

Extraction, Optical Properties and Bio-Imaging of Fluorescent Composition From Moso Bamboo Shoots

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Abstract: A novel fluorescent composition was firstly isolated from natural winter fresh Moso bamboo shoots, and its optical properties were fully investigated by fluorescence spectroscopy. It could emit strong blue light both in solid and solution state, providing high fluorescence intensity in ethanol. The solution's concentration and addition of water greatly affected the fluorescence intensity, high concentration and addition of much water could quench fluorescence. Apoptosis results showed that the fluorescent extract (0-25 mg/L) could not induce apoptosis of Hela cells. Confocal fluorescent microscopic imaging in human hepatocellular carcinoma cells (HepG2) was realized using the fluorescent extract, it could dye the whole cell well which was different from 4', 6-diamidino-2-phenylindole (DAPI) only dyeing cell nucleus. The fluorescent extract may be candidate for future natural fluorescent bio-imaging agent.

Keywords: Bamboo shoots; extraction; fluorescent composition; optical properties; bio-imaging

1 Introduction

Organic fluorescent compounds have received much attention due to their increasing application on the biological imaging field [1-5], but many of them were prepared by a way of synthesis. A new polythiophene derivative (PT-HMDA) with low cytotoxicity was synthesized and used as imaging agent in A549 cells [6], as well as, a 1,8-naphthalimide-based organic luminescent molecule was prepared for application in fluorescence images of live Hela cells [7]. In addition, new imidazo[1,5-a]pyridine and imidazo[5,1-a]isoquinoline derivatives were synthesized for cell imaging applications on *Arabidopsis thaliana* seedlings [8], and a low cytotoxicity fluorescent NTR probe based on the iminocoumarin-benzothiazole fluorophore (KC-NTR) was developed to monitor nitroreductase in the living HepG2 cells [9]. At the same time, a few of turpentine (natural product) derivatives had been synthesized for an imaging application in A549 cells and watermelon peel veins [10,11].

Some dyes from natural plants also had fluorescence characteristics, such as chalcone, coumarin, flavonoid [12], protein [13], chlorophyll a [14], pollen and spores [15], dyes extracted from *Plumeria rubra* flowers [16], eumelanin [17], indicaxanthin dyes [18], apple juice [19], etc. But using for cell imaging was rarely reported till now.

Bamboo is a useful plant which is widely distributed in both temperate and tropical regions of the world and offers a rich yield of bamboo shoots [20]. The eighth national forest resources inventory results shows that Moso bamboo covers an area of 4.43 million hm² in China, which provides abundant Moso bamboo shoots [21-23]. Bamboo shoots are well known as an excellent health food with dietary fiber, proteins, amino acids, carbohydrates, and minerals [24]. Owing to their large annual output, many studies have made a tremendous progress in this field. A study on bamboo fiber showed that it could lower cholesterol levels and improve bowel functions [25]. Six novel nucleosides and two unique amino acids

were also isolated and identified from bamboo shoots of *Gramineae Phyllostachys prominens* [26]. In addition, two water-soluble polysaccharides were extracted from bamboo shoots (*Phyllostachys praecox*) using hot-water extraction [27], and a β -pyran polysaccharide of bamboo shoot shell had natural anti-diabetic activity [28]. Carboxymethylcellulose was prepared from bamboo shoot cellulose via chemical modification [29]. The volatile aromatic components were obtained from shoots of *Phyllostachys edulis*, with fresh green aroma ingredients of methoxy-phenyl oxime, byn-hexanol and 3Z-hexenal [30]. However, to the best of our knowledge, there are no fluorescent substance from bamboo shoots reported today. Meanwhile, we have been interested in the fields of bamboo shoots, and think that it has utility for extracting fluorescent composition from Moso bamboo shoots to image cells.

In this paper, a novel fluorescent extract was successfully separated from natural fresh winter Moso bamboo shoots by a simple solvent extraction method, and its optical properties were studied comprehensively. In the end, a new approach to confocal fluorescence imaging of HepG2 cells was also delicately designed.

2 Materials and Methods

2.1 Materials and Instruments

Winter fresh Moso bamboo shoots were collected from Yuhang district, Hangzhou city. Reagents and solvents were purchased from commercial suppliers and used without further purification. The IR spectra were recorded on Nicolet 380 FT-IR infrared spectrometer. The fluorescence spectra were determined on a Hitachi F-4600 fluorescence spectrophotometer, excitation wavelength at 230.0 nm, scan from 200.0 nm to 550.0 nm with a 240 nm/min of scan speed. The UV-visible absorption spectra were measured on a UV-2550 spectrophotometer. Fluorescent microscopic images were photographed with a fluorescent microscopic imaging system (BX51 Olympus). The fluorescence lifetime was measured by an FM-4P-TCSPC transient fluorescence spectrometer.

