ultrasonically treated for 20 min, and then the obtained extraction was placed in a centrifuge tube sealed with tin foil and stored in a refrigerator at −4 °C. Afterward, the sapwood was treated in the same way, using only deionized water as the extraction solvent.

2.2.2. Obtaining Isocratic pH Samples

In order to research the effect of pH value on the fluorescence intensity of the sample, it is necessary to configure the extract solution with equal pH gradient for testing. First, 5 mL of deionized water and ethanol heartwood extract solutions were placed in 12 test tubes, and 0.005 mol/L NaOH and 0.005 mol/L HCl were added dropwise to them. After the pH was tested, samples with an equal pH gradient (1 to 12) were obtained. The obtained solution was ultrasonically treated for 20 min and was put into test tubes sealed with tin foil for future use. Moreover, to exclude the interference of ions, pH was adjusted and tested using the same H^+ and OH^- concentrations of acids and bases (H_2SO_4, HNO_3, and KOH); and salt solutions (NH_4Cl and $NaHCO_3$).

2.2.3. Acquisition of Samples with Different Concentrations

The concentration of fluorescent molecules in the extract also affects the intensity of fluorescence, so samples with different concentrations need to be tested. First, 5 mL of the extract from Mill-Q and ethanol were placed in a foil sealed tube. The extract solution was mixed with the corresponding extraction solvents (deionized water and ethanol), and an extraction was obtained, with concentration of 100% of the original solution. Other

solutions of concentration percentages (50%, 10%, and 1%) were obtained using the same method. The obtained extractions were ultrasonically treated for 20 min and then placed in test tubes sealed with tin foil for later use.

2.2.4. Different Centrifugation Speeds and Temperature for Samples

In addition to the above conditions, the effects of temperature and external forces on the fluorescence intensity also need to be explored. First, 3 mL of Mill-Q water extract were placed in a foil-sealed test tube. They were centrifuged at 7000 rad/min, 9000 rad/min, 11,000 rad/min, 13,000 rad/min, and 15,000 rad/min for 5 min with the centrifuge. The liquids were put into test tubes sealed with tin foil and kept for later use. Then, 100 mL of deionized water extract was placed in an oil bath, treated at 40, 50, 60, 70, and 80 for 1 h, and immediately tested after being taken out.

2.2.5. Preparation for LC-MS Analysis

Afterward, LC-MS analysis of the extract is required to determine the molecular composition in the extract. The preparation for LC-MS was as follows: 360 μL of the extracts from different systems were placed into the test tube, and 1000 ppm of ribitol internal standard solution were added to a total of 60 μL. Afterward, a filter (0.2 μm) was used for filtration. Then, the mixed solution was transferred to a vial, waiting for the test by the machine.

2.3. Representation

2.3.1. Characterizations

Liquid Chromatograph Mass Spectrometer (Xevo G2-XS Q-Tof, WATERS, Shanghai, China) was used to analyze the substances present in the extract. Vortex (vortex-genie 2, Scientific Industries, Beijing, China) was used for uniform mixing of solutions. Benchtop high-speed freezer centrifuge (5427 R, Eppendorf AG) was used to roughly analyze the molecular weight of fluorescent molecules. Fluorescence spectrometer (Horiba FluoroMax 4, HORIBA Scientific, Palaiseau, France) was used to quantify fluorescence intensity and obtain images. Deionized water machine (Milli-Q Academic A10, ai research biological technology co., LTD, Shanghai, China) was used to obtain all pure water in the experiment. Oil bath was used for temperature control of the system. All photos were taken using an iPhone 12 Pro (Apple, Cupertino, CA, America).

2.3.2. LC-MS Conditions

The main conditions for LC-MS testing are listed below, and the final experimental conditions were as follows: liquid phase system: ACQUITY UPLC (WATERS, Shanghai, China); column: BEH C18, 2.1 × 100 mm, 1.7 μm; mobile phase A: H_2O (with 0.1% methanoic acid); mobile phase B: acetonitrile (with 0.1% methanoic acid); column temperature: 45 °C; flow rate: 0.4 mL/min; injection volume: 2 μL.

The conditions for mass spectrometry are as follows: mass spectrometry system: Xevo G2-XS Q-Tof offset: 80 V; source temperature: 120 °C; desolvation temperature: 450 °C; desolvation gas flow: 800 L/h; cone gas flow: 50 L/h; mass range: 50–1000 amu; scan time: 0.1 s; collision energy (low energy): 6; ramp collision energy (high energy): 20–35; scan type: MSE Profile.

2.3.3. LC-MS Data Processing

The obtained data were collected using Mass Lynx 4.0 software, and then Progenesis QI V2.3 software was used to perform data peak alignment, convolution solution, normalization analysis, and database search. HMDB and METLIN were used for this experiment. In the standard of metabolite identification, when the molecular weights are matched, the secondary fragments must also be matched with each other. Based on this, various substances in the extract system were obtained.

