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Comparison of Phenolic Compound, Colour Value, and Antioxidant Activity of Roselle Calyces Extract Between Modified Supercritical Carbon Dioxide and Conventional Extraction

Zuhaili Idham[1,*], Noor Azwani Mohd Rasidek[2], Nicky Rahmana Putra[2], Dwila Nur Rizkiah[2], Nur Husnina Arsad[2], Mohd Azizi Che Yunus[2]

[1] Ibnu Sina Institute for Scientific & Industrial Research (ISI-SIR), Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia
[2] Centre of Lipids Engineering & Applied Research (CLEAR), ISI-SIR, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia

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ABSTRACT

Supercritical carbon dioxide (SC-CO_2) is a clean and green technology for extracting polyphenols and antioxidants from roselle (Hibiscus Sabdariffa) as nutraceutical ingredients and high-value co-products development. To increase the SC-CO_2 fluid affinity towards polar compounds, general regard as a safe (GRAS) solvent, ethanol-water was added in a small amount as a modifier. Dry roselle calyces were extracted using modified SC-CO_2 and conventional methods (Soxhlet and Shaking Water Bath) to determine whether the SC-CO_2 technology may improve the extraction of phenolic content and antioxidant activity. The results showed that the SC-CO_2 extract had a significantly higher yield of total phenolic and flavonoid content than the conventional extraction method. The conventional method produces a redder but lighter extract than SC-CO_2 extraction. SC-CO_2 also had a higher antioxidant activity as measured by DPPH radical scavenging. These results suggest that SC-CO_2 technology increases the quantity and quality of roselle calyces' extract. The findings increased the reliability of using this technique to produce high-value products from this high-value plant. Roselle as a low-cost sustainable local crop source, as well as SC-CO_2 as a clean energy process with low environmental impact, excellent solvent recyclability, and reduced chemical use, could increase the market value of antioxidant-rich extract.

1. Introduction

Roselle, or its scientific name, Hibiscus sabdariffa Linn. from the Malvaceae family, is an annual, erect, bushy, herbaceous sub-shrub that grows to 8 feet in height and typically consists of a red calyx with five large sepals. Though its origin is uncertain, the plant is cultivated in many tropical and subtropical countries, including Malaysia, India, Saudi Arabia, China, Indonesia, Philippines, Sudan, Egypt, Nigeria, and Mexico [1]. The red calyces could provide a natural colorant through the anthocyanins pigment. The red calyces also contain high antioxidants from the phenolic and

* *Corresponding author.*
E-mail address: zuhaili@cheme.utm.my

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flavonoid compounds. The antioxidant capacity of phenolic compounds implies that they might be exploited as natural food additives.

Phenolic compounds have been extracted for decades using conventional methods such as Soxhlet, maceration, infusion, and digestion [2, 3]. Soxhlet extraction and maceration generally use high solvent/feed (S/F) ratios (above 20) and long extraction times for the exhaustive extraction of all compounds from a matrix [4]. Thus, these procedures are often utilized as a reference against which the efficacy of advanced alternative methods may be measured.

Supercritical carbon dioxide extraction (SC-CO_2) is an eco-friendly extraction method that favors the extraction of the most bioactive compounds due to the solvent's eco-friendliness and the recovery method's effectiveness [5]. Even though SC-CO_2 has significant associated costs as one of its key limitations, it does not cause thermolabile compound degradation as badly as other extraction methods [6]. Nonetheless, SC-CO_2 has limits when it comes to extracting slightly polar and non-polar molecules. To address this constraint, a polar modifier is added to the SC-CO_2 system to improve polar analyte extraction [7]. Ethanol and water were used as co-solvents, in this case, to alter the solvent power or selectivity of CO_2 for a better result in the extraction of polar pigment compounds.

Therefore, the objectives of this study were to investigate the performance of modified SC-CO_2 compared with two conventional extraction methods on polyphenol compounds, red color characteristics, and the antioxidant activity of roselle calyces.

