Bioluminescence Study of Red Dragon Fruit (*Hylocereus polyrhizus*) Extract in Various Solvents

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ABSTRACT

Special attention has been given to red dragon fruit (*Hylocereus polyrhizus*) because it contains unique natural products and exhibits wide biological activities. However, their bioluminescence properties and their potential use as photonic material have not been addressed yet. In the present work, simple extraction of red dragon fruit was carried out to study the bioluminescence properties of the corresponding extracts in various solvents. At first, the flesh and peel of red dragon fruit were separated, dried, and macerated for 24 hours using distilled water, ethanol, and acetone, separately. The results demonstrated that the red dragon fruit exhibited different luminescence properties depending on the fruit part and the used solvent. It was revealed that the ethanolic extract of red dragon fruit either of flesh or peel gave the highest fluorescence intensity among the others. The flesh of the red dragon fruit extract showed two excitation peaks at 228 and 293 nm, yielding only a single emission peak at 335 nm when monitored at both excitation wavelengths. Meanwhile, the peel of red dragon fruit extract showed two excitation peaks at 290 and 359 nm, yielding different emission properties. Excitation at 290 nm gave one emission peak at 339 nm,

while the excitation at 359 nm gave an emission peak at 436 nm. Such strong bioluminescence properties observed in a wide range of UV and visible regions demonstrated the potential use of the red dragon fruit extract as a photonic material.

Keywords: bioluminescence, fluorescence, maceration, red dragon fruit, solvent

1. Introduction

A great concern is on synthetic pigments because they have been widely used in daily life, especially in food and textile industries. Several synthetic pigments such as methylene blue, congo red, acid red 88, naphthol, *etc.* have been utilized from the 18th century. However, they generate serious effects in human health, such as allergic, liver dysfunction and cancer (Hassan and Nemr 2017; Pirkarami and Olya 2017; Liu and Zhu 2017). Because of that, finding and developing some environmentally-friendly pigments from our nature are important approaches, especially in Indonesia, which is one of the biggest biodiversity countries in the world (Liaotrakoon *et al.* 2013; Wresdiyati *et al.* 2015; Sapitri *et al.* 2019).

Dragon fruits are abundantly available in Indonesia and they are well known for their intense color. In nature, there are three species of dragon fruits distinguished based on their peel and flesh color, *i.e*. red dragon fruit (*Hylocereus polyrhizus*), white dragon fruit (*Hylocereus undatus*), and yellow dragon fruit (*Selenicereus megalanthus*) (Mercado-Silva *et al.* 2018; Lee *et al.* 2013). Among them, red dragon fruit has the strongest color intensity due to high betacyanin contents (Stintzing et al. 2002). Besides that, special attention has been made to the red dragon fruit, because it contains several bioactive natural products, *e.g.* flavonoid, carotenoid and polyphenol, and has been thoroughly evaluated for their anti-inflammatory, antioxidant, antibacterial, antidiabetic and anticancer activities (Jafaar *et al.* 2009; Rebecca *et al.* 2010; Kim *et al.* 2011; Hanifa *et al.* 2016).

While there are many studies on the stepwise isolation and biological activity of bioactive products from dragon fruits, the chemiluminescence properties of the dragon fruit extracts have not been addressed yet, whereas they are critical for understanding the potential use of the dragon fruit extract as one of the photonic materials. Nowadays, photonic materials, especially dye-sensitized photocatalyst and dye-sensitized solar cell,

are attracting many attentions because of their unique phenomena and high quantum efficiency (Wang *et al.* 2014; Zhang *et al.* 2016; Freitag *et al.* 2017; Wang and Lang 2018). From its phytochemical content, red dragon fruit is believed as one of the potential dye sources for dye-sensitized materials because of its strong color intensity.

In the present work, we reported a chemiluminescence study on the extract of red dragon fruit in polar (distilled water), semi-polar (ethanol) and non-polar (acetone) solvents. At first, the flesh and peel of the dragon fruit were macerated using distilled water, ethanol, and acetone, separately for 24 h. The extracted natural pigments were predicted from the ultraviolet-visible (UV-Vis) spectrum, while the chemiluminescence properties of the extracts were recorded by fluorescence spectroscopy to determine the main excitation and emission peak from their three-dimensional fluorescence spectrum.

