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Research Article

Natural Pigments from *Gardenia jasminoides* J. Ellis and *Peristrophe bivalvis* (L.) Merr for Food Coloring

Pham Thi Luong Hang,[1] Le Thuy Ngan,[2] Tran Thi Lan Phuong,[3] Nguyen Lai Thanh,[3] Yuki Ishikawa-Ishiwata,[4,5] Dang Minh Hieu,[5] and Nguyen Dinh Thang[5]

[1]*HUS High School for Gifted Students, University of Science, Vietnam National University, Hanoi 100000, Vietnam*
[2]*Intellectual Property Office of Vietnam (IP Vietnam), Thanh Xuan, Hanoi 100000, Vietnam*
[3]*Faculty of Biology, University of Science, Vietnam National University, Hanoi 100000, Vietnam*
[4]*Global and Local Environment Co-creation Institute (GLEC), Ibaraki University, Mito 316-0036, Japan*
[5]*Faculty of Advanced Technology and Engineering, Vietnam-Japan University, Vietnam National University, Hanoi 100000, Vietnam*

Correspondence should be addressed to Nguyen Dinh Thang; nd.thang@vju.ac.vn

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Food coloring not only provides an attractive appearance but also aids in the quality of the food. Currently, colorants derived from natural sources, especially plants, are widely used for food coloring, with many benefits for health and the environment. This study used yellow and purple pigments extracted from *Gardenia* fruits and *Peristrophe* leaves for chemical component analysis and antioxidant and toxicity tests. Results from LC/MS analysis demonstrated that the major compounds contributing to the yellow color of the *Gardenia* extract and purple color of the *Peristrophe* extract were Crocin-1 ($C_{44}H_{64}O_{24}$) and Perisbivalvine B ($C_{13}H_{10}N_2O_4$), respectively. Moreover, the physiochemical tests showed that yellow and purple pigments were stable in a wide temperature range from 40 to 100°C. The yellow pigment was stable in a pH range of 1.0–12.0, while the purple pigment was not stable in acid media. These extracts also possessed moderate antioxidant activities with IC_{50} values of 0.33 g/L and 0.31 g/L for *Gardenia* and *Peristrophe* extract, respectively. In addition, toxicity tests on zebrafish embryos indicated that these extracts had low toxic activities with high LD_{50} values of 1.31 g/L for *Gardenia* extract and 0.74 g/L for *Peristrophe* extract. These tested extracts had also no acute toxicity on mice. Conclusively, this study provided important information about the major chemical components, which create yellow and purple colors for the *Gardenia* and *Peristrophe* extracts, and strongly suggested that the pigments extracted from *Gardenia* and *Peristrophe* can be safely used as food colorants.

1. Introduction

Food coloring is one of the crucial processes that help enhance the quality and the attractive appearance of the foodstuffs. Pale-colored foodstuffs are likely rejected by customers. Food colorants can be originated from either natural or synthetic sources. Synthetic colorants are diverse and have many advantages, especially heat, acid/base stabilities, and low cost. However, several synthetic colorants have different negative impacts on human health such as tumor development, organ damage, and hyperactivity; thus, a number of synthetic colorants are banned from the list of agents that can be applied in food coloring by different governments [1, 2]. In another way, natural colorants are made from renewable sources, especially plants with common natural pigment groups such as anthocyanins, anthoxanthins, chlorophylls, and carotenoids. These pigment groups produce diverse colors, including red, purple, blue, yellow, green, orange, and white, with different color levels [1, 2]. In addition, natural pigments also supply additional nutrients, such as minerals (potassium, calcium, magnesium,...), vitamins (vitamin C, vitamin K, folic

acid,. . .), and omega-3 fatty acids, which have many benefits for human health aspects [3, 4]. Therefore, natural colorants are now being used, especially in home kitchens, as clean and safe food additives to partly replace synthetic colorants. Zion Market Research estimated that the global natural food colorant market was USD 1.77 billion in 2021, with the annual growing rate of around 5.2%.

Asian countries have a long history of using plant pigments as food coloring because of their biological diversity. Various pigments derived from different plants are widely applied in food coloring. *Peristrophe bivalvis* leaves producing purple color and *Gardenia jasminoides* fruits bearing yellow color are two common materials for food colorants in Vietnam. In addition, these two plants are considered as medicinal plants. Particularly, fruit extract of *Gardenia jasminoides* expressed various pharmacological effects such as anticonvulsion, neuroprotection, anti-inflammation, antioxidant, and antitumor formation [5–7], while leaf extract of *Peristrophe* has been used to treat cough, dysentery, diarrhea, and bronchitis [8–11]. Thus, using *Gardenia* and *Peristrophe* extracts as food colorants is not only to improve the appearance of the food but also to improve the health.

It is well documented that crocins are the primary pigments responsible for the yellow color of the *Gardenia* fruit [12, 13] and phenoxazine alkaloids are the major purple pigments in the *Peristrophe* leaves [9, 14, 15]. Although several studies have focused on optimization extraction methods [16, 17] and the biological activities of crocins and phenoxazine alkaloids [6, 8, 10, 12, 18], there were no reports about the toxicity of two these colorants on a susceptible test model of zebrafish embryos and color stability in the wide range pH of 1 to 12. In this study, we conducted a comprehensive study on the identification of significant pigment ingredients, physical and chemical properties, biological activity (antioxidant activity), and their biological effects on health based on toxicity tests on animals (zebrafish and mice) of yellow (from *Gardenia* fruits) and purple (from *Peristrophe* leaves) colorant preparation. Further, lyophilization technology used for converting natural pigment extracts into powder forms gave advantages to the natural colorants due to retaining pigment color and long-term use without supplementing preservatives.

2. Materials and Methods

2.1. Preparation of Pigment Powder. *Peristrophe bivalvis* (L.) Merr leaves and *Gardenia jasminoides* J. Ellis fruits were collected from the northwestern mountainous areas of Vietnam in the dry season in 2019. They were identified, coded (with Vietnamese and scientific names), and placed at the Museum of Biology, VNU University of Science, Vietnam National University, Hanoi, Vietnam. 1 kg of fresh *Peristrophe* leaves was extracted in two liters of boiling water for 30 minutes and filtered to obtain purple pigment extract [9]. In parallel, 300 g of fresh *Gardenia* fruits was extracted in 2 liters of boiling water for 30 minutes, followed by filtration to obtain yellow pigment extract [9]. Each extract was concentrated into one liter by oven at 40°C and frozen at −80°C following the guidelines for food storage of the "U.S. Department of Health and Human Services" [19] before lyophilizing by using BenchTop 2K ES Freeze Dryer (VirTis, USA). The purple and yellow pigment powders were stored at 4°C until use.