2. Methodology

2.1 Materials

The dried roselle calyces from Ladang Setiu Terengganu were bought from Ekomekar resources. A warning blender was used to completely crush the dried materials. The ground sample was sieved (Retshch, Germany), and particle sizes in the range of 250- 300 µm were chosen. The ground sample was maintained in the freezer (-20°C) until it was needed. Ethanol, Folin–Ciocalteau, sodium carbonate, aluminium nitrate, potassium acetate, sodium chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and hydrochloric acid were purchased from Sigma-Aldrich (Selangor, Malaysia). Meanwhile, the standard reagent, gallic acid, and quercetin were purchased from Merck (Sigma-Aldrich (M) Sdn Bhd). These compounds were utilized without being purified or treated in any way.

2.2 Supercritical Carbon Dioxide (SC-CO_2) Extraction

The experimental work was carried out using an SC-CO_2 unit fabricated at the Centre of Lipids Engineering and Applied Research, Universiti Teknologi Malaysia. The equipment comprises an oven with a 3 mL stainless steel extraction vessel. A back-pressure (Jasco, BP-2080 Plus) valve was added to the line between the extraction vessel and the separator to manage the pressure in the vessel. A water circulation bath (WiseCircu) aids depressurization by converting CO_2 from a supercritical to a gaseous state, which then separates CO_2 from the extract. Using a cooled bath circulator (Daihan Scientific. Co Ltd, Korea) and a carbon dioxide liquid pump, a consistent flow rate of carbon dioxide gas (Kras Instrument Sdn Bhd) at 3.7 mL/min and 99.99 % purity was liquidized and pumped to an extractor (Tokyo, Japan). A modifier pump was used to apply 75:25 mixtures of ethanol and water at a flow rate of 0.3 ml/min.

1.5 g of dry calyces were subjected to pressures of 10, 20, and 30 MPa and temperatures of 55°C for 2 hours' extraction time. The samples were fed into the extraction cell, sealed tightly, and then placed in the extraction chamber to allow the system to attain the desired temperature. After the system has reached the desired state, the extraction process begins. To remove CO_2 gas from the

separator, it was depressurized. At 38°C, the extract was passed through a centrifugal vacuum evaporator (Mivac concentrator) to remove the co-solvent. Until further investigation, the concentrated extract was kept in the freezer (-20 °C).

2.3 Soxhlet Extraction (SE)

5g of ground roselle calyces was combined with 160 mL of 75% ethanol. It takes roughly 6 hours to complete the Soxhlet extraction until clear extract. The solvents were then extracted using a rotary evaporator (Heidolph, Germany) at a 38°C. The remaining extract was then frozen (at -20 °C) for further examination

2.4 Shaking Water Bath Extraction (SWB)

A shaking water bath instrument (NE5-28D Series Clifton, Nickel-Electro Limited, United Kingdom, UK) was used for maceration extraction (ME). The extraction was carried out at 55°C for 6 hours using 75% ethanol. The extract was filtered and re-extract until the calyces were pale. The extract was then evaporated using a rotary evaporator at 38°C and stored in a freezer (-20°C) for analysis.

2.5 Extraction Analysis

The analysis of phenolic compounds, antioxidant activity, and colour characteristics used UV-Vis Spectrophotometer (Jasco, Japan). Before analysis, all the concentrated extracts were diluted with 1 mL of distilled water for the consistency of samples. The TPC was calculated as mg of gallic acid (GAE) per 100g of dried roselle calyces extract using a modified Folin-Ciocalteu method from [8]. The TFC of the roselle calyces extract was analysed according to the published method [9] with slight modifications. TFC was expressed as mg of quercetin (QUE) per 100g of dried calyces. Meanwhile, the radical scavenging ability in the DPPH activity of the roselle extracts was analysed using the method by Li *et al*., [10]. Radical scavenging ability was calculated using Eq. (1);

$$\text{DPPH radical-scavenging activity (\%)} = \frac{(A0-(A1-A2))}{A0} \times 100 \quad (1)$$

where A0 is the absorbance of the control (ethanol instead of the sample solution), A1 is the absorbance of the sample, and A2 is the absorbance of the sample under identical conditions as A1 with ethanol instead of DPPH solution.