2. Material and Methods

2.1. Materials and Instruments

Red dragon fruits were purchased from a traditional market in Malang ~~(28 March 2019)~~, East Java, Indonesia. The solvents, *i.e*. ethanol and acetone were purchased from Labware Medical and Laboratory Supplies in technical grade. The UV-Vis and fluorescence spectra of the extracts were recorded using Jasco Spectrophotometer V-760 and Jasco Spectrofluorometer FP-8500, respectively.

2.2. Maceration procedure

First of all, flesh and peel of red dragon fruit (2 kg) were separated and washed by distilled water. The flesh and peel of red dragon fruit were chopped and dried at room temperature overnight. Afterwards, either flesh or peel of red dragon fruit (5.0 g) was macerated with distilled water, ethanol and acetone (50 mL) separately for 24 h in a similar procedure as previously reported (Kurniawan *et al.* 2019; Purnomo *et al.* 2020; Kurniawan *et al.* 2020). After 24 h, the extracts were filtered to obtain a clear filtrate.

2.3. Extract Characterizations

The obtained extract was characterized using UV-Vis spectrophotometer and spectrofluorometer. The UV-Vis spectrum of each extract was measured with the condition of data interval of 1 nm, bandwidth of 1 nm, response of 0.06 s, and scan speed of 1000 nm min^{-1} using D2 and Wl lamps as the light sources. Meanwhile, the fluorescence spectrum

of each extract was recorded with low sensitivity and the condition of data interval of 1 nm, bandwidth of 5 nm, response of 0.2 s, and scan speed of 1000 nm min^{-1} using Xe lamp as the light source.

3. Results

3.1. Maceration of red dragon fruit using various solvents

As dozens of reports have been focused on the isolation and purification of secondary metabolites from red dragon fruit, this work mainly focused on the spectroscopy studies of the red dragon fruit extracts. The photographic images of the extracts from flesh and peel of red dragon fruit in various solvents are shown in Figure 1. From Figure 1, it was shown that the extract color of the flesh of red dragon fruit were red, colorless, and orange when using distilled water, acetone, and ethanol solvents, respectively. On the other hand, the extract color of the peel of red dragon fruit using distilled water, acetone, and ethanol as the solvent were pale yellow, colorless, and yellow.

Figure 1. Photograph images of (a) flesh and (b) peel of red dragon fruit extract using (1) distilled water, (2) acetone and (3) ethanol.

3.2. UV-Vis spectra of red dragon fruit using various solvents

The UV-Vis spectrum of each extract was recorded without any dilution and shown in Figures 2 and 3 for extracts from red dragon fruit flesh and peel, respectively. As expected from the color appearance, the absorbance value of acetone extracts was the lowest while the absorbance value of distilled water extracts was the highest in the visible region. The extract of the red dragon fruit flesh in distilled water (Figure 2(a)) showed strong absorption peaks at 271 and 538 nm, in good agreement with its red color, while the extract of the red dragon fruit from flesh part in acetone (Figure 2(b)) showed weak absorption peaks at 337, 447, and 476 nm. Different from the extracts in distilled water and acetone, the extract in ethanol (Figure 2(c)) showed a very strong absorption below 320 nm

and strong absorption peaks at 351 and 417 nm, which is in good agreement with its orange color.