2.2 Extraction of Fluorescent Composition

A new fluorescent mixture was directly extracted from winter fresh Moso bamboo shoots. 200.0 g small pieces of fresh bamboo shoots were added to a 2000 mL beaker with 1000 mL distilled water. Then it was smashed and extracted for 30 min by a high speed homogenizer, and the suspension was centrifuged for 20 min to provide a residue (1). The residue (1) was washed twice with 100 mL distilled water to remove water soluble substances, then provided residue (2) after finishing a process of centrifuging. Residue (2) was mixed with 800 mL NaCl water solution (3%) and extracted for 30 min by the high speed homogenizer. Centrifuged for 20 min, washed twice with 100 mL distilled water, the residue (3) was obtained after centrifuging. The residue (3) was mixed with 500 mL ethanol to extract fluorescent mixture (30 min) at the condition of a high speed homogenizing. The supernatant (450 mL) was obtained after centrifuging, and blended with 1350 mL distilled water, then it was stored in a refrigerator for 48 h at a temperature of 4°C to produce a precipitate. A fluorescent extract was provided after centrifuging and drying. Fluorescent extract: viscous substance; yield of 0.08% (fresh bamboo shoots with a water content of 90.5%); FT-IR (KBr) ν (cm^{-1}): 3417, 3006, 2956, 2923, 2851, 1738, 1649, 1555, 1466, 1380, 1166, 1069.

2.3 HepG2 Cell Culture and Imaging

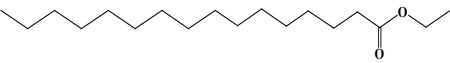
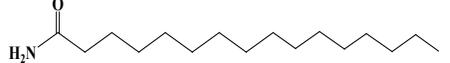
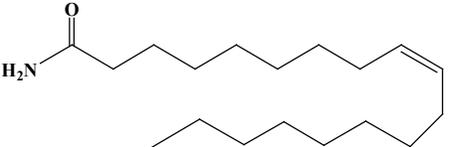
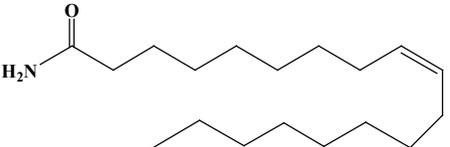
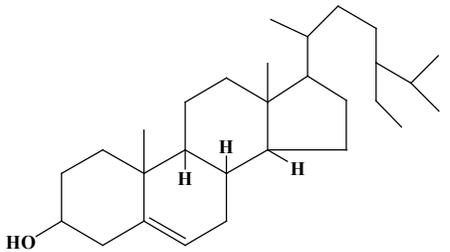
HepG2 cells in logarithmic growth phase were digested with 2% trypsin and 0.15% EDTA, then diluted into 4×10^4 cells/mL, with 1 mL added to the 24-well plates. After that, it was incubated for 24 h at 37°C, and washed twice with PBS. Cold poly formaldehyde (4%) was used to fix it for 20 min, and it was washed three times with PBS. After finishing cell permeability for 10 minutes with Triton X-100 (0.2%), it was washed three times with PBS for cell-staining. The fluorescent dye in PBS in a humidified atmosphere with 5% CO₂ was incubated with HepG2 cells for 20 min at 37°C, and excess dye was removed by washing with PBS prior to record on a confocal laser fluorescent microscopy.

3 Results and Discussion

3.1 GC-MS Analysis

To find the possible chemical compounds in the extract of bamboo shoots, a GC-MS method (America Agilent 7890A gas chromatograph and 5975c mass spectrometer) was used to analyze it, as shown in Tab. 1. The six major compounds accounted for 88.50%, and two compounds (RT = 21.69, 24.23 min) were the most important compounds which accounted for 80.39%. The compound (21.69 min) could be fatty amide derivative of 9-octadecenamide due to the similarity of 90.24%. In addition, the compound showed peak at 24.23 min might be a sterol derivative due to the 58.98% similarity with ζ -sitosterol. Thus, it can be deduced that the amide derivatives and sterol derivatives were the core components in the six major compounds, which of the shoots extract might emit fluorescence.