The CIELAB or CIE L*, a*, b* colour space diagram was used to determine the colour of the red colour extract from roselle calyces with a D65 light source and 10° observation angle. The colour characteristic was expressed as L* (100 = white; 0 = black) is an indication of lightness; a* measures chromaticity, with positive values indicating redness and negative values indicating greenness; and b* measures chromaticity, with positive values indicating yellowness and negative values indicating blueness. RGB colour was calculated using OpenRGB software (logicol).

3. Results

The extraction of roselle calyces in this research was done using three extraction methods, namely SC-CO_2, SE, and SWB. The results obtained for the extract were compared between these three methods for the total phenolic content, total flavonoids, red colour characteristics, and antioxidant activity.

3.1 Comparison of Total Phenolic Content and Total Flavonoid Content

Figure 1a and 1b show the TPC and TFC values of roselle calyces in SWB, SE, and modified SC-CO_2 extraction at three different pressure conditions. It can be seen that using modified SC-CO2 at 20MPa gives the highest percentage yield of TPC and TFC among other extraction conditions. The lowest extraction yield of TPC was obtained using SWB (70.08 mg GAE/100g), while the lowest extraction yield of TFC was detected in Soxhlet extraction (546.71 mg QUE/100g).

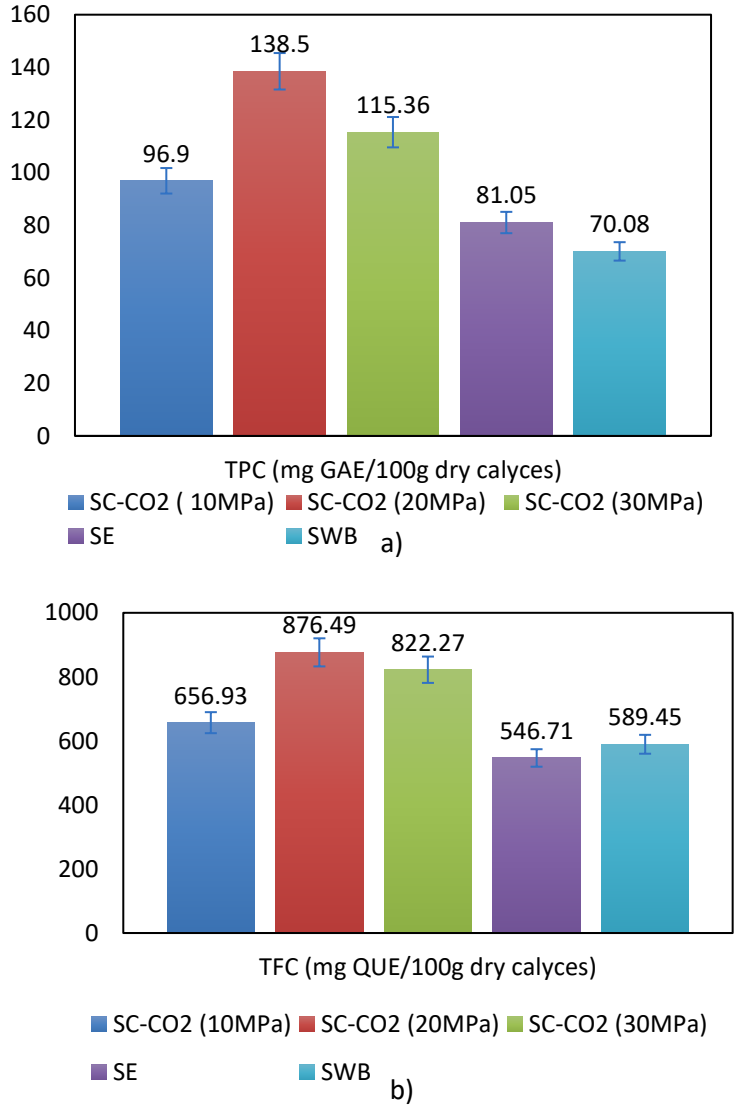

Fig. 1. Comparison of SC-CO_2 and conventional extraction of a) TPC and b) TFC

This study shows the ability of modified SC-CO_2, especially at higher pressure, to accelerate the mass transfer of bioactive compounds from the cell into the solvent. When there is an increase in extraction pressure, the intermolecular distance decreases, resulting in a higher degree of interaction between the soluble compounds and CO_2, consequently resulting in an improvement in extraction yield. However, the decrease in TPC and TFC was observed at 30 MPa. An increase in pressure beyond a critical limit decreases SC-CO_2 diffusion ability, owing to increased compaction of the samples at higher pressure, which causes the SC-CO_2 to channel around it rather than diffusing through it [11].