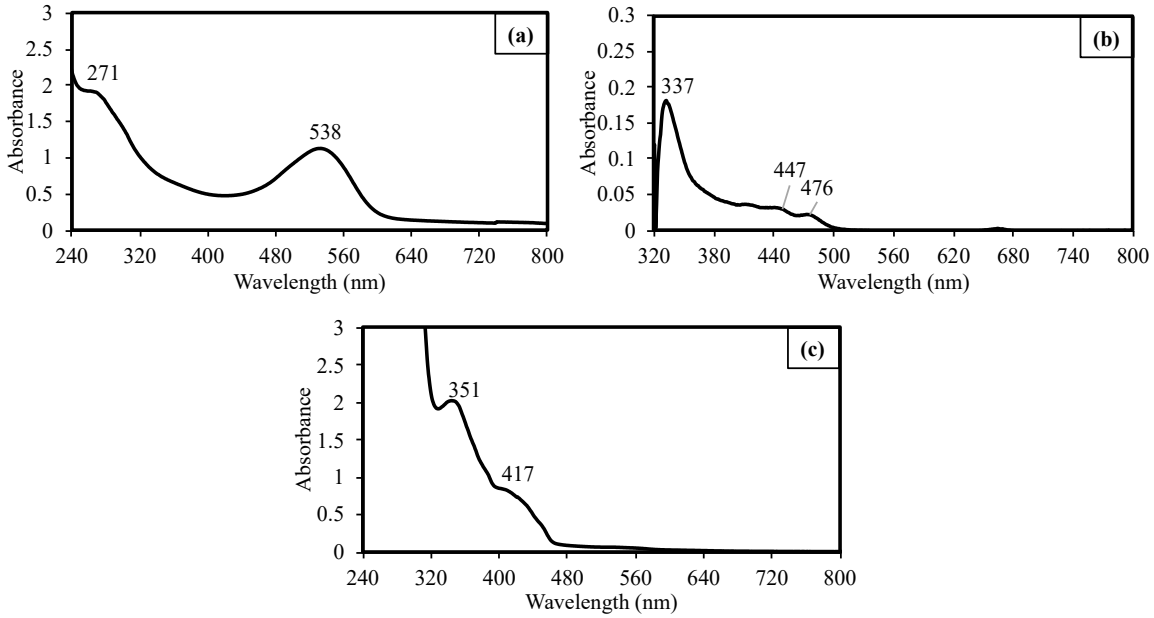

Figure 2. UV-Vis spectra of flesh of red dragon fruit extract using (a) distilled water, (b) acetone, and (c) ethanol.

On the other hand, the peel extract of red dragon fruit in distilled water only gave strong absorbance in the UV region without obvious absorption in the visible region as shown in Figure 3(a). The peel extract of red dragon fruit in acetone (Figure 3(b)) shows weak absorption peaks at 331, 452, and 480 nm. The pattern of the UV-Vis spectrum of the red dragon fruit peels' extract is similar to the red dragon fruit flesh indicating that the contained pigments are similar in both extracts. Meanwhile, the peel extract of the red dragon fruit in ethanol (Figure 3(c)) shows strong absorption peaks at 263 and 346 nm with background absorption in the visible region which is in good agreement with its yellow color.

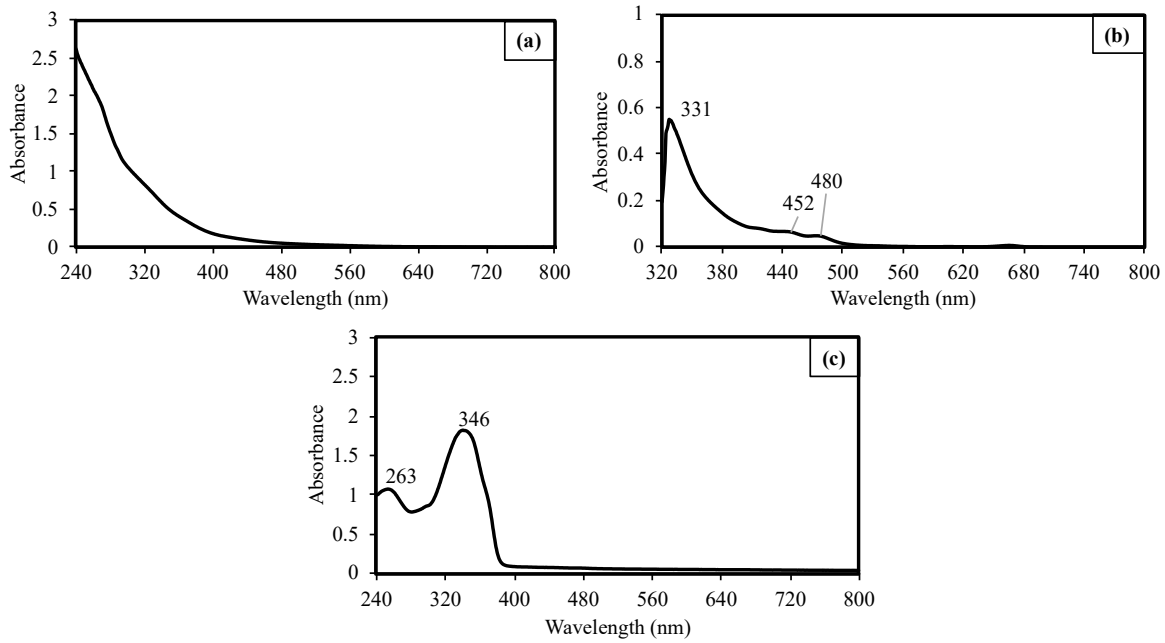

Figure 3. UV-Vis spectra of peel of red dragon fruit extract using (a) distilled water, (b) acetone and (c) ethanol.

