EASR

Engineering and Applied Science Research

https://www.tci-thaijo.org/index.php/easr/index

Published by the Faculty of Engineering, Khon Kaen University, Thailand

Green extraction of anthocyanins from roselle: A comparative evaluation of extraction techniques and solvents

Raihanah Roslan[1)], Muhammad Azri Mohamad Fauzi[1)], Natashah Emira Ahmad Amran[1)], Syamila Mansor[2)], Kiki Adi Kurnia[3)] and Muhammad Syafiq Hazwan Ruslan*[1, 4)]

[1)]School of Chemical Engineering, College of Chemical Engineering, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[2)]Faculty of Science and Technology, Universiti Sains Islam Malaysia, Bandar Baru Nilai, 71800 Nilai, Negeri Sembilan, Malaysia
[3)]CICECO - Aveiro Institute of Materials, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
[4)]Integrated Separation Technology Research Group (i-STRONG), Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

Received 10 October 2024
Revised 21 January 2025
Accepted 5 February 2025

Abstract

Due to the abundance of roselle in nature, the potential of the roselle to be a source of natural colorant with high anthocyanin content was explored. Moreover, in conjunction with the 12 principles and practices of Green Chemistry, one of the promising solvents that can offer greenness is deep eutectics solvents (DESs) with broad tunability and high selectivity as an alternative to volatile organic solvents. DESs are solvents comprising a combination of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) with lower melting points than their parents' salts. Therefore, this study explored the potential of DESs as an additive to water as solvents for the extraction of total anthocyanins content (TAC) from roselle extracts. By optimizing extraction parameters, including the best DESs, solid-to-solvent ratio, particle size, and extraction method, this study identified the most effective conditions. Based on the results obtained, the best solvents were choline chloride and triethylene glycol at a ratio of (1:1). Meanwhile, the best solid-to-solvent ratio is 1:15 (g/g). Furthermore, this study obtained the best extraction condition at 750 µm of average particle size using ultrasonic-assisted extraction (UAE) with a yield of 119.02 mg/L cyanidin-3-glucoside equivalence (GCE). As a result, UAE is a promising way to get anthocyanins out of roselle, using DESs as an extra solvent and following the set experimental rules. This study highlights the potential of DESs and the UAE to recover valuable compounds from natural sources and promote sustainable and environmentally friendly practices.

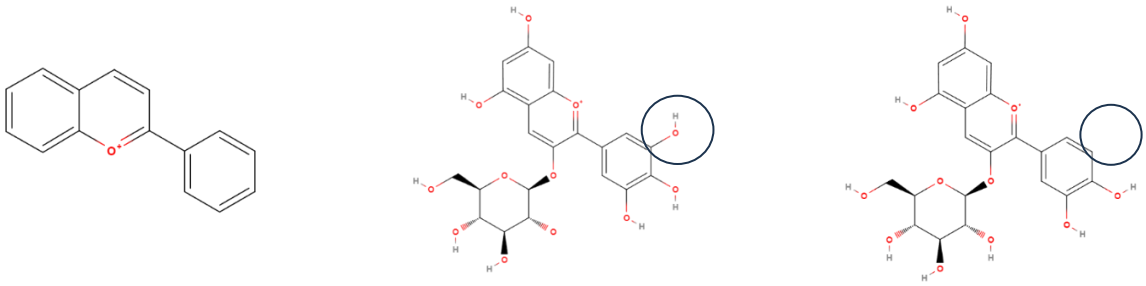
Keywords: Deep eutectics solvents, Irradiation assisted extraction, Anthocyanins content, Particle sizes

1. Introduction

As a member of the *Malvaceae* family, *Hibiscus sabdariffa*, also known as roselle, is gaining more attention as a natural red colorant due to its natural abundance, mainly in tropical regions such as Southeast Asia and West Africa [1]. The bright red calyces of the roselle, are mainly sold as commodities in processed delicacies such as jams, jellies, and drinks [2]. Roselle is rich in bioactive compounds such antioxidants, vitamins, and minerals, which could improve health for those who consume it. An analysis reveals that roselle contains 1484.3 mg of vitamin B per 100g, surpassing the recommended daily allowance suggesting its potential to serve as a significant source of vitamins [3]. Furthermore, the vibrant red color of the roselle can be attributed to the presence of anthocyanins as the major bioactive compounds in roselle. There are mainly two types of anthocyanins which are delphinidin-3-sambubioside and cyanidin-3-glucoside, that are present in the vacuoles of the calyces which differ only based on their hydroxyl groups, as seen in Figure 1 [4]. Additionally, studies have examined these anthocyanins for their potential to mitigate the risk of cancer and exhibit antigenotoxic properties [5]. Besides, the consumption of roselle juice as a supplement was found to be safe in low dosage, posing no risk to the liver and kidneys upon consumption [6]. This encourages the exploitation of these anthocyanins from roselle via various extraction methods.

Traditionally, anthocyanins were extracted from roselle using conventional maceration extraction. As depicted in Figure 2(a), for this method, the plant samples undergo a 24-hour soak in the solvent at room temperature with continuous stirring, resulting in the extraction of the desired compounds [7]. However, this method takes a long time, eventually leading to high energy consumption, thus contributing to a higher carbon footprint. Therefore, innovative modern extraction technologies such as irradiation-assisted extraction methods are gaining attention due to their potential to reduce the time taken and energy for each extraction. These methods are in line with the 12 principles of Green Chemistry that Paul Anastas proposed [8].

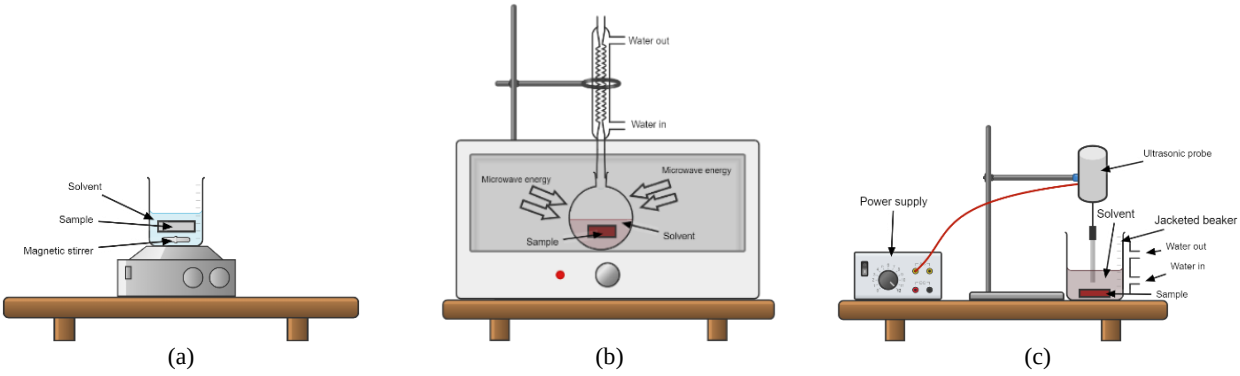
*Corresponding author.
Email address: syafiqhazwan@uitm.edu.my
doi: 10.14456/easr.2025.12



(a) (b) (c)

Figure 1 The molecular structures of anthocyanins (a), delphinidin-3-sambubioside (b), and cyanidin-3-glucoside (c). (b) and (c) can be differentiated based on the presence of the hydroxyl groups as circled in both diagrams.

These new irradiation-assisted extraction methods include microwave-assisted extraction (MAE) and UAE, which have different mechanisms that utilize radiation energy. MAE method utilizes microwave energy to extract the solutes from the solids, as seen in Figure 2 (b). The samples soaked in solvent are submitted to an enclosed microwave oven, allowing the sample solution to absorb all the radiated microwave energy. The extraction process could be explained through the localized superheating mechanism, wherein the samples absorb microwave energy, generating internal heat. This heat causes the degradation of solid cell walls, facilitating the secretion of solutes into the solvent [9]. Meanwhile, for the UAE, as illustrated in Figure 2 (c), the samples undergo exposure to ultrasonic energy. This exposure triggers a series of rarefaction and compression cycles, along with cavitations of bubbles within the solvents. These phenomena collectively enhance the rate of the mass transfer of the solutes into the solvents [10]. Hence, each extraction method will have a different effect on the extraction products; therefore, selecting a suitable method is a crucial factor in determining the extraction process.



(a) (b) (c)

Figure 2 Simple stirring maceration extraction at room temperature (a), rapid Microwave-assisted extraction (MAE) relying on superheating for faster extraction (b), and mechanical ultrasound-assisted extraction (UAE) relying on cavitation effect for enhanced extraction (c) setup.

Another critical element of efficient extraction is solvent selection. A solvent is the medium in which mass transfer of the solutes out of the solids occurs. Conventionally, the most utilized solvents are the volatile organic solvents and carbon-based solvents such as methanol and ethanol [11]. In the extraction of anthocyanins from strawberries, methanol was deemed to be the best extraction solvent when compared with chloroform-methanol magnesium oxide solvents with over 60% higher yield [12]. However, it's worth noting that these solvents are also classified as volatile organic compounds (VOCs), which have been identified as potentially hazardous to both handlers and the environment over the long term. Hence, the use of conventional solvents is discouraged as it contradicts the principles of green chemistry advocated by John Warner. These principles emphasize the elimination of toxic chemicals in processing and ensuring the safety of handlers [8, 13]. Concurrently, green solvents such as deep eutectics solvents (DESs) are gaining interest as novel solvents that can replace these organic solvents with better performances.