Song *et al.,* [12] found that as pressure increased from 15 to 25 MPa, the extraction of flavonoids from the *Ziziphus jujuba* Mill. leaves increased. This might be attributed to an increase in SC-CO_2 density. Meanwhile, as the pressure increased to 45 MPa, the flavonoid content decreased. When compared to the flavonoid, an increase in pressure above 25 MPa may significantly impact the extraction of other molecules. This trend is due to a decrease in SC-CO_2 selectivity, which reduces flavonoid content due to the diluting effect [12]. A different study on the extraction of the phenolic compound epigallocatechin-3-gallate (EGCG) from green tea showed that the yield increased as pressure rose from 10 to 20 MPa. Nonetheless, a further increase to 30 MPa has decreased the concentration of EGCG [13].

Regardless, SWB may take a long time and a high temperature before the compound can be released into the solvent. The decreased value of TFC and TPC was presumably due to the degradation of quercetin and gallic acid at a longer extraction period and a high boiling point of ethanol/water solvent (greater than 78°C) during the SE, even though the exhaustive extraction occurred.

3.2 Comparison of Colour Characteristics

Table 1 shows the colour characteristics of roselle calyces extracted by a different method of extraction. From the results, CIE LAB colour was measured with the following colour coordinates: lightness (L*), redness (a*, red-green), and yellowness (b*, yellow-blue). The L*, a*, and b* values of Roselle extracted by Soxhlet were 41.63, 60.03, and 42.71, respectively. The L*, a*, and b* values of Roselle extracted by SWB were 39.63, 63.04, and 52.45, respectively. A slightly lower value of L* and a* values of Roselle extracted by modified SC-CO_2 are in the range of 22.91-32.69 of L* and 50.77-57.51 of a*. The results from this study showed more red colour intensity from roselle extracted by the conventional method (SE and SWB). RGB colour was observed to demonstrate the colour of the extract. It was found that the SC-CO_2 has a dark red colour of extract as compared with conventional, which has a redder colour but is brighter. Some literature showed that the roselle calyces contained other natural constituents of an organic acid such as malic, citric, and 3-indolyl acetic acids, which played an important role in giving the brilliant red colour of the sample extract [14, 15]. This compound was probably extracted by conventional extraction as a less selective extraction method.

Table 1
Colour characteristics of different extraction methods

Extraction condition	Color characteristics			RGB Colour
	L*	a*	b*	
SC-CO_2 (10MPa)	32.69	56.01	51.59	
SC-CO_2 (20MPa)	22.91	50.77	39.49	
SC-CO_2 (30MPa)	29.44	57.51	50.38	
SE	41.63	60.03	42.71	
SWB	39.63	63.04	52.45	