3.3. Fluorescence spectra of red dragon fruit using various solvents

As shown in Figures 4 (a), distilled water extract of the red dragon fruit flesh gave medium fluorescence intensity as indicated with orange color in the graph. In contrast, the acetonic gave poor fluorescence phenomena (Figure 4 (b)), which could be related to fewer pigment contents in acetone as compared to those extracted in distilled water. On the other hand, the ethanolic extract gave strong fluorescence phenomena with higher fluorescence intensity than the distilled water extract.

The pair of excitation and emission peaks of each extract was determined from two-dimensional fluorescence spectrum and shown in Figure 5. The extract from the flesh of red dragon fruit in distilled water (Figure 5(a)) showed medium excitation and emission peaks at 292 and 357 nm, respectively. The extract from the flesh of red dragon fruit in acetone (Figure 5(b)) gave weak excitation and emission signals at 334 and 387 nm, respectively, while the extract from the flesh of red dragon fruit in ethanol (Figure 5(c)) exhibited strong excitation peaks at 228 and 293 nm and strong emission peak at 335 nm.

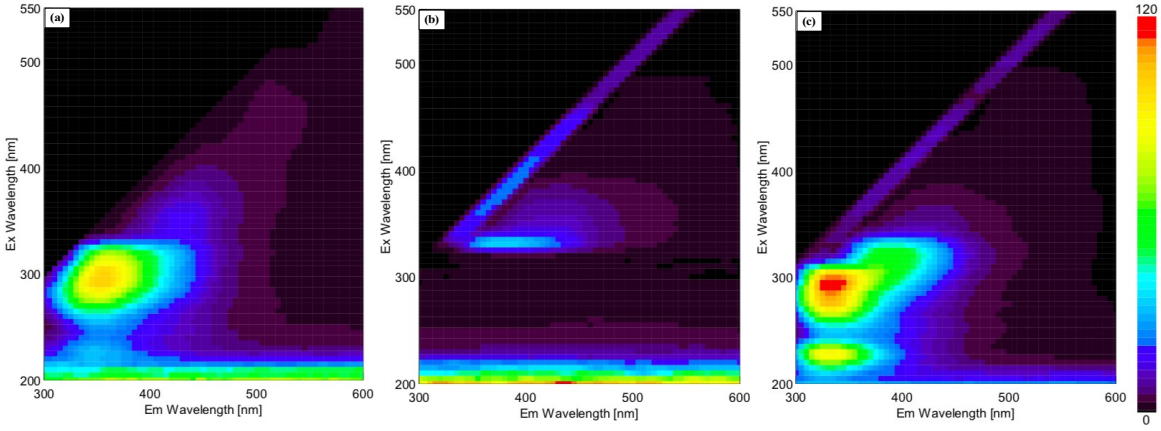

Figure 4. 3D Fluorescence spectra of flesh of red dragon fruit extract using (a) distilled water, (b) acetone and (c) ethanol.