2.2. LC-MS Analysis. The molecular masses of pigment components were measured by liquid chromatography coupled with high-resolution mass spectrometry (Agilent Technology, USA). Particularly, 1.0 mg pigment powder from *Gardenia* fruit extract or *Peristrophe* leaf extracts was dissolved in 1.0 mL of deionized water. The components of each extract were separated using the VertiSep GES C18 (150 × 4.6 mm, 5 μm) HPLC-column, eluted in the gradient of 40–100% methanol in deionized water at a flow rate of 0.5 mL/minute, and detected by a diode array detector. The sample was injected in 5.0 μL and analyzed in a cycle of 25 minutes. The ESI-ion trap mass spectra of each compound were recorded in the positive mode in the range of 50–1600 m/z [20]. The accurate masses were searched for possible pigments in two updated websites, https://dnp.chemnetbase.com and https://pubchem.ncbi.nlm.nih.gov, in March 2024.

2.3. Effects of Physiochemical Conditions on Stability of the Yellow and Purple Pigments

2.3.1. Water Solubility of Pigment Powders. Each pigment powder (yellow or purple) was dissolved in distilled water at five concentrations: 0.2, 0.4, 0.6, 0.8, and 1.0 g/L. All solutions were then centrifuged at 10000 × g for 10 minutes at 10°C in order to find out the highest content, which produced the homogeneous solution.

2.3.2. Effect of pH. The yellow pigment of *Gardenia* extract and the purple pigments of *Peristrophe* extract at a concentration of 0.4 g/L were separately dissolved in distilled water before adjusting with certain amounts of NaOH 0.5N or HCl 0.5N to get the solutions with pH values varying in the range of 1.0–12.0. The color levels of the solutions were defined by measuring the optical absorbance by UV/Vis spectrophotometer at the wavelengths of 440 nm (maximum absorbance of crocins) and 492 nm (wavelength absorbance of phenoxazine alkaloids), respectively [12, 21]. The color retention % of yellow or purple of solution at each pH was calculated by comparing the absorbance value to that of pigment solution at pH neutral (pH = 7) in which the absorbance of pigments at pH neutral was appointed as 100% [22].

2.3.3. Effect of Temperature. Thermal stabilities of the yellow and purple pigment powders at a concentration of 0.4 g/L and neutral pH were tested at various temperatures ranging from 40 to 100°C. At each temperature, samples were kept in a water bath for 5 min before measuring the optical absorbance by UV/Vis spectrophotometer at the wavelengths of 440 nm for yellow pigments and 492 nm for purple pigments. The color retention % of yellow or purple at each

temperature was calculated by comparing the absorbance value to that of pigment solution at room temperature (RT) in which the absorbance of pigments at RT was appointed as 100% [22].

2.3.4. Effect of the Storage Time. Stability over time is an important property of food products and additives, especially when they do not contain any food preservatives. For the color stability evaluation, *Gardenia* and *Peristrophe* powders were stored at 4°C for 24 months and the optical absorbance of aqueous solution at 0.4 g/L was frequently determined at months 0, 6, 12, 18, and 24 by UV/Vis spectrophotometer. The color retention % of yellow or purple at each time point was calculated by comparing the absorbance value to that of pigment solution at month zero, in which the absorbance of pigments at the first time point (month Rezo) was appointed as 100% [22].

2.4. Antioxidant Activity Test. The free radical scavenging activities of the extracts were analyzed by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. This method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that can decolorize the DPPH solution. The antioxidant activity is determined by measuring the decrease in optical absorption at 517 nm, the maximum optical absorbance of DPPH [23]. In this experiment, 1 mL aqueous solution of yellow and/or purple pigment at various concentrations ranging from 0.01 to 2.0 μg/mL was added into 1 mL DPPH 0.2 mM (dissolved in ethanol) to obtain the final concentrations ranging from 0.005 to 1.0 μg/mL. The mixture was incubated at room temperature for 30 minutes in the dark before measuring optical absorbance at 517 nm. A decrease in the optical absorbance of the reaction mixture indicates the free radical scavenging activity of the sample. Measurements were performed in triplicate. Ascorbic acid, a well-known antioxidant, was used as a reference standard, ensuring the validity and reliability of the experiment.

The inhibition ratio was calculated according to the following equation:

$$\text{Inhibition (\%)} = \left[1 - \frac{\left(A_i - A_j\right)}{A_0}\right] \times 100, \quad (1)$$

where A_0 is the absorbance of the blank sample (DPPH), A_i is the absorbance of the sample containing DPPH and the sample at different concentrations after 30 minutes, and A_j is the absorbance of the sample only (yellow or purple pigment extracts).

2.5. Acute Toxicity Test on Mice. Swiss mice at 5-week age with an average weight of 20–22 grams purchased from the National Institute of Hygiene and Epidemiology were used in this study. Mice were cared for in cages at a temperature of 24°C ± 3°C and humidity of 55% and fed twice/day. Acute toxicity was characterized by the detrimental effects of the extracts on mice for a short period of time (in the range of 24–96 hours). In this experiment, 36 healthy mice were divided into six groups (six mice per group), including one control plot and five test plots. Mice were completely deprived 16 hours prior to oral administration of a single dose. The dose concentrations of the extracts are shown in Table 1. After administration of the *Gardenia* extract, *Peristrophe* extract, and water (negative control) for 1 to 2 hours, mice were fed as usual and monitored continuously for 72 hours to determine the number of dead mice in each batch and to calculate the LD_{50} values. All procedures involving animals were conducted following the Principles of Laboratory Animal Care (NIH publication no. 85–23, revised 1996) [24, 25]. The animal experiments in this study were approved by JICA-VJU, Vietnam National University.