3.3 Comparison of Antioxidant Activity

Roselle anthocyanins have been demonstrated to have a high antioxidant capacity [1, 14, 16]. In particular, the level of hydroxylation on the 3' and 4' positions of the B-ring structure is a fundamental determinant of their radical scavenging activity [16]. DPPH is a known and widely used spectrophotometric method for evaluating the antioxidant capacities of compounds. This approach is based on the free radical's (DPPH) propensity to react with a hydrogen donor (AH+) [17]. The

antioxidant donates electrons to neutralize the DPPH radical. This free radical is stable, but when the electron delocalizes, it produces a purple hue, resulting in an intense absorption in the UV–vis spectral region at 517 nm. When DPPH reacts with a hydrogen donor, the reduced form, DPPH, is produced, and the violet colour fades. As a result, the decrease in DPPH provides an index for assessing the extract's ability to capture radicals [18].

Figure 2 indicates that roselle calyces extract obtained via SC-CO_2 with EtOH/H2O as co-solvent has a high capability in scavenging the DPPH free radicals with the highest value of 89.55% at 55°C and 30MPa. When using lower pressure values, the antioxidant activity data still showed higher with 87.37% and 88.89% at 20 MPa and 10 MPa, respectively. The result was not associated with TPC and TFC, thus it's possible that other substances such as anthocyanins and other phenolic compounds were responsible for the antioxidant value. As compared with SE and SWB, 83.27% and 86.21% of antioxidant activity were calculated in this study. Wu *et al.,* [19] observed the DPPH radical scavenging activity was 80% when the sample of roselle concentration was 7.5 mg/mL while Suryaningsih *et al.,* [20] reported 80.68% of antioxidant activity in roselle calyces extract. Both extractions were performed using the conventional maceration extraction method.

Similar findings by Jiao and Kermanshahi pour [22] reported that SC-CO_2 extraction of haskap berry had a DPPH scavenging effect of 82.4 - 90.7%, which was higher than the 72.2 - 78.0% found in conventional extracts. Guedes *et al.*, [23] also discovered that the antioxidant activity of Synadenium grantii associated with anthocyanins content was higher in SC-CO_2 than in the conventional method. The increased acidity in extract due to CO_2 solubilization was attributed to the increased antioxidant activity of extracts obtained from plant materials using SC-CO_2 [24, 25].

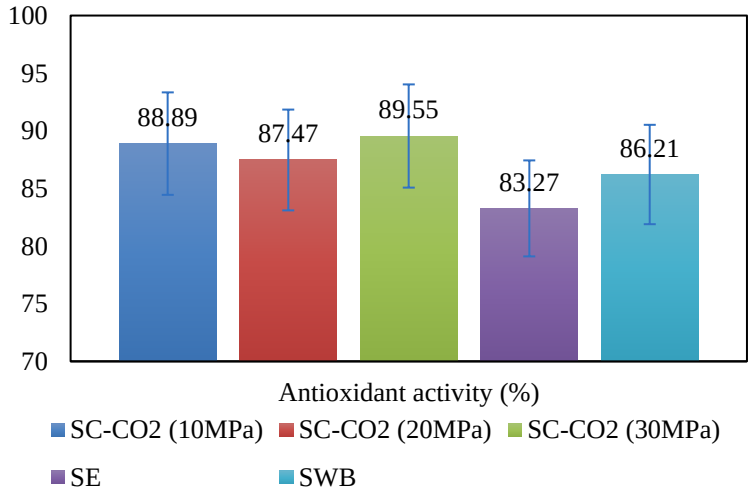

Fig. 2. Antioxidant activity of SC-CO_2 and conventional extraction

4. Conclusions

Roselle calyces extracted with SC-CO_2 contained high phenolic and flavonoid compounds and exhibited high antioxidant activity compared to SWB and SE methods. The findings of this investigation indicated that SC-CO_2 at 20 MPa could be employed for the extraction of phenolic and colorant compounds from roselle calyces owing to the effective mass transfer of than conventional extraction method. Due to the modest quantity and high purity of phenolic compounds, it was recommended that SC-CO_2 extract be used as an ingredient for the high-value product in the nutraceutical and cosmetic industries, or as a natural food colorant.

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