Thus, in the extractions and processing that involve natural products, DESs emerged as promising candidates to replace these toxic ILs by offering greener solutions that have the same capability and capacity. DESs are homogenous liquids comprising hydrogen bond donors and hydrogen bond acceptors, characterized by a lower melting point than their parent salts [14, 15]. Notably, DESs, particularly those consisting of choline chloride paired with carboxylic acids, have been utilized in the extraction of phenolic compounds from kinkeliba *(Combretum micranthum G. Don)*, demonstrating practical and enhanced extraction when compared with conventional solvents. The results showed that the total phenolic contents (TPC) obtained ranged from 23.61 to 17.44 mg GAE/g, higher than the TPC obtained by ethanol at 4.60 mg GAE/g of TPC [16]. This performance can be attributed to the high selectivity of the DESs, as they can be tailor-made following the characteristics of the solutes of interest. In the study, DESs have a more substantial hydrogen bonding capability to extract bioactive compounds than ethanol. The strong hydrogen bonding capability is due to more hydroxyl groups from the carboxylic acids of the DESs, which interact with each other without affecting other protons, thus indicating a strong hydroxyl attraction from the chlorine anion [17-19]. Moreover, due to the abundance of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) that exist in nature, the combinations of DESs are ample. This attribute enhances the versatility of DESs, as they can be customized to suit the specific requirements of the target products. Moreover, the implementation of DESs as an additive in organic solvents such as water has also shown a potential to obtain optimum yield [20]. Furthermore, the ratio of the HBA and HBD is also crucial in determining the extraction capacity of the DESs [21]. Thus, the right combination of the HBA and HBD and its ratio must be determined carefully.

Other extraction parameters, such as solid-to-solvent ratio and particle sizes, will also affect the yield. This is because each parameter will determine the mass transfer rate during the extraction process. An optimum solid-to-solvent ratio will determine the concentration gradient between the solid and solutes, determining the mass transfer rate. For instance, in the extraction of antioxidants in the form of total phenolic content and TAC from *Centella asaitica*, the best solid-to-solvent was the middle ratio at 1:15 instead of 1:20 and 1:25 with the best yield of 967.2 mg GAE/100g DW and 908.3mg CE/100g [22]. This is because, for the sample, the best ratio that could accommodate an effective concentration in which the solvent could solubilize all solutes is at the ratio of 1:15. Therefore, it is imperative to investigate the optimal solid-to-solvent ratio for each sample to achieve the highest yield possible.

Moreover, the particle sizes of the samples will also affect the yield of the product as the mass transfer rate of the solutes from the solids into the solvents will be influenced. Typically, the lower the particle size, the higher the total surface area available for the solvent to capture [23]. Thus, the mass transfer between the solutes and the solvent could be enhanced as the solvent can penetrate effectively into the solids and eventually dissolve all the target compounds within the solid matrix. Nevertheless, the particle size of each sample plant varies for the extraction of individual compounds. Therefore, it is necessary to determine the ideal particle sizes of the roselle samples to achieve optimal extraction efficiency and yield.

Additionally, the interactions between the parameters could be explained through statistical analysis, which serves to validate the significance of the obtained results [24]. In this case, the findings can be supported with statistical analyses such as analysis of variance (ANOVA) and desirability analysis. Therefore, the best experimental parameters can be determined by using a statistical approach. Furthermore, a mathematical model could also be established using statistical tools to predict the responses within the provided range further. Besides, the impact of the extraction methods on the sample matrix is also investigated by conducting a scanning electron microscopy (SEM) analysis. The difference in the surface morphology of the calyces after the extractions with three different methods could be studied to find the best extraction method.

Consequently, roselle has a broad potential to be utilized as a natural colorant. While traditional methods such as maceration and Soxhlet extraction are exhaustive and time-consuming, modern technologies such as MAE and UAE have emerged as efficient alternatives. However, the usage of conventional organic solvents raises environmental concerns. Hence, DESs as green and tunable solvents offer a promising solution. The synergistic effect of DESs and irradiation extraction techniques specifically to extract anthocyanins from roselle focusing on factors such as solvent composition, solid-to solvent ratio, and average particle sizes are still scarce. Therefore, by directly addressing the limitations of traditional solvents and extraction methods, this study not only highlights the potential of DESs as green and tunable solvents but also establishes UAE as a superior non-thermal extraction technique. The study aimed to provide a scalable and sustainable methodology for the recovery of high-value compounds from natural sources, contributing to greener industrial processes and environmental sustainability.

2. Materials and methods

2.1 Sample preparation and deep eutectic solvents preparations

The roselle was obtained from a local supplier located in Kuala Terengganu, Terengganu, Malaysia. The sample was then reduced into average particle sizes by steeping them with liquid nitrogen. The brittle samples were then crushed, grounded, and sieved to 1840, 1340, and 750 µm of average particle sizes by using a commercial blender. The average particle sizes chosen were based on literature ranging from 500 to 2000 µm [25-31]. A sieve shaker (GB/T 6003.1-2012, Endecotts Octagon 2000, United Kingdom) was used to separate the calyces into the specified particle sizes. Choline chloride (ChCl), triethylene glycol (TEG), levulinic acid (LevA) and oxalic acid (OA) (Sigma Aldrich, America), pH 1.0 and pH 4.5 buffer (System, Malaysia), and distilled water were obtained from the laboratory in UiTM Shah Alam. A 5 ± 0.05 g portion of each particle size sample was individually packed into a teabag.

The DESs were initially screened with a conductor screening model for real solvent (COSMO-RS) software with anthocyanins as the compounds of interest [32]. The range of ratios of HBA and HBD screened were 1:1, 1:2, and 2:1 was obtained from the literature. After the screening process, the best DESs were ChCl paired with betaine (Be), triethylene glycol (TEG), levulinic acid (LevA), and oxalic acid (OA). However, preparing the Be and ChCl DESs was difficult as both are quaternary ammonium salts, which are stable at high temperatures. Therefore, the DESs that were selected to be used in this extraction were ChCl paired with TEG, LevA, and OA (Merck, Germany). The DESs were synthesized as an additive of 5% in water. ChCl were mixed with TEG, LevA, and OA separately to form DESs with 1:1, 1:2, and 2:1 ratio of HBA and HBD by mass. The HBA and HBD were mixed at 80°C with continuous stirring for 2 hours until a homogenous transparent liquid was formed and water was added to make 5% DESs in water [14, 33].

2.2 Determination of total anthocyanins contents

For the extraction process results, the extracts were assessed using a total anthocyanin content analysis based on an AOAC official pH-differential method [34]. The extracts were first diluted with distilled water to obtain a dilution factor of 2. Then, 0.5 ml of each diluted extract was added with pH 1.0 and pH 4.5 buffer separately and left at room temperature for 30 minutes. After 30 minutes, the samples were analyzed in the UV-Vis spectrophotometry (Cary 60 UV-visible spectrometer, Varian, Inc, Palo Alto, CA, USA) at wavelengths of 520nm and 700nm for both pH buffer solutions. The absorbances of the samples were calculated as equation (1), and the total anthocyanin content (TAC) was calculated as equation (2).

$$A = (A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5} \quad (1)$$

$$TAC = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times L} \quad (2)$$

Where *MW* is the molecular weight of the cyanidin-3-glucoside, which is 449.2, *DF* is the dilution factor, which is 2, 10^3 is the conversion from g to mg, $\mathcal{E}$ is the molar extinction coefficient, which is 26900 L.mol^{-1}.cm^{-1} and *L* is the pathlength which is 1 cm. The TAC is expressed as mg/L cyanidin-3-glucoside equivalent (CGE).

2.3 Determination of the best ratio of DESs and optimum solid-to-solvent ratio

The experimental parameters were determined by stages. The first stage is to find the best ratio of the DESs and the solid-to-solvent ratio. In this attempt, the MAE method, as shown in Figure 2(b) was used to determine these parameters as it can accommodate various ratios of the solid-to-solvent ratio. For this extraction, 5 g ± 0.05 g of the sample was added into a round bottom flask with different ratios of 1:05, 1:10, 1:15, 1:20, and 1:25 (g of sample/ g of solvent). The range for the solid-to-solvent ratio was determined from the literature [4, 26-30]. The sample solvent mixture was then placed into a microwave oven at 400 W for 30 minutes. The microwave oven was also connected to a Liebig condenser to ensure the solvent would not evaporate. The microwave was modified by drilling a hole in the middle of the upper rack. Then, a connector was installed to extend the neck of the round-bottomed flask to connect with the condenser. The condenser was secured vertically by using a retort stand.

2.4 Determination of the best method and average particles sizes by using statistical tool

For the extraction parts, 5 g ± 0.05 g of sample was added with the predetermined solid-to-solvent ratio with the best DESs that were also predetermined. For the MAE method, the same power was used constantly at 400W; meanwhile, for the maceration (ME) and UAE, the stirring rate for the ME was kept constant at 400rpm, and the amplitude was kept constant at 40%. The extraction process was also kept steady at 30 minutes for all extraction methods. Meanwhile, the average particle sizes of the calyces studied were 1840 (A), 1340 (B), and 750 (C) µm. The experiments were conducted according to the design of the experiments generated by the software Design Expert with 13 runs and 4 repetitions. Design Expert software offers efficient experimental design and robust statistical analysis as the number of experimentations can be reduced and the statistical analysis is accurate to gain an insight into the interaction of the factors [35]. Moreover, a mid-point was added as repetition in the design to check for repeatability and reliability of the data produced.

$$y_k = \beta_o + \sum \beta_i x_i + \sum \beta_{ii} x_{ii}^2 + \sum \beta_{ij} x_i x_j + e \quad (3)$$

y is the response(s), k is respective to the response, β_0 is the mean response, βi is the linear coefficient for factor $x1$, βii is the quadratic coefficient for factor xi, βij, is the interaction coefficient for factor xij.

The interactions between the variables could be explained by finding the mathematical modeling for the extraction process. This is because, in establishing a mathematical model for the extraction process, equations are formed to describe the connection of the defined variables based on the analysis of the results as seen in equation (3). Therefore, to obtain an efficient extraction, the experimental parameters must be determined thoroughly, and the reliability of the results can be validated with the use of statistical tools.

