PROGRESS IN APPLIED SCIENCE AND TECHNOLOGY
Faculty of Science and Technology
Rajamangala University of Technology Thanyaburi
Prog Appl Sci Tech. 2023; 13(2):38-44
ISSN (Print): 2730-3012
ISSN (Online): 2730-3020
https://ph02.tci-thaijo.org/index.php/past

Research Article

Received: January 27, 2023
Revised: April 23, 2023
Accepted: May 22, 2023

DOI: 10.14456/past.2023.7

Optimum Conditions and Comparison of Extraction Methods of Red Roselle Water on the Contents of Anthocyanin, Total Phenolic, Total Flavonoid, and Anti-Radical Scavenging Activity

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Abstract

Red roselle (*Hibiscus sabdariffa* L.) is an herb that is widely used. Because it has a wide variety of properties and also contains the main substance such as anthocyanin. Which is a substance that has two times higher antioxidant activity than Vitamin C and Vitamin E. This research aimed to study the optimum conditions for extracting red roselle water on the contents of anthocyanin, total phenolic, total flavonoid, and anti-radical scavenging activity. Red roselle with moisture contents of 19.92 percent was used and extracted with water in a ratio of 1:10 w/v (Red roselle from Non-Sung, Nakhon Ratchasima). It was extracted by using 4 methods, there Microwave extraction method at 100 (100 W ME) and 200 watts (200 W ME), the Boiling method (BE), the Maceration method (MA) at 25ºC and room temperature (METR) and Sonication extraction method (SE) for 7 15 30 45 60 120 180 and 240 minutes. The results showed the optimum conditions for extraction of Red roselle water on anthocyanin content were boiling extraction for 15 minutes. showed anthocyanin content cyanidin-3-glucoside and pelargonidin-3-glucoside 35.58±1.07 and 21.01±0.63 mg/L respectively. Extraction methods with the highest total phenolic content and total flavonoid content were extracted with microwave 100 watts for 30 minutes showing total phenolic content was 1,234±12.09 µg GAE/mL extract. The total flavonoid content was 141.71±1.06 µg QE/mL extract. The extraction methods with the highest anti-radical scavenging activity were sonication extraction for 7 min. The anti-radical scavenging activity was 134.02 ±1.85 µg Vit.CE/mL extract. Red roselle water extract of each condition showed a pH in the range of 2.00-2.50.

Keywords: *Hibiscus sabdariffa* L., Extraction Method, Anthocyanin, Phenolic, Flavonoid, Anti-Radical Scavenging Activity

1. Introduction

Red roselle is classified as a Malvaceae family plant. It is scientifically known that *Hibiscus sabdariffa* L. is a widely used herb due to its variety of medicinal properties such as antibacterial, antifungal, diuretic, anti-cough, and prevent bladder stones (1). It is also a source of many important nutrients, including vitamins, magnesium, calcium, phosphorus, and iron (2). It is also found in a wide range of antioxidants, such as gallic acid, catechin, delphinidine-3-sambucoside, cyanidine-3-glucoside, and that found. The most is anthocyanin (3).

Anthocyanin is pigment or a natural colorant. It is categorized in the flavonoid group. It has a C6-C3-C6 structure (4). Nowadays, anthocyanin is a pigment that has received great attention from researchers because is an antioxidant making it possible to prevent the

occurrence of various chronic diseases such as cardiovascular disease, cancer, and diabetes (5). In addition, anthocyanins are effective in twice as high in antioxidants as vitamin C and vitamin E. Help strengthens the body's immunity and makes the body healthy. reduce the occurrence of cancer, anti-germs, and helps to enhance the function of red blood cells (6).

Therefore, the study of the optimum conditions and compared extraction methods of red roselle water. which provide a high content of important substances including Anthocyanin content total phenolic content and total flavonoid content and anti-radical scavenging compare the easy, convenient, and fast methods is interested to be further developed in the future of product making.

