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Potentiality of Roselle and Onion (*Allium cepa*) peel as Raw Materials for Producing Protocatechuic Acid in Tropical Malaysia: A Comparative Study

Mohamad Osman[1], Nuha Nazim Hasan Sazalli[2], Deny Willium[3], Faruq Golam[4*] and Arash Nezhadahmadi[4]

[1]Faculty of Plantation and Agro technology, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia
[2]Department of Biotechnology, Faculty of Science, International Islamic University, Kuantan, Malaysia
[3]Department of Applied Chemistry, Faculty of Science, International Islamic University, Kuantan, Malaysia
[4]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603, Malaysia; faruqwrc@gmail.com

Abstract

Protocatechuic acid from natural sources are widely used in human health. This study focuses on the presence of protocatechuic acid (PCA) in Rosselle (*Hibiscus sabdariffa*) and onion peel (*Allium cepa*). Collection of onion peel is difficult and amount is less than 1% in onion. Moreover, Malaysia is importing 100% from overseas. On the contrary, roselle is very common in tropical Malaysia and its calyx can be dried and preserved. Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) were used to identify protocathechuic acid (PCA) from the samples. TLC was performed on 20×20 cm Silica gel 60 F_{254} aluminum sheets as TLC plates with mixtures of water-methanol-formic acid as mobile phase. TLC recorded the presence of protocatechuic acid in both of the samples. HPLC analysis was performed on C-18 in 250×4.6 mm steel column with methanol–water–formic acid in 25:75:0.5 ratio as mobile phase. The flow rate of the mobile phase was 1.0 ml/min and protocatechuic acid was detected at 260 nm of wavelength. The quantities of protocatechuic acid determined by HPLC in rosselle and onion peel were 0.014 mg in 30 g of dried samples extract and 0.25 mg in 30 g of dried extract, respectively. The amount of protocatechuic acid in onion peel extract was eighteen times higher compared to Rosselle extract but considering availability of raw materials in Malaysia, roselle might be used as good source of PCA production with potential economic feasibility.

Keywords: Protocatechuic Acid, Roselle, Hibiscus sabdariffa, Onion Peel

1. Introduction

Roselle belongs to *Hibiscus sabdariffa* L. (family Malvaceae), which is cultivated for its jute-like fiber and can be found in countries such as India, Saudi Arabia, Indonesia and Vietnam. The origin of roselle is uncertain, while it is believed that its home country is India and Saudi Arabia. The most exploited part of roselle plant is the calyx which is obtained by removing seeds of the flower from its capsule[8]. Roselle calyces are used for the preparation of herbal drinks, cold and warm beverages, jams and jellies.

Protocatechuic acid is one of the isomeric compounds of dihydrobenzoic acid which have two hydroxyl groups and one carboxylic group attached on the benzene ring. This naturally active compound in herbal plants is always biologically stable, not accumulated in the body, and able to neutralize the harmful effect of other compounds[16]. The amount of chemical constituents in plants are varying and depended on the plant organs such as flower, root, or stem. Protoatechuic acid can be found in the calyces (flower) part of the rosselle sample[11]. This active compound in roselle can treat hypertension[12], and heart disease[6]. It is

*Author for correspondence

also proved that protoatechuic acid can protect humans' body from liver damage[1]. Moreover, this active compound can also provide protection from cardiovascular disease[4], and induce prolification of abnormal cell[2]. It is believed that protocatechuic acid is present in the onion peel[3], and responsible for the antioxidant activities in the body[13]. Previous studies showed that the samples which contained protocatechuic acid were extracted through one solvent extraction method[17] or two solvents which were used separately in the extraction method[15]. Detection of PCA has also been utilized by Szaufer et al.[17] in the analysis of phenolic acids from *Aquilegia vulgaris* or columbine flower. In this study, it was tried to determine and compare the amount of Protocatechuic acid (PCA) between rosselle (*Hibiscus sabdariffa*) and onion peel (*Allium cepa*) samples and evaluation the potentiality of roselle and onion peel as raw material for producing PCA in Malaysia..

