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Optimization of Green Ultrasound-Assisted Extraction of Carotenoids from *Tagetes erecta* L. using Natural Deep Eutectic Solvents (NADES)

Ni Luh Febby Andriani, Anak Agung Gede Rai Yadnya-Putra*, and Luh Putu Mirah Kusuma Dewi

Department of Pharmacy, Faculty of Mathematic and Natural Science, Universitas Udayana, Bali, Indonesia
*Corresponding author: Anak Agung Gede Rai Yadnya-Putra | Email: agungryp@unud.ac.id

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Abstract: Carotenoids from marigold flowers (*Tagetes erecta* L.) are commonly extracted using conventional solvents like hexane and ethanol, which may pose environmental and health risks. Based on these considerations, this study aimed to evaluate the potential of Natural Deep Eutectic Solvents (NADES) as greener alternatives and to determine the optimal NADES composition and extraction conditions using Ultrasound-Assisted Extraction (UAE). The most effective NADES was selected based on total carotenoid content, statistically analyzed using one-way ANOVA. Optimization was carried out using Response Surface Methodology (RSM) with a Box–Behnken Design (BBD) to assess the effects of ultrasonic power, extraction time, and temperature. Total carotenoid content was determined by UV-Vis spectrophotometry. Among the tested solvents, the hydrophobic NADES composed of menthol:lactic acid (8:1) showed the highest efficiency (98.48 ± 0.20 mg β-carotene equivalent (BCE)/g dry sample (DS)), outperforming the hydrophilic NADES, hexane, and 80% ethanol. Optimization using Response Surface Methodology with a Box–Behnken Design identified optimal UAE conditions at 100% ultrasonic power, 40 minutes, and 60°C, yielding 304.01 ± 1.60 mg BCE/g DS. These results confirm the effectiveness of NADES as sustainable and efficient solvents for carotenoid extraction from marigold flowers.

Keywords: carotenoid; NADES; *Tagetes erecta*; UAE

1. INTRODUCTION

Marigold flowers (*Tagetes erecta* L.) are among the most popular plants from the Asteraceae family and are widely recognized as a natural source of carotenoid compounds. The carotenoids found in marigold flowers consist of two major groups: carotenes (α- and β-carotene), which are nonpolar, and xanthophylls (lutein and zeaxanthin), which are generally more polar [1]. According to Tokas et al. (2018), marigold flowers contain 0.73-68.43 mg/100 g of carotenes and 29.36- 317.09 mg/100 g of xanthophylls in dried form. This composition highlights marigold flowers as a valuable candidate for the production of high-value bioactive compounds, particularly carotenoids [2].

As scientific evidence supporting the health benefits of carotenoids continues to grow, carotenoid extracts from natural sources like marigold flowers are gaining increasing interest and are being developed into functional products and dietary supplements [3]. In the health sector, carotenoids serve as precursors for vitamin A synthesis, function as physiological antioxidants, and can enhance immune response. Lutein and zeaxanthin, the two primary types of xanthophylls, play a critical role in forming macular pigment in the retina and protecting the eyes from oxidative damage caused by UV and blue light exposure [4].

Traditionally, carotenoids are extracted using organic solvents. Nonpolar carotenes are commonly extracted with hexane, while polar xanthophylls are typically extracted using ethanol [5]. However, the long-term use of conventional solvents can pose environmental and health risks. Therefore, the use of environmentally friendly solvents such as Natural Deep Eutectic Solvents (NADES) has emerged as a promising alternative. NADES are non-toxic, biodegradable, cost-

effective, and capable of maintaining the stability of target compounds without the need for solvent removal [6], [7].

Natural Deep Eutectic Solvents (NADES) are green solvent systems formed by mixing two or more natural compounds that interact mainly through hydrogen bonding, resulting in a liquid mixture with a melting point lower than that of the individual components. A NADES is typically composed of a hydrogen-bond acceptor (HBA) and a hydrogen-bond donor (HBD). The HBA is the component that accepts hydrogen bonds, commonly an organic salt such as choline chloride, whereas the HBD donates hydrogen bonds and is usually a compound such as an organic acid, sugar, alcohol, or amino acid. Based on their polarity, NADES are classified into hydrophilic and hydrophobic types. Hydrophilic NADES have higher polarity and are therefore more suitable for dissolving polar compounds, while hydrophobic NADES have lower polarity and are generally used for the extraction of nonpolar compounds [7], [8], [9],[10], [11], [12].