2.6. Toxicity Test on Zebrafish Embryos. Adult zebrafish wild-type strain AB (*Danio rerio*) (ZIRC, Eugene, OR) (OECD, 2002) [24] was maintained in the zebrafish facility of the Animal Laboratory, Center for Life Sciences Research, Faculty of Biology, University of Science. The fish were cultured in glass rectangular pools measuring 40 cm (wide) x 50 cm (long) x 30 cm (high). Several pools of adult fish were bred individually for each assay. After sorting, embryos from pools with high fertility (≥80%) were mixed and used for subsequent experiments. Experiments were validated only when the survival rate of the controls was ≥90% at 4 days post fertilization (dpf). Fish were reared in a Tecniplast recirculating system under 14 : 10 h light/dark photocycle. Before using, eggs were screened and sorted under a stereoscope to remove the unfertilized and/or abnormal ones. Healthy embryos that showed normal cleavage were distributed into 6-well plates at 25 embryos/well for the toxic tests. Fish Embryo Acute Toxicity (FET) was determined according to OECD test guidelines of OECD [24, 25]. In brief, embryos were transferred into each well of 6-well plate and exposed to *Gardenia* or *Peristrophe* extracts at different concentrations. The development of embryos was observed and compared with the control at 24-, 48-, 72-, and 96-hpf (hours post fertilization).

2.7. Statistical Analysis. Statistical analysis was performed according to the method previously described in a publication [26, 27]. Results from three independent experiments in each group were statistically analyzed by Student's *t*-test. The SPSS (version 22) software package (SPSS Japan Inc.) was used, and the significance level was set at $p < 0.05$. Excel software and GraphPad Prism 6.0 software were used to analyze data and calculate the IC_{50} and LD_{50} values.

3. Results and Discussion

3.1. Gardenia and Peristrophe Pigment Powders Exhibited High Water Solubility. In this study, pigments were isolated by using the HPLC system. And the spectra of pigments were analyzed by both UV-Spectra and mass spectra (MS). The UV-Spectra cannot be used for identifying of chemical structure; however, mass spectra are strong and reliable for chemical analysis. Many previous studies have also applied

Table 1: The dose concentration of *Peristrophe* and *Gardenia* pigment powder.

Samples	Doses (g/kg body weight)				
Peristrophe pigment power	2.500	5.000	10.000	20.000	25.000
Gardenia pigment power	2.062	4.125	8.250	16.500	25.000

HPLC to isolate chemicals and used MS to identify chemical structures in the yellow pigment extracted from the *Gardenia* fruit [28–31] and the purple pigment extracted from the of the *Peristrophe bivalvis* (L.) Merr leaves [32, 33].

Water solubility is a key factor in determining the suitability of a pigment as a food colorant. In this study, *Gardenia* pigment powder (Figure 1(a)) and *Peristrophe* pigment powder (Figure 1(b)) were dissolved in water to create five solutions with concentrations ranging from 0.2 to 1.0 g/L. The absolute solubility of the yellow pigment powder and the purple pigment powder was found to be 0.8 gram/L and 0.6 g/L, respectively (Figures 1(c) and 1(d)). We also conducted a primary examination of the UV-VIS spectra of the yellow and purple pigments. Our findings revealed that the yellow pigments absorb a strong wavelength region of 400–480 nm with a peak around 440 nm (Figure 1(e)), while the purple pigments absorb both UV light and visible light in the wavelength regions of 210–260 nm and 420–540 nm with the peaks around 230 nm and 490 nm, respectively (Figure 1(f)).

Although it was well mentioned that ethanol, methanol, and water were the suitable solvents for extraction of yellow and purple pigments of *Gardenia* fruits and *Peristrophe* [9, 14–17], in this study, we appointed boiled water for extraction due to several benefits such as low-cost solvent, eco-friendly to environment, and safety of colorant. After extraction, water was evaporated and then sublimated to obtain the pigment powders. The major components and their biological activities of the yellow and purple pigment powders from the *Gardenia* fruits and *Peristrophe* leaves, respectively, were investigated.

3.2. The Major Compounds Corresponding to the Yellow Color of Gardenia Extract Powder and the Purple Color of Peristrophe Extract Powder. The *Gardenia* pigment powder was dissolved in deionized water at 1 mg/mL concentration and analyzed through the LC-MS system. The occurrence of natural compounds in the extract was detected by a diode array spectrophotometer with wavelengths from 190 nm to 1100 nm. By the UV-VIS spectrum result (Figure 1(e)), the maximum absorption of compounds was found at 440 nm with five peaks appearing at different retention times of 19.89, 20.83, 22.03, 22.25, and 23.76 minutes on the HPLC chromatogram (Figure 2). The major compound corresponding to the highest peak (at the retention time of 19.89 minutes) had a mass to charge of 976.2 m/z in negative $[M–H]^-$ mode and an estimated molecular weight of 977.2 Dalton (Da) (Figure 3). The UV/Vis and mass spectra of this compound were 100% core fit to Crocin-1 (C44H64O24), a compound that bears the yellow color for the *Gardenia* extract that had been reported in the previous studies [12, 34].

Similarly, the major components of *Peristrophe* pigment powder (at a concentration of 1 mg/mL) were analyzed by using the LC-MS system. Consistent with the UV-VIS spectrum analysis (Figure 1(f)), the maximum absorption of the compound bearing the purple color was found at 492 nm with a sharp peak appearing at a retention time of 22.35 minutes on the HPLC chromatogram (Figure 4). In addition, the mass spectrum of this compound showed the highest peak at 256.9 m/z in negative $[M–H]^-$ mode, corresponding with a molecular weight of 257.9 Dalton (Figure 5). Based on the previous studies on *Peristrophe bivalvis*, this compound could be Perisbivalvine B ($C_{13}H_{10}N_2O_4$) [14, 32], a phenoxazine alkaloid that contributed to purple color of *Peristrophe bivalvis* leaf extract [9, 21].

In Asian countries, the fruit of *Gardenia jasminoides* is one of the most common sources bearing the natural color of yellow, which is widely utilized as a food colorant for sticky rice, noodles, pastry, desserts, and confections [12, 22]. Besides, *Gardenia* fruits have been medicinally used for curing various inflammatory diseases, including diabetes, hepatitis, and cystitis, and used as an antiphlogistic, diuretic, and hemostatic drug in traditional medicine [5–7]. Previous studies have shown that the primary active components of *Gardenia* fruit were iridoid glycosides, chlorogenic acid, and ursolic acid. Among these components, Crocin-1 (one of the iridoid glycoside compounds) was the primary yellow pigment that was also obtained from saffron [35]. In addition, although *Peristrophe bivalvis* (L.) Merr is a typical herb rich in anthocyanin, the *Peristrophe* leaf extract has been used as a natural colorant for staining several traditional foods in Vietnam [33]. It was reported that Perisbivalvine B, a phenoxazine alkaloid, contributed to the purple color of the *Peristrophe bivalvis* [14, 32].