2. Materials and Methods

2.1 Plant Materials

Calyxes of Roselle were collected from Universiti Kebangsaan Malaysia (UKM) and were identified as UKMR-2 varieties. Onion peel (Outer skin of bulb) and dry Roselle calyx were used for PCA quantification.

2.2 Methods

2.2.1 Extraction and Phytochemical Investigation

PCA extraction method had been established since the 19th century. The first extraction method was initiated using warm water extract in order to isolate the compound[10]. Nowadays, PCA can be extracted from the herbal plants using wet solvent such as acetone, methanol or ethanol. A total amount of 30 grams of Roselle calyces were extracted with boiling ethanol on a water bath under reflux for 18 hours. Then, the extracts were combined and concentrated under reduced pressure until complete removal of the ethanol. After that, the residue was diluted with hot distilled water and left at room temperature for 24 hours. The precipitate was filtered and the filtrate was subsequently extracted with petroleum ether and diethyl ether respectively. The extract was purified using Amberlite XAD-2 resin to remove anthocyanin. After that, extracts were put in fridge and dried using freeze dryer. In the next step, the residue was collected and dissolved in methanol in a total volume of 10 ml. These procedures were also repeated for onion peel samples. Both of the roselle and onion peel extracts were concentrated using freeze dryer. The remaining solid residues were measured and yield percentages were calculated. Final products and yield percentages of the samples are shown in Tables 1 and 2 respectively.

2.2.2 Preparation of Protocatechuic Acid (PCA) Standard

The stock solution was prepared by dilution of 10 mg of Protocatechuic acid standard into methanol HPLC grade using 10 ml volumetric flask. The methanol HPLC grade was added until it reached to graduation marks. Then, 1000 ppm of stock solution was successfully prepared in 10 ml labeled volumetric flask. Highly diluted solution was provided from stock solution and 4 different standard concentrations including 100, 80, 40, and 16 ppm were prepared using serial dilution techniques.

3. Results and Discussion

3.1 TLC Analysis

TLC was performed on 20×20 cm Silica gel F_{254} aluminum sheet TLC plates. Before use, the plates were washed with methanol and dried for 3-4 hours in 40°c. Then,

Table 1. Final product weight roselle and onion peel samples

Sample	Weight of flask (g)	Weight of flask (g) + sample	Weight of sample (g)
Roselle	96.51	96.78	0.27
Onion peel	127.76	127.35	0.41

Table 2. Yield Percentages of roselle and onion peel samples

Sample	Mass Sample (g)	Mass of extraction (g)	Percentages yield (%)
Roselle	30.0	0.27	0.9
Onion peel	30.0	0.41	1.36

1000 ppm of standard PCA was used in this test. Solvent system was prepared with water, methanol, and formic acid in 75:25:0.5 ratio. Both of the samples were spotted next to the standard PCA in the TLC plate. Spotted TLC plate was put in slanted position into developing tank that was saturated in solvent system. The TLC plate was left until solvent reached the TLC front. TLC plate result was viewed under the UV light at 254 or 365nm. Identified spots were marked using pencil and TLC plate was left in the dryer to produce more significant result. The result of the TLC was determined by Retention factor (Rf) value in both of the samples and standard (Table 3).

3.2 HPLC Analysis of PCA Standard

Quantitative analysis was performed by Perkin Elmer S200 High Performance Liquid Chromatography instruments. Reversed phase HPLC with C-18 250×4.6 mm steel column was used to allow separation of extract and detection of PCA. The flow rate of the mobile phase (methanol-water-formic acid 25:75:0.5) was 1.0 ml/min. Protocatechuic acid was detected at 260 nm of wavelength and the analysis was performed at 25°C. 1.0 ml of protocatechuic acid standards was pipetted and filtered. Filtered standard solutions were placed in the HPLC valve. All five protocatechuic acid standards were injected into the HPLC and all of the retention times were recorded. The standard curve was plotted using the peak area of the standard protocatchuic chromatograms and the concentrations (Figure 1).