The application of NADES in carotenoid extraction has been widely reported in the literature, with their effectiveness largely depending on the polarity of the target compounds [10], [11], [12]. For example, Silva et al. (2019) showed that a hydrophobic NADES composed of menthol:lactic acid (8:1) was effective for extracting carotenes from tomato pomace, whereas Roy et al. (2021) reported that a hydrophilic NADES consisting of choline chloride:lactic acid (1:2) was more effective for extracting xanthophylls from shrimp waste. In these systems, lactic acid is a promising hydrogen-bond donor (HBD) because it contains both carboxyl (–COOH) and hydroxyl (–OH) groups, which support the formation of a stable hydrogen-bond network within the NADES system. Moreover, its short carbon chain may facilitate interactions with the polyene carbon chain of carotenoid compounds, thereby enhancing their solubilization during extraction [11], [12].

The extraction performance of NADES can be further enhanced when combined with Ultrasound-Assisted Extraction (UAE), due to the synergistic effects between ultrasonic cavitation and the solvent's affinity for target molecules [13]. UAE is well known for accelerating diffusion processes and promoting extensive cell wall disruption, thereby facilitating the release of intracellular bioactive compounds [14]. Moreover, the combined use of NADES and UAE aligns with the principles of green extraction, which emphasize reducing energy consumption, utilizing renewable and natural products, and ensuring the safe and high-quality extraction of bioactive compounds [7].

Based on these considerations, this study aims to evaluate the ability of NADES to extract carotenoids from marigold flowers and to determine the optimal NADES composition and extraction conditions using UAE. Optimization was conducted using Response Surface Methodology (RSM) with a Box-Behnken Design (BBD) to assess the effects of ultrasonic power, extraction time, and temperature on total carotenoid content. This research is expected to contribute to the development of more sustainable and efficient extraction methods for carotenoids from marigold flowers.

2. MATERIALS AND METHODS

2.1. Place and time of research

This study was carried out at the Phytochemistry Laboratory, Universitas Udayana Jimbaran, between January and April 2025.

2.2. Chemical and materials

The chemicals used in this study included choline chloride (Himedia), 90% food-grade lactic acid (Subur Kimia Jaya), menthol (Kimia Jaya Abadi), distilled water (technical), ethanol p.a. (Smart-Lab), n-hexane p.a. (Smart-Lab), methanol p.a. (Smart-Lab), and β-carotene standard (Sigma). Additional materials included aluminium foil and labeling tape.

The equipment employed consisted of an analytical balance (Newtech), grinder, sieve no. 60, laboratory glassware (Iwaki Pyrex), micropipette (Joanlab®), pH meter (Kedida CT-6020A), Ostwald viscometer (Pyrex), pycnometer (Pyrex), hot plate with magnetic stirrer (Cimarec+), centrifuge and centrifuge tubes (Eppendorf), ultrasonic bath (Branson 1510E-DTH), cuvettes, and a UV-Vis spectrophotometer (Shimadzu UVmini-1240).

2.3. Sample preparation

Marigold flower (*Tagetes erecta* L.) samples were collected from local farmers at Mayungan Village, Baturiti District, Tabanan Regency, Bali Province. The flowers were separated from their base, and only the orange-colored petals were used. The selected petals were then cleaned and dried manually under direct sunlight. Once dried, the samples were ground using a grinder and sieved through a No. 60 mesh to obtain fine marigold flower powder and stored in light tight container.

2.4. NADES preparation and characterization

NADES were prepared by mixing the appropriate molar ratios of the hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) components (Table 1). The mixture was placed in a beaker, stirred using a magnetic stirrer at 300 rpm, and heated at 80°C until a clear and homogeneous liquid was obtained. For hydrophilic NADES, 10% (v/v) distilled water was added and the solution was stirred again until fully homogenized [12]. The resulting NADES were then cooled to room temperature and subjected to characterization. The pH was measured using a digital pH meter; density was determined using a pycnometer; and viscosity was measured using an Ostwald viscometer.