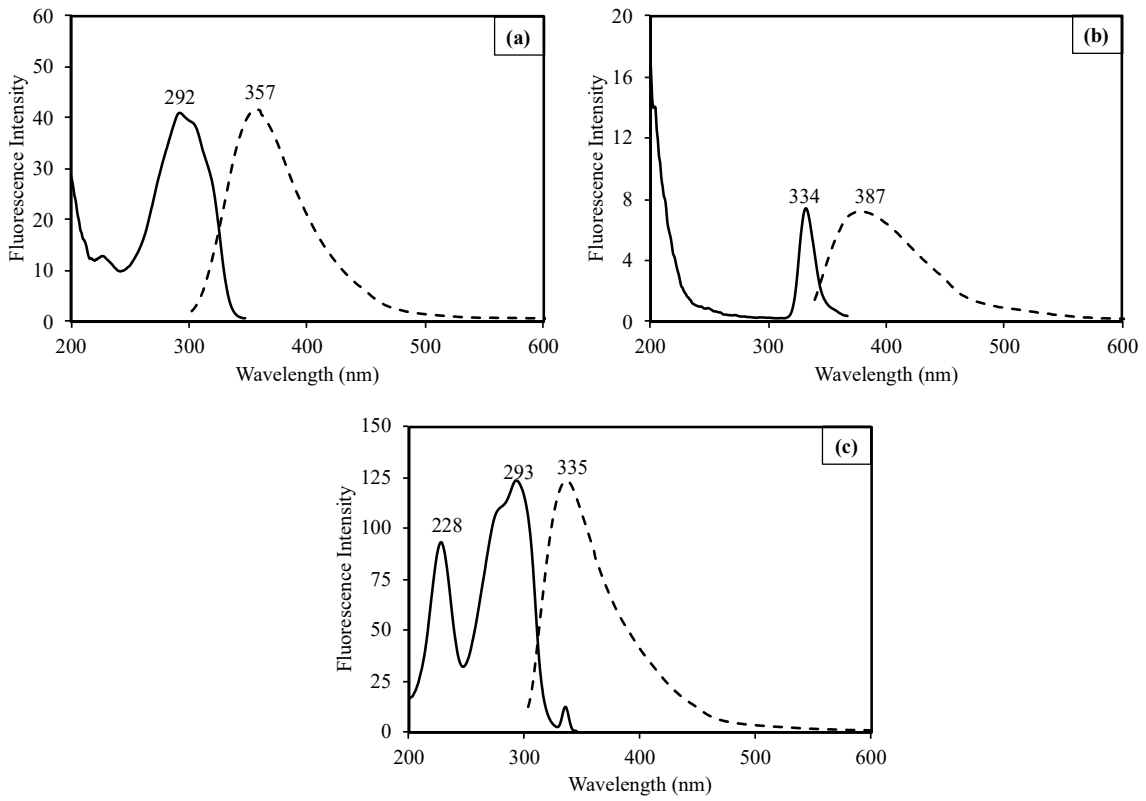

Figure 5. 2D Fluorescence spectra of flesh of red dragon fruit extract using (a) distilled water, (b) acetone and (c) ethanol.

The three-dimensional fluorescence spectrum of each peel of red dragon fruit extracts in various solvents was also recorded and shown in Figure 6. Similar to the results obtained in flesh extracts, the peel extracts gave medium fluorescence signals when using distilled water as the solvent as depicted in Figure 6 (a). The peel extract in acetone almost

did not show any peaks as shown in Figure 6 (b), while the peel extract in ethanol as shown in Figure 6 (c) gave medium fluorescence signal, which was stronger than the peel extract in distilled water.

The pair of excitation and emission peaks of each extract is shown in the two-dimensional fluorescence spectrum (Figure 7). The distilled water extract from the peel of red dragon fruit gave two pairs of excitation and emission peaks. As shown in Figure 7 (a1), the first pair of excitation and emission wavelengths were 291 and 353 nm, respectively. Meanwhile, the second pair is shown in Figure 7 (a2), where the excitation wavelengths were 326 and 378 nm and the emission wavelength was 437 nm. As expected, the acetone extract from the peel of red dragon fruit gave only one pair of weak excitation and emission peaks at 333 and 383 nm, respectively. On the other hand, the ethanolic extract gave two pairs of excitation and emission signals. The first pair gave excitation and emission at 290 and 339 nm, while the second pair gave 359 nm as excitation wavelength and 436 nm as the emission wavelength, which could be observed in Figure 7 (c1) and (c2), respectively.

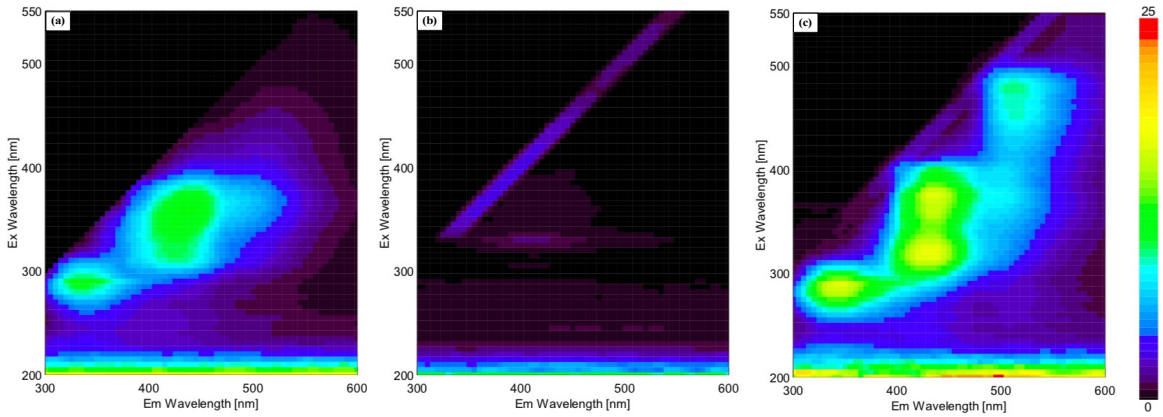

Figure 6. 3D Fluorescence spectra of peel of red dragon fruit extract using (a) distilled water, (b) acetone and (c) ethanol.