Table 1: Identification of major compounds of the fluorescent extract from bamboo shoots

Code	RT (min)	Area (%)	Compound name	Structure	Probability (%)
a-1	18.08	1.47	Hexadecanoic acid ethyl ester		41.69
a-2	19.05	2.87	Heneicosane		17.48
a-3	19.90	1.92	Hexadecanamide		52.54
a-4	21.69	10.03	9-Octadecenamide		90.24
a-5	21.93	1.85	9-Octadecenamide		34.51
a-6	24.23	70.36	ζ -Sitosterol		58.98

3.2 Fluorescence in Solid State

Some fluorescents can emit fluorescence in solution, but quench fluorescence in a solid state, thus it is very necessary to know the solid state light-emitting properties and fluorescence spectra. In order to determine fluorescence spectra, a superfine powder mixture was prepared for use, with amount of fluorescent extract and KBr grinding for 30 min by hand. The fluorescence spectra of the bamboo shoots' fluorescent extract with a concentration of 2×10^{-3} mg/g was shown in Fig. 1. Fig. 1 depicts the fluorescence spectra of the fluorescent extract from bamboo shoots in the solid state, inset is a picture of the extract with an enhanced fluorescence and emitting blue light. It was clearly to see that there was a significant peak giving an intensity of 1758 at 384.0 nm.

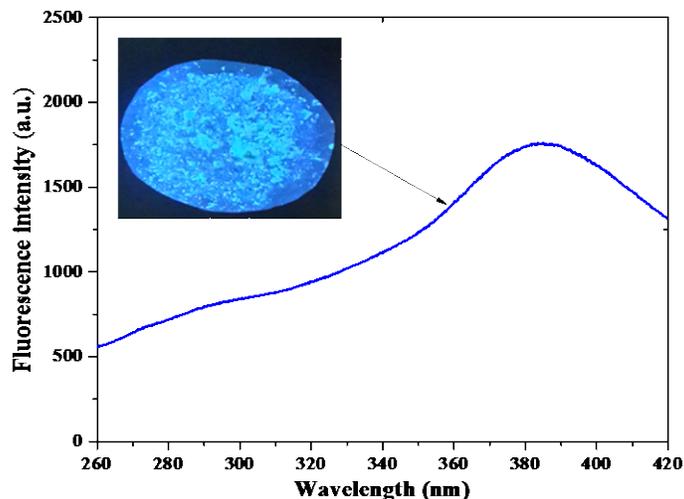


Figure 1: Fluorescence spectra of the fluorescent extract from bamboo shoots in a form of KBr powder ($\lambda_{\text{ex}} = 230.0$ nm), inset is a picture of the fluorescent extract under 365 nm UV light

3.3 Optical Properties in Solution

The fluorescent extract of bamboo shoots was readily soluble in ethanol, methanol, cyclohexane, isopropanol, dichloromethane and ethyl acetate, and the fluorescence spectra in these solvents were shown in Fig. 2(a), the UV-visible absorption spectra in ethanol was seen in Fig. 2(b).

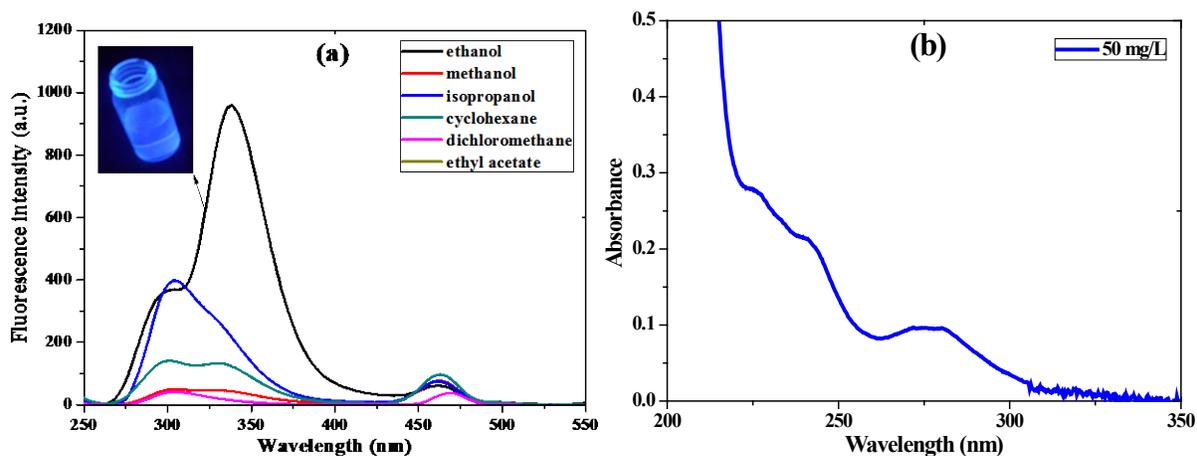


Figure 2: (a) Fluorescent spectra of the fluorescent extract from bamboo shoots (0.5 mg/L) in different solvents, $\lambda_{\text{ex}} = 230.0$ nm. (b) UV-visible absorption spectra of the fluorescent extract from bamboo shoots (50 mg/L) in ethanol