3.3. Effects of Physiochemical Conditions on the Stability of the Yellow and Purple Pigments. Color stability with pH is a fundamental property of any coloring product. The *Gardenia* and *Peristrophe* pigment powders were dissolved in different distilled water with pH values ranging from 1 to 12. It revealed that the yellow color of *Gardenia* pigment was absolutely stable in the pH range of 1–12 (Figure 6(a)). The color retention of yellow pigment stayed at a very high rate of 92–100% in all the tested pH values (Figure 6(c)), while the purple color of *Peristrophe* pigments was only stable in the high pH range of 6–12, with color retention in the range of 90–95% (Figure 6(b)). At the low pH values, the color retention of the purple pigment was dramatically reduced and reached about 22% at a pH of 1 (Figure 6(c)). These results suggested that Crocin-1, the primary pigment in *Gardenia* pigment powder, could retain its yellow color in acid or base medium with a pH range of 1.0–12.0. In comparison, Perisbivalvine B, the major pigment in the *Peristrophe* pigment powder, gave the purple color stability only in the neutral or basic medium but lost the purple color in the acid medium. A previous study just reported that the yellow pigment from *Gardenia* was pH-independent in the range of 3.5-7.5 [22]. Interestingly, although the purple pigment powder lost its color in the acid medium, it could

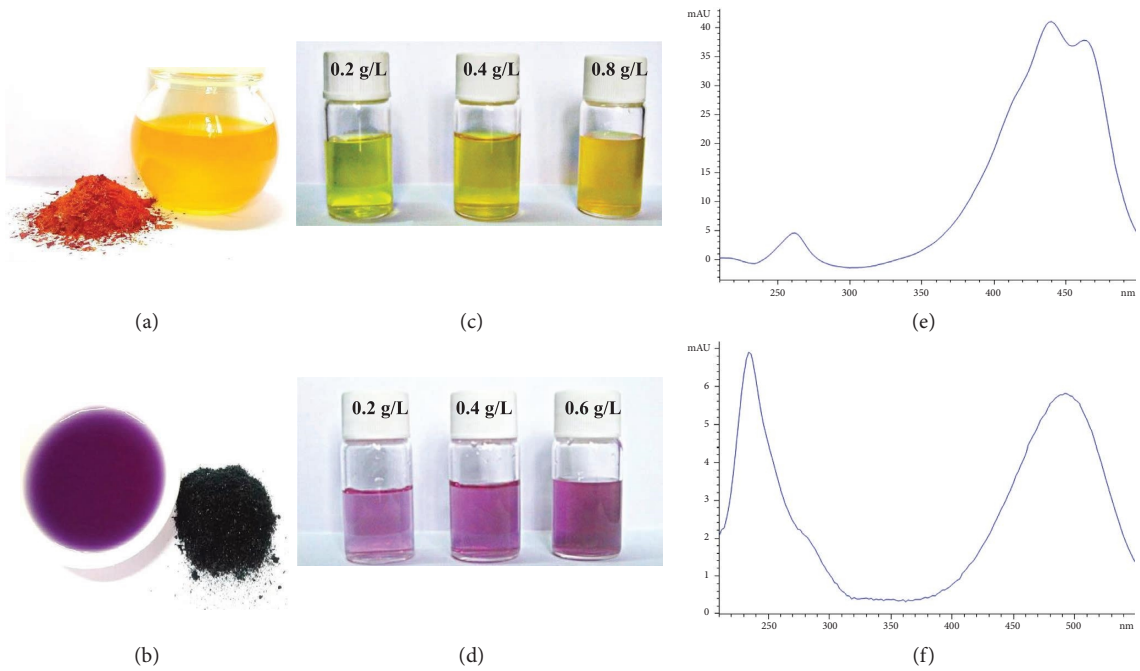


Figure 1: Yellow and purple pigment from *Gardenia* fruits (a) and *Peristrophe* leaves (b), solubility of the yellow (c) and purple (d) pigments at different concentrations, and UV-VIS spectra of the yellow (e) and purple (f) pigments measured by UV-VIS spectrophotometer.

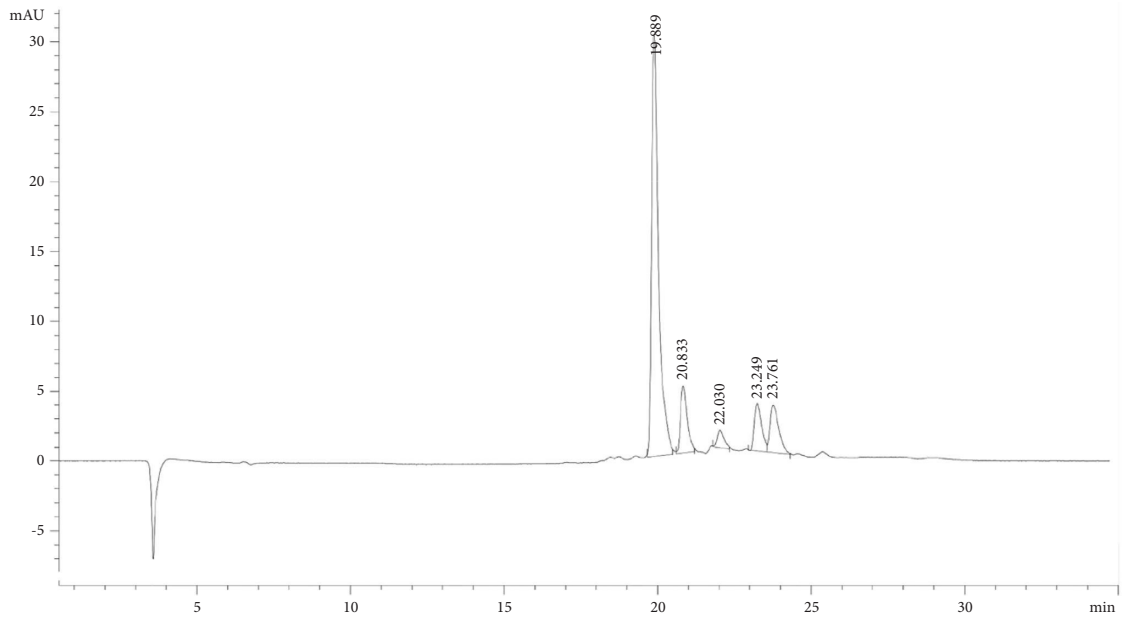


Figure 2: HPLC chromatogram of *Gardenia* pigment powder at 440 nm.

recover its original purple color when shifting the pH of the medium from acidic to basic (Supplemental Figure 1). This result suggested that Perisbivalvine B could bear different colors depending on the pH of the solution due to its protonated or deprotonated form rather than its degradation caused by an acidic medium.

