Evaluation of polyphenol, anthocyanin content and antioxidant capacity of some Vietnamese fruits

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Abstract. Polyphenol-rich fruits are plant materials of interest due to the high anthocyanin content and number of antioxidant compounds. Besides, anthocyanins also possess other various beneficial properties, including antioxidant activity and mitigation of risk of circulatory diseases. In this study, different polyphenol-rich fruit species including Ficus racemosa, Fragaria ananassa, Passiflora edulis, Carissa carandas Linn, and Rhodomyrtus tomentosa were harvested and compared for total anthocyanin content, total phenolic, and antioxidant activity. Extraction conditions included solvent of 50% ethanol, material/solvent ratio of 1:5, temperature of 60°C temperature and extraction time of 30 minutes. In terms of total phenolic content, Fig fruit achieved 136.08 mg GAE/g. Among examined fruits, strawberries contained the largest amounts of anthocyanin (355.27 mg cy-3glu L−1). Passion fruit showed the highest antioxidant activity (409.89ug/mL). The results of this study are expected to contribute to further development in utilization of the said fruits and open new pathways in enhancing value of agricultural products in Vietnam.

1. Introduction
The focus on research and innovation in the food and materials sector in the world will increase over the next few years [1-7]. The berries or red fruits have been receiving a great deal of public attention due to their antioxidant properties. Besides, the red fruits are not only used fresh but also applied in different fields such as cosmetics, pharmaceuticals. Recently, different researches focus on the composition and antioxidant properties of typical red fruits, which published frequently. Optimal extraction methods were carried out to obtain rich antioxidant products from different sorts of berries. Many studies have been reported for the anthocyanin content, Total phenolic content, and antioxidant activity in fruit. Recent studies have investigated and shown many of the fruits that contain these ingredients. The red berries of Vitis vinifera L. are abundant in flavonols and anthocyanins [8]. Punica granatum L. is often called pomegranate, originating from the Middle East [9] are reported as a good source of natural antioxidants. Carissa carandas Linn. fruits are rich in phenolic compounds including...
anthocyanins [10]. Polyphenolic compounds play an essential role in the quality of grapes and wines. These components can be separated into two groups: non-flavonoid and flavonoid compounds (anthocyanins, flavonols). The function of anthocyanins is immediately responsible for color in grapes and young wines [11–12]. Notably, the anthocyanins play a vital function for the color of polyphenol-rich fruit species [13–15]. Besides its coloring properties, anthocyanins also have a link to a variety of biological, pharmacological, chemotherapy, anti-toxin and anti-inflammatory properties [16–18]. This article investigates the phenolic composition of red berries in Vietnam including *Ficus racemosa*, *Fragaria ananassa*, *Passiflora edulis*, *Carissa carandas* Linn. and *Rhodomyrtus tomentosa* and compares them with previous results to show the influence of geographic location, growth characteristics on phenolic content, anthocyanins, and their antioxidant activity.

2. Materials and methods

2.1. Fruit sample

The fruits such as Fig (*Ficus racemosa*), Strawberry (*Fragaria × ananassa*), Passion fruit (*Passiflora edulis*), Karanda (*Carissa carandas* Linn.) and Sim fruit (*Rhodomyrtus tomentosa*) were obtained in Vietnam (Southeast region). The fresh samples were washed thoroughly using the distilled water, then chopped or grinded before extraction. Each experimental condition will be tested individually. The sample (10g) was placed in the two necks round bottom flask and was extracted by ethanol with concentration at 50. The liquid/solid ratio in this experiment was 1:5. The extraction temperature was 60°C in 30 min. Then, centrifugation took place at 4000 rpm for 15 min by high-speed centrifuge Model LACE16 (from COLO lab expert). The pH scanning of supernatant ranges from 400 nm to 700 nm (Figure 1).