2. Materials and Experiment

2.1 Materials and Experiment

Dried Red roselle from Non-Sung District, Nakhon Ratchasima Province, Potassium chloride, Sodium acetate, Hydrochloric acid, DI water, Sodium carbonate, Distilled Water, Sodium nitric, Aluminium chloride, Sodium hydroxide, Gallic acid, Ethanol, 2, 2-diphenyl-1-picrylhydrazyl, Quercetin, Ascorbic acid, Folin-ciocalteu.

The extraction of Red roselle water was used at a ratio of 1:10 (w/v) of Dried Red roselle (g) per water (mL). They were extracted for 7 15, 30, 45, 60, 120, 180, and 240 minutes extracted by using 4 methods, there is Microwave extraction method at 100 (100 W ME), and 200 watts (200 W ME), Boiling extraction method (BE), Maceration extraction method at 25ºC (MA), room temperature (MERT) and sonication extraction method (SE). Filter with filter paper No. 1 and adjust the volume. Packed into clean bottles. the Red roselle water was kept at 4ºC for further analysis.

2.2 Determination of the Moisture Content of Dried Red roselle

Moisture is determined by AOAC method (7).

2.3 Determination of the pH value of red roselle water

The pH value was determined by using calibrated pH meter in accordance with AOAC methods (7).

2.4 Determination of Anthocyanin Content

Anthocyanin determination by the pH-differential method using a UV-Visible spectrophotometer to measure the absorbance to calculate the anthocyanin content (8). Pipet 0.4 mL of 4 times diluted red roselle water extract into tubes 1 and 2, In the first tube, was diluted with 3.6 mL of pH 1.0 buffer (Potassium chloride, 0.025M), and the second tube was diluted with 3.6 mL of pH 4.5 buffer (sodium acetate, 0.4M) and shaken well. Absorbance was measured at wavelengths of 520 and 700 nm. Calculate the anthocyanin content relative to cyanidin-3-glucoside and pelargonidin-3-glucoside from the following equation:

$$\text{AC (mg/L)} = \frac{\text{A} \times \text{MW} \times \text{DF} \times 10^3}{\varepsilon \times 1} \qquad (2.1)$$

where AC = Anthocyanin content A = ($A520_{nm}$ – $A700_{nm}$) pH 1.0 – ($A520_{nm}$ – $A700_{nm}$) pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside and 306.7 g/mol for pelargonidin-3-glucoside; DF = dilution factor established in D; l = pathlength in cm; ε = molar extinction coefficient. 26,900 for cyanidin-3-glucoside, 31,100 for pelargonidin-3-glucoside in $L \times mol^{-1} \times cm^{-1}$; and 10^3 = factor for conversion from g to mg.

2.5 Determination of Total Phenolic Content (TPC)

Determination of total phenolic content by Folin-Fiocalteu's used a microplate reader to measure the absorbance and gallic acid was used as a standard solution (9). Dilute red roselle water extract with distilled water (200 µL Red roselle water/mL). Pipet 12.5 µL of the diluted extract into a 96-well plate, add 50 µL of DI water, and 12.5 µL of Folin-Ciocalteu (50% v/vin DI water) After 10 min, 125 µL of 7% Na_2CO_3 and 100 µL of DI water were added. The mixture was allowed to stand for 15 min at 45°C and the absorbance was determined at 765 nm. Total phenolic content was calculated from the gallic acid standard curve with linear relation of $r^2 = 0.998$. Data were expressed as µg of gallic equivalent (GAE) per 1 mL of extract.

2.6 Determination of Total Flavonoid Content (TFC)

Determination of total flavonoid content by the colorimetric method used a microplate

reader to measure the absorbance and quercetin was used as a standard solution (9). Dilute red roselle water extract with distilled water (250 µL Red roselle water/mL). Pipet 100 µL of the diluted extract into a 96-well plate, and 100 µL of 2% $AlCl_3$ were added and mixed thoroughly. The reaction mixture was kept at room temperature for 15 min and the absorbance was determined at 435 nm. Total flavonoid content was calculated from the quercetin standard curve with linear relation of r^2 = 0.998. Data were expressed as µg of quercetin equivalent (QE) per 1 mL of extract.