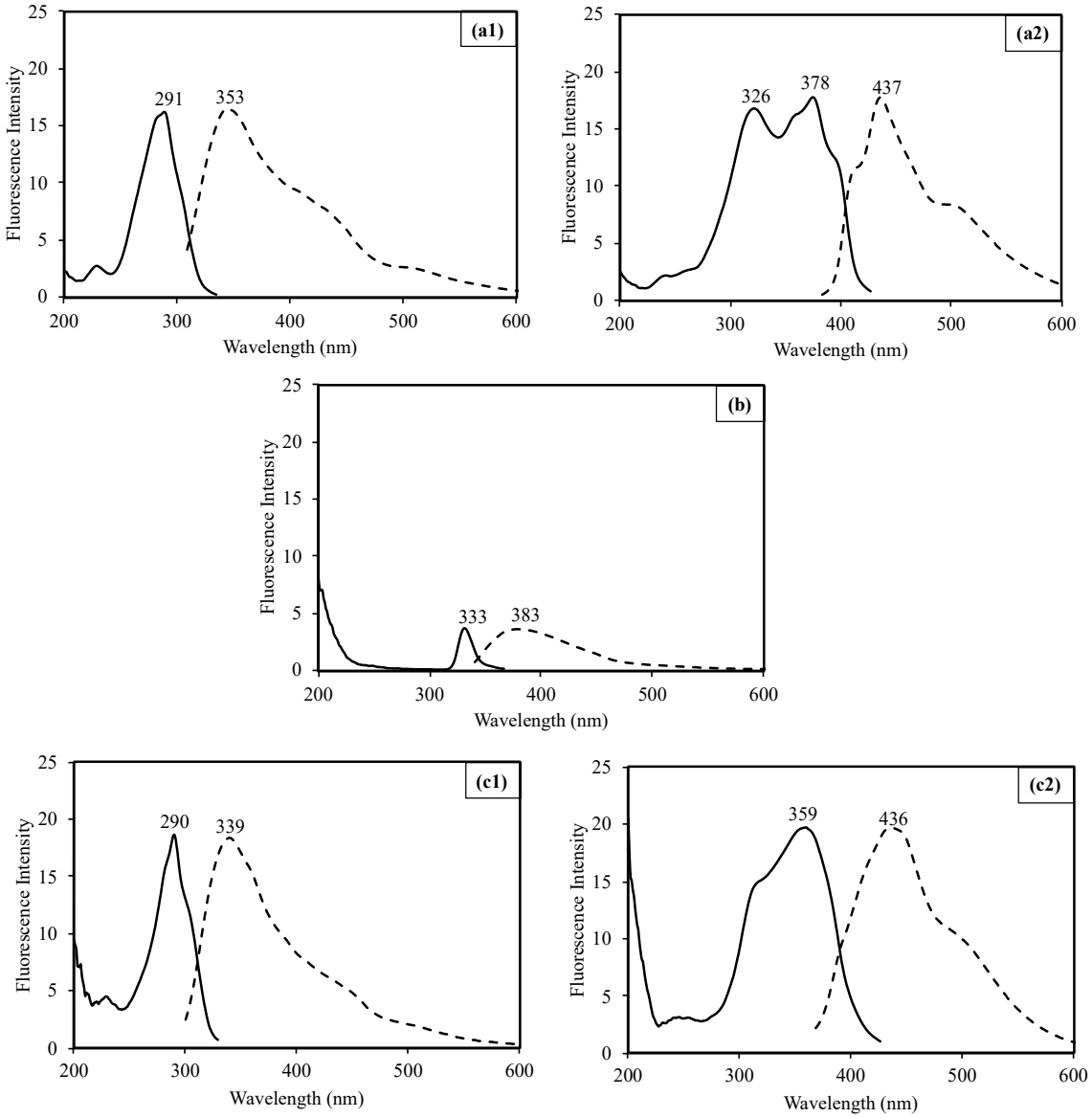

Figure 7. 2D Fluorescence spectra of peel of red dragon fruit extract using (a1-3) distilled water, (b) acetone and (c1-2) ethanol.