Table 1. Composition of NADES Used in This Study

Code	Type	Composition		Ratio molar
		HBA	HBD	
NADES-1	Hydrophilic	Choline chloride	Lactic acid	1:2[10]
NADES-2	Hydrophobic	Menthol	Lactic acid	8:1[9]

2.5. Screening of NADES for the Extraction

A preliminary experiment was conducted to select the optimal NADES for extracting carotenoids from marigold flowers. The prepared NADES components were evaluated to experimentally compare the performance of hydrophilic and hydrophobic solvents in terms of extraction yield. The extraction was carried out under the same conditions, i.e. with a solvent to solid ratio of 20:1 (mL/g); at 60°C for 30 min with ultrasonic bath assisted 40 kHz frequency at 100% power. The resulting extracts were centrifuged at 4000 rpm for 20 minutes, and the supernatant was collected for further analysis [12]. Extraction was also performed using conventional solvents 80% ethanol and n-hexane as a control to compare the extraction efficiency. Each treatment was carried out in triplicate, and the results were expressed as mean ± standard deviation. The total carotenoid content (TCC) in each liquid extract was determined using a UV-Vis spectrophotometric method.

2.6. Determination of Total Carotenoid Content (TCC) by UV-VIS Spectrophotometry

The TCC of the marigold flower extracts was determined using a UV-Vis spectrophotometer, following the method by Koutsoukos et al. (2019). A stock solution of β-carotene (1000 μg/mL) was prepared by dissolving 10.0 mg of β-carotene in 10 mL of methanol. Standard β-carotene solutions were then prepared at concentrations of 70, 100, 130, 160, and 190 μg/mL. The maximum wavelength (λ max) was determined using the 100 μg/mL standard solution and its blank (methanol), by scanning absorbance in the range of 400–500 nm. The obtained λ max was used to measure the absorbance of all standard solutions. The absorbance values were used to construct a calibration curve, and the resulting linear regression equation was used to calculate the β-carotene concentration (μg/mL) in each test solution.

For extraction yield evaluation, 600 μL of marigold flower extracts were diluted in 4 mL of methanol and measured at the determined λ max. Blanks for each treatment were prepared using the same procedure, substituting 600 μL of the corresponding solvent. All measurements were performed in triplicate. The total carotenoid content (TCC) of the marigold flower extracts was

calculated using Equation (1) and expressed as mg β-carotene equivalent per gram of dried sample (mg BCE/g DS) [8].

$$\text{TCC (mg BCE/g DS)} = \frac{C \times V \times DF \times 10^{-3}}{m} \quad \text{.....(1)}$$

Where:

C = β-carotene concentration in the test solution (μg/mL)
DF = Dilution factor
m = Mass of dried sample (g)
V = Total volume of the test solution (mL)

2.7. Optimisation of UAE

Response Surface Methodology (RSM) using Design Expert software version 13.1 was employed to analyze the optimization parameters for UAE conditions. A Box–Behnken Design was applied to determine the optimal extraction conditions using three independent variables: ultrasonic power (X_1, 40-100%), extraction time (X_2, 20-40 min), and extraction temperature (X_3, 30-60°C). The extraction of marigold flower powder was carried out using the selected optimal NADES with a solvent-to-solid ratio of 20:1 (mL/g), and the extraction conditions followed the experimental matrix generated by the RSM-BBD in Design Expert. A total of 17 experimental runs were conducted as describe in table 3, and the response variable was measured as total carotenoid content (dependent variable). The determination of total carotenoid content was performed according to the procedure described in Section 2.6.

2.8. Data Processing and Data Analysis Methods

Statistical analysis was performed using one-way ANOVA followed by Post Hoc LSD (Least Significant Difference) tests to determine the optimal NADES for use in the optimization step. All statistical analyses were conducted at a 95% confidence level ($p = 0.05$). The optimal NADES was selected based on the highest total carotenoid content that was significantly different from other solvents.

Response data obtained from the optimization procedure were analyzed using Design Expert software version 13.1. The results were fitted to response function models corresponding to the independent variables used. Model selection was evaluated using ANOVA, considering the significance level ($p \leq 0.05$), lack of fit ($p > 0.05$), and the coefficients of determination (Predicted R-squared and Adjusted R-squared). The most optimal UAE conditions were identified from the best-fitting model based on the highest desirability (D) value. Verification of the model was then conducted under the predicted optimal conditions.