2.5 Determination of surface morphology to study the effect of each method

For the scanning electron microscopy method, the samples were initially kept in a drying oven at 35ºC after the extraction process. Before the SEM analysis, the samples were coated with iridium for 37 sec with 30 mA of current. After the coating, the samples were submitted to an SEM machine (Hitachi, SU3500) for magnifications at 500, 1000, and 2500x at 15kV of electron acceleration voltage and a working distance of 10mm.

3. Results and discussions

3.1 The best solid-to-solvent ratio and the best DESs

The selection of an optimum solid-to-solvent ratio plays a vital role in influencing the concentration gradient between the solid material and the solvent, thereby inducing mass transfer efficiency [22]. In this study, as depicted in Figure 3, the data clearly shows that as the ratio increased from 1:05 to 1:15, there was a rise in the TAC values correspondingly. However, the TAC values were diminished after the ratio was increased to 1:20 and 1:25. The highest TAC value was achieved with a combination of ChCl and TEG at a ratio of 1:1 of HBA and HBD, along with the solid-to-solvent ratio of 1:15, yielding a significant TAC of 73.54 mg/L GCE. From the dynamics of the TAC yield at the ratio of 1:05, an insight that could be unveiled was that insufficient solvent volume fails to attract all solutes adequately. This limitation can be supported by previous research, where an increased solid-to-solvent ratio enhanced the solid-solute interactions, leading to higher antioxidant yield in the extraction of antioxidants from *Moringa Olifeira* leaves [36]. In the study, a solid-to-solvent ratio of 1:60 (g/mL) was found to be more favorable in achieving a higher yield of antioxidants when compared with the ratio of 1:30 (g/mL) [36]. Increasing the solvent volume could improve the solvent-solute interaction as the solubilization capacity is more facilitated. Therefore, in this study, the solid-to-solvent ratio of 1:15 facilitates the development of all solid particles by the solvent and enhances mass transfer mechanisms, as the ratio is enough to provide optimal saturation and concentration gradient, which is crucial for an effective mass transfer.

This can be explained by the fact that at the ratio of 1:15, there is enough solvent to envelope all the solid particles covering the whole surface area of the solutes [37]. Thus, the mass transfer rate could be enhanced, and more TAC could be achieved. Previously, in the extraction of curcuminoids from turmeric, the optimal solid-to-solvent ratio obtained was 1:15, yielding the highest amount of curcumin [38]. A similar trend could be observed in the extraction of anthraquinones from *Rheum palmatum*; when the solid-to-solvent ratio was increased, the yield of the anthraquinones obtained was higher, however; after the ratio reached 1:20 (g/mL), the yield decreased [39]. This is due to the solvent's ability to completely cover all the solutes in the anthraquinone extraction process at a ratio of 1:20, resulting in adequate saturation and concentration for effective mass transfer. As a result, different compounds require different ideal solid-to-solvent ratios for extraction; in this study, a high extraction of TAC can be achieved at a ratio of 1:15.

Meanwhile, for the solid-to-solvent ratio of 1:20 and 1:25 in this study, there is an excess of solvent to envelope the solids, thus causing an oversaturation in which there are not enough solutes to be extracted, eventually reducing the rate of mass transfer. This is due to the lowering of the solid-weight ratio and the decline in the density of the extraction solutes declining density as the solvent proportion increased [40]. Furthermore, a study on the extraction of sugars and polyols from coffee berries concluded that when there is a disproportionate amount of solvent exceeding the optimum ratio, the microwave will be absorbed excessively by the polar solvents, causing lower energy reaching the sample, thus lowering the yield of extraction [41]. Thus, the optimal solid-to-solvent ratio for the

DESs was 1:15, as the solvent was sufficient to provide enough saturation and concentration gradient to reach the sample, yielding a high extraction value.

Additionally, the incorporation of 5% DESs as an additive in water enhanced the selectivity of water towards anthocyanins in this study. However, due to its high polarity, water as traditional solvent lacks selectivity and specificity, thus leading to the extraction of all polar components indiscriminately [27]. Furthermore, DESs, in nature, are highly viscous due to the strong hydrogen bonding between the HBA and HBD [14, 42]. The high viscosity of the solvent can increase the mass transfer resistance between the solute and solvent, making the extraction inefficient. Therefore, DESs as an additive in water could reduce the overall viscosity, enabling a higher mass transfer rate between the solvent and the solid as the solubilization capacity of the solvent could be enhanced. Therefore, DESs as an additive can offer selectivity and customizability tailored to target specific solutes, especially in the extraction of anthocyanins. This can be attributed to the tunability and designability of the DESs, which can be tailor-made per the solutes of interest [14, 18, 43-45]. Notably, in this study, DESs formulated with ChCl and TEG at a ratio of 1:1 with a solid-to-solvent ratio of 1:15 can be seen to outperform water extraction methods, yielding significantly higher TAC values, as seen in Figure 3. The efficacy of TEG as HBD in the DESs can be attributed to its polarity and abundant hydrogen bonding capacity, which further enhances the solute attraction and thus increases the extraction capacity.

Consequently, the impact of HBD ratios in the DES formulation on the TAC yield has different insights. No visible trend could be inferred as the variation of the HBA and HBD have different influences on the TAC for each formulation. From Figure 3, for ChCl: TEG, the highest TAC was obtained at the ratio of 1:1 at 73.54 mg/L GCE, closely followed by a 1:2 ratio at 58.10 mg/L GCE, highlighting the TEG role in enhancing the TAC extraction. Conversely, ChCl: LevA, at a ratio of 2:1 of HBA to HBD, yielded the highest TAC at 53.91 mg/L GCE, indicating a superior effect of HBA content on extraction efficiency. Additionally, for ChCl: OA, an equal ratio of 1:1 of HBA and HBD was found to be the best for TAC yield at 60.60 mg/L GCE.

Thus, carboxylic acids such as LevA and OA exhibit distinct chemical characteristics compared to TEG, an alcohol rich in OH groups and hydrogen atoms. This difference influences the hydrogen bonding interactions within DESs. Furthermore, anthocyanins rich in HBD characteristics compete for hydrogen bonding with the chloride anion against TEG, LevA and OA. The abundance of HBD groups in TEG facilitates effective TAC extraction by enveloping the chloride anion, thereby preventing any interference with the anthocyanin bonding, and consequently enhancing the solute attraction, ultimately leading to increased TAC extraction [46, 47]. In this study, the application of ChCl: TEG at a ratio of 1:1 with a solid-to-solvent ratio of 1:15 was found to achieve a notable TAC of 73.54 mg/L GCE which is higher than when water was used as the solvent (20.9 ± 2.1 mg/100g of roselle) [48]. This shows the enhanced selectivity of DESs for anthocyanins when DESs were used as an additive in water. Furthermore, TEG's distinct polarity and hydrogen bonding capacity make it a superior HBD in the DES formulations, which outperform LevA and OA due to its abundance of OH groups and hydrogen bonding potential with the chloride anion [49, 50]. This finding underscores the influence of the HBD ratio and DESs component on the TAC extraction efficiency, allowing the tailored DESs formulation to optimize an effective extraction and enhanced selectivity for target solutes.

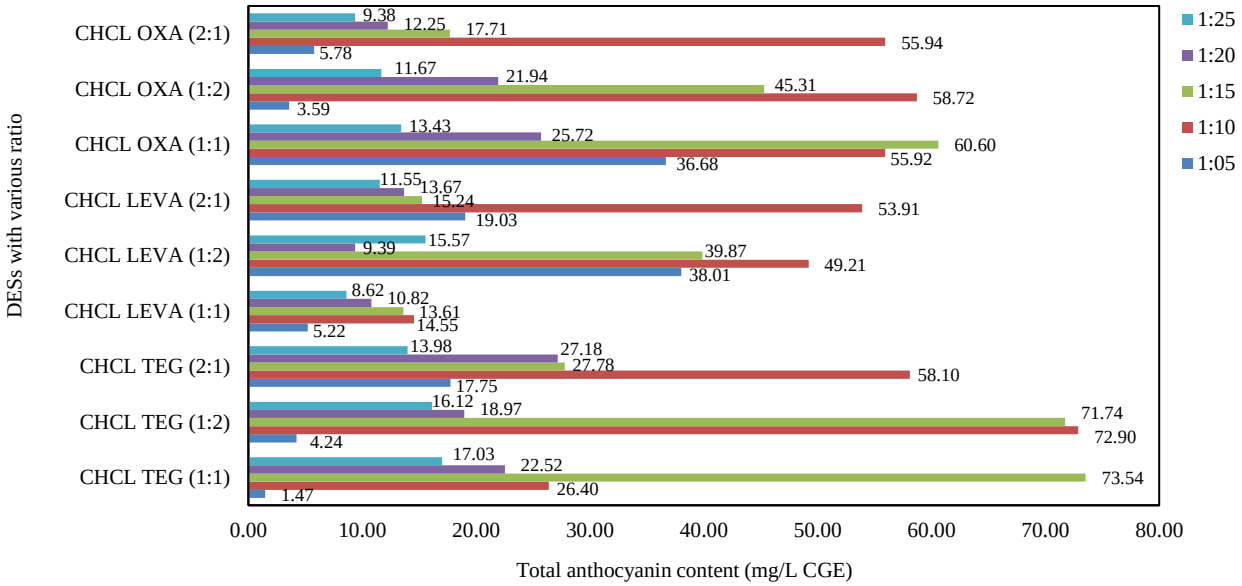


Figure 3 TAC (mg/L CGE equivalence) of roselle extracts obtained with various DESs at various ratio and solid to solvent ratios.