Fig. 2(a) showed that the ethanol solution of the fluorescent mixture could enhance fluorescence at the emission wavelength of 336.0 nm and gave an intensity of 954.6, and it emitted strong blue light under a 365 nm UV light in solution. When it was dissolved in isopropanol, a blue-shift from 336.0 to 304.6 nm had happened, giving a weaker fluorescence intensity of 399.3. At the same time, cyclohexane, methanol and dichloromethane quenched fluorescence a lot, and nearly no fluorescence appeared when ethyl acetate was used as a solvent. It was concluded that the fluorescent intensity of bamboo shoot fluorescent extract was greatly affected by these solvents, with the best fluorescent intensity in ethanol. In comparison with the results of solid (KBr) state (peak intensity appeared at 384.0 nm), a 48.0 nm blue shift appeared when the shoot fluorescence substance was dissolved in ethanol. Thus, ethanol was an

ideal solvent for enhancing fluorescence in a solution state. As shown in Fig. 2(b), it had an absorption peak at 280.0 nm and gave an absorbance of 0.0959.

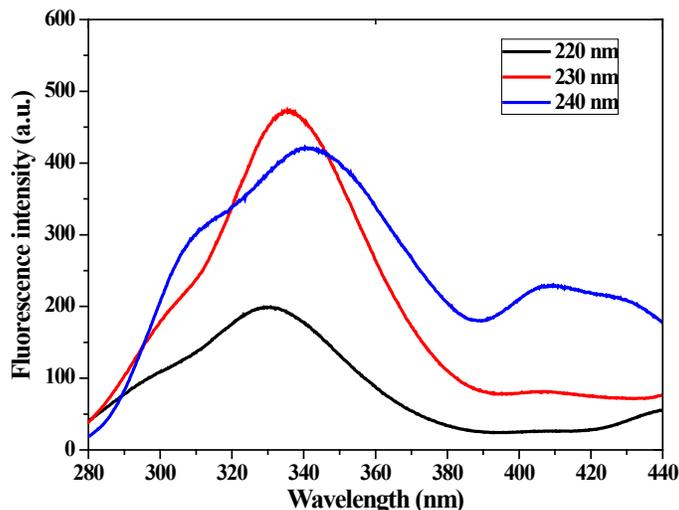


Figure 3: Fluorescent spectra of the fluorescent extract from bamboo shoots (50 mg/L) in ethanol with different excitation wavelength

Excitation wavelength also has an influence on fluorescence spectra, so these fluorescent spectra of the fluorescent extract of bamboo shoots in ethanol at the excitation wavelength of 220.0 nm (230.0 or 240.0 nm) were finished in Fig. 3.

From Fig. 3, it was clearly to know that it had a maximal intensity (198.9) at 330.4 nm when the excitation wavelength was 220.0 nm. A 5.0 nm red-shift had happened when the excitation wavelength changed to 230.0 nm, with a maximal fluorescence intensity of 473.5. Using excitation wavelength at 240.0 nm, differently from a peak at the excitation wavelength of 220.0 nm and 230.0 nm, there were two peaks at 340.4 and 409.6 nm, giving fluorescence intensity of 421.7 and 229.7. A 230.0 nm excitation wavelength gave the best intensity, that was to say that it could enhance fluorescence with a 230.0 nm excitation wavelength.

The solution’s concentration is very important for fluorescence light-emitting, so the fluorescence spectra of the fluorescent extract (bamboo shoots) in ethanol with different concentrations were fully studied, as shown in Fig. 4.