2.2. Determination of total anthocyanins (TAs)

Monomeric anthocyanin content was determined using the pH differential method. Sodium acetate buffer pH 4.5 and potassium chloride buffer pH 1 were used for analysis. The samples were diluted with pH 1 and 4.5 buffer and equilibrated for 15 min, then absorbances were measured at 510 nm and 700 nm by using a spectrophotometer calibrated with distilled water as the blank. The total monomeric anthocyanin a eas calculated as follows:
\[ a = \frac{A \cdot MW \cdot DF \cdot 10^3}{\varepsilon \cdot l} \quad (1) \]

\[ A = (A_{\lambda \text{vis max}} - A_{700\text{nm}}) \text{pH1.0} - (A_{\lambda \text{vis max}} - A_{700\text{nm}}) \text{pH4.5} \]

With \( A \) is the absorbance, \( V \) is the solvent volume (ml), \( MW \) is the molecular weight of cyanidin-3-glucoside (449.2g/mol), \( DF \) is the dilution factor, \( a \) is the molar absorptivity (26900), \( l \) is the cell path length (1cm).

### 2.3. Determination of antioxidant activity (AAs)

The antioxidant activity of the individual essential oil was tested using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay with 600 µL of DPPH (OD 517 nm = 0.0403 ± 0.013) into 500 µL solution sample. The sample solution with a specified concentration was added to DPPH solution and allowed to stabilize at room temperature in the dark within 37 min. The optical measurement of the mixture by UV/VIS - 1800 Shimadzu Spectrometer at 517 nm. The blank was 500 µL solution replaced with EtOH 99.7%. Standard sample: Vitamin C (0.1g ÷ 0.01) was dissolved EtOH 99.7% into volume flask 100mL, in the dark (C = 100 µL/mL). The percent DPPH scavenging effect calculated as follows.

There are different techniques for estimating the antioxidant activity of both synthetic compounds and natural. The DPPH assay was a rapid and low-cost method, which usually used for evaluation of the antioxidative potential of different natural stocks. The DPPH scavenging assay is broadly applied to assess the free radical scavenging of plant extracts thanks to its sensitive, simple, rapid Antioxidants can remove the radical by hydrogen donation, which results in a decrease of DPPH absorbance at 517 nm. The IC50 value was the concentration of the sample which inhibited percentage reaches 50%. Therefore, IC50 values are negatively correlated to the antioxidant activity, the lower IC50 value means the highest antioxidant activity of the tested sample.

\[ \text{DPPH scavenging activity (\%)} = \left( \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100 \]

### 2.4. Determination of the total phenolic (TPs) content

TPC in pulp extract were evaluated using Folin–Ciocalteu’s colorimetric assay, as modified by Sinanoglou. The absorbance was spectrophotometrically measured at 765 nm with equipment (Thermo Scientific™ GENESYS™ 10S UV-Vis Spectrophotometer). The total phenolic content was expressed as mg gallic acid equivalents (GAE) per 100g dry weight, using a standard curve with 2-10 mg/L gallic acid (\( y = 0.01697x + 0.02266, R^2 = 0.99951 \)).

### 2.5. Data Analysis

All experiments were conducted in duplicate. Experiment data was analyzed using one-way analysis of variance (ANOVA) test in SPSS program (IBM Company, USA) with the level of significance at 5%.

### 3. Results and discussion

#### 3.1. Total Phenolic contents (TPC) of VietNamese polyphenol-rich fruit

In this study, different kind of fruits is carried out for their Total Phenolic contents, which belong to four different botanical families (Fig, Strawberries, Passion fruit, Karanda, Sim fruit). The Folin-Ciocalteu method is applied to determine the TPC in different sorts of fruits and foods. Figure 2 illustrated the TPC in the extracts of Vietnam fruits including Fig, Strawberry, Passion fruit, Karanda, and Sim fruit. As can be seen from figure 2, the TPC of Fig achieved 136.08 mgGAE/g, followed by Passion fruit (91.12mgGAE/g), and Rhodomyrtus (91.12mgGAE/g). Moreover, Strawberry and Karanda obtained at 57.66mgGAE/g and 43.07mgGAE/g, respectively. This study is consistent with previous studies of Johora (2013), in which Ficus racemosa has been analyzed [19]. However, this is the first time Fig has been harvested in Vietnam and analyzed and compared with other polyphenol-rich materials. It shows
a significant difference between Fig and the remaining materials (about 20-60 mg GAE g\(^{-1}\)). Besides, Karanda fruit exhibit the lowest TPs contents (43.07 mg GAE g\(^{-1}\)) when compared with other materials in this survey, this result is in good with Pewlong’s previous research (2014) on TPs of *Carissa carandas Linn*. Vietnam is a region of the tropical climate, where there is a diverse and abundant source of fruit. These research results help to expand the extraction of phenolics compounds, thereby increasing the use value of Vietnamese agricultural products.