3.3 Validation of HPLC Method

The concentration of protocatechuic acid in both of the samples were calculated by regression equation y = 1.32x + 1.45 obtained from the calibration curve of standard protocatechuic acid (Figure 1). The correlation coefficient of calibration curve of protocatechuic acid was found to be 0.998. Thus, it exhibited a good linear relationship between concentrations and peak areas.

Table 3. Determination of PCA by comparing Retention factor (R_f) value of standard using solvent system Methanol HPLC grade

Sample	Distance of spot	Distance of Solvent	R_f value
Standard	4.6	8.4	0.548
Roselle	4.7	8.4	0.559
Onion peel	4.8	8.4	0.571

3.4 HPLC Analysis

Polar mobile phase was used to separate the compounds and the retention time was recorded at 7.87 minutes. In other studies, the retention time to detect the PCA was at 6.37 minutes[13] and 5.87 minutes[5]. The retention time was varied due to the pressure in the column and composition of the mobile phase. In chromatogram of roselle extract, 34 peaks were formed during the analysis. It was observed that rosselle sample showed two major peaks that were separated in the chromatogram. The first major peak was recorded at 3.909 min and the second one was formed at 7.898 min. The second major peak was highly recognized as protocatechuic acid compound as the retention time of sample was 7.898 min and protocatechuic acid standard peak was 7.816 min which were matched (Table 4). So, it was accepted that protocatechuic acid was presented in the roselle extract. From onion peel extract chromatogram (Figure 3), there were 3 peaks that were formed during the analysis. The chromatogram showed two major peak separations in the chromatogram. The first major peak

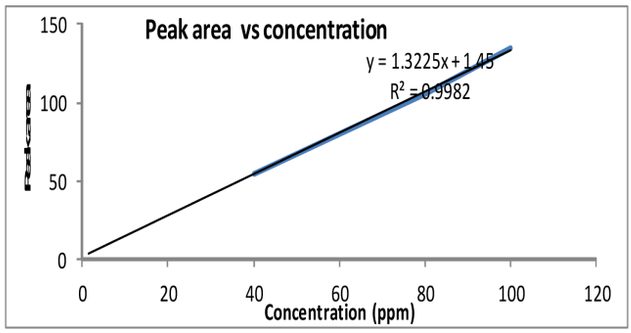

Figure 1. Calibration curve of the standard PCA with peak area and concentration.

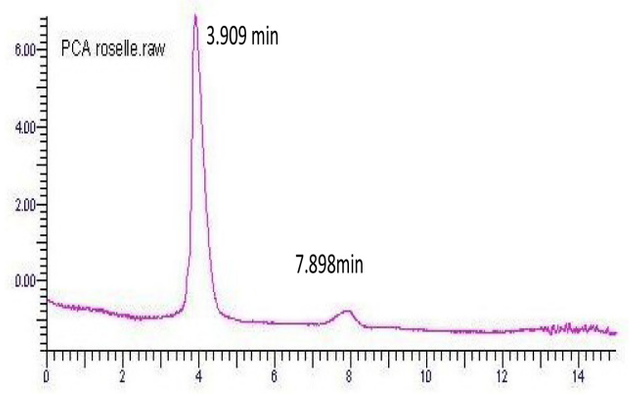

Figure 2. Chromatogram of roselle extract in different durations of time.

was recorded at 3.886 minutes and the second one was formed at 7.864 min. The retention time of peak sample was 7.864 min and protocatechuic acid standard peak was 7.816 min that were matched. So, the second major peak was highly recognized as protocatechuic acid compound according to the standard retention time (Table 4).