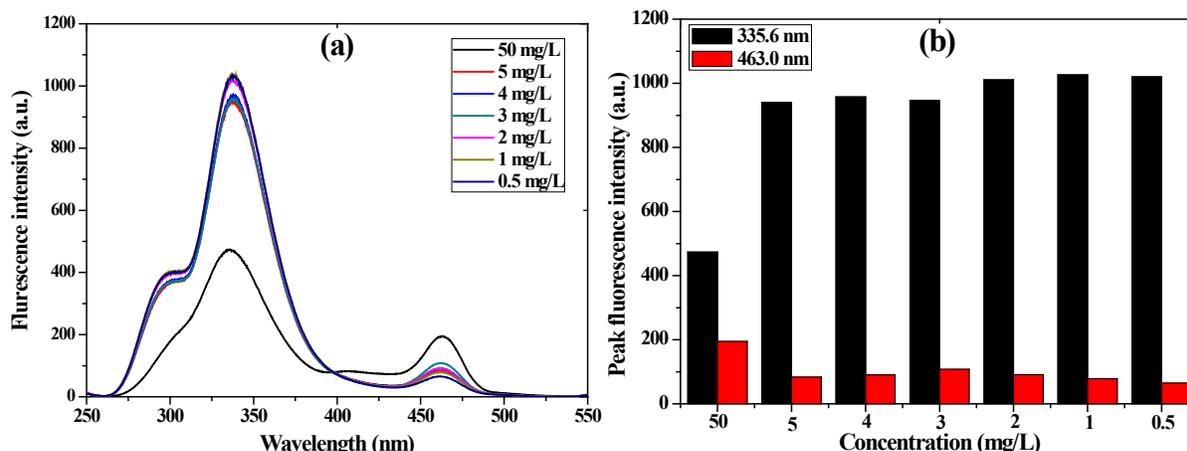


Figure 4: Fluorescence spectra (a) and peak intensity (b) of the fluorescent extract from bamboo shoots in ethanol with different concentrations ($\lambda_{ex} = 230.0$ nm)

From Fig. 4, we noted that the fluorescence intensity was in connection with concentration. A high concentration (50 mg/L) could quench fluorescence, meanwhile, it had made an enhancement when the concentration decreased to 5 mg/L. When the concentration continually reduced to 2 mg/L, the fluorescence increased a little, but with a concentration of 0.5 mg/L, the fluorescence intensity did not change. Thus, 0.5 mg/L was chosen as a good concentration for a further study.

Water is another factor to affect the changes of fluorescence intensity, so we obtained fluorescence spectra of the fluorescent extract from bamboo shoots in ethanol/distilled water mixtures with different volume fractions of distilled water, keeping the final concentration constant at 0.5 mg/L, these data appear in Fig. 5.

Shifts in fluorescence spectra (200.0-550.0 nm) were shown in Fig. 5(a) at a 230.0 nm excitation wavelength, and there were three peaks (231.8, 337.6, 462.4 nm). As shown in Fig. 5(b), peak (231.8 or 462.4 nm) intensity of the fluorescent mixture gradually increased with an increasing volume of distilled water until the volume ratio of distilled water and ethanol was 3:2. After that, the intensity began to decrease when the amount of distilled water was continuously added. It could be seen that the changes of two peaks intensity at 231.8 and 462.4 nm had the similar variation trend. However, the change of another peak (337.6 nm) intensity differed from the above two peaks. When the volume fraction of distilled water was added from zero to 20%, it enhanced fluorescence rapidly to a maximum step by step, keeping adding up to 40%, only a little fluorescence quenching occurred. The fluorescence intensity reduced sharply after the volume of water was added with more than 50%, and there was nearly no fluorescence appeared using 100% distilled water as a solvent. Data showed that the fluorescence intensity of shoot fluorescent extract was affected greatly by water, a small amount of water could enhance fluorescence, but a large number of water quenched fluorescence a lot.

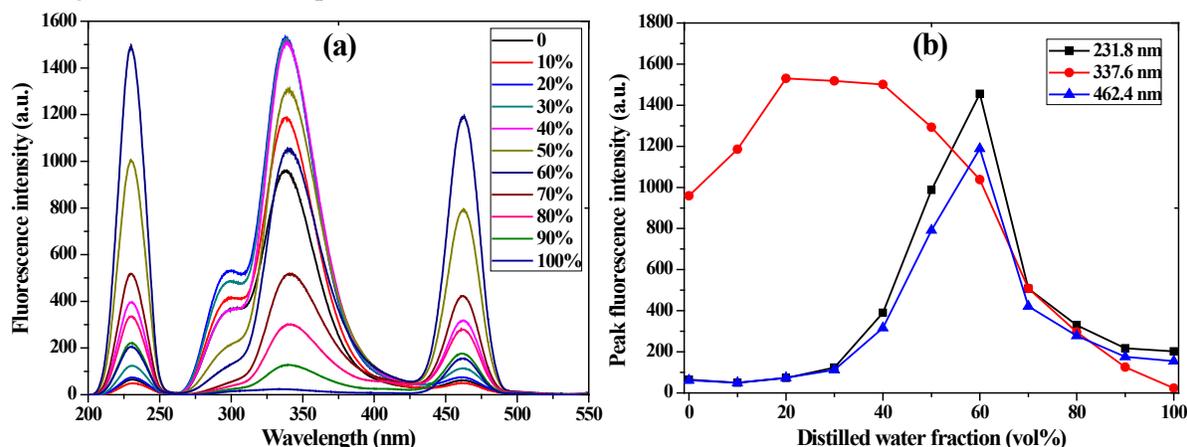


Figure 5: Fluorescence spectra (a) and changes in peak fluorescence intensity (b) of the fluorescent extract from bamboo shoots in ethanol/distilled water mixtures with different volume fractions of distilled water ($\lambda_{\text{ex}} = 230.0$ nm)