3.2. Total anthocyanin contents (TAs) of Vietnam polyphenol-rich fruit

Anthocyanins are phenolic compounds which plays a vital role in contributing the red-purple or blue coloration of different fruits. The pH differential method applied for the quantification of anthocyanins due to the rapidly performed procedure [20]. Figure 3 revealed the content of anthocyanin in the different extraction of fruits. In this study, Strawberry and Karanda fruit and strawberry exhibited wealth in anthocyanin content with 355.27 mg Cy-3-glu L\(^{-1}\) and 267.27 mg Cy-3-glu L\(^{-1}\), respectively. The total anthocyanin content was not directly correlated with the TPs contents in all of the species. While the TAs contents of Strawberry extraction was highest (355.27 mg Cy-3-glu L\(^{-1}\)), the TPC was lowest (57.66 mg GAE g\(^{-1}\)). The high TAs contents in strawberry fruits may be explained by the presence of other types of anthocyanin. Besides, the previous study also carried out that fully-ripe fruits exhibited the highest anthocyanin content, which contributed to their TPC. [19].
3.3. Antioxidant activity of Vietnam polyphenol-rich fruit

Figure 4 illustrates shows the DPPH radical scavenging activity of the difference extracts fruits in Vietnam. As can be seen from the figure, the free radical scavenging capacities of the strawberry and passion fruit were superior to other samples. The highest free radical scavenging capacity of passion fruit with an IC$_{50}$ value of 409.89 µg/mL, while the other had activities with IC$_{50}$ values ranging from 93.25–371.09 µg/mL. By comparing the IC$_{50}$ of the extracts, we can evaluate their ability to neutralize DPPH. The smaller IC$_{50}$ shows, the stronger the ability to catch free radicals, and vice versa. The results showed that passion fruit and strawberry could catch free radicals less than vitamin C, achieved 91 times and 82 times, respectively. Meanwhile, Fig shows that the ability to capture free radicals outperforms the remaining ingredients, performed 4.3 times better than passion fruit. This results is in line with a previous study with different types of fruits and vegetables consumed in Thailand and using DPPH method for investigation [22]. The antioxidant content of Rhodomyrtus tomentosa was similar to the results of Maskam (2014) (186.39ug/mL compared with 200ug/mL) [23]. Johora (2013) revealed that the antioxidant of bark Fig in Bangladesh has significantly higher free radical scavenging activity compared to the leaf extract. [19]. Oxidation resistance also depends on the characteristics and extraction conditions of each type of material. Nevertheless, this result also shows great potential in exploiting the antioxidant capacity of fruits in Vietnam.

![Figure 4. Antioxidant activity of the extracts of Vietnamese fruits](image)

4. Conclusion

In this study, different polyphenol rich fruit including Ficus racemosa, Fragaria x ananassa, Passiflora edulis, Carissa carandas Linn. and Rhodomyrtus tomentosa are carried out to determine their total anthocyanin content, Total Phenolic Content, and antioxidant activity. In general, the study results concluded that strawberries contained large amounts of cyanidin 3-glucoside (355.27 mg Cy-3-glu L$^{-1}$) and showed high antioxidant activity (371.09 µg/mL) compared to other tested extracts. Moreover, Fig fruit shows the highest total phenolic content (136.07 mg GAE g$^{-1}$). However, anthocyanins and their antioxidant activity are also affected by storage conditions as well as various climatic and geographical factors. Therefore, it is necessary to make a list of anthocyanin content and free radical scavenging properties of all commonly used natural sources such as fruits, flowers and vegetables to provide detailed knowledge of the content and distribution of anthocyanin as well.

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