3.5 Amount of Protocatechuic Acid (PCA) in Samples

There were significant differences in quantitative proportion of protocatechuic acid which were observed in this study. The amount of protocatechuic acid in onion peel was eighteen times higher than roselle. According to HPLC analysis, the amount of protocatechuic acid in roselle and onion peel was 0.014 mg per 30 g of dried calyces and 0.25 mg per 30 g of dried onion peel respectively (Table 5). The amount of protocatechuic acid can be measured by converting the concentration of ppm into the amount of PCA (in mg) that was dissolved with methanol HPLC grade until 10 ml. In this study, protocatechuic acid concentration in roselle sample was lower as some of the protocatechuic acid compound may be lost during the purification of the compound and also in the liquid-liquid extraction process. Compared to the result of other studies, the content of protocatechuic acid in roselle was 0.012 mg per 50 g of dried sample[7] compared to 2.1 mg of protocatechuic acid that could be isolated from roselle calyces in 30 g dried sample[18]. On the other hand, phenolic compound in onion showed that there were 0.56 mg of protocatechuic acid per 30 g of onion peel sample[13] and 2.3 mg of protocatechuic acid presented in 30 g of onion sample in other studies[5]. Different studies produced different results which involved factors such as the types of extraction. Some of the studies used water extraction[18] and others used solvent extraction[9] which can have effect on the results. Moreover, other influencing factors can be related to the type of the sample in which Yin and Chao[18] used fresh sample and Sayago et al.[14] utilized dried samples in the extraction method. Therefore, the amount of protocatechuic acid was varied in different studies in which the protocatechuic acid in onion peel extract was higher compared to calyces roselle extract. Thus, in the current investigation, the results were significant when the amount of protocatechuic acid in onion peel was eighteen times higher than roselle.

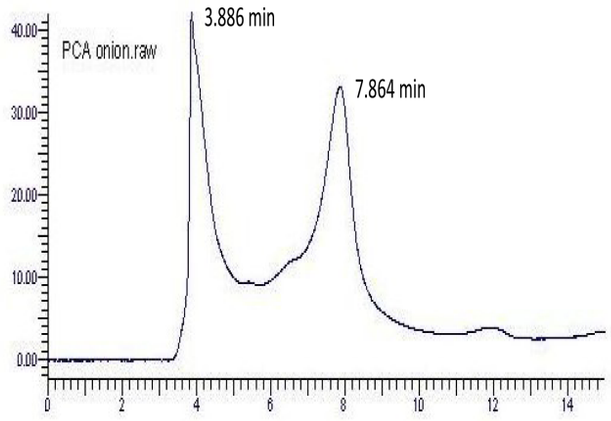

Figure 3. Chromatogram of onion peel extract.

Table 4. Standard PCA concentration and peak retention time

Standard concentration (ppm)	Peak retention time (min)
16	7.883
40	7.731
80	7.883
100	7.786
1000	7.797
Total retention time	39.08
Mean retention time	7.816
Standard deviation	0.06

4. Conclusion

Protocatechuic acid is widely used in human health which can treat hypertension, heart disease, provide protection from cardiovascular disease, and induce reduction of abnormal cells. The present investigation indicated that Roselle and onion were appropriate sources for natural antioxidants as they showed the presence of hydrobenzoic acid. Quantitative analysis confirmed the existence

Table 5. Sample concentrations in roselle and onion peel

Sample	Peak height (cm)	Baseline (cm)	Peak area (cm^2)	Concentration (ppm)
Roselle	1.6	2.5	2.0	1.4
Onion	38.0	1.8	34.2	25.0

of protocatechuic acid in Roselle and onion peel in significant amounts. From the results obtained, both of samples contained the phenolic compound but in different amounts in which the amount of protocatechuic acid in onion peel was eighteen times higher compared to Roselle. However, considering availability and economic feasibility Roselle might be a suitable source of PCA production in tropical Malaysia.

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