2.7 Antioxidant Capacity Assay

Determination of the anti-radical scavenging activity by using the dipheny l-2-picrylhydrazyl (DPPH) method. The assay was performed with a microplate reader (9). Dilute red roselle water extract with distilled water (250 µL Red roselle water/mL). Pipet 100 µL a 96-well plate, mixed with 100 µL of the DPPH-radical (100 µg/mL in methanol) and left to stand at room temperature for 15 min in the dark. The absorbance was measured at 517 nm. The percentage of DPPH-radical scavenging was calculated from Equation 2 and the antioxidant capacity of the extracts was expressed as vitamin C equivalent antioxidant capacity (VCEAC).

$$\% \text{ Radical scavenging activity} = [((A_{control} - A_{sample})/A_{control}) \times 100] \quad (2.2)$$

Where, A_{sample}: The absorbance of the extracts or standards mixed with DPPH. $A_{control}$: The absorbance of the MeOH mixed with DPPH

3. Results and Discussion

3.1 Moisture of Dried Red roselle

Analysis of moisture content of dried red roselle. 2 g of dried Red roselle to bake at 105°C for 5-6 hours. The dry red roselle's average moisture content accounted for 19.92±0.11% of the dry weight.

3.2 The pH value of red roselle water

pH analysis of Red roselle water extracts was in the range of 2.00-2.50, which is acidic. In addition, increasing extraction time resulted in a decrease in pH value. Because the extraction time causes cell tissue rupture and the release of important substances, namely anthocyanin, which is acidic and the main substance found in roselle flowers or other organic acids substances, such as ascorbic acid, citric acid, malic acid, and tartaric acid (10). The analysis results are shown in Table 1.

Table 1 Results of the pH analysis of red roselle water under different conditions

Time	pH value of Red roselle water					
	100W ME	200W ME	BE	MA	MERT	SE
7	2.42	2.34	2.38	2.53	2.49	2.50
15	2.39	2.40	2.33	2.41	2.44	2.42
30	2.38	2.45	2.42	2.32	2.30	2.42
45	2.36	2.49	2.35	2.30	2.38	2.42
60	NT	NT	NT	2.12	2.21	2.14
120	NT	NT	NT	2.09	2.05	2.08
180	NT	NT	NT	2.00	2.08	2.14
240	NT	NT	NT	2.07	2.04	2.06

100W ME; Microwave extraction method at 100W, 200W ME; Microwave extraction method at 200W, BE; Boiling extraction method, MA; Maceration extraction method at 25ºC, MERT; Maceration extraction method at room temperature, SE; sonication extraction method and NT; Not test.

3.3 Results of Total phytochemical contents of red roselle water

The results of the experimental extraction of red roselle water by various methods, including microwave extraction method at 100 (100 W ME), and 200 watts (200 W ME), Boiling extraction method (BE), Maceration extraction method at 25ºC (MA), and room temperature (MERT), and sonication extraction method (SE) for 7 15, 30, 45, 60, 120, 180, and 240 minutes on the anthocyanin content, total Phenolic content and total flavonoid content was found that the extraction with Microwave at 100 watts for 45 min and at 200 watts for 30 min and boiling extraction for 30 min, the amount of this substance was significantly reduced. It may be related to higher temperatures and long times causing the decomposition of substances (11). Maceration extraction at 25°C, maceration extraction at room temperature, and sonic extraction showed that the content of active substances increased continuously. This is because the extraction time affects the breakdown of the sample's cell tissue and the release of the active substance (12).

Effect of extraction of red roselle water on anthocyanin content. Microwave extraction at 100 watts for 30 min and boiling extraction method for 15 minutes. It was found that showed almost identical the anthocyanin contents (cyanidin-3-glucoside; 37.61, 35.58 mg/L

and pelargonidin-3-glucoside; 22.21, 21.01 mg/L). Therefore, boiling extraction for 15 minutes is considered a better extraction method due to the shorter time and lower cost of extraction compared to microwave. The results are shown in Table 2 and 3.