3.2 The best method and particle sizes

The irradiation methods used in this study were MAE and UAE to determine the optimal conditions for extracting the solutes of interest. Additionally, a statistical analysis was employed to enhance the significance of the results obtained, which will be discussed elaborately in the next section. Initially, this study focuses on identifying the best solvent and optimum solid-to-solvent ratio. Subsequently, the most effective irradiation method and average particle sizes were selected as the final parameters. A clear trend could be observed for average particle sizes optimization, as the particle sizes decreased, the TAC increased consistently across both MAE and UAE methods, as evident in Figure 4. For instance, in the UAE, for the lowest particle sizes at 750 µm, the TAC yielded 36% higher than the highest in 1840 µm. Similarly, with MAE, an average particle size of 750 µm yielded a 22% higher TAC compared with 1840 µm. Moreover, a 31% increase of TAC was obtained for particle size of 750 µm than 1840 µm for ME. This highlights the consistency of the pattern for all the methods, with a noticeable trend in UAE, where smaller average particle sizes consistently correlate

with higher TAC values in smaller particle sizes since there is a larger total surface area that can come into contact with the solvent thus increasing the solvent penetration into the cell walls [30] .

Consequently, the highest TAC was obtained for the roselle extracted with the UAE method with an average particle size of 750 µm, with the highest yield obtained at 119 mg/L CGE, as seen in Figure 4. This is because, at lower particle sizes, the solvent can cover more surface area of the solute particles at the right solid-to-solvent ratio [51]. Therefore, in this study, the lower the particle sizes, the higher the surface area of the solutes available for diffusion and solubility of in the solvent [52].

Furthermore, as seen in Table 1, it is evident that when the particle sizes reach their maximum at 2000 µm, the corresponding TAC value is at its lowest [25]. This highlights a hypothesis: smaller particle sizes correlate with higher TAC values. However, it is also important to note that despite having the lowest particle sizes of 125 µm for roselle, the TAC value obtained in the study was lower than anticipated [30]. This difference can be pointed out as a significant factor: the potential drawbacks associated with excessive fine particle sizes. Too small of particle sizes can hinder the solvent circulation, thus causing the separation and drainage of solids from the solvents to be more difficult [53, 54]. This increases mass transfer, consequently leading to lower TAC yield. Moreover, higher energy input is needed to produce finer particle sizes, thus risking the loss of bioactive compounds during post-production due to heat-induced degradation [55]. Furthermore, using finer average particle sizes poses additional safety concerns, such as the risk of dust explosion [56]. These factors underscore the need to optimize the particle sizes used to ensure maximum extraction efficiency while mitigating potential drawbacks and safety hazards.

Moreover, the extractions of antioxidant and phenolic compounds from Acai berries using MAE and UAE were also conducted [57]. In the study, MAE yielded the highest antioxidant activity while having the lowest phenolic contents; meanwhile, UAE yielded the highest phenolic content but with the lowest antioxidant activity. Both extraction methods were performed at the same temperature and same period. However, each response obtained showed a different reaction towards the extraction method applied. Therefore, each method will produce a different yield; thus, the selection of an efficient extraction method must be considered. Besides, to further support the performance of each extraction method using ChCl: TEG DESs in this study, the anthocyanins obtained from various extractions of roselles were also tabulated in Table 1. Based on Table 1, it could be seen that in ChCl: TEG DESs obtained higher TAC at 119.02 mg/L when compared with water, 39.1% ethanol, and ChCl and ethylene glycol solvents at 70.97 mg/L, 51.76 mg/L, and 17.87 mg/L respectively when UAE is applied [14, 58]. This can be attributed to the higher polarity of the ChCl when compared with ethanol and water due to the chlorine anion [49]. However, the lowest TAC obtained with the use of ChCl:EG DESs as solvents could be explained by the other factor, which is the particle size, which is at its highest at 1000 µm. The particle size is too high, causing the extraction to be lower. Furthermore, in comparing the MAE and ME methods, it could be seen that due to the involvement of rapid heating in MAE, the TAC obtained was lower than the ME method as ME is operated in room temperature conditions. Therefore, the solvent and method used in this study perform better than the currently reported data on the extraction of TAC from roselle.

Furthermore, in determining the best extraction methods to extract TAC in this study, it could be seen, based on Figure 4, that the highest yield was obtained by the UAE method, followed by ME and lastly, MAE at 119.02 mg/L, 91.51 mg/L, and 80.24 mg/L in that order. By comparing the extraction methods, UAE was proven to perform better than MAE at the same average particle size, this can be attributed to the nature of the mechanism. UAE is a method that utilizes sound waves in the form of ultrasound, which when submitted into the solvent containing the sample, will propagate a series of compression and rarefaction waves in the form of acoustic pressure [10]. This will induce the molecules to oscillate around their position in the liquid medium, causing the formation of cavitation bubbles. These bubbles will cavitate after repeated application of waves in the form of compression and expansion waves, causing the bubbles to grow and contract and eventually implode after they reach their critical sizes. The cavitation of these bubbles in the solvents will then cause the cell walls of the solids to disintegrate and thus secrete the solutes into the solvent medium. Furthermore, heating and cooling also occur due to the compression and expansion of the solvent bubbles. However, due to the usage of a jacketed beaker throughout the procedure, the heating of the solvents could be reduced as the water was being supplied continuously around the beaker. Therefore, the degradation of the thermolabile TAC could be avoided in the UAE.

Likewise, a similar study was conducted to compare the extraction performance of UAE with ME [27]. It was found that the TAC for UAE was ± 65 mg/L CGE equivalence while the TAC for ME was ± 15 mg/L CGE equivalence. This shows an apparent difference between the two methods, proving that UAE is superior in the extraction process. This is also parallel to a study on the extraction of anthocyanins from roselle to compare the UAE and ME methods [30]. It was found that the yield for the UAE was 70.97 mg/L CGE equivalence, which is higher than the ME at 65.29 mg/L CGE equivalence. The two results might show that there is just a slight difference between the values; however, if the time of the extraction is taken into consideration, the gap between the performance of the two methods is visible. UAE consumed only an hour to yield such content, while ME took 3 hours to obtain the TAC mentioned.

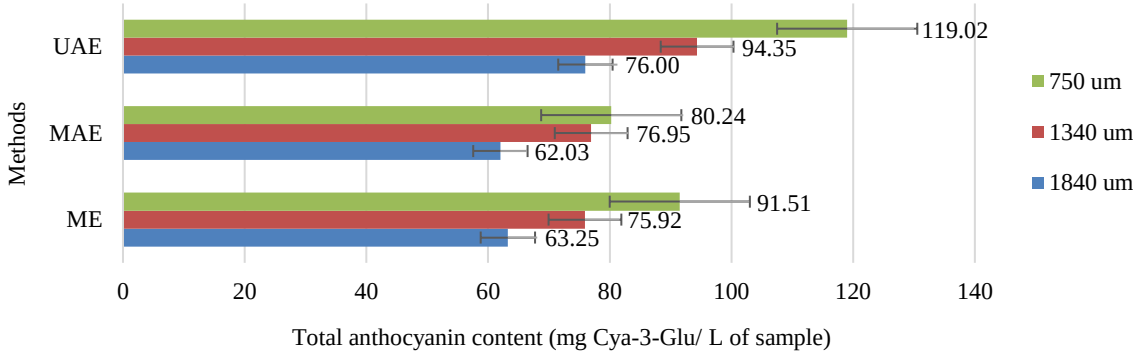


Figure 4 TAC (mg/L CGE equivalence) for roselle extracts obtained via different methods.

Furthermore, the usage of the MAE, which produced lower TAC than UAE and ME, could be explained due to the high heat involved in the extraction process [59]. MAE utilized the mechanism of localized superheating in which when the samples are submitted to the microwave energy, the solvents start to heat up internally due to the radiation of the microwave energy directly into the solvent.

Rapid heating causes the pressure inside the cells to build up and eventually collapse, thus secreting its solutes into the solvent medium. However, due to the thermolabile nature of the anthocyanins, the TAC might be degraded after a period of microwave submission. This explains why the TAC obtained with ME is comparatively higher than MAE, as no heat is involved in the extraction. A similar study could furthermore explain the situation in which antioxidants were extracted from strawberries to compare the performance of MAE and ME [60]. It was found that ME yielded a 5.77% yield, which is higher than the MAE yield, which yielded 1.55%. Given the numbers obtained, it was concluded that ME is a better extraction method than ME due to the yield obtained. However, the ME took 24 hours, while the MAE took only 5 minutes to obtain the yield. Therefore, considering the time taken for the processing, which would make a lot of difference if it were to be scaled up industrially, MAE could perform better. This is because a longer extraction time would consume more energy, thus causing this irradiation method to be not green.

Additionally, in the extraction of TAC from Acai berries, it was found that MAE yielded higher TAC when compared to UAE, in which MAE yielded 5.09 mg CGE/dm sample while UAE yielded 4.68 mg CGE/g sample [57]. This can be attributed to the fact that the solvent used in the extraction is ethanol; therefore, ethanol possessing a high dielectric constant is suitable for the MAE, thus enhancing the mass transfer rate. However, the content TAC obtained is higher than the values obtained, therefore proving the performance of DESs as a better solvent than ethanol. Likewise, UAE was also compared with MAE in extracting phenolics from grape skin using DESs [61] DESs made up of ChCl and OA were utilized as a solvent with 25% water when submitted into UAE, yielding ±31 mg/g dry weight of a sample of TAC, which is higher than MAE that yielded ± 27 mg/g dry weight of a sample of TAC. This is parallel with this study in which the difference between the UAE and MAE was shown numerally to have a big gap. This can be supported by statistical data.

Table 1 Comparison of the TAC values for roselle extracts obtained via various experimental parameters.