2.3.4. Draw the Two-Dimensional Fluorescence Spectra

In order to intuitively depict the fluorescence intensity and peak wavelength, it is necessary to draw a two-dimensional fluorescence spectrum diagram. The obtained samples were classified according to the type of comparison, and the samples were put into the cuvettes and tested on the machine. After debugging, the sapwood extraction system, heartwood concentration gradient, and heartwood pH gradient of Milli-Q water were obtained under 380 nm excitation light, and the emission range was 400–750 nm. Corresponding to the system of Milli-Q water, the extraction system of ethanol solution was obtained under the excitation light of 420 nm, and the emission range was 425 nm to 750 nm. The excitation range of aqueous solution fluorescence response to excitation wavelength is 320–420 nm, and the emission range is 425–600 nm. A series of fluorescence spectra were obtained under these conditions.

3. Results and Discussion

3.1. Acquisition of Fluorescence Phenomena and Collation of LC-MS Results

3.1.1. Fluorescence in Different Solvents

After obtaining the extract of *Pterocarpus* wood powder, which was extracted with different solvents, a 400 nm UV lamp was used to observe the fluorescence of the extract, and compare it with visible light (Figure 1a,b). It can be seen that the fluorescence wavelength produced by the extract in different solvents has a great difference, which has nothing to do with the color of the extract itself. Moreover, the fluorescent color of the solution does not correlate well with the color exhibited by the original solution in natural light. This conclusion was obtained by a comparison of n-hexane (NH) and Milli-Q water–heartwood (MQW–H). By observing the extract of heartwood and sapwood under ultraviolet light (both are extracted using deionized water), it can be concluded that the fluorescent molecule exists in the heartwood extract of *Pterocarpus* wood (Figure 1a). However, it is still not certain that the fluorescence is caused by individual fluorescent molecules. This requires the separation and analysis of the substances in the extracts.

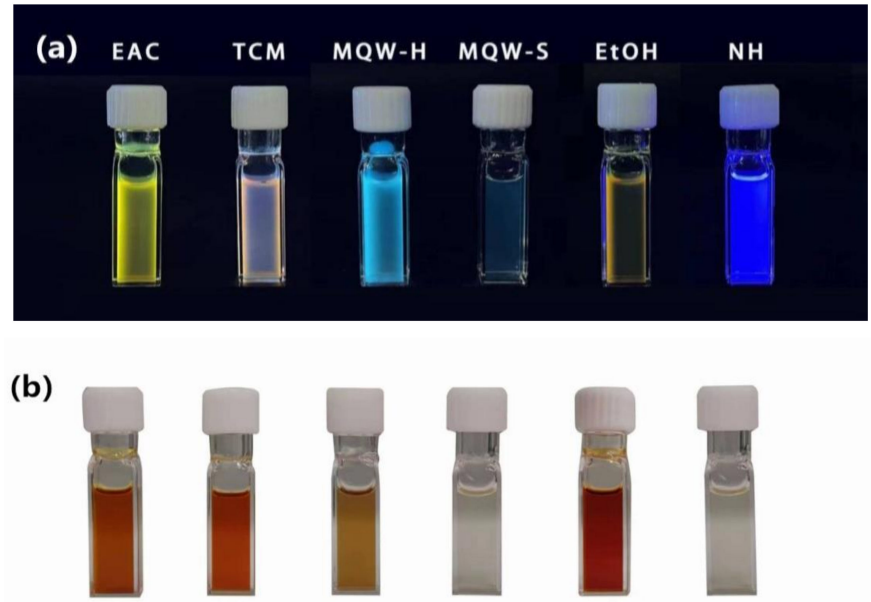


Figure 1. Photos of solutions obtained from different extraction systems: (**a**) The photo of samples obtained under fluorescence: ethylacetate (EAC), trichloromethane (TCM), Milli-Q water–heartwood (MQW–H), Milli-Q water–sapwood (MQW–S), ethanol (EtOH), and n-hexane (NH); (**b**) photos of samples obtained under normal light, with solvents corresponding to **(a)** (all unspecified samples are heartwood).