Table 2 Results of anthocyanin contents type cyanidin-3-glucoside of red roselle water

Time	cyanidin-3-glucoside (mg/L)		
	100 W ME	**200 W ME**	**BE**
7	18.20±0.07	18.33±0.82	28.75±0.52
15	22.52±1.81*	25.58±0.98*	35.58±1.07
30	37.61±1.12	13.86±0.65*	31.94±0.85
45	27.77±0.10	7.82±0.05	24.06±0.46
60	NT	NT	NT
120	NT	NT	NT
180	NT	NT	NT
240	NT	NT	NT
Time	**cyanidin-3-glucoside (mg/L)**		
	MA	**MERT**	**SE**
7	2.99±0.08	3.63±0.13*	3.36±0.15*
15	9.02±0.06**	7.74±0.18**	9.54±0.81*,**
30	12.69±0.85^{a,*,**}	10.30±0.24	12.18±0.54**
45	13.45±0.26^{a}	12.07±0.32	16.63±0.31
60	16.43±0.04	15.50±0.45	19.12±1.01
120	19.07±0.83^{b,*}	18.80±0.43*	25.65±1.98^{d,e}
180	21.84±1.11^{c}	22.93±0.60*	23.50±0.46^{d,f,*}
240	19.95±0.57^{b,c}	25.03±0.29	23.91±0.29^{e,f}

A column with English letters raised to the power (a,b,c,d,e,f,g) and rows 7 15 30 45 60 120 180 and 240 min with the same asterisk symbol (*,**) indicate that the obtained values were not significantly different at 95 percent confidence (P-Value > 0.05). 100 W ME; Microwave extraction method at 100 watts, 200 W ME; Microwave extraction method at 200 watts, BE; Boiling extraction method, MA; Maceration extraction method at 25ºC, MERT Maceration extraction method at room temperature, SE; sonication extraction method, NT; Not test and ND; Not detected.

Table 3 Results of anthocyanin contents pelargonidin-3-glucoside of red roselle water

Time	pelargonidin-3-glucoside (mg/L)		
	100 W ME	**200 W ME**	**BE**
7	13.95±0.62	10.28±0.48	16.98±0.31
15	17.94±0.44	15.11±0.58	21.01±0.63
30	22.21±0.66	8.19±0.39	18.86±0.50
45	16.40±0.06	4.62±0.03	14.21±0.27
60	NT	NT	NT
120	NT	NT	NT
180	NT	NT	NT
240	NT	NT	NT
Time	**pelargonidin-3-glucoside (mg/L)**		
	MA	**MERT**	**SE**
7	1.76±0.05	2.14±0.08	1.99±0.09
15	5.33±0.04	4.57±0.11	5.64±0.48
30	7.49±0.50^{a}	6.09±0.14	7.20±0.32
45	7.94±0.15^{a}	7.13±0.19	9.82±0.18
60	9.71±0.02	9.16±0.26	11.29±0.59
120	11.26±0.49^{b,*}	11.10±0.25*	15.15±1.17^{d,e}
180	12.90±0.65^{c,*}	13.54±0.35*	13.88±0.27^{d,f}
240	11.78±0.34^{b,c}	14.78±0.17	14.12±0.17^{e,f}

A column with English letters raised to the power (a,b,c,d,e,f,g) and rows 7 15 30 45 60 120 180 and 240 min with the same asterisk symbol (*,**) indicate that the obtained values were not significantly different at 95 percent confidence (P-Value > 0.05). 100 W ME; Microwave extraction method at 100 watts, 200 W ME; Microwave extraction method at 200 watts, BE; Boiling extraction method, MA; Maceration extraction method at 25ºC, MERT Maceration extraction method at room temperature, SE; sonication extraction method, NT; Not test and ND; Not detected.

The optimum conditions for extracting Red roselle water with total phenolic content were microwave extraction at 100 watts for 30 minutes. Provides amounts of total phenolic content 1234.53 µg GAE/mL extract and followed by extraction boiling method for 15 min provides amounts of 1117.47 µg GAE/mL extract. The results are shown in Table 4.