Method	Solvent	Experimental parameters				TAC	Ref
		A	B	C	D		
UAE	ChCl: TEG: H_2O 2.5%: 2.5%: 95%	750 µm	40% amplitude	1:15 (g/g)	30 min	119.02 mg/L	This work
UAE	H_2O	125 µm	20% amplitude	1:10 (g/ml)	15 min	70.97 mg/L	[30]
UAE	39.1 % EtOH (v/v)	850 µm	296.6 W	1: 30 (g/ml)	26.1 min	51.76 mg/L	[62]
UAE	ChCl:EG (1:4)	1000 µm	25% amplitude	0.25:20 (g/ml)	45 min	17.87 mg/L	[58]
MAE	ChCl: TEG: H_2O 2.5%: 2.5%: 95%	750 µm	400 W	1:15 (g/g)	30 min	80.24 mg/L	This work
MAE	ChCl: OA: H_2O 45: 55%	2000 µm	700 W	1:30 (ml/mg)	20 min	3.64 mg/g	[25]
ME	ChCl: TEG: H_2O 2.5%: 2.5%: 95%	750 µm	400 rpm stirring rate	1:15 (g/g)	30 min	91.51 mg/L	This work
ME	H_2O	125 µm	-	1:10 (g/ml)	15 min	65.29 mg/L	[30]

A is the average particle size of samples, *B* represents the amplitude which defines the intensity of the ultrasonic waves, W is the power applied, rpm is the stirring rate for ME, *C* is the solid-to-solvent ratio, and *D* is the time taken for extraction. *TAC* is expressed as mg cyanidin-3-glucoside equivalence for every 1L of extract or gram of sample.

3.3 Statistical analysis

Furthermore, Table 2 contains the regression analysis to establish the reliability and significance of the experimental factor's statistical analysis. There are two factors in which the particle sizes (A_1) are considered numerical factors, and methods (B_2) are included in the categorical factor. The methods compared were ME, MAE, and UAE, while the particle sizes were 750, 1340, and 1840 µm with the response as TAC. There were 12 experiments with three repetitions on a point for the UAE method at 750 µm. From the runs, the highest TAC was method UAE with particle size 750 µm yielding 119.02 mg/L. From the results shown in Figure 4, among all the several models that could be fitted, the regression analysis could be precisely described via a linear model. Based on Table 2, a significant value of the F-model shows that the model is significant at a 95% confidence level. This is because there is only a 0.01% chance that this model of F-value could occur due to noise. Meanwhile, all the models were highly significant as the Probability (prob>F) value is less than 0.0001. The model terms which are the particle size (A_1) and methods (B_2) are significant as the prob>F value is lower than ($p < 0.05$). Therefore, the two factors are both significant in the linear model and have an impact on the extraction efficiency of the TAC. The significant F-value indicates that the model as a whole is reliable and can explain a substantial portion of the variability of the response TAC. Moreover, for the model, the lack of fit F-value is 1.32, implying that the lack of fit is insignificant relative to the pure error hence the linear model adequately fits the experimental data. Therefore, the model fits because the lack of fit is not significant.

Additionally, the R^2 obtained was 0.9173, which shows that the regression model is significant, indicating a strong correlation between the model and experimental data. For any data to be substantial, the R^2 must be higher than 0.85 [24]. The predicted R^2 and adjusted R^2 were also in reasonable agreement with each other, further supporting the model's reliability. Eventually, the factors could not be included in the same equation to demonstrate their significance. This is because there are two different types of factors: numeral particle sizes and categorical methods. Thus, there are three distinct individual equations for each method in relation to the particle size *(A_1)*, as seen in equations (4), (5), and (6) for ME, MAE, and UAE, respectively. These models demonstrate the significant influence of particle size on TAC. Therefore, there is no interaction between the different types of methods as the methods cannot be enumerated. However, in Figure 5, the interaction graph shows that all the data for the UAE (blue line) were in the upper limit of the TAC as compared to the MAE (green line) which are scattered in the lower limit of the graph. The error bar also shows that the responses for UAE did not overlap with the other two methods, emphasizing the statistical significance of these differences.

TAC_{ME} = 104.11912 – 0.020783 (A_1) (4)

TAC_{MAE} = 103.91428 – 0.020783 (A_1) (5)

TAC_{UAE} = 116.06816 – 0.020783 (A_1) (6)

Moreover, a validation study was conducted to ensure the capability of the proposed equation. The MAE method with various particle sizes was completed in this validation process, as seen in Table 3. Based on the equation obtained previously, the response values were predicted at a certain validation point. The predicted values based on the equations were then compared with the experimental values obtained, the average errors were calculated, and the average error of 4.288% was achieved. With an error margin below 5%, the predictive model is highly reliable and aligns closely with the experimental values. Low margin error implies that the model generated can effectively capture the essential factors influencing the TAC yield. Thus, the results obtained for comparing the extraction methods and average particle sizes are significant, with a low possibility of error of 5%, proving the validity of the results in which the model is applicable for other experimentations.

Table 2 Analysis of variance (ANOVA): Effect of particle sizes (A) and methods (B) on the TAC yield.

ANOVA for Response Surface Linear Model						
Analysis of variance table [Partial sum of squares]						
Source	**Sum of Squares**	**DF**	**Mean Square**	**F Value**	**Prob > F**	
Model	1932.8	3	644.27	29.59	0.0001	significant
A	974.66	1	974.66	44.76	0.0002	
B	394.65	2	197.32	9.06	0.0088	
Residual	174.2	8	21.78			
Lack of Fit	119.7	5	23.94	1.32	0.4366	not significant
Pure Error	54.5	3	18.17			
Cor Total	2107	11				
R-square table						
Std. Dev.	4.667		R-Squared	0.917		
Mean	85.727		Adj R-Squared	0.886		
C.V.	5.443		Pred R-Squared	0.820		
PRESS	378.279		Adeq Precision	12.920		

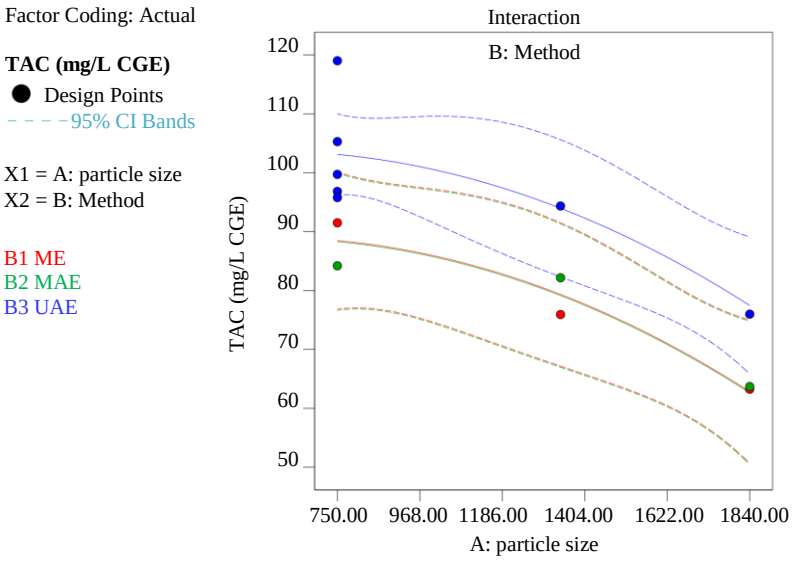


Figure 5 Interaction graph for the particle size and methods.

Table 3 Validation study.

Method	**Particle sizes (µm)**	**Experimental TAC (mg/L CGE)**	**Predicted TAC (mg/L CGE)**	**Error bar**
MAE	1840	62.026	62.639	0.978
MAE	1340	76.945	79.139	2.773
MAE	750	80.241	88.289	9.115
			Average error	4.288

3.4 SEM analysis

Furthermore, a scanning electron microscope analysis on the surface of the roselle calyces with various average particle sizes of 1840, 1340, and 750 µm was also conducted to investigate further the morphological effects of each of the treatments on the structure. Figure 6 shows the raw materials before the extraction process, with an average particle size of 1840µm, meanwhile, Figure 7 for

1340 µm, and Figure 8 for 750 µm. From Figures 6 to 8, the rough threadlike fibers on the morphological surface of the calyces become finer, longer, and more abundant forming layered structures as the average particle sizes become lower. Generally, before the extraction, most of the cellulose and hemicellulose are abundant in the form of small granules on the surface of the fibers. Cellulose is a soluble fiber, while hemicellulose is an insoluble fiber. This can be due to the mechanical shearing effect of particle size reduction as grinding and sieving were involved in the sample preparation [59, 63]. These layered threadlike fibers with increasing length as the particle sizes become smaller (as seen in Figures 6 to 8) and are formed via the cellulose and hemicellulose that envelop the solutes between them [26]. Therefore, when various extraction processes are applied, the effect on the surfaces is visible.

Figures 9 to 11 show the morphological surfaces of the calyces after ME treatment, the threadlike fibers are still abundant and long, with smaller granules between the thick fibers. For Figure 11, with a smaller particle size of 750 µm, the threadlike fibers are much thinner than the larger particle sizes of 1840 µm in Figure 9. This can be attributed to the continuous stirring in the extraction in which the soluble fibers in the calyces were dissolved into the solvent, leaving a few granules on the surface. Moreover, after the extraction, the fibers are no longer layered on one another, but they are more arranged in a sense that they form thick fibers with parallel distances between each other, as seen in Figures 9, 10, and 11 when compared with Figures 6, 7, and 8 before the extractions. For Figure 11, at smaller particle sizes, most of the small granules disappear, leaving behind the fibers intact. This can be explained as the washing step in the extraction phase, in which the solvent in the extraction washed away the granules that enveloped the solutes [64]. This is because more surface area of the solutes is available for the solvent to cover, therefore enhancing the mass transfer of the solutes into the solvent. However, as ME did not involve heat and was done at room temperature, the cell walls of the fibers that contain the remaining solutes could not be disrupted. Hence, the removal of the granules primarily relies on the washing mechanism to extract the solutes. That is why, as seen in Figures 9, 10, and 11, the fibers are still intact, with the granules disappearing when compared with Figures 6, 7, and 8. The lack of significant cell wall disruption limits the extraction efficiency. Therefore, the lower extraction of TAC when macerated, as discussed in the previous subtopic, is justified in that the extraction of the solutes mainly depends on the washing of the solutes physically.