4. Discussion

The absorption properties of the extracts represent the natural compounds successfully extracted in the respective solvents. As shown in Figures 2 and 3, different solvents gave different characters of absorption spectra, suggesting that different compounds were extracted when using different solvents. The absorption spectra of the extracts were also different to each other depending on the part taken from the red dragon fruit. In other words, the compounds in the flesh were different from those in the peel of the red dragon fruit.

Based on the absorption spectrum shown in Figure 2 (a), the extract from the flesh of the red dragon fruit in distilled water gave strong absorption peaks at 271 and 538 nm, which could be due to the presence of betacyanin pigment (Stintzing *et al.* 2002). This is reasonable due to the polar properties of betacyanin that can be dissolved in water well. When using acetone as the solvent, the non-polar compound could be extracted. As shown in Figure 2 (b), the flesh extract in acetone showed weak absorption peaks at 337, 447, and 476 nm, which corresponded to the presence of carotenoid pigment content as reported by Britton *et al.* (1995). Figure 2 (c) revealed that when using ethanol as the solvent, the main compound extracted would be flavonol compound as the absorption peaks were observed at 351 and 417 nm (Liu *et al.* 2013). Therefore, it could be suggested that the flesh of red dragon fruit consisted of betacyanin, carotenoid, and flavonoid compounds based on these three solvents.

The extracts from the peel of red dragon fruit showed different absorption properties from those of the extracts from the flesh. As shown in Figure 3 (a), there was no clear absorption peak in the visible region that could be observed when distilled water was used as the solvent. This could be due to the low amount of the pigment in the peel of the red dragon fruit that could be extracted by the distilled water, and thus, no valid pigment identification could be made. The peel extract in acetone (Figure 3 (b)) showed absorption peaks at 331, 452 and 480 nm, which were very similar to the flesh extract in acetone (Figure 2 (b)), suggesting the similar carotenoid pigment existed in both peel and flesh extracts. Judging from intensity of the absorption spectra, the content of the carotenoid in the peel extract was much lower than in the flesh extract. The ethanolic extract from the peel of red dragon fruit gave strong absorption peaks at 263 and 346 nm, which indicated the presence of polyphenol derivative (Venter *et al.* 2013). Based on these three solvents, the detected compounds in peel of the red dragon fruit were carotenoid and polyphenol derivatives.

The fluorescence properties of the extracts from the flesh of the red dragon fruit could be explained in analogy to their absorption spectra. As the detected fluorescence spectra only have one pair excitation and emission spectra, the spectra could be assigned directly to the compounds detected in their absorption spectra. However, it was noted that the excitation at visible regions for betacyanin and carotenoid pigments could not be detected in this work, which might be originated to the low content of the pigments. In this case, the analogy of the peak assignment was made based on the excitation at UV region.

As shown in Figure 5 (a), the flesh extract in distilled water gave excitation and emission wavelength at 292 and 357 nm, respectively, which would correspond to the betacyanin pigment. The flesh extract in acetone exhibited excitation and emission spectra at 334 and 387 nm, respectively, as shown in Figure 5 (b), and this could be assigned to the presence of carotenoid pigment. As for the ethanolic extract of red dragon fruit flesh, the excitation at 228 and 293 nm as well as emission at 335 nm shown in Figure 5 (c) would correspond to flavonol compound.

