



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 Rasidek², Nicky Rahmana Putra², Dwila Nur Rizkiah², Nur Husnina Arsad², Mohd Azizi Che Yunus²

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

ARTICLE INFO

Article history:

Received 6 October 2022

Received in revised form 29 November 2022

Accepted 23 December 2022

Available online 14 January 2023

Keywords:

Roselle; Modified supercritical carbon dioxide; Phenolic; Antioxidant

ABSTRACT

Supercritical carbon dioxide (SC-CO₂) 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-CO₂ 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-CO₂ and conventional methods (Soxhlet and Shaking Water Bath) to determine whether the SC-CO₂ technology may improve the extraction of phenolic content and antioxidant activity. The results showed that the SC-CO₂ 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-CO₂ extraction. SC-CO₂ also had a higher antioxidant activity as measured by DPPH radical scavenging. These results suggest that SC-CO₂ 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-CO₂ 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

<https://doi.org/10.37934/araset.29.2.204211>

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-CO₂) 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-CO₂ 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-CO₂ 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-CO₂ 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 CO₂ 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-CO₂ 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 (Retsch, 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–Ciocalteu, 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-CO₂) Extraction

The experimental work was carried out using an SC-CO₂ 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 CO₂ from a supercritical to a gaseous state, which then separates CO₂ 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 CO₂ 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);

$$\text{DPPH radical-scavenging activity (\%)} = \frac{(A_0 - (A_1 - A_2))}{A_0} \times 100 \quad (1)$$

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 (logical).

3. Results

The extraction of roselle calyces in this research was done using three extraction methods, namely SC-CO₂, 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-CO₂ 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).