Moreover, with the involvement of heating in MAE, in contrast, the cell walls were disrupted more severely when compared with ME. Based on Figures 12, 13, and 14, the fiber structures are fragmented and distorted with no long and threadlike fibers. This can be due to the rapid heating in the inner cells caused by the microwave which then causes pressure to build up inside the cells and eventually leads to the disruption of the cell walls [65]. Therefore, most of the granules on the surfaces disappear, and consequently, the remaining long fibers become shorter with various sizes and thicknesses.

Two major phenomena that occur in the MAE can be seen based on the SEM results. First, as MAE involves microwave radiation when the sample solution is subjected to microwave irradiation, the temperature of the sample solution increases rapidly as localized superheating occurs. Localized superheating occurs as the polar solvent absorbs the microwave energy quickly and directly without any convection through the sample vessel. The polar solvent molecules will then vibrate due to the dipole moment interaction. Polar solvents with high dipole moments will vibrate and rotate with each other, causing the temperature to increase rapidly [66-68].

Consequently, the rise of the solvent temperature causes pressure to build up inside the cells, as the water molecules inside the cells also absorb the microwave energy. Then, after a certain period, the cell wall will eventually rupture as the pressure increases due to the excessive pressure built inside and releasing the solute content [65, 66]. Therefore, as seen in Figures 12-14, the fibers become shorter and more clustered with more visible cavities. This can also increase the amount of the extract obtained due to a higher amount of solute accessible by the solvent. To further explain, in the conventional extraction method, the heating of the solvent occurs through convection, in which the heat is transferred to the solvent from the vessels that absorb the heat. On the other hand, microwave radiation was absorbed directly by the sample solution without the sample vessel absorbing the energy. Thus, localized superheating occurs in which the sample solution reaches a boiling point higher than its normal boiling point rapidly [9].

Furthermore, the second phenomenon that can be seen is that due to the temperature increase, the kinetic movement of the solvent molecules also increased. Thus, this causes the fluidity of the solvent to be higher as higher energy is produced. The solvent then washed away all the soluble fibers on the surface of the calycles, which is why, for most of the MAE reaction, the gradient for the washing stage is higher than the gradient for the diffusion stage [64, 66]. Therefore, MAE extraction mechanism is in-to-out, in which the sample cells burst due to the pressure from the inside. Consequently, the localized superheating and high pressure generated during MAE led to the rupture of cell walls, facilitating the release of intracellular compounds.

In contrast, in Figures 15-17, the morphological surfaces of the calyces become more porous with more visible long fibers after UAE. UAE resulted in a more porous structure with numerous micropores on the surface of the calyces. The grooves of the thin fibers are also more minor and smoother, as compared to the before extraction in Figures 6 to 8. This can be attributed to the enhancement of the mass transfer rate as ultrasonic waves were applied to the samples. The ultrasonic waves, when submitted into the solvent, cause cavitation bubbling in the form of microbubbles in the solvents, which then cause the cell walls of the solid to collapse. This enables the penetration of the solvents into the solid and eventually secures the solutes [69]. The microbubbles come in the form of compression (the bubbles become smaller) and rarefaction (the bubbles become bigger), and after several cycles, they eventually implode [70]. The implosion will then cause the cell walls to be crushed due to the shear stress of the pressure from the bubbles and will also form micropores on the surface morphology of the cell walls. That explains the formation of the fibers after UAE possesses little pores but with long strands of fibers. The cavitation phenomenon generated by the ultrasonic waves enhanced the mass transfer by disrupting the cell walls and increasing solvent penetration.

Additionally, the sonocapillary effect of UAE on plant morphology can also be explained as ample micropores, which will cause the solvent to enter the solid quickly and attract the solutes. Therefore, the extraction for the UAE occurs from out of the cells and into the cells, which is the opposite of MAE. Furthermore, as a jacketed beaker with continuous running water is used as a cooling system for the extraction process, the temperature increment of the process can be minimized, therefore reducing the bioactive compounds degradation risk. This offers an advantage, in which UAE can offer the prevention of degradation of the cell structure as compared to MAE due to the continuous cooling system and operation at a lower temperature of UAE with the high yield of UAE when compared to MAE to prove. Overall, the SEM analysis supports the superior performance of UAE in extracting anthocyanins from roselle. The combination of physical and chemical effects, including cavitation, increased mass transfer, and reduced thermal degradation, contributes to the higher extraction yield observed with the UAE.

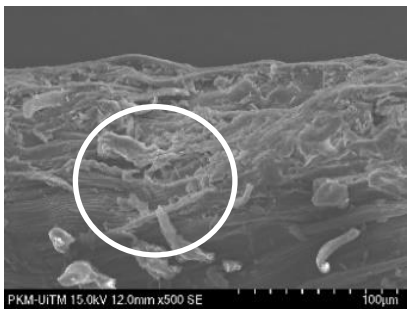


Figure 6 1840µm before extraction with rough threadlike fibers and granules on the surfaces

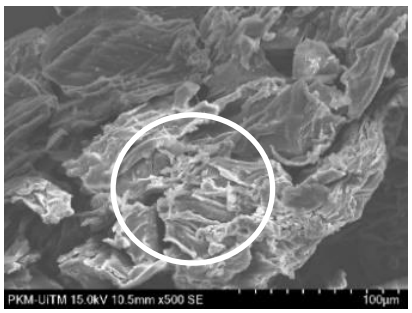


Figure 7 1340µm before extraction with finer and rough threadlike fibers

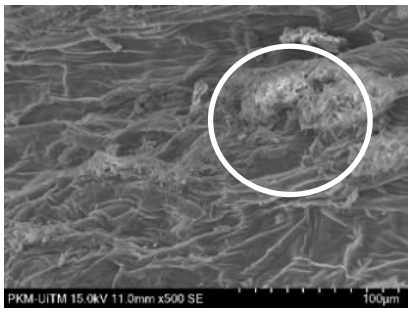


Figure 8 750µm before extraction with finer, longer, and rough threadlike fibers

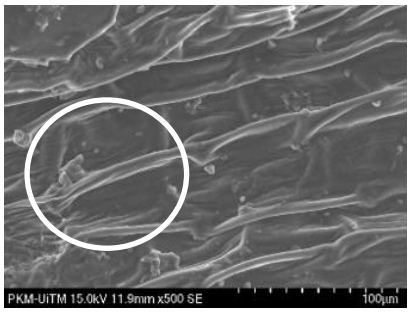


Figure 9 1840µm after ME with thick fibers distanced parallelly with some leftover granules

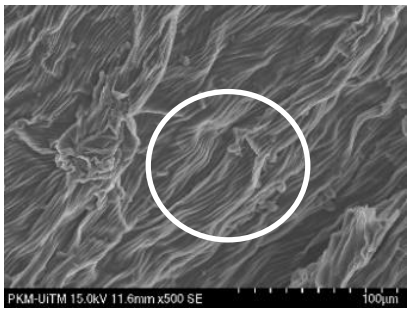


Figure 10 1340µm after ME with thick and finer fibers distanced parallelly

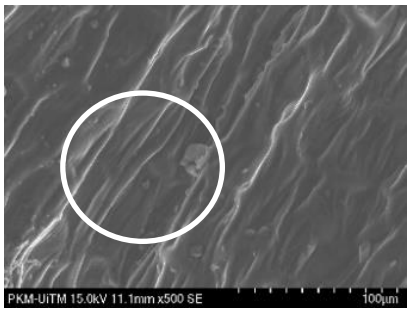


Figure 11 750µm after ME with thick and longer intact fibers distanced parallelly