3.1.2. Chemical Mechanism of Fluorescent Substances

Understanding the mechanism of fluorescence production is important for the exploration of the nature of fluorescence in wood extracts of the genus *Pterocarpus* wood. It helps with the screening of the huge number of molecules in the extracts. The currently recognized fluorescence mechanism is mainly based on the energy-level transition theory. The main transition type is the transition of π electrons. In the extraction system studied in this experiment, the conjugated structure of various aromatic molecules (such as tannins), C=O double bonds, and C=C double bonds connected by single bonds can promote the generation of fluorescence and affects the intensity of fluorescence. Another important factor is the degree of rigidity of the fluorescent molecule. For example, the structural unit compositions of fluorescein and phenolphthalein are very similar, and the reason for the former's high-intensity fluorescence is mainly due to the rigidity-enhancing mechanism brought about by its oxo bridges; the latter's lack of oxo bridges makes its molecules unable to maintain a relatively stable plane. Consequently, it cannot produce stable fluorescence. Molecules with a rigid structure do not produce the various intramolecular vibrations (bending vibrations, stretching vibrations, etc.). The motion of electrons in rigid molecules has a simpler pattern. This results in a more direct way of releasing energy from the electrons, thus enhancing the intensity of the fluorescence emitted during the process. In addition, the existence of electron-rich groups ($-NH_2$, $-OH$, $-OCH_3$, $-CN$, etc.) also enhances the intensity of fluorescence and shifts the wavelength of the emitted light [26].

3.1.3. Processing and Analysis of LC-MS Results

Based on the above fluorescence phenomena and the mechanisms affecting fluorescence, a Liquid Chromatograph Mass Spectrometer (LC-MS) was used to separate the extracts obtained by several different extraction agents. The extracts can then be screened one by one for substances that may be fluorescent, according to the chemical mechanism described above.

The results obtained by LC-MS were checked against the molecules in the database to obtain the names and structures of the molecules in the extracts. Both TCM and EAC had a high number and variety of extracts. The results obtained by Basudeb [21], based on computational chemistry, indicate that the fluorescent molecule is the epimer of coatline A. By comparing the chromatographic results with the database, we found that the molecules described in the experiments of Basudeb were not present in this system. It is shown that the substances that produce fluorescence in the extract may be different.

Different wavelengths of incident wave excitation give different intensities of fluorescent emission waves (Figure 2a,b). The fluorescence intensity reaches a very high level under the excitation of the optimum wavelength. The fluorescence intensity is concentration-dependent over a range. This indicates that the molecules that produce fluorescence are present at high levels in the extracts. In fact, the variety of molecules obtained from the LC-MS results was very large, so substances with high levels in the LC-MS needed to be screened to reduce the difficulty of analysis. Thus, molecules with relative levels greater than 5×10^4 in the test results were listed and analyzed one by one. Considering the effect of Aggregation-Caused Quenching (ACQ), the relative content of 5×10^4 did not cause fluorescence quenching. At the same time, the chemical structure of the molecules had to be analyzed in conjunction with the fluorescence mechanism mentioned in Section 3.1.2. Taking water as an example, we obtained more than 20 kinds of molecules with strong conjugated systems. Afterward, the five molecules that were most likely to have strong fluorescence properties were identified and listed in combination with the planarity characteristics of the molecules and the number of electron-rich groups (Table 1). Then, the wavelength of the fluorescence emission of the extract in different solvents was studied (Figure 2e). According to the Stokes shift, the wavelength of the emission wave generated by excitation continually increases with the increase in quantity in the conjugated system. The fluorescence emission wavelength of the TCM and EAC systems is obviously larger, which is consistent with the more diverse extracts obtained by LC-MS. It can be seen

that the conjugation degree in the extraction system affects the wavelength of fluorescence to a great extent. The above results also give more accurate proof of the different color behaviors of the extract fluorescence shown in Figure 1. It is worth mentioning that the fluorescence emission peak wavelength of the ethanol system is relatively large for the results shown in Figure 2d,e. This may be due to the partial volatilization of the fluorescent substance in the extraction system with the solvent, resulting in a decrease in the degree of conjugation in the solution. This results in a reduction in the peak emission wavelength in the system, as shown in Figure 2d,e.