The yellow and purple pigment extracts were heated to different temperatures ranging from 40 to 100°C for 5 minutes to investigate the thermal effect on color stability. It demonstrated that both yellow and purple pigments were stable in the tested temperature range, even at the high temperature of 100°C (Figures 6(d), 6(e), and 6(f)). The heat-resistant abilities of yellow and purple pigments were expressed as a percentage of color retention. It showed that yellow pigments were more heat-stable than purple pigments, with color retention varying in the range of 95–100% at all tested temperature values (Figure 6(f)). Although the purple pigments were also heat-stable with the color retention of 88–100% in the temperature of 40–90°C, at the high temperature of 90–100°C, the purple pigments were

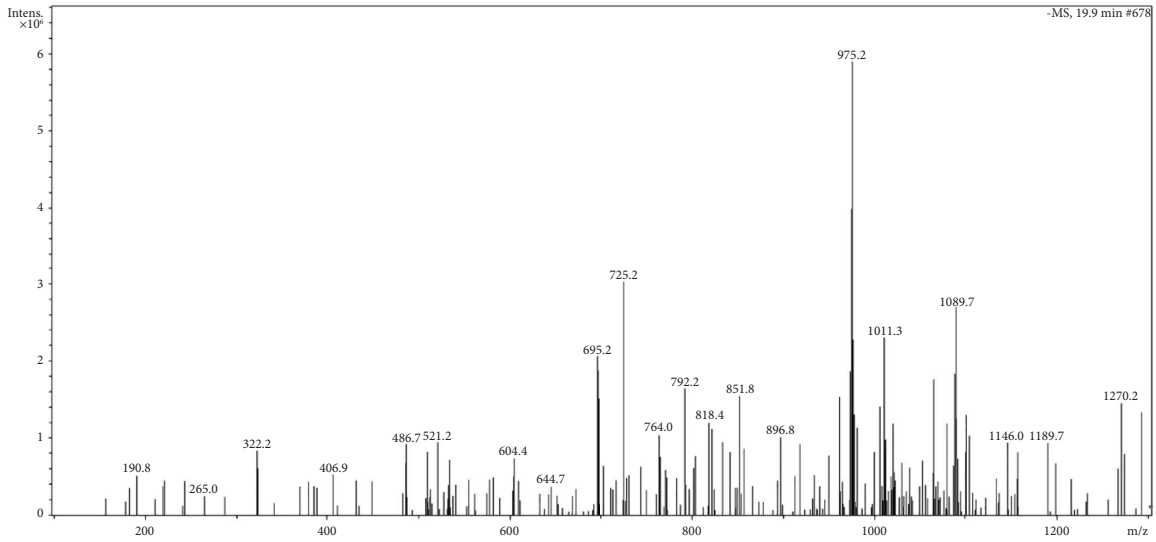


Figure 3: Mass spectrum of the main compound (corresponding to the yellow color of the *Gardenia* pigment powder) eluted at the minute of 19.9 on HPLC.

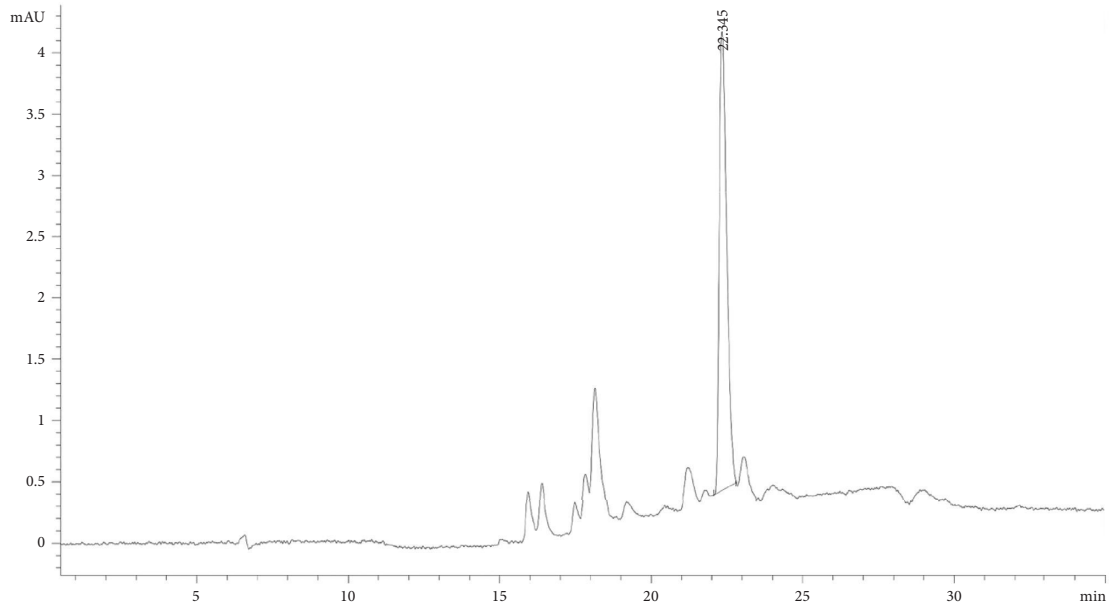


Figure 4: HPLC chromatogram of *Peristrophe* pigment powder at 492 nm.

slightly lost (about 5–10% lower compared with the color at the room temperature) (Figure 6(f)). The extreme stability of yellow pigment from *Gardenia* fruits was consistent with a previous report by Ichi et al. [36]. The stability in high temperatures is an essential criterion of food coloring to maintain color of the products during food processing. Compared with almost natural colors such as anthocyanin and betanin [37], which are not so stable in high temperatures, the yellow color from *Gardenia* and purple color from *Peristrophe* were much better in heat stability.