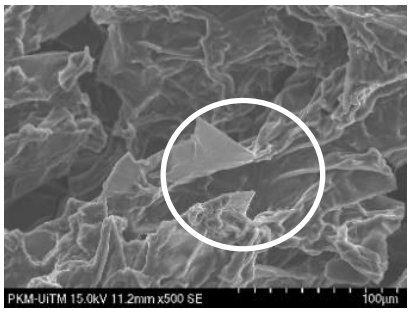


Figure 12 1840µm after MAE with fragmented and distorted threadlike fibers

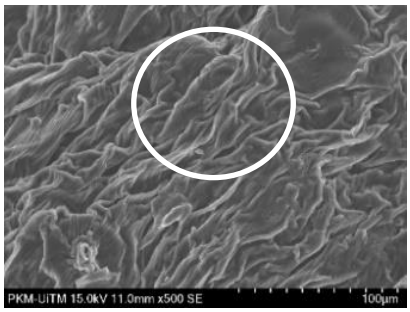


Figure 13 1340µm after MAE with more fragmented and clustered fibers

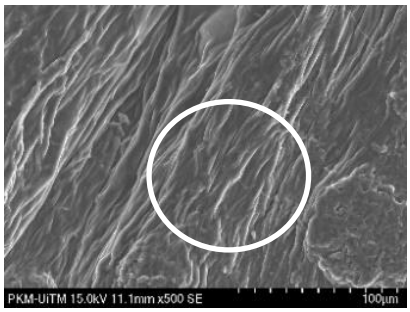


Figure 14 750µm after MAE with thinner, fragmented and more visible fibers

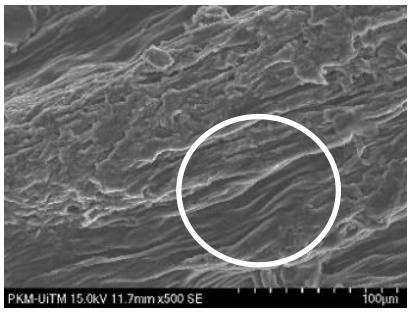


Figure 15 1840µm after UAE with more porous fibers

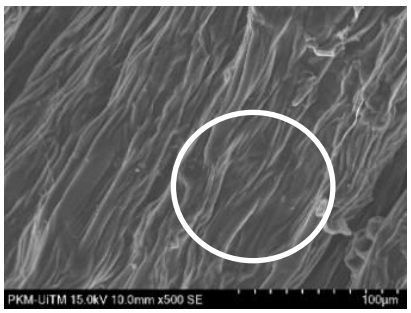


Figure 16 1340µm after UAE with more porous and smooth fibers

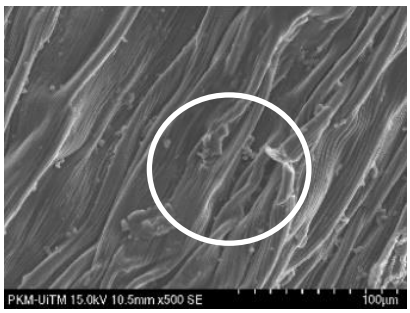


Figure 17 750µm after UAE with the smoothest and most porous fibers

4. Conclusions

In conclusion, the extraction of anthocyanins from roselle by using DESs and UAE has been optimized successfully. The best DESs that can yield the highest TAC was the combination of ChCl and TEG at a ratio of 1:1. Optimal parameters such as the solid-to-solvent ratio of 1:15 (g/g) and the average particle size of 750 µm were proven to enhance the extraction efficiency. The usage of DESs as versatile green solvents offers various advantages such as broad tunability, high biodegradability, and reduced toxicity. Using DESs, even as an additive to water, effectively obtained a higher TAC. Furthermore, the utilization of UAE as a non-thermal extraction technique, minimizes thermal degradation of the anthocyanins. This is also supported by the analysis of SEM in which the usage of UAE has a less severe impact on the degradation of the cellulose structure of the samples as the extraction of the solutes occurs from out of the cells and into the cells, promoting an efficient mass transfer. The synergistic effect of DESs and UAE resulted in significantly higher anthocyanin yield compared to the traditional method. Hence, the prospect of using DESs as an additive to water as solvents could be further explored by manipulating experimental parameters for the UAE setup for future research. A study on the optimization of the UAE amplitudes and the best concentration of DESs as an additive in water can be optimized to enhance the TAC yield. Additionally, exploring the scalability of the process and conducting economic assessments would be crucial for industrial applications. A comprehensive study investigating the stability of anthocyanin extracts obtained using DES-based extraction would also be valuable. By addressing these aspects, the potential of DES-based extraction can be fully realized for the recovery of high-value compounds from natural sources.

5. Acknowledgements

This work is supported by Fundamental Research Grant Scheme funded by ministry of higher education (MOHE) under the FRGS 2021-1 with the code of FRGS/1/2021/TK0/UITM/03/15.

6. References

[1] Aminul Islam AKM, Jamini TS, Mominul Islam AKM, Yeasmin S. Roselle: A functional food with high nutritional and medicinal values. Fund Appl Agric. 2016;1(2):44-9.
[2] Shruthi VH, Ramachandra CT, Nidoni U, Hiregoudar S, Naik N, Kurubar AR. Roselle (*Hibiscus Sabdariffa* L.) as a source of natural color: a review. Plant Arch. 2016;16(2):515-22.
[3] Riaz G, Naik SN, Garg M, Chopra R. Phytochemical composition of an underutilized plant sorrel/roselle (*Hibiscus Sabdariffa* L.) cultivated in India. Lett Appl NanoBioScience. 2021;10(2):2138-47.
[4] Chin KL, Zhen J, Qi Y, Chin SL, Breithaupt M, Wu QL, et al. A comparative evaluation: Phytochemical composition and antioxidant capacity of three roselle (*Hibiscus sabdariffa* L.) accessions. Acta Hortic. 2016;1125:99-108.
[5] Pozos GIP, Ruiz-López MA, Nátera JFZ, Moya CÁ, Ramírez LB, Silva MR, et al. Antioxidant capacity and antigenotoxic effect of *Hibiscus sabdariffa* L. extracts obtained with ultrasound-assisted extraction process. Appl Sci. 2020;10(2):560.
[6] Riaz G, Chopra R. A review on phytochemistry and therapeutic uses of *Hibiscus sabdariffa* L. Biomed Pharmacother. 2018;102:575-86.
[7] Abubakar AR, Haque M. Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. J Pharm Bioallied Sci. 2020;12(1):1-10.
[8] Gałuszka A, Migaszewski Z, Namieśnik J. The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices. TrAC Trends Anal Chem. 2013;50:78-84.
[9] Dean JR. Microwave extraction. In: Pawliszyn J, editor. Comprehensive sampling and sample preparation volume 2. Amsterdam: Elsevier; 2012. p. 135-49.
[10] Lavilla I, Bendicho C. Fundamentals of ultrasound-assisted extraction. In: González HD, Muñoz MJG, editors. Water extraction of bioactive compounds. Amsterdam: Elsevier; 2017. p. 291-316.
[11] Joshi DR, Adhikari N. An overview on common organic solvents and their toxicity. J Pharm Res Int. 2019;28(3):1-18.
[12] Taghavi T, Patel H, Rafie R. Comparing pH differential and methanol-based methods for anthocyanin assessments of strawberries. Food Sci Nutr. 2021;10(7):2123-31.
[13] Warner JC, Cannon AS, Dye KM. Green chemistry. Environ Impact Assess Rev. 2004;24(7-8):775-99.
[14] Smith EL, Abbott AP, Ryder KS. Deep Eutectic Solvents (DESs) and their applications. Chem Rev. 2014;114(21):11060-82.
[15] Abbott AP, Barron JC, Ryder KS, Wilson D. Eutectic-based ionic liquids with metal-containing anions and cations. Chem - Eur J. 2007;13(22):6495-501.
[16] Zannou O, Pashazadeh H, Ibrahim SA, Koca I, Galanakis CM. Green and highly extraction of phenolic compounds and antioxidant capacity from kinkeliba (*Combretum micranthum* G. Don) by natural deep eutectic solvents (NADESs) using maceration, ultrasound-assisted extraction and homogenate-assisted extraction. Arab J Chem. 2022;15(5):103752.