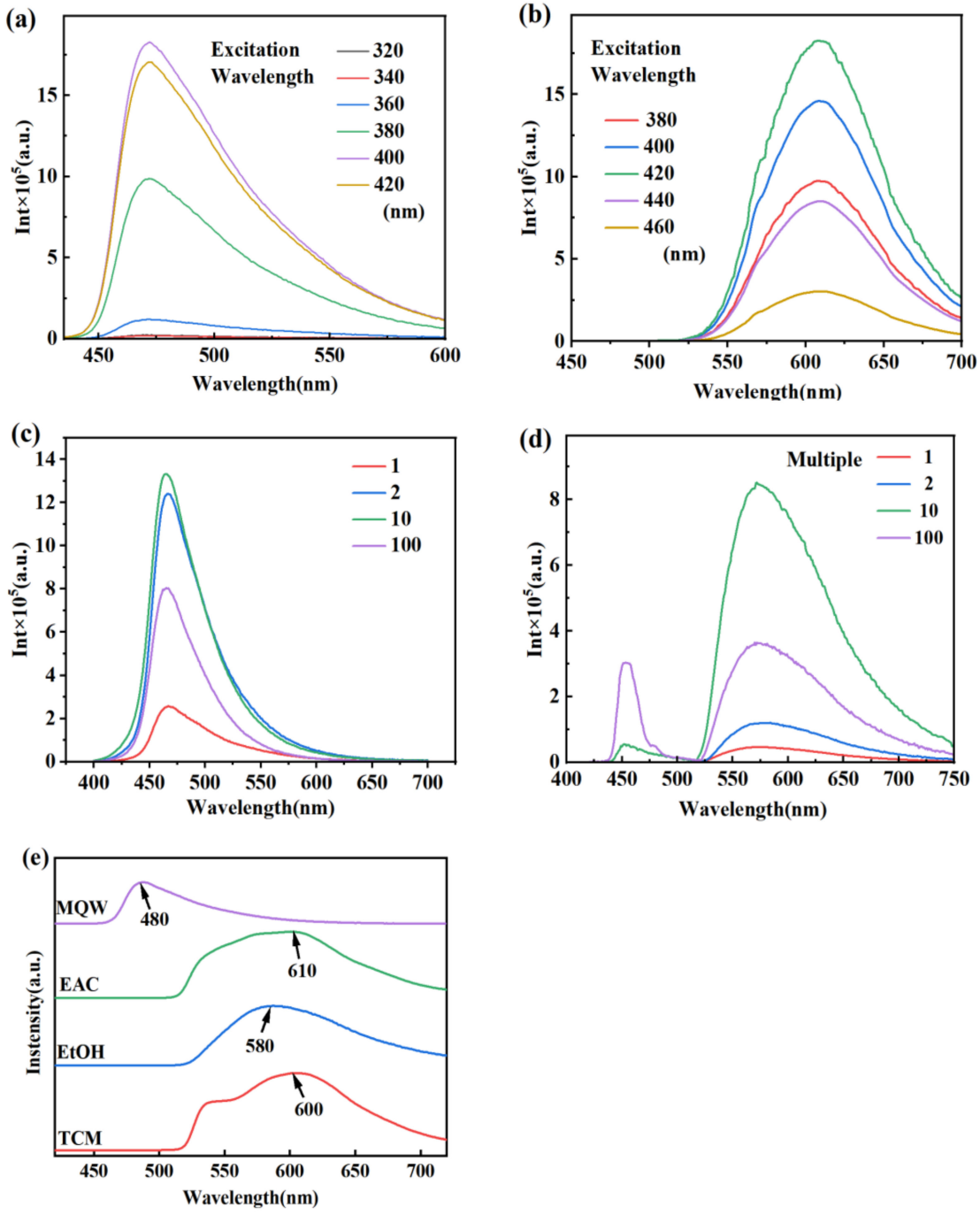
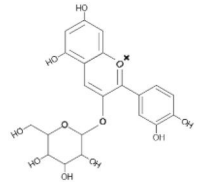
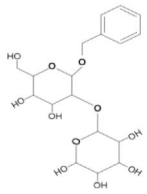
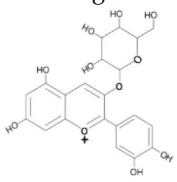
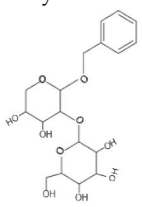
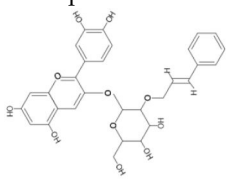
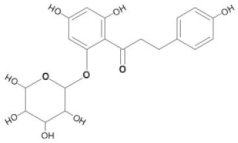
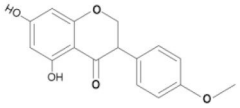
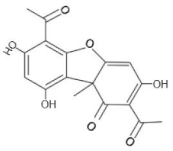
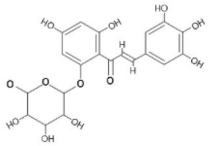
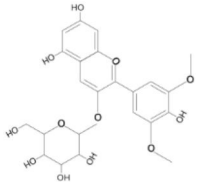
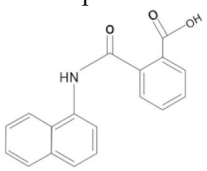
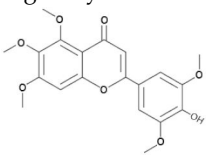
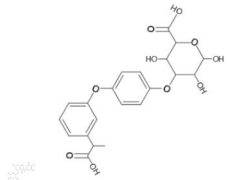
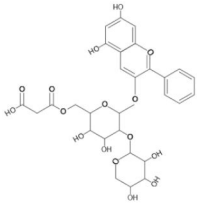
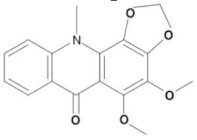
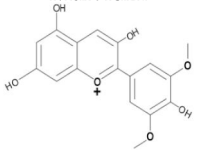


Figure 2. Spectra obtained under different conditions: (**a**) spectra of Milli-Q water system under different excitation wavelengths, (**b**) spectra of ethanol system under different excitation wavelengths, (**c**) spectra of Milli-Q water system at different dilution levels, (**d**) spectra of ethanol system at different dilution times, and (**e**) spectra corresponding to 380 nm optical excitation in different solvents.