The storing-time stabilities of the pigment powders were also investigated. The yellow and purple pigment powders, stored at 4°C for 24 months, were examined every 6 months during the storing time. The result (Supplemental Table 1) revealed that both the yellow color of *Gardenia* pigment powder and the purple color of *Peristrophe* pigment powders retained over 95% compared with their original color at the starting point. This fact indicates that these pigments are time-stable and can keep their high quality as food colorants even for long time storing.

Generally, colorants are added very early in the food coloring process. Thus, the colorants must be color-stable with changes in temperature, pH, oxygen, light, and storage time. Results from this study demonstrated that the yellow color met all requirements regarding chemo-physical properties of pH, heat, and time-stableness for a food

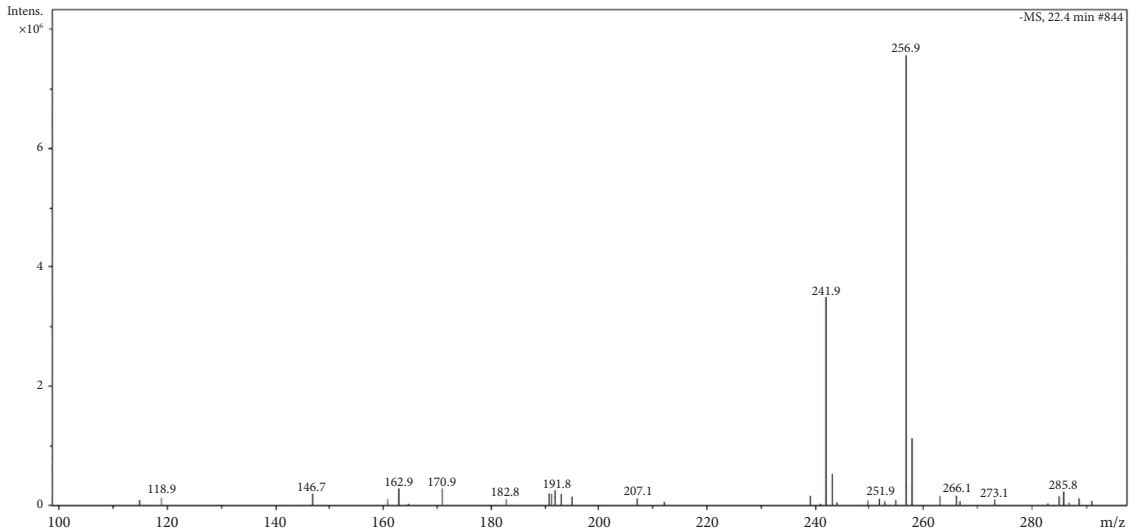


Figure 5: Mass spectrum of the main compound (corresponding to the purple color of the *Peristrophe* pigment powder) eluted at the minute of 22.4 on HPLC.

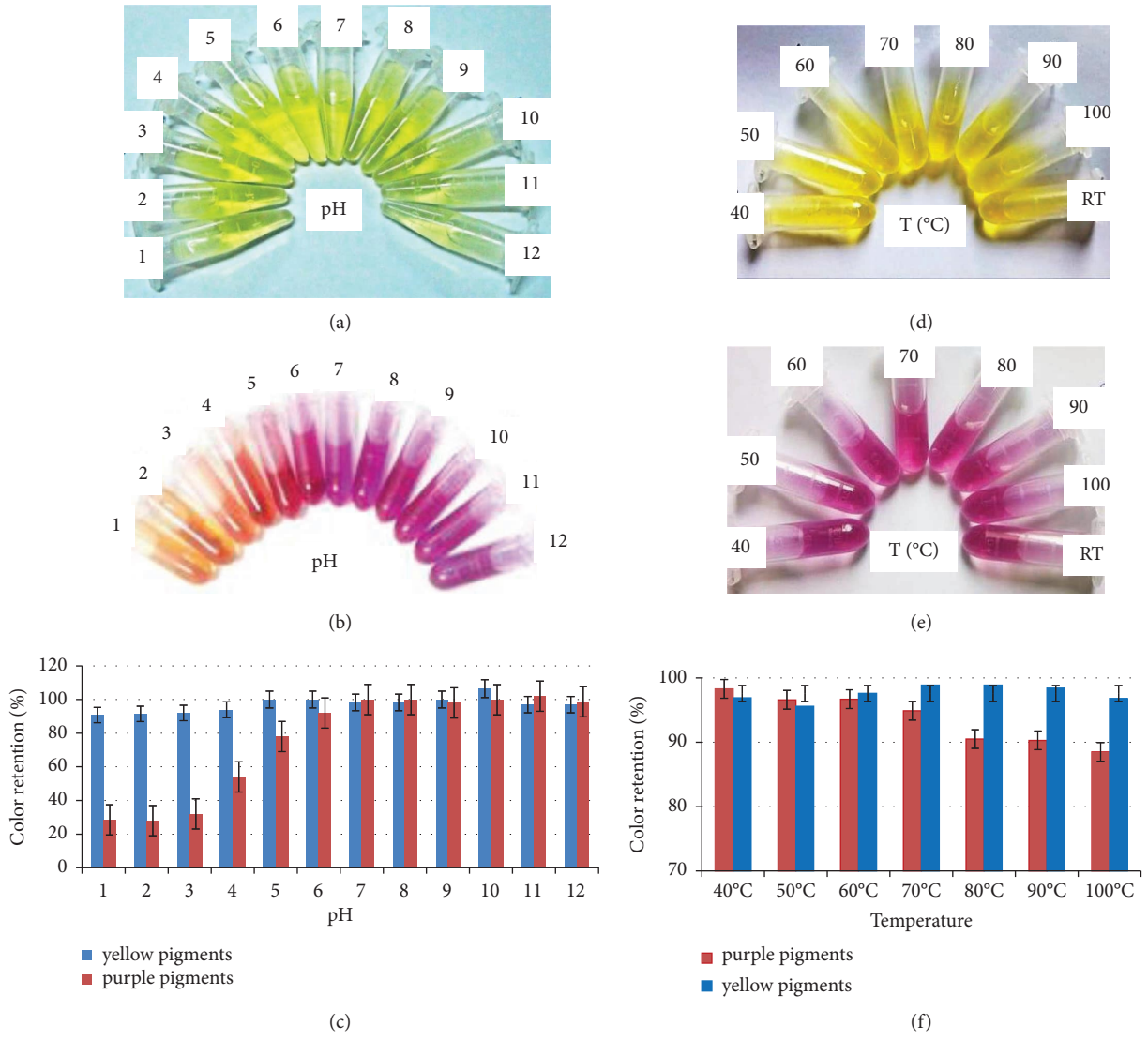


Figure 6: Effects of pH (a–c) and temperature (d–f) on color stability of *Gardenia* and *Peristrophe* pigment powders.

colorant, and our results were consistent with a previous report [22]. On the other hand, the purple color was stable with heat and storing and neutral to alkali pH, but it would lose its color in an acidic medium.