[17] Abbott AP, Capper G, Davies DL, Rasheed RK, Tambyrajah V. Novel solvent properties of choline chloride/urea mixtures. Chem Commun. 2003;(1):70-1.
[18] Dai Y, Witkamp GJ, Verpoorte R, Choi YH. Tailoring properties of natural deep eutectic solvents with water to facilitate their applications. Food Chem. 2015;187:14-9.
[19] Dai Y, Rozema E, Verpoorte R, Choi YH. Application of natural deep eutectic solvents to the extraction of anthocyanins from *Catharanthus roseus* with high extractability and stability replacing conventional organic solvents. J Chromatogr A. 2016;1434:50-6.
[20] Roy D, Miller L. Exploring the utility of natural deep eutectic solvents as additives in super/subcritical fluid chromatography-insights into chiral recognition mechanism. Anal Chim Acta. 2022;1200:339584.
[21] Omar KA, Sadeghi R. Database of deep eutectic solvents and their physical properties: a review. J Mol Liq. 2023;384:121899.
[22] Tan PW, Tan CP, Ho CW. Antioxidant properties: effects of solid-to-solvent ratio on antioxidant compounds and capacities of Pegaga (*Centella asiatica*). Int Food Res J. 2011;18:557-62.
[23] Azmi SN, Ruslan MSH. Extraction of essential oil from kaffir lime (*Citrus hystrix*) leaves using microwave-assisted extraction. Malaysian J Chem Eng Technol. 2022;5(2):84-93.
[24] Minitab Inc. What are response surface designs, central composite designs, and Box-Behnken designs?, Response Surface Design [Internet]. 2010 [cited 2022 Jan 27]. Available from: https://support.minitab.com/en-us/minitab/18/help-and-how-to/modeling-statistics/doe/supporting-topics/response-surface-designs/response-surface-central-composite-and-box-behnken-designs/.
[25] Alañón ME, Ivanović M, Pimentel-Mora S, Borrás-Linares I, Arráez-Román D, Segura-Carretero A. A novel sustainable approach for the extraction of value-added compounds from *Hibiscus sabdariffa* L. calyces by natural deep eutectic solvents. Food Res Int. 2020;137:109646.
[26] Amaya-Cruz DM, Perez-Ramirez IF, Ortega-Diaz D, Rodriguez-Garcia ME, Reynoso-Camacho R. Roselle (*Hibiscus sabdariffa*) by-product as functional ingredient: effect of thermal processing and particle size reduction on bioactive constituents and functional, morphological, and structural properties. J Food Meas Charact. 2018;12:135-44.
[27] Aryanti N, Nafiunisa A, Wardhani DH. Conventional and ultrasound-assisted extraction of anthocyanin from red and purple roselle (*Hibiscus sabdariffa* L.) calyces and characterization of its anthocyanin powder. Int Food Res J. 2019;26(2):529-35.
[28] Aneke NN, Okonkwo WI, Ezeoha SL, Okafor GI, Anyanwu CN. Optimization of anthocyanin extraction from roselle (*Hibiscus sabdariffa*) calyces: RSM, kinetic modelling, mass transfer and thermodynamic studies. J Res Innov Food Sci Technol. 2023;11(4):437-50.
[29] Selim KA, Khalil KE, Abdel-Bary MS, Abdel-Azeim NA. Extraction, encapsulation and utilization of red pigments from roselle (*Hibiscus sabdariffa* L.) as natural food colourants. Alex J Food Sci Technol. 2008;5(2):7-20.
[30] Redzuan S, Ho CY, Idham Z, Yusuf S, Putra NR, Yunus MAC, et al. Optimization of anthocyanins extracts from roselle (*Hibiscus sabdarifa*) petals using ultrasonic-assisted extraction method. In: Zaini MAA, Jusoh M, Othman N, editors. Proceedings of the 3rd International Conference on Separation Technology. Lecture Notes in Mechanical Engineering. Singapore: Springer; 2021. p. 295-309.
[31] Cid-Ortega S, Guerrero-Beltran JA. Roselle calyces particle size effect on the physicochemical and phytochemicals characteristics. J Food Res. 2014;3(5):83-94.
[32] Roslan R, Kurnia KA, Hasanudin N, Hilmy NIMF, Ruslan MSH. Screen and design of deep eutectic solvents (DESs) for the extraction of delphinidin-3-sambubioside from *Hibiscus sabdariffa* via COSMO-RS. AIP Conf Proc. 2024;3041(1):050003.
[33] Benvenutti L, Zielinski AAF, Ferreira SRS. Which is the best food emerging solvent: IL, DES or NADES?. Trends Food Sci Technol. 2019;90:133-46.
[34] Lee J, Durst RW, Wrolstad RE. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. J AOAC Int. 2005;88(5):1269-78.
[35] Anderson MJ, Whitcomb PJ. DOE simplified - practical tools for effective experimentation. 3rd ed. Boca Raton: CRC Press; 2015.
[36] Pollini L, Tringaniello C, Ianni F, Blasi F, Manes J, Cossignani L. Impact of ultrasound extraction parameters on the antioxidant properties of *Moringa oleifera* leaves. Antioxidants. 2020;9(4):277.
[37] Pan X, Xu L, Meng J, Chang M, Cheng Y, Geng X, et al. Ultrasound-Assisted deep eutectic solvents extraction of polysaccharides from *Morchella importuna*: optimization, physicochemical properties, and bioactivities. Front Nutr. 2022;9:1-11.
[38] Kisanthia R, Hunt AJ, Sherwood J, Somsakeesit LO, Phaosiri C. Impact of conventional and sustainable solvents on the yield, selectivity, and recovery of curcuminoids from turmeric. ACS Sustain Chem Eng. 2022;10(1):104-14.
[39] Wang J, Jing W, Tian H, Liu M, Yan H, Bi W, et al. Investigation of deep eutectic solvent-based microwave-assisted extraction and efficient recovery of natural products. ACS Sustain Chem Eng. 2020;8(32):12080-8.
[40] Izirwan I, Munusamy TD, Hamidi NH, Sulaiman SZ. Optimization of microwave-assisted extraction of anthocyanin from *Clitoria ternatea* flowers. Int J Mech Eng Robot Res. 2020;9(9):1246-52.
[41] Setyaningsih W, Putro AW, Fathimah RN, Kurnia KA, Darmawan N, Yulianto B, et al. A microwave-based extraction method for the determination of sugar and polyols: application to the characterization of regular and peaberry coffees. Arab J Chem. 2022;15(3):103660.
[42] Yadav A, Trivedi S, Rai R, Pandey S. Densities and dynamic viscosities of (choline chloride+glycerol) deep eutectic solvent and its aqueous mixtures in the temperature range (283.15-363.15)K. Fluid Phase Equilib. 2014;367:135-42.
[43] Abbott AP, Boothby D, Capper G, Davies DL, Rasheed RK. Deep eutectic Solvents formed between choline chloride and carboxylic acids: versatile alternatives to ionic liquids. J Am Chem Soc. 2004;126(29):9142-7.
[44] Abbott AP, Ahmed EI, Prasad K, Qader IB, Ryder KS. Liquid pharmaceuticals formulation by eutectic formation. Fluid Phase Equilib. 2017;448:2-8.
[45] Dai Y, van Spronsen J, Witkamp GJ, Verpoorte R, Choi YH. Natural deep eutectic solvents as new potential media for green technology. Anal Chim Acta. 2013;766:61-8.
[46] Rashid Z, Wilfred CD, Gnanasundaram N, Arunagiri A, Murugesan T. Screening of ionic liquids as green oilfield solvents for the potential removal of asphaltene from simulated oil: COSMO-RS model approach. J Mol Liq. 2018;255:492-503.
[47] Zhao BY, Xu P, Yang FX, Wu H, Zong MH, Lou WY. Biocompatible deep eutectic solvents based on choline chloride: characterization and application to the extraction of rutin from *Sophora japonica*. ACS Sustain Chem Eng. 2015;3(11):2746-55.

[48] Salazar-González C, Vergara-Balderas FT, Ortega-Regules AE, Guerrero-Beltrán JÁ. Antioxidant properties and color of *Hibiscus sabdariffa* extracts. Cien Inv Agr. 2012;39(1):79-90.
[49] Aissaoui T, Benguerba Y, AlNashef IM. Theoretical investigation on the microstructure of triethylene glycol based deep eutectic solvents: COSMO-RS and TURBOMOLE prediction. J Mol Struct. 2017;1141:451-6.
[50] Hayyan M, Aissaoui T, Hashim MA, AlSaadi MAH, Hayyan A. Triethylene glycol based deep eutectic solvents and their physical properties. J Taiwan Inst Chem Eng. 2015;50:24-30.
[51] Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: a comprehensive review. Chinese Med. 2018;13:1-26.
[52] Tchabo W, Ma Y, Kwaw E, Xiao L, Wu M, Apaliya MT. Impact of extraction parameters and their optimization on the nutraceuticals and antioxidant properties of aqueous extract mulberry leaf. Int J Food Prop. 2018;21(1):717-32.
[53] Richardson JF, Harker JH, Backhurst JR. Chapter 10 - Leaching. Chemical engineering volume 2. Amsterdam: Elsevier; 2002. p. 502-41.
[54] Chanioti S, Liadakis G, Tzia C. Solid–Liquid extraction. In: Varzakas T, Tzia C, editors. Food Engineering Handbook. Boca Raton: CRC Press; 2014. p. 253-86.
[55] Hu J, Chen Y, Ni D. Effect of superfine grinding on quality and antioxidant property of fine green tea powders. LWT - Food Sci Technol. 2012;45(1):8-12.
[56] Amyotte PR, Eckhoff RK. Dust explosion causation, prevention and mitigation: an overview. J Chem Health Saf. 2010;17(1):15-28.
[57] Hanula M, Wyrwisz J, Moczkowska M, Horbańczuk OK, Pogorzelska-Nowicka E, Wierzbicka A. Optimization of microwave and ultrasound extraction methods of açai berries in terms of highest content of phenolic compounds and antioxidant activity. Appl Sci. 2020;10(23):8325.
[58] Şahin S, Pekel AG, Toprakçı İ. Sonication-assisted extraction of *Hibiscus sabdariffa* for the polyphenol's recovery: application of a specially designed deep eutectic solvent. Biomass Conv Bioref. 2022;12:4959-69.
[59] Chaves JO, de Souza MC, da Silva LC, Lachos-Perez D, Torres-Mayanga PC, da Fonseca Machado AP, et al. Extraction of flavonoids from natural sources using modern techniques. Front Chem. 2020;8:1-25.
[60] Fitri N, Halimatussa'diah, Fitriastuti D. Comparison between maceration and microwave extraction techniques of strawberry fruit (*fragaria sp*) and antioxidant activity test. IOP Conf Ser: Mater Sci Eng. 2019;523:012024.
[61] Cvjetko Bubalo M, Ćurko N, Tomašević M, Kovačević Ganić K, Radojcic Redovnikovic I. Green extraction of grape skin phenolics by using deep eutectic solvents. Food Chem. 2016;200:159-66.
[62] Pinela J, Prieto MA, Pereira E, Jabeur I, Barreiro MF, Barros L, et al. Optimization of heat- and ultrasound-assisted extraction of anthocyanins from *Hibiscus sabdariffa* calyces for natural food colorants. Food Chem. 2019;275:309-21.
[63] Oliveira G, Marques C, de Oliveira A, de Almeida dos Santos A, do Amaral W, Ineu RP, et al. Extraction of bioactive compounds from *Curcuma longa* L. using deep eutectic solvents: In vitro and in vivo biological activities. Innov Food Sci Emerg Technol. 2021;70:102697.
[64] Kate A, Singh A, Shahi N, Pandey JP, Prakash O. Modeling and kinetics of microwave assisted leaching based oil extraction from Bhat. J Food Process Eng. 2020;43(10):e13503.
[65] Xiaokang W, Lyng JG, Brunton NP, Cody L, Jacquier JC, Harrison SM, et al. Monitoring the effect of different microwave extraction parameters on the recovery of polyphenols from shiitake mushrooms: comparison with hot-water and organic-solvent extractions. Biotechnol Rep. 2020;27:e00504.
[66] Alara OR, Abdurahman NH. Microwave-assisted extraction of phenolics from *Hibiscus sabdariffa* calyces: kinetic modelling and process intensification. Ind Crops Prod. 2019;137:528-35.
[67] Alara OR, Abdurahman NH, Ukaegbu CI, Kabbashi NA. Extraction and characterization of bioactive compounds in *Vernonia amygdalina* leaf ethanolic extract comparing soxhlet and microwave-assisted extraction techniques. J Taibah Univ Sci. 2019;13(1):414-22.
[68] Olalere OA, Abdurahman HN, Yunus R bin M, Alara OR, Ahmad MM, Zaki YH, et al. Parameter study, antioxidant activities, morphological and functional characteristics in microwave extraction of medicinal oleoresins from black and white pepper. J Taibah Univ Sci. 2018;12(6):730-7.
[69] Dias ALB, Arroio Sergio CS, Santos P, Barbero GF, Rezende CA, Martínez J. Effect of ultrasound on the supercritical CO_2 extraction of bioactive compounds from dedo de moça pepper (*Capsicum baccatum* L. var. *pendulum*). Ultrason Sonochem. 2016;31:284-94.
[70] Chemat F, Rombaut N, Sicaire AG, Meullemiestre A, Fabiano-Tixier AS, Abert-Vian M. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrason Sonochem. 2017;34:540-60.