Table 1. The substance that was most likely to be fluorescent in each system.

Milli-Q Water (MQW)	Ethanol (EtOH)	Ethyl Acetate (EAC)	Trichloromethane (TCM)
Cyanidin 3-galactoside 	Zizvbeoside I 	Cyanidin 3-galactoside 	Zizybeoside 
Epicatechin 	Phloridzin 	Biochanin A 	(−)-Usnic acid 
Hexahydroxychalc-one 2′-glucoside 	Oenin 	Naptalam 	Ageconyflavone A 
4′-Hydroxyfenoprofen glucuronide 	Pelargonidin 	Melicopine 	Malvidin 

3.2. Standard Control Based on LC-MS Results

In order to further identify molecules that fluoresce in *Pterocarpus* wood extracts, standard samples of the substances listed in Table 1 were used for fluorescence testing. Based on the previous description of the fluorescence mechanism and the basic properties of each substance, the four most probable substances were first selected based on the chemical structure of each substance for the standard control. The results are shown in Figure 3. These four substances are phloridzin, epicatechin, naptalam, and (−)-usnic acid. The fluorescence intensities of each substance in different solvents are shown in Figure 3a–d. According to the results shown in the spectrogram, it can be found that the same substance has different fluorescence effects in different solvents. This is mutually confirmed by the color and intensity of the fluorescence generated by each extraction solution during excitation. The results show that the (−)-usnic acid solution has no fluorescence effect in various solvent systems. According to the analysis of the cause and mechanism of the fluorescence, this is caused by the non-coplanarity of its molecules. As for the naptalam solution, it has strong fluorescence characteristics in various systems, which matches its molecular structure. However, as a component of herbicides [27], no research shows that this substance exists in the heartwood of *Pterocarpus* wood. The existence of this substance was detected in a large amount in the extract, which needs further study. The fluorescence intensities of the other two substances differ greatly in different solvents, though they both have high intensity in TCM solvent. This seems to suggest that there is a facilitative effect of TCM solvents on the fluorescence effect of the molecules, which of course needs

to be confirmed by future studies. In order to analyze the fluorescence effect of different substances in the same solvent, we compared the spectral patterns of the four substances under MQW and TCM systems (Figure 3e,f). They were selected because MQW is the most common solvent in the experiment, and TCM is the solvent with the highest fluorescence intensity in our research. Through experimental research, more than one substance in the extract of *Pterocarpus* wood contains strong fluorescence. Phyloridzin and epicatechin, which are abundant in *Pterocarpus* wood, play a great role in enhancing the fluorescence intensity. (−)-Usnic acid does not have strong fluorescence intensity in several systems, so its contribution to the fluorescence of the system can basically be ruled out. All systems contain naptalam, which has a great fluorescence intensity, although whether it exists in *Pterocarpus* wood needs further study. However, it is valuable to note that the strong fluorescent properties of naptalam were discovered during the control process, allowing it to have many more applications in the future as a molecule with high fluorescence.