It also affects the fluorescence intensity changes when the light-emitting solution is added with some acid or alkali, so the fluorescence spectra of fluorescent extract from bamboo shoots in ethanol (0.5 mg/L) affected by trifluoroacetic acid (TFA) and potassium *tert*-butoxide were provided for a further study (Fig. 6). Fig. 6 showed the fluorescence spectra of the fluorescent extract from bamboo shoots with adding TFA (1.0 mmol/L) and potassium *tert*-butoxide (1.0 mmol/L), it was clear to see that the fluorescence intensity decreased a lot with adding TFA or potassium *tert*-butoxide. The reason for quenching might be the interaction of the fluorescent extract with H^+ or OH^- [31]. It was concluded that the addition of TFA or potassium *tert*-butoxide could lead to fluorescence quenching of the fluorescence extract of bamboo shoots in a solution state.

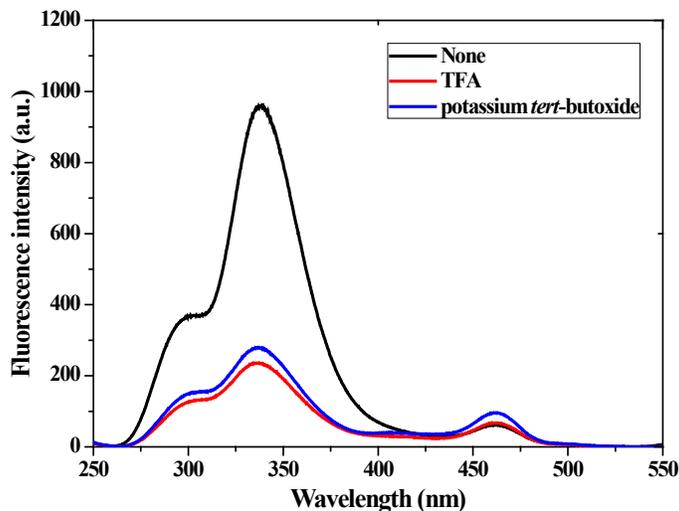


Figure 6: Fluorescence spectra of the fluorescent extract from bamboo shoots in ethanol with addition of TFA and potassium *tert*-butoxide ($\lambda_{\text{ex}} = 230.0 \text{ nm}$)

3.4 Fluorescence Lifetime

Fluorescence lifetime (τ) was further determined after the extract was dissolved in ethanol (excitation wavelength = 265.0 nm, emission wavelength = 338.0 nm), the results were shown in Fig. 7. The results proved that the shoots' fluorescent extract had a fluorescence lifetime of 1.93 ns.

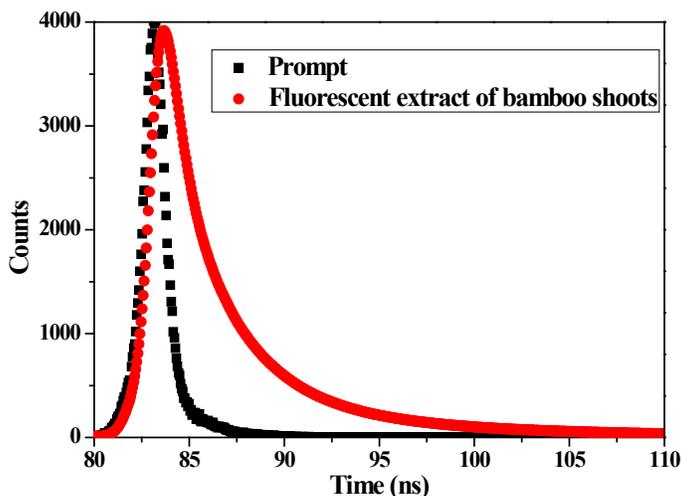


Figure 7: Decay curves of the fluorescent extract of bamboo shoots in ethanol solution

3.5 Apoptosis

In order to investigate cell apoptosis induced by the fluorescent extract of bamboo shoots, the extract-treated Hela cells were stained with Annexin V-FITC/PI and analyzed by a flow cytometry method [32], as shown in Fig. 8.