Table 4 Results of the total phenolic content of red roselle water

Time	Total phenolic content (µg GAE/mL extract)		
	100 W ME	**200 W ME**	**BE**
7	681.24±9.03	594.35±5.85	987.24±10.24
15	878.59±6.58	850.47±11.67	1117.47±4.07
30	1234.53±12.09	785.88±0.62	1086.24±7.35
45	971.47±7.80	429.29±5.49	936.47±1.15
60	NT	NT	NT
120	NT	NT	NT
180	NT	NT	NT
240	NT	NT	NT
Time	**Total phenolic content (µg GAE/mL extract)**		
	MA	**MERT**	**SE**
7	154.88±4.50	240.29±3.48	167.59±0.82
15	427.24±7.04	456.88±6.73	528.59±11.24
30	608.18±19.70^{a,*}	546.06±5.59	639.94±15.88*
45	648.12±17.91^{a,*}	680.59±2.77*	733.12±7.59
60	962.47±12.08^{b}	768.71±9.12	1014.35±8.88
120	1043.06±15.42	925.18±4.65	1320.29±7.83
180	1110.7±24.93	1160.94±10.51	1351.18±11.62
240	956.05±22.16^{b}	1219.00±2.75	1263.06±6.99

A column with English letters raised to the power (a,b,c,d,e,f,g) and rows 7 15 30 45 60 120 180 and 240 min with the same asterisk symbol (*,**) indicate that the obtained values were not significantly different at 95 percent confidence (P-Value > 0.05). 100 W ME; Microwave extraction method at 100 watts, 200 W ME; Microwave extraction method at 200 watts, BE; Boiling extraction method, MA; Maceration extraction method at 25ºC, MERT Maceration extraction method at room temperature, SE; sonication extraction method, NT; Not test and ND; Not detected.

The optimum conditions for extracting Red roselle water with total flavonoid content were microwave extraction at 100 watts for 30 minutes. Provides amounts of total flavonoid content 141.71 µg QE/mL extract and followed by extraction boiling method for 15 min provides amounts of total flavonoid content 122.52 µg QE/mL extract. The results are shown in Table 5.

Table 5 Results of total flavonoid content of red roselle water

Time	Total flavonoid content (µg QE/mL extract)		
	100 W ME	**200 W ME**	**BE**
7	70.70±0.41	61.49±0.62	88.76±0.17
15	98.11±0.77	88.51±0.55	122.52±1.31
30	141.71±1.06	70.64±0.37	110.34±2.02
45	86.64±2.01	39.67±0.07	99.09±1.33
60	NT	NT	NT
120	NT	NT	NT
180	NT	NT	NT
240	NT	NT	NT
Time	**Total flavonoid content (µg QE/mL extract)**		
	MA	**MERT**	**SE**
7	16.62±0.27*	19.78±1.39	17.01±0.54*
15	42.43±0.71	37.49±0.83	46.55±0.35
30	60.62±1.05*	52.83±0.27	60.17±0.58*
45	64.13±0.82	62.83±1.20	73.24±1.38
60	91.22±0.82a	74.22±1.19	99.69±1.09
120	91.11±1.45a	101.00±0.74	142.42±7.83a
180	116.49±1.42	120.66±0.67	137.80±0.94
240	103.98±2.14	135.03±3.43	144.58±2.41a

A column with English letters raised to the power (a,b,c,d,e,f,g) and rows 7 15 30 45 60 120 180 and 240 min with the same asterisk symbol (*,**) indicate that the obtained values were not significantly different at 95 percent confidence (P-Value > 0.05). 100 W ME; Microwave extraction method at 100 watts, 200 W ME; Microwave extraction method at 200 watts, BE; Boiling extraction method, MA; Maceration extraction method at 25ºC, MERT Maceration extraction method at room temperature, SE; sonication extraction method, NT; Not test and ND; Not detected.

3.4 Results of anti-radical scavenging activity of red roselle water

The results of the experimental extraction of red roselle water by various methods, including microwave extraction method at 100 (100 W ME), and 200 watts (200 W ME), Boiling extraction method (BE), Maceration extraction method at 25ºC (MA), and room temperature (MERT) and sonication extraction method (SE) for 7 15, 30, 45, 60, 120, 180 and 240 minutes. It was found that microwave extraction at 100 and 200 watts and extraction boiling method for 15 and 30 minutes. The anti-radical scavenging activity was decreased, but at 45 minutes the anti-radical scavenging activity was increased. Therefore, the extraction temperature may not have an effect on the increase or decrease of the anti-radical scavenging activity. it may have an effect on structural changes of antioxidants that act against DPPH (13). Maceration extraction at 25°C, maceration extraction at room temperature, and sonic extraction were found that the anti-radical scavenging activity of red roselle water continued to decrease at 15 min because the time of extraction may affect the structure of antioxidants in roselle juice against DPPH (3).