3.4. Antioxidant Activity of Gardenia and Peristrophe Pigment Powders. The antioxidant properties will provide more benefits to food colorants. It showed that the *Gardenia* and *Peristrophe* pigment extracts exhibited antioxidant activity in a concentration-dependent manner (Figure 7). Particularly, the highest antioxidant activities of *Gardenia* and *Peristrophe* pigments were 92% and 94% at concentrations of 1.0 μg/mL and 0.8 μg/mL, respectively. The IC_{50} values of the *Gardenia* pigment and *Peristrophe* pigment powders were 0.33 μg/mL and 0.31 μg/mL, respectively, while the IC_{50} value of ascorbic acid was 0.02 μg/mL. Previous studies also consistently reported that the yellow and purple pigments extracted from *Gardenia* fruits and *Peristrophe* leaves expressed their antioxidant at different levels by different assays [8, 13]. Although both *Gardenia* and *Peristrophe* extracted pigments moderately displayed antioxidant activities compared with ascorbic acid, they are good enough to protect foodstuffs from oxidation and to keep the quality of the foods.

3.5. Effect of Gardenia and Peristrophe Extracts on the Development of Zebrafish Embryos. Furthermore, the toxicity of the yellow and purple pigments on zebrafish embryos had been investigated. Toxic effects of *Gardenia* pigment powder at various concentrations of 0.1, 0.2, 0.5, 1.0, 1.5, and 2.0 g/L on zebrafish embryos at different hours post fertilization (hpf) times of 24-, 48-, 72-, and 96-hpf were evaluated (Figures 8(a), 8(b), and 8(c)). Based on the calculation, the LC_{50} value of *Gardenia* pigment powder on zebrafish embryos was 1.31 g/L (Figure 8). Notably, at low concentrations of 0.1 and 0.2 g/L, this *Gardenia* pigment powder did not affect the development of zebrafish embryos even at 96-hpf. At a concentration of 0.5 g/L, *Gardenia* pigment powder started to exhibit its toxicity on embryos at 96-hpf with a 10% lethality rate. At a concentration of 1.0 g/L, *Gardenia* pigment powder caused the lethality rates of 20% and 50% at 72-hpf and 96-hpf, respectively. At a concentration of 1.5 g/L, *Gardenia* pigment powder led to the death rates for zebrafish embryos of 41% and 71% at 72-hpf and 96-hpf, respectively (Figure 8(a)). In addition, there were several malformations, especially yolk edema, that occurred on zebrafish embryos when treated with *Gardenia* pigment powder at high concentrations of 1.5 g/L and 2.0 g/L (Figures 8(b) and 8(c)), or the lethality rate increased from 55% to 100% of embryos at 72-hpf to 96-hpf at a concentration of 2 g/L (Figure 8(a)).

The toxic effects of *Peristrophe* pigment powder on the development and lethality of zebrafish embryos have also been investigated. Concentrations of *Peristrophe* pigment powder in the range of 0.1–2.0 g/L were applied, and the calculated LC_{50} value of *Peristrophe* pigment powder on zebrafish embryos was 0.74 g/L (Figures 8(d), 8(e), and 8(f)). Similar to *Gardenia* pigment powder, the *Peristrophe* pigment powder at low concentrations of 0.1 and 0.2 g/L had no effect on the development of zebrafish embryos. However, at concentrations above 0.5 g/L, *Peristrophe* pigment powder showed toxicity in zebrafish embryos. Remarkably, at the concentration of 0.5 g/L, it caused about 20% and 30% lethality rates of zebrafish at 72-hpf and 96-hpf, respectively. At a concentration of 1.0 g/L, *Peristrophe* pigment powder led to lethality rates of 10%, 50%, and 85% at 48-hpf, 72-hpf, and 96-hpf, respectively. At high concentrations of 1.5 g/L and 2.0 g/L, *Peristrophe* pigment powder exhibited its toxicity at earlier times, with the lethality rates of embryos at 24-hpf, 48-hpf, 72-hpf, and 96-hpf to be about 10%, 30%, 58%, and 95%, respectively (Figure 8(d)). In addition, at the concentration of above 1.0 g/L, *Peristrophe* pigment powder significantly induced retardation in the development of the embryos at 96-hpf (Figures 8(e) and 8(f)).

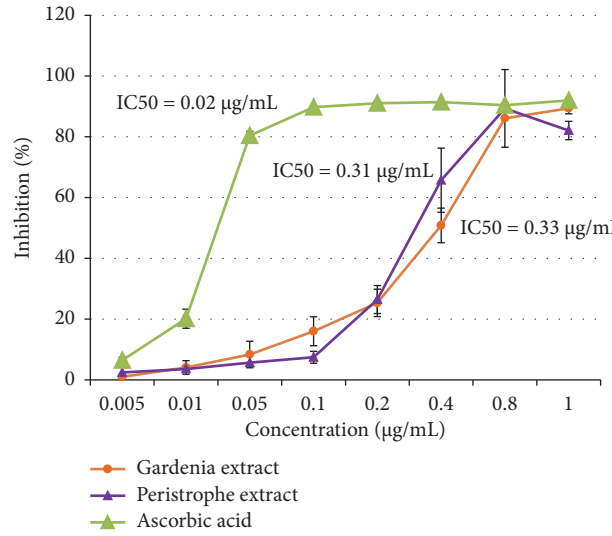


Figure 7: Antioxidant activities of *Gardenia* and *Peristrophe* pigment powders. Ascorbic acid was used as positive control.