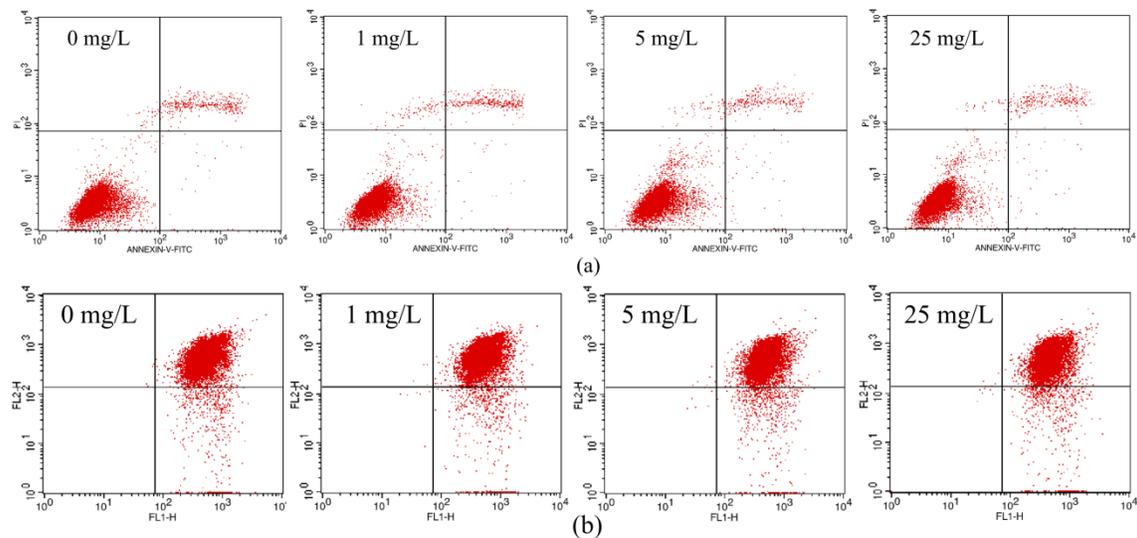


Figure 8: Apoptosis (a) and fluorescence (b) in HeLa cells treated with various concentrations of the fluorescent extract of bamboo shoots for 24 h

As shown in Fig. 8(a), after HeLa cells were treated for 24 h with different concentrations (0, 1, 5, 25 mg/L) of the extracted fluorescent mixture, the percentages of apoptotic cells were 5.55%, 5.29%, 4.37%, 4.18%, respectively. The results showed that the addition of fluorescent mixture could not induce apoptosis of HeLa cells in a dose-dependent manner.

Another method of mitochondrial membrane potential was also used to analyze apoptosis of HeLa cells for a further study, using 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide (JC-1) as a cationic lipid fluorescent dye [33], as shown in Fig. 8(b). When HeLa cells were separately conducted for 24 h with various concentrations (0, 1, 5, 25 mg/L) of bamboo shoot fluorescence extract, the green fluorescence accounted for percentages of 4.19%, 4.45%, 5.05%, 4.62%. There was nearly no change of the data after adding amount of shoot fluorescent substance, that was to say that no apoptosis occurred, and the apoptosis had no concern with the added fluorescence extract.

Thus, it was proved that the shoot fluorescent extract had almost no influence on HeLa cells and could be further used in cell imaging.

3.6 Cell-Staining

For cell imaging, 25 mg/L bamboo shoot fluorescence extract and contrast fluorescent dye DAPI (4',6-diamidino-2-phenylindole, a common cell dye) [34] were respectively used to dye HepG2 cells, the results were shown in Fig. 9.

From Fig. 9(a), it could be seen that the bamboo shoot fluorescence substance was internalized by living HepG2 cells, and the extracted dye permeated the whole cell well and offered bright intracellular fluorescence. Differently, as shown in Fig. 9(b), the contrast fluorescent dye could only dye cell nucleus. Thus, the fluorescent mixture extracted from bamboo shoots may have a different use with fluorescent microscopic imaging of HepG2 cells by contrast with DAPI.