The extraction of red roselle water with the highest anti-radical scavenging activity was sonication extraction for 7 min. The anti-radical scavenging activity was 134.02±1.85 µg Vit.CE/ mL extract. The results are shown in Table 6.

Table 6 Results of Anti-radical scavenging activity of red roselle water

Time	Anti-radical scavenging activity (µg Vit.CE/mL extract)		
	100 W ME	**200 W ME**	**BE**
7	85.38±2.06a	109.94±0.61	59.23±3.15b
15	74.56±3.03	59.74±1.84	12.84±2.01
30	40.06±2.76	80.18±0.77	29.21±2.68
45	87.24±1.37a	118.92±1.03	59.11±2.50b
60	NT	NT	NT
120	NT	NT	NT
180	NT	NT	NT
240	NT	NT	NT
Time	**Anti-radical scavenging activity (µg Vit.CE/mL extract)**		
	MA	**MERT**	**SE**
7	103.79±0.89	123.33±2.62e	134.02±1.85
15	83.82±2.10	125.67±1.72e	112.62±1.04
30	76.85±0.81c	108.00±2.04f	91.36±1.47
45	73.65±2.51c	105.03±1.74f	85.02±0.49
60	56.37±1.51	78.05±2.22	68.20±2.23
120	38.53±3.13d	57.07±2.85	18.11±2.82g
180	35.90±1.82d	40.68±2.06	18.01±1.04g
240	13.12±1.41	10.73±2.63	ND

A column with English letters raised to the power (a,b,c,d,e,f,g) and rows 7 15 30 45 60 120 180 and 240 min with the same asterisk symbol (*,**) indicate that the obtained values were not significantly different at 95 percent confidence (P-Value > 0.05). 100 W ME; Microwave extraction method at 100 watts, 200 W ME; Microwave extraction method at 200 watts, BE; Boiling extraction method, MA; Maceration extraction method at 25ºC, MERT Maceration extraction method at room temperature, SE; sonication extraction method, NT; Not test and ND; Not detected.

In each extraction method, the result is that the phytochemical content increases with the extraction time but after a prolonged period, the phytochemical content started decreasing due to the loss and degradation of components (14). On the other hand, for the antioxidant activity of each extraction method, the obtained values were largely independent of the extraction time. Except for sonication extraction. The relationship between the phytochemical content and antioxidant activity of each extraction method may depend on the structure of the compounds (15). As a result, the effect obtained varies according to the extraction period.

In a previous report, Red Roselle from Indonesia with a moisture content below 10% was extracted by using water as a solvent at pH 7 and stirred at 200 rpm for 5 hr. at 5, 30, and 55ºC. Extracts of each condition extraction at a concentration of 5000 ppm were analyzed for the total anthocyanin content. The results indicated that the estimation of the total anthocyanin was 50, 30, and 20 mg/l, respectively (16). Previous research provides a greater amount of anthocyanin than this research which might involve the moisture content of Red Roselle before extraction and the concentration of Red Roselle extract for total anthocyanin analysis

4. Conclusions

In this research, Dried Red roselle with a moisture content of 19.92 percent, and the pH of red roselle water extracts were in the range of 2.00-2.50. Red roselle water extraction results on anthocyanin content showed that the optimum conditions were boiling extraction for 15 minutes. The extraction method that gave the highest total phenol and total flavonoid content was microwave extraction at 100 watts for 30 minutes. The extraction method with the highest anti-radical scavenging activity was sonication extraction for 7 minutes.

Acknowledgments

We would like to thank the Chemistry Major, Faculty of Science, Technology, and Agriculture, Yala Rajabhat University.

Declaration of conflicting interests

The authors declared that they have no conflicts of interest in the research, authorship, and this article's publication.

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