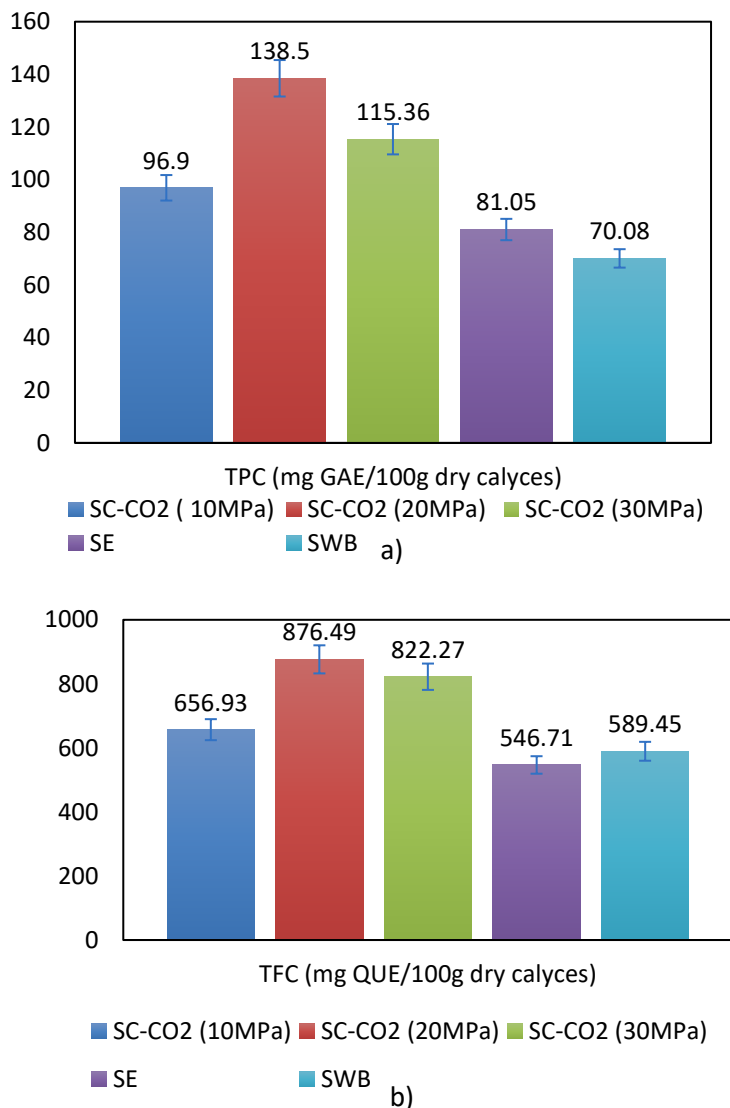


Fig. 1. Comparison of SC-CO₂ and conventional extraction of
a) TPC and b) TFC

This study shows the ability of modified SC-CO₂, 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 CO₂, 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-CO₂ diffusion ability, owing to increased compaction of the samples at higher pressure, which causes the SC-CO₂ 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-CO₂ 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-CO₂ 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-CO₂ 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-CO₂ 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-CO₂ 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-CO₂ 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-CO₂ than in the conventional method. The increased acidity in extract due to CO₂ solubilization was attributed to the increased antioxidant activity of extracts obtained from plant materials using SC-CO₂ [24, 25].

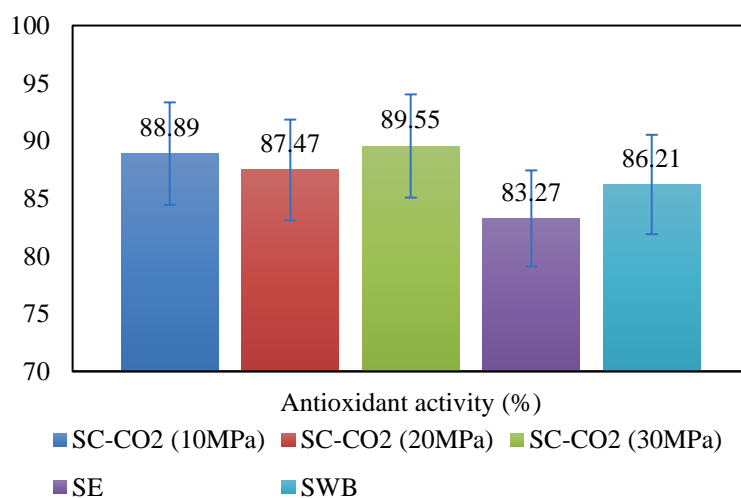


Fig. 2. Antioxidant activity of SC-CO₂ and conventional extraction

4. Conclusions

Roselle calyces extracted with SC-CO₂ 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-CO₂ 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-CO₂ extract be used as an ingredient for the high-value product in the nutraceutical and cosmetic industries, or as a natural food colorant.

Acknowledgement

We would like to thank UTM ICONIC RA Grant (Vote No. Q. J130000.4351.09G56) and also the Centre of Lipids Engineering and Applied Research (CLEAR), UTM for the instruments provided.