Based on the above assignments, even though the peel extract in distilled water did not have obvious absorption peak, the presence of betacyanin pigment could be confirmed from the excitation wavelength at 291 nm and emission wavelength at 353 nm as shown in Figure 7 (a1). The second pair of excitation and emission wavelengths shown in Figure 7 (a2) was close to chlorogenic acid, which was reported to show excitation at 330 nm and emission at 440 nm in methanol (Morales *et al.* 2005). The chlorogenic acid was reported to be present in the dragon fruit peel (Lourith and Kanlayavattanakul 2013). However, as the fluorescence signals were very weak, the content of those compounds would be very low. As shown in Figure 7 (b), the peel extract in acetone showed very similar fluorescence properties to those of the flesh extract in acetone. This result was in good agreement to their respective UV-Vis spectra. The excitation peak at 333 nm and emission peak at 383 nm could be then assigned to the carotenoid pigment. The ethanolic peel extract gave two pairs of excitation and emission wavelengths. The first pair with excitation at 290 nm and emission at 339 nm as depicted in Figure 7 (c1) could be assigned to the presence of polyphenol in accordance to its UV-Vis spectrum. The second pair of excitation and emission wavelengths as shown in Figure 7 (c2) could be related to the presence of chlorogenic acid (Morales *et al.* 2005). The summary of the predicted main compounds in each extract as well as their absorption and biochemiluminescence properties is listed in Table 1.

Table 1. Comparison of flesh and peel of red dragon fruit extracts using distilled water, acetone and ethanol

Used solvent	Flesh Extract	Peel Extract
Distilled water	Main compound: betacyanin Abs: 271 and 538 nm Ex: 292 nm	Main compound: betacyanin, chlorogenic acid Abs: < 200 nm Ex: 291; 326 and 378 nm

	Em: 357 nm Fluorescence intensity: Medium	Em: 353; 437 nm Fluorescence intensity: Weak
Acetone	Main compound: carotenoid Abs: 337, 447, and 476 nm Ex: 334 nm Em: 387 nm Fluorescence intensity: Weak	Main compound: carotenoid Abs: 331, 452, and 480 nm Ex: 333 nm Em: 383 nm Fluorescence intensity: Weak
Ethanol	Main compound: flavonol Abs: 351 and 417 nm Ex: 228 and 293 nm Em: 335 nm Fluorescence intensity: Strong	Main compound: polyphenol, chlorogenic acid Abs: 263 and 346 nm Ex: 290; 359 nm Em: 339; 436 nm Fluorescence intensity: Medium

As discussed above, different solvents and red dragon fruit parts gave different compounds, and therefore the selection of solvent and fruit part shall be considered if one would use the extract for certain application. As the absorption and biochemiluminescence properties of the extracts from the flesh of the red dragon fruit were better than the peel one, it is suggested to use the flesh extracts for photonic material applications. Nishimura *et al.* (2003) and Calogero *et al.* (2012) reported a possible application of betacyanin and anthocyanin pigments for dye-sensitized solar cell and dye-sensitized photocatalyst materials. Therefore, the distilled water extracts would be the most suitable ones for such uses. In addition, the ethanolic extracts gave the strongest fluorescence intensity among other extracts that could open new possibility to be explored.

Conclusions

Extraction of natural pigments from flesh and peel of the red dragon fruit was successfully carried out through a simple maceration method for 24 h. The absorption and fluorescence properties of flesh extracts were better than those of the peel extracts. From the UV-Vis and fluorescence spectra, it was predicted that the flesh of red dragon fruit extract using distilled water, acetone and ethanol mainly contained betacyanin, carotenoid and flavonol, respectively, while the peel of red dragon fruit extract in acetone and ethanol

mainly contained carotenoid and phenolic compounds. Biochemiluminesce study revealed that acetone extracts gave poor fluorescence intensity indicating a weak chemiluminescence property. On the other hand, distilled water and ethanolic extracts gave medium to strong fluorescence intensity, which could be further explored for wider applications for functional materials.

Conflict of Interest

The authors declare no conflict of interest.

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Figure Captions

Figure 1. Photograph images of (a) flesh and (b) peel of red dragon fruit extract using (1) distilled water, (2) acetone and (3) ethanol.

Figure 2. UV-Vis spectra of flesh of red dragon fruit extract using (a) distilled water, (b) acetone and (c) ethanol.

Figure 3. UV-Vis spectra of peel of red dragon fruit extract using (a) distilled water, (b) acetone and (c) ethanol.

Figure 4. 3D Fluorescence spectra of flesh of red dragon fruit extract using (a) distilled water, (b) acetone and (c) ethanol.

Figure 5. 2D Fluorescence spectra of flesh of red dragon fruit extract using (a) distilled water, (b) acetone and (c) ethanol.

Figure 6. 3D Fluorescence spectra of peel of red dragon fruit extract using (a) distilled water, (b) acetone and (c) ethanol.

Figure 7. 2D Fluorescence spectra of peel of red dragon fruit extract using (a1-3) distilled water, (b) acetone and (c1-2) ethanol.