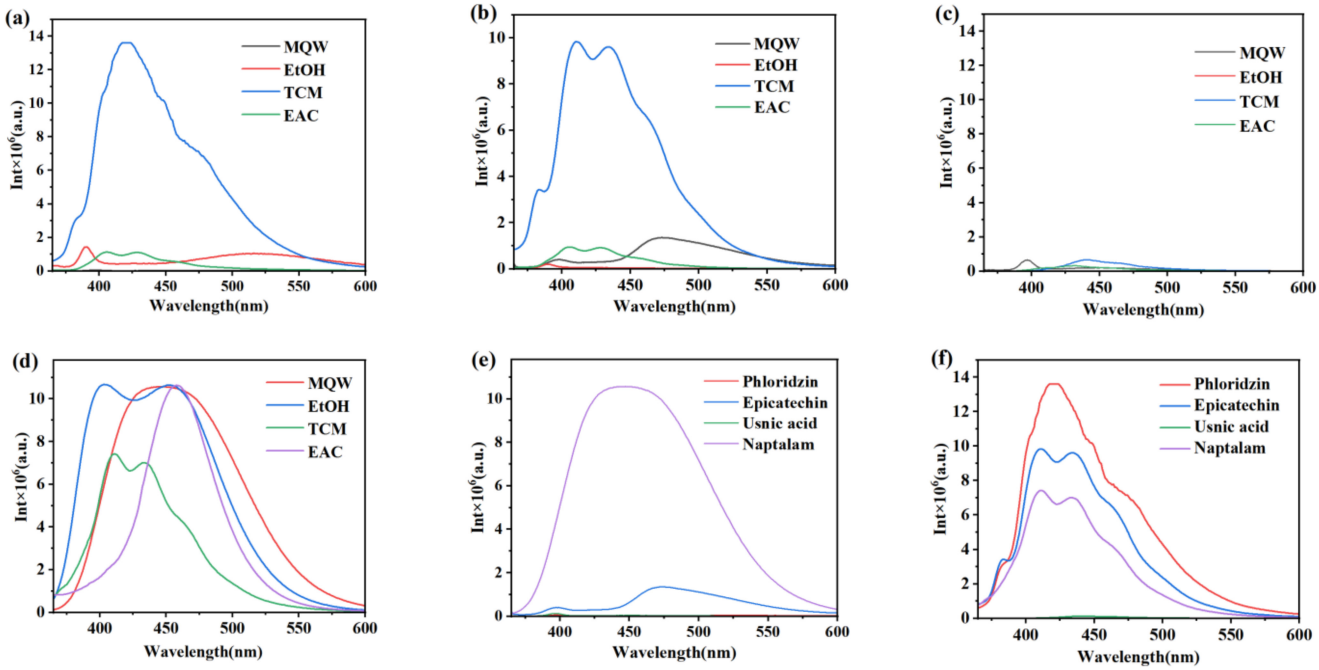


Figure 3. Fluorescence spectra of extracts in different solvent systems: (**a**–**d**) fluorescence spectra of phloridzin (**a**), epicatechin (**b**), (−)-usnic acid (**c**), and naptalam (**d**) in different solvent systems; (**e**) fluorescence spectra of four substances in MQW (**e**) and TCM (**f**) solvent systems.

3.3. Response of Fluorescent Substances of Red Sandalwood Species to External Forces and Temperature

Firstly, the effects of external forces and temperature conditions on the fluorescence intensity in this extract were investigated. The effect of external forces was achieved by centrifugation at different speeds. The fluorescence intensity was tested after centrifugation at different speeds, and no significant effect was found. This phenomenon reflects the small molecular weight of the molecule, which is consistent with previous experiments. The effect of temperature on the fluorescence intensity was also investigated. The fluorescence intensity was tested after designing different temperature gradients below the boiling point of the solvent, and there was almost no effect on the fluorescence intensity when the solution temperature was changed. This indicates that the fluorescent molecules in this extract have good thermal stability.

3.4. Response of Fluorescent Substances of Red Sandalwood Species to pH

Our previous studies showed that there is more than one substance that emits fluorescence. Therefore, we carried out more experiments to find out the macroscopic response of

the fluorescence intensity of the extraction solution to the change in pH (Figure 4). After comparing 12 sets of samples with equal pH gradients, the results showed that the fluorescence intensity of the extract was significantly different with the change in pH. At the same time, the substance was more sensitive to acids than alkalis, and acidic conditions had more obvious effects on its fluorescence.