In general, it demonstrated the correlations of higher mortality and malformations associated with higher concentrations of both *Gardenia* and *Peristrophe* extracts; however, high values of LD_{50} of *Gardenia* pigment powder (1.31 g/L) and *Peristrophe* pigment powder (0.74 g/L) indicated the low toxicity of these colorants. More impressively, it showed that the toxicities of *Gardenia* and *Peristrophe* extracts on zebrafish embryos were lower than that of Tartrazine, a synthetic yellow color widely used in food coloring. Tartrazine might cause several typical malformations of yolk edema and head edema/necrotic and hematoma at concentrations of above 1.0 g/L [13, 38].

3.6. Acute Toxicity of Gardenia and Peristrophe Pigment Powders on Mice. Mice were treated with *Peristrophe* pigment powder at a wide range doses of 2.0 to 25.0 g/kg body weight. It revealed that all mice survived without any abnormal sign after 72 hours treatment even at a very high dose of 25.0 g/kg (body weight). Therefore, the LD_{50} value of *Peristrophe* extract was not determined. Besides, mice were treated with *Gardenia* extract, and it showed that all animals survived with no

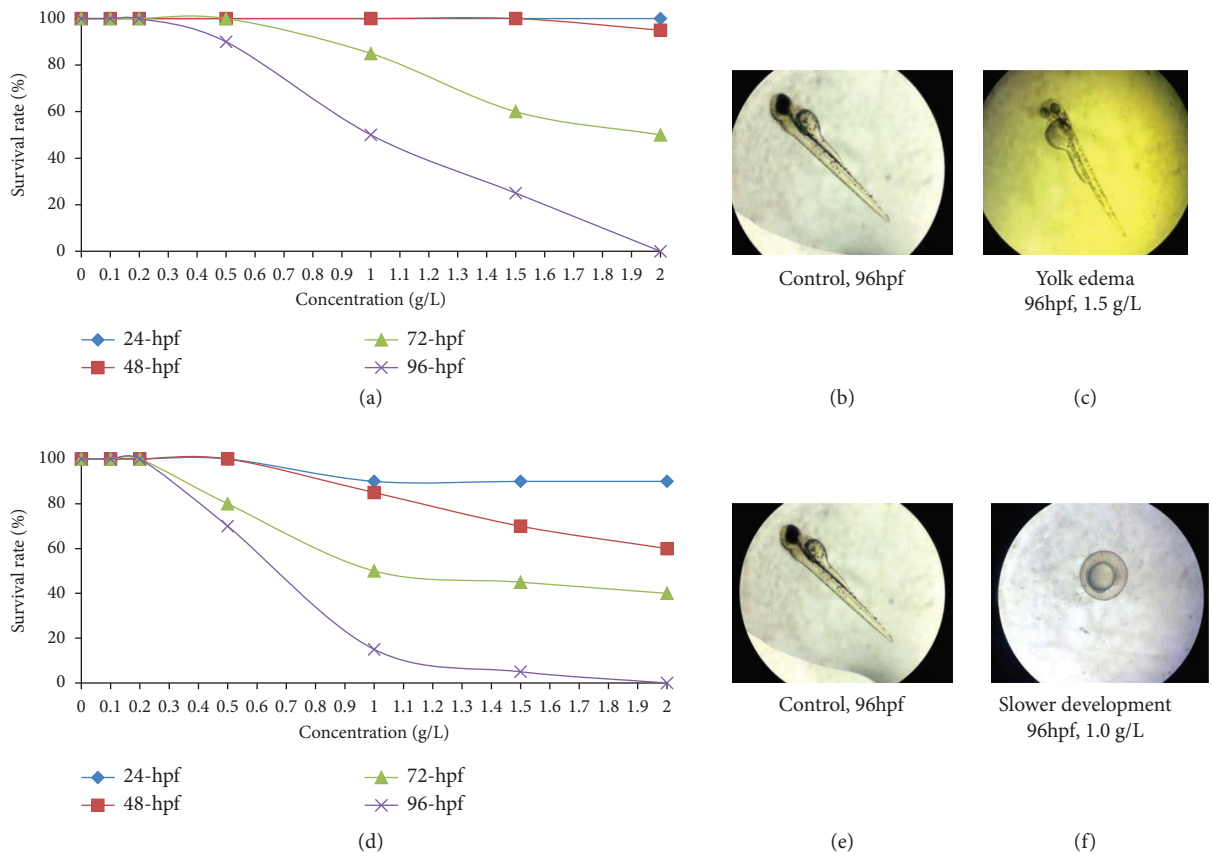


Figure 8: Effects of *Gardenia* (a–c) and *Peristrophe* (d–f) pigment powders on development of zebrafish embryos were investigated.

abnormal signs after 72 hours at the high dose of 25.0 g/kg (body weight). Similar to the *Peristrophe* extract, the LD_{50} value of *Gardenia* extract was not determined.

For food application, acute toxicities of the pigment powders on mice were investigated. Impressively, the *Peristrophe* pigment powder did not cause any signs of acute toxicity, even at a very high oral dose of 25.0 g/kg. A previous study also reported that *Peristrophe* pigment had a very low acute median lethal dose (LD_{50}) in mice at 26.50 g/kg [9]. Although the previous study demonstrated that *Gardenia* pigment, which was extracted in ethanol, might cause several acute symptoms in Wistar rats, such as diarrhea, increased hemorrhage in the liver, vacuolar degeneration, and necrosis of hepatocytes [39], in this study, we did not find any acute toxicity of *Gardenia* pigment on mice with no appreciable toxic-related signs at an oral dose of 25.0 g/kg. This difference might be due to modification in the solvent used for pigment extraction. By using water, we extracted the major yellow and purple pigments and reduced the possible toxic components in both pigment powders.

4. Conclusion

In this study, we for the first time investigated the safety of the yellow pigment powder extracted from the *Gardenia* fruits and the purple pigment powder extracted from the *Peristrophe* leaves on zebrafish embryos, a well-known model for toxicological test. The results obtained from this study suggest that both the yellow and purple pigment powders meet the requirements for food colorants, including safety for human health, stability with heat, pH, and storage time, and thus can be applied for food coloring to improve the attraction and quality of foods.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Supplementary Materials

Supplemental Figure 1: the rehabilitation of purple color due to change of pH. Supplemental Table 1: effect of storing time (at 4°C) on color of the pigments. (*Supplementary Materials*)

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