References

- [1] Da-Costa-Rocha, Inês, Bernd Bonnlaender, Hartwig Sievers, Ivo Pischel, and Michael Heinrich. "Hibiscus sabdariffa L.—A phytochemical and pharmacological review." *Food chemistry* 165 (2014): 424-443. <https://doi.org/10.1016/j.foodchem.2014.05.002>
- [2] Fiori, L., D. De Faveri, A. A. Casazza, and P. Perego. "Grape by-products: extraction of polyphenolic compounds using supercritical CO₂ and liquid organic solvent—a preliminary investigation Subproductos de la uva: extracción de compuestos polifenólicos usando CO₂ supercrítico y disolventes orgánicos líquidos—una investigación preliminar." *Cyta-Journal of Food* 7, no. 3 (2009): 163-171. <https://doi.org/10.1080/11358120902989715>
- [3] Jauhar, Safiullah, Mohammad Rashedi Ismail-Fitry, Gun Hean Chong, Mahmud Ab Rashid NorKhaizura, and Wan Zunairah Wan Ibadullah. "Polyphenol compounds from Pomegranate (*Punica Granatum*) extracted via various methods and its application on meat and meat products: a review." *Journal of Advanced Research in Applied Sciences and Engineering Technology* 12, no. 1 (2018): 1-12.
- [4] Tyśkiewicz, Katarzyna, Marcin Konkol, and Edward Rój. "The application of supercritical fluid extraction in phenolic compounds isolation from natural plant materials." *Molecules* 23, no. 10 (2018): 2625. <https://doi.org/10.3390/molecules23102625>
- [5] Aris, Noor Aiysah, Nor Faadila Mohd Idrus, Lee Nian Yian, Muhammad Syafiq Zuhaili Idham, Hazwan Ruslan, and Mohd Azizi Che Yunus. "The Effect of Fluid Flow Rate and Extraction Time in Supercritical Carbon Dioxide." *Journal of Advanced Research in Applied Sciences and Engineering Technology* 16 (2019): 26-34.
- [6] Da Silva, Rui PFF, Teresa AP Rocha-Santos, and Armando C. Duarte. "Supercritical fluid extraction of bioactive compounds." *TrAC Trends in Analytical Chemistry* 76 (2016): 40-51. <https://doi.org/10.1016/j.trac.2015.11.013>
- [7] Mohamed, Masniza, W. R. W. D. Mahmood, and Masturah Markom. "Cosolvent selection for supercritical fluid extraction (SFE) of bioactive compounds from orthosiphon stamineus." *Sains Malaysiana* 47, no. 8 (2018): 1741-1747. <https://doi.org/10.17576/jsm-2018-4708-13>
- [8] Kupina, Steve, Chris Fields, Mark C. Roman, and Sharon L. Brunelle. "Determination of total phenolic content using the Folin-C assay: single-laboratory validation, first action 2017.13." *Journal of AOAC International* 101, no. 5 (2018): 1466-1472. <https://doi.org/10.5740/jaoacint.18-0031>
- [9] Matejić, Jelena S., Ana M. Džamić, Tatjana M. Mihajilov-Krstev, Vladimir N. Ranđelović, Zoran A. Krivošej, and Petar D. Marin. "Total phenolic and flavonoid content, antioxidant and antimicrobial activity of extracts from *Tordylium maximum*." *Journal of Applied Pharmaceutical Science* 3, no. 1 (2013): 055-059.
- [10] Li, Jing, Huige Song, Nan Dong, and Guohua Zhao. "Degradation kinetics of anthocyanins from purple sweet potato (*Ipomoea batatas* L.) as affected by ascorbic acid." *Food Science and Biotechnology* 23, no. 1 (2014): 89-96. <https://doi.org/10.1007/s10068-014-0012-9>
- [11] Reverchon, Ernesto, and Iolanda De Marco. "Supercritical fluid extraction and fractionation of natural matter." *The Journal of Supercritical Fluids* 38, no. 2 (2006): 146-166. <https://doi.org/10.1016/j.supflu.2006.03.020>
- [12] Song, Lijun, Pengzhan Liu, Yinzhuo Yan, Ying Huang, Bingyao Bai, Xujie Hou, and Li Zhang. "Supercritical CO₂ fluid extraction of flavonoid compounds from Xinjiang jujube (*Ziziphus jujuba* Mill.) leaves and associated biological activities and flavonoid compositions." *Industrial Crops and Products* 139 (2019): 111508. <https://doi.org/10.1016/j.indcrop.2019.111508>
- [13] Ghoreishi, S. M., and E. Heidari. "Extraction of Epigallocatechin-3-gallate from green tea via supercritical fluid technology: Neural network modeling and response surface optimization." *The Journal of Supercritical Fluids* 74 (2013): 128-136. <https://doi.org/10.1016/j.supflu.2012.12.009>
- [14] Salmerón-Ruiz, Mayra L., J. Abraham Domínguez-Avila, J. Fernando Ayala-Zavala, Emilio Alvarez-Parrilla, Mónica A. Villegas-Ochoa, Sonia Guadalupe Sáyago-Ayerdi, Martín Valenzuela-Melendez, and Gustavo A. González-Aguilar. "Optimization of total anthocyanin content and antioxidant activity of a *Hibiscus sabdariffa* infusion using response surface methodology." *Biotechnia* 21, no. 2 (2019): 114-122. <https://doi.org/10.18633/biotechnia.v21i2.937>
- [15] Frimpong, Grace, Joseph Adotey, Kwabena Ofori-Kwakye, Samuel Lugrie Kipo, and Yaw Dwomo-Fokuo. "Potential of aqueous extract of *Hibiscus sabdariffa* calyces as colouring agent in three paediatric oral pharmaceutical formulations." *Journal of Applied Pharmaceutical Science* 4, no. 12 (2014): 001-007.
- [16] Obouayeba, A. P., N. B. Djyh, S. Diabate, A. J. Djaman, J. D. N'guessan, M. Kone, and T. H. Kouakou. "Phytochemical and antioxidant activity of roselle (*Hibiscus sabdariffa* L.) petal extracts." *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 5, no. 2 (2014): 1453-1465.
- [17] Machado, Ana Paula Da Fonseca, Miriam Rueda, Gerardo Fernández Barbero, Ángel Martín, Maria José Cocero, and