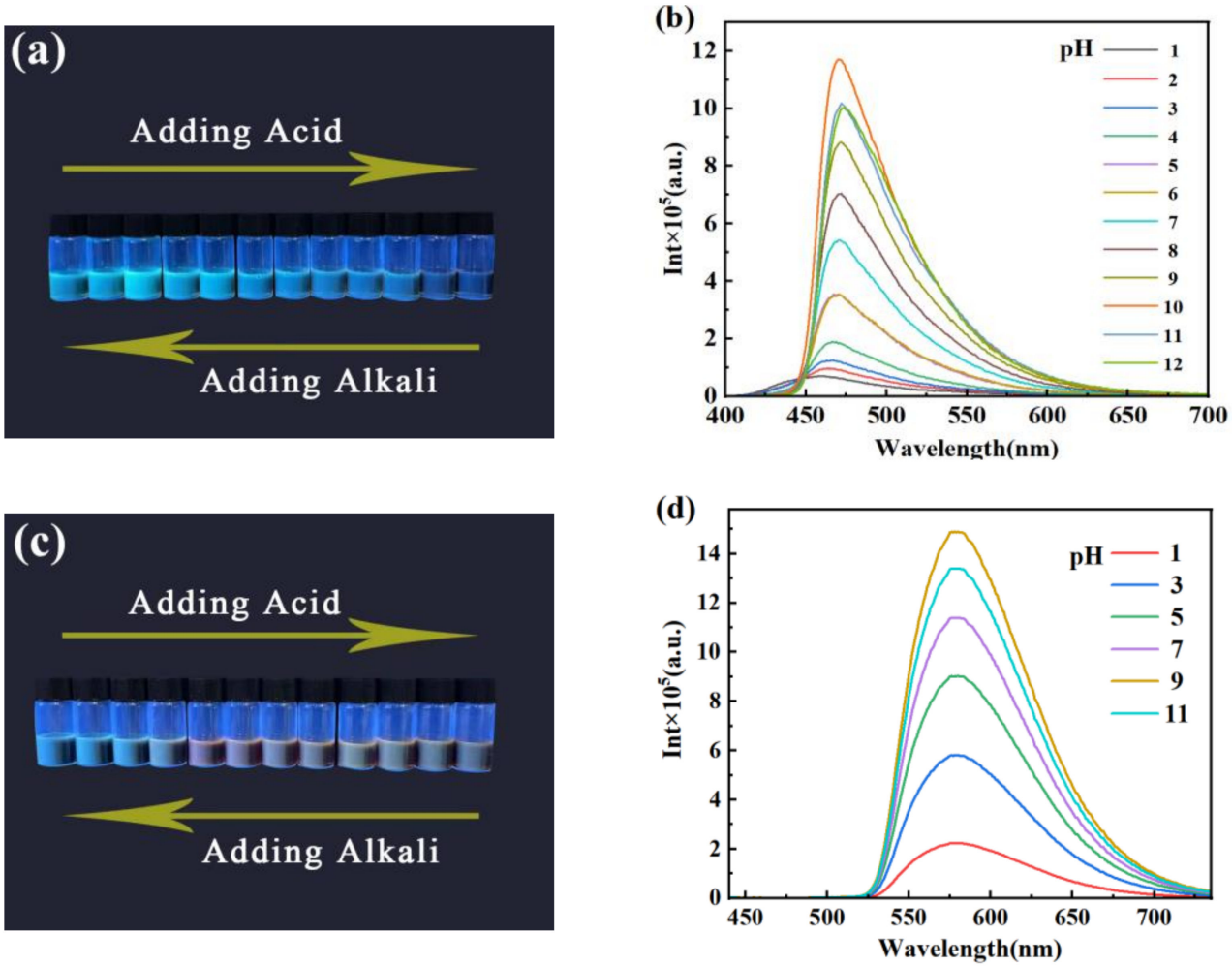


Figure 4. The fluorescence intensity varied according to the pH value in different systems: (**a**) photo of UV excitation in Milli-Q water system with variable pH value; (**b**) photo of corresponding spectrogram with the change in pH gradient in Milli-Q water system; (**c**) photo of UV excitation of EtOH system with pH change; (**d**) photo of corresponding spectrogram with the change in pH gradient in EtOH system.

There are three possible ways to analyze the influence of pH on fluorescence [28]: First, pH may promote the hydrolysis of fluorescent molecules in the extract solution, resulting in changes in the fluorescence properties of the fluorescent molecules. Second, the protonation–deprotonation reaction caused by pH changes also has some influence. This leads to the complexation–decomplexation reaction of molecules to ions, which significantly affects the fluorescence intensity of the extract. Finally, the change in the fluorescence intensity comes from the change in the molecular orbital of excitable electrons, and the ionization of fluorescent molecules occurs after the pH value changes. The fluorescence of the extract changed with the pH, and the change in the fluorescence in the *Pterocarpus* wood extract with pH was mostly due to the acid–base hydrolysis of the fluorescent molecule, which indicated that the fluorescent molecule might be unstable under acid–base conditions. At the same time, due to the complexation of ions (especially metal ions) and organic matter in

the wood processing process or growth environment, the fluorescence intensity increases. The process of protonation leads to a reduction in this complexation, resulting in a more pronounced effect on the fluorescence intensity under acidic conditions.

In order to explore the stability of the fluorescence properties of this substance, the pH of the substance was repeatedly adjusted and restored (i.e., the acidity and basicity of the same sample was changed several times and then restored to test whether the acid–base conditions would cause damage to the fluorescence properties of the molecule). When the pH value is continuously changed and restored, the fluorescence intensity can be restored to the original value after pH restoration (as shown in Figure 4), which indicates that the response of this fluorescence to pH is reversible, and the change in pH value does not cause any change in the conjugate system of the solution. In order to exclude the interference of the metal cations and non-metal anions introduced by the pH adjustment, we used different acid–base substances for the pH adjustment (H_2SO_4, HNO_3, KOH and NH_4Cl, and $NaHCO_3$), and the fluorescence intensity was not different. This provides a new direction for reasonable application.