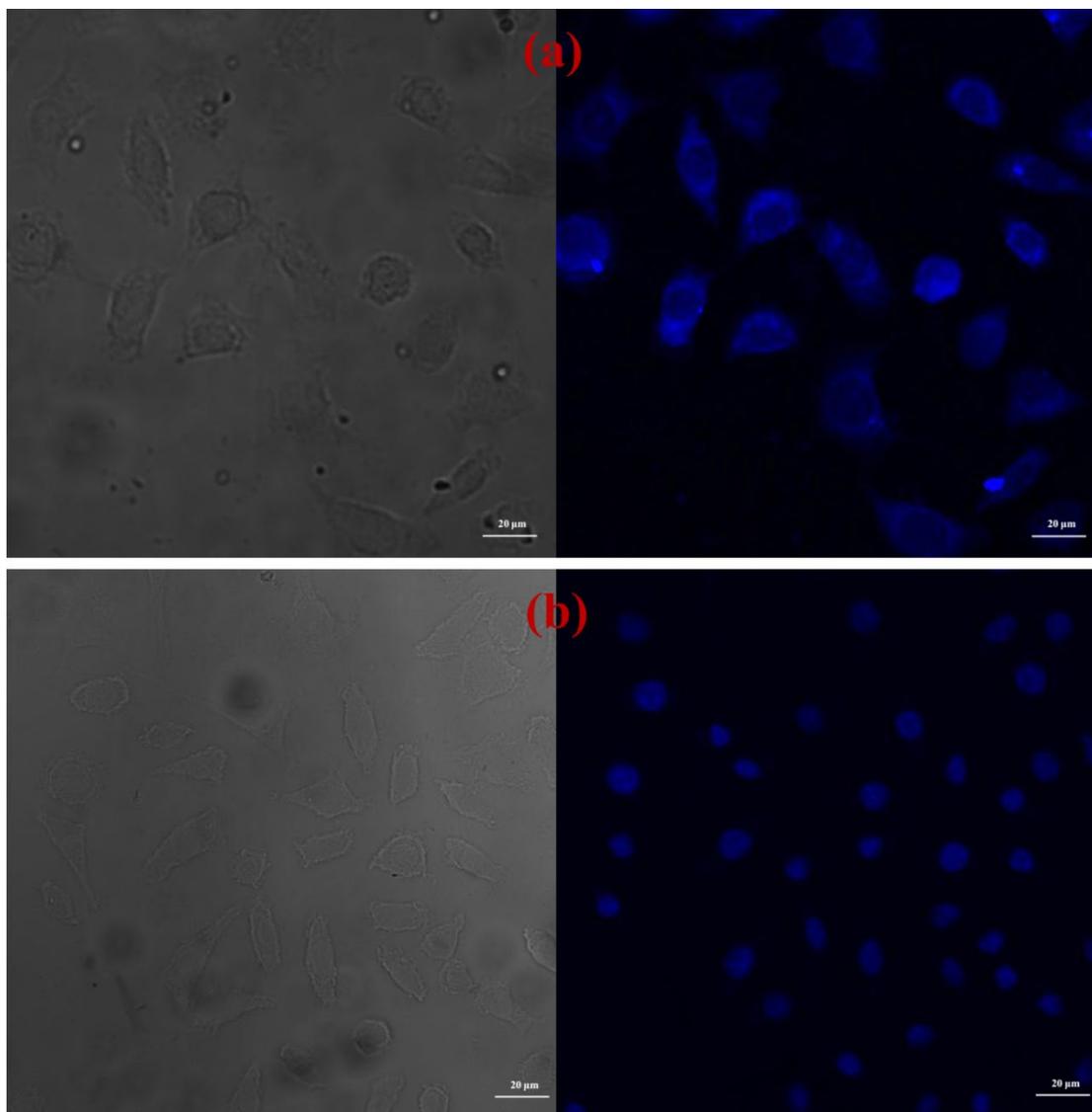


Figure 9: Confocal fluorescent microscopic images (right) and bright-field transmission images (left) of the fluorescent extract of bamboo shoots (a) and DAPI (b) in HepG2 cells

4 Conclusion

A new fluorescent extract was successfully isolated from natural winter fresh Moso bamboo shoots by a simple solvent extraction and precipitation method. The extract had good light-emitting properties and could emit strong blue light both in solid and solution state. Ethanol was an ideal solvent for it to provide strong fluorescence intensity (954.6) in solution. The fluorescence intensity was not very good when its concentration was 50 mg/L in ethanol, providing an excellent intensity with a concentration of 0.5-2.0 mg/L. In addition, the addition of water in the light-emitting system caused tremendous impact on fluorescence intensity, the intensity gradually added to a maximum along with an increasing water, then reduced step by step. The shoot fluorescence extract had a 1.93 ns fluorescence lifetime in ethanol. The fluorescent extract could not induce apoptosis of Hela cells. The natural fluorescent extract could dye the whole cell (HepG2) well while DAPI could only image cell nucleus. The bamboo fluorescent extract will have a different use in fluorescent bio-imaging. Thus, the new bio-imaging agent from natural Moso bamboo shoots could be further used in cell imaging, and added the value of bamboo shoots.

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