- Julian Martínez. "Co-precipitation of anthocyanins of the extract obtained from blackberry residues by pressurized antisolvent process." *The Journal of Supercritical Fluids* 137 (2018): 81-92. <https://doi.org/10.1016/j.supflu.2018.03.013>
- [18] Rahim, Nur Fazira Abdul, Norhayati Muhammad, Norazlin Abdullah Abdullah, Balkis A. Talip, and Nur Jihan Shahirah Dusuki. "Optimization of the antioxidant properties of the polyherbal formulations." *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences* 50, no. 1 (2018): 16-25.
- [19] Ariyabukalakorn, Varitha, Sumalee Panthong, and Arunporn Itharat. "Effects and chemical contents of hydrolysis modification of aqueous roselle extract to reflect the antioxidant and anti-inflammatory effects." *Science & Technology Asia* (2019): 115-125.
- [20] Wu, Hai-Yao, Kai-Min Yang, and Po-Yuan Chiang. "Roselle anthocyanins: Antioxidant properties and stability to heat and pH." *Molecules* 23, no. 6 (2018): 1357. <https://doi.org/10.3390/molecules23061357>
- [21] Suryaningsih, S., B. Muslim, and M. Djali. "The antioxidant activity of Roselle and dragon fruit peel functional drink in free radical inhibition." In *Journal of Physics: Conference Series*, vol. 1836, no. 1, p. 012069. IOP Publishing, 2021. <https://doi.org/10.1088/1742-6596/1836/1/012069>
- [22] Jiao, Guangling. "Extraction of anthocyanins from haskap berry pulp using supercritical carbon dioxide: Influence of co-solvent composition and pretreatment." *Lwt* 98 (2018): 237-244. <https://doi.org/10.1016/j.lwt.2018.08.042>
- [23] Guedes, Amanda Roman, Ariádine Reder Custódio de Souza, Rafaela Cristina Turola Barbi, Estephanie Laura Nottar Escobar, Éverton Fernando Zanoello, and Marcos Lúcio Corazza. "Extraction of *Synadenium grantii* Hook f. using conventional solvents and supercritical CO₂+ ethanol." *The Journal of Supercritical Fluids* 160 (2020): 104796. <https://doi.org/10.1016/j.supflu.2020.104796>
- [24] del Pilar Garcia-Mendoza, Maria, Faber Ariel Espinosa-Pardo, Andressa Mara Baseggio, Gerardo Fernández Barbero, Mário Roberto Maróstica Junior, Mauricio Ariel Rostagno, and Julian Martínez. "Extraction of phenolic compounds and anthocyanins from juçara (*Euterpe edulis* Mart.) residues using pressurized liquids and supercritical fluids." *The Journal of Supercritical Fluids* 119 (2017): 9-16. <https://doi.org/10.1016/j.supflu.2016.08.014>
- [25] Pereira, Paula, Maria-João Cebola, M. Conceição Oliveira, and M. Gabriela Bernardo-Gil. "Supercritical fluid extraction vs conventional extraction of myrtle leaves and berries: Comparison of antioxidant activity and identification of bioactive compounds." *The journal of supercritical fluids* 113 (2016): 1-9. <https://doi.org/10.1016/j.supflu.2015.09.006>