3.5. Response of Fluorescent Substances of Red Sandalwood Species to Concentration

During the exploration of fluorescence, the strength of the fluorescence intensity is also interfered with by another factor, which is called the molecules' aggregation degree. At present, there are two theories in this regard: Aggregation-Caused Quenching (ACQ) and Aggregation-Induced Emission (AIE) [29,30]. For general conjugated molecules, their molecular structures are usually rigid planar molecules. Once the concentration is too high, these planar molecules pile up with each other. This leads to two results: one is that the fluorescent molecules located in the center of the stack after stacking cannot be effectively excited by ultraviolet light; another is that the energy from high-energy molecules was transferred to low-energy molecules, since the molecules stacked together had different excitation energies. This is the cause of the ACQ phenomenon. In both cases that cause ACQ, the energy dissipates gradually inside the system, releasing energy to the outside instead of luminosity, and then the fluorescence intensity began to decrease [31]. On the basis of our derived conjugated molecular structure, the molecule has the characteristics of Aggregation-Caused Quenching [32]. The effect of molecular concentration on the fluorescence intensity was studied by us. Different dilution concentrations were set (expressed as multiple of dilution relative to the original solution) (Figure 2c,d), and the result indicated that the sample revealed extremely strong ACQ characteristics. However, when the concentration of the extract was too low, the fluorescence intensity also falls. Since the number of fluorescent molecules per unit volume of the solution is small at this time, it is not enough to produce high-intensity fluorescence. The results show that the concentration of the extraction solution when the maximum fluorescence intensity is obtained is worthy of further study.

In addition, the interpretation of fluorescence attenuation must consider the inner filter effect of fluorescence. This is a phenomenon that occurs when the concentration of fluorescent molecules is large or coexists with other light-absorbing substances, and the fluorescence may be absorbed by other substances in the solution and weakened. Firstly, according to the uniform excitation wavelength before and after dilution, the influence of excitation light being absorbed is eliminated. Under the Milli-Q water system, the two-dimensional fluorescence spectrum of the solution always only presents a single peak, and the emission wavelength remains unchanged during the dilution process. Under this system, the possibility of the inner filter effect is extremely small. In the EtOH system, the two-dimensional fluorescence spectrum of the solution is more complicated. Considering that the emission wavelength is larger than the excitation wavelength, the weakened peak at 570 nm cannot be attributed to the enhanced emission peak at 450 nm due to the dilution of the extract. This is because fluorescent molecules with an emission peak at 450 nm are not excited by the emission wave at 570 nm. Instead, the enhancement of the peak at 450 nm coincides with the decrease at 570 nm. This shows that the 450 nm emission wave does not excite molecules with the 570 nm emission wave (otherwise, both should

be enhanced at the same time). This seems to indicate that the system is not applicable to explain the inner filter effect. According to the molecular planar structure inferred in the experiment, the fluorescence quenching caused by molecular aggregation and stacking can more reasonably describe this phenomenon.

What is more, from the figure we can determine that fluorescence of different wavelengths would be produced after the ethanol system is diluted to a certain extent, which again indicates that there may be more than one fluorescent substance in the extract. Although it is difficult to use AIE characteristics to enhance the fluorescence intensity, this fluorescence, as a substance with an active response to concentration, can be used as a probe in other fields through concentration changes.

4. Conclusions

In this study, through some analysis and research, the fluorescent substances in the wood extract of *Pterocarpus* wood were expounded. After obtaining and observing the fluorescence in the extract, we separated the substances in the extract by LC-MS. Then, the chemical structure of the molecule was analyzed by using the mechanism of fluorescence generation, further narrowing the scope of research. The inferred molecules were enumerated to facilitate subsequent studies. After selecting representative substances for standard reference and considering whether the molecules obtained by LC-MS exist in *Pterocarpus* wood, it was found that there is more than one fluorescent molecule in the system. Moreover, the intensity and wavelength of fluorescence vary in different systems. This is due to the different types and numbers of fluorescent molecules in the different systems. When analyzing the environmental response characteristics of the fluorescence intensity of the extract, we researched the effects of external forces, temperature, pH, and concentration on the fluorescence intensity and wavelength. An increase in the concentration of the molecule led to a decrease in the intensity of this fluorescence. After excluding the fluorescence self-absorption phenomenon, the fluorescent molecule had the characteristic of aggregation-induced quenching. The fluorescence intensity of the molecule is reduced by both acids and alkalis. The reasons for the fluorescence change under acid–base conditions are multiple, most likely the hydrolysis of the fluorescent molecule due to the acid–base. Temperature and external forces have little effect on its fluorescence intensity. This study contributes to the investigation of the nature of fluorescence in Pterocarpus wood and its future rational application.

Author Contributions: Conceptualization, R.L., Z.L. and J.S.; methodology, R.L. and Z.L.; software, R.L. and J.L.; validation, J.S., Y.Z. and Y.S.; formal analysis, R.L.; investigation, J.L. and Y.C.; resources, J.S.; data curation, R.L. and Y.C.; writing—original draft preparation, R.L.; writing—review and editing, J.L., Y.S. and Z.L.; visualization, Y.Z.; supervision, J.S. and Z.L.; project administration, Y.Z.; funding acquisition, J.S. All authors have read and agreed to the published version of the manuscript.

Funding: The authors would like to thank the Innovative Training Program for Chinese College Students (2022NFUSPITP0447) and the Qing Lan Project of Jiangsu Province (2022) for the financial support.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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