3. RESULTS AND DISCUSSION

3.1. NADES Preparation and Characterization

NADES in this study were prepared using the stirring and heating method, which involved mixing two separate components followed by continuous stirring and heating until a clear and homogeneous liquid was obtained. This method was chosen for its simplicity, ease of application, and ability to produce solvents in large volumes [15]. NADES-1 was formulated with the addition of 10% (v/v) distilled water, while NADES-2 was prepared without water. The addition of water in NADES-1 was intended to reduce the viscosity of hydrophilic NADES, which is known to limit extraction efficiency due to its high viscosity [12]. The preparation results showed that both NADES types successfully produced clear and homogeneous liquids (Figure 1a).

Following the preparation, the NADES were characterized based on pH, density, and viscosity—critical physicochemical properties that influence extraction performance. The pH value reflects the degree of ionization of NADES components and can affect their interaction with target compounds,

such as through electrostatic forces that enhance solubilization. Hydrogen bonding between NADES components creates a compact and stable liquid structure, which increases both density and viscosity. However, excessively high viscosity and density may hinder molecular diffusion and reduce solute dissolution efficiency [7]. Thus, proper characterization of these parameters is essential.

As shown in Table 2, NADES-1 exhibited a lower pH (0.93 ± 0.01) compared to NADES-2 (2.03 ± 0.01). The density of NADES-1 was (115.18 ± 1.05) × 10^{-2} g/mL, while NADES-2 had a lower density of (90.36 ± 0.06) × 10^{-2} g/mL. These results align with previous studies reporting that hydrophobic NADES typically exhibit lower densities than water [9]. In terms of viscosity, NADES-1 showed a higher value of (1.33 ± 0) × 10^{-2} kg/m·s compared to NADES-2, which measured (1.18 ± 0) × 10^{-2} kg/m·s. This finding is consistent with Mišan et al. (2019), who reported that hydrophobic NADES generally have lower viscosities than hydrophilic NADES.

Table 2. Results of NADES Characterization

NADES	Characteristics*		
	pH	Density (g/mL)	Viscosity (kg/m.s)
NADES-1	0.93 ± 0.01	(115.18 ± 1.05) × 10^{-2}	(1.33 ± 0) × 10^{-2}
NADES-2	2.03 ± 0.01	(90.36 ± 0.06) × 10^{-2}	(1.18 ± 0) × 10^{-2}

*Values shown are mean ± standard deviation (n=3); measured at 32°C

3.2. Selection of the optimal NADES for Extraction

Evaluating the solubility of carotenoids in both hydrophilic and hydrophobic NADES was the initial step in identifying the most promising NADES for carotenoid extraction from marigold flowers. This experimental evaluation was essential because marigold flowers contain two major groups of carotenoids, namely carotenes and xanthophylls, which differ in polarity [2]. Total carotenoid content (TCC) was determined by UV–Vis spectrophotometry at 448 nm, which corresponds to the absorption maximum of the standard solution. The calibration curve showed good linearity, with the regression equation y = 0.0034x + 0.0714 (x denotes the carotenoid concentration in the test solution (μg/mL), whereas y denotes the absorbance value of the solution) and a correlation coefficient (R^2) of 0.99, indicating that the method was reliable for TCC quantification. This equation was then used to calculate the carotenoid content of each extract. Differences in extraction performance among the tested NADES can be attributed to variations in their physicochemical properties, particularly polarity and viscosity, which are governed by their constituent components and directly influence solvation capacity. The use of an appropriately selected NADES may improve the extraction of plant metabolites by enhancing the solubility of target bioactive compounds [8]. The visual appearance of NADES before and after extraction is presented in Figure 1, while the TCC values obtained from each extract are shown in Figure 2.

(**a**) (**b**)

Figure 1. The appearance of NADES (a) before and (b) after extraction

As shown in Figure 2, NADES-2 produced the highest total carotenoid content (98.48 ± 0.20 mg BCE/g DS), which was significantly higher than that obtained with NADES-1 (71.71 ± 0.33 mg BCE/g DS), n-hexane (43.35 ± 0.19 mg BCE/g DS), and 80% ethanol (14.95 ± 0.05 mg BCE/g DS). This difference is closely related to the chemical nature of the carotenoids in marigold flowers, which are predominantly present as nonpolar esterified lutein and zeaxanthin [16], even though xanthophylls are generally polar in their free form [1], [2]. According to the "like dissolves like" principle, nonpolar solvents such as the hydrophobic NADES-2 are more effective for dissolving nonpolar compounds. Conversely, the hydrophilic NADES-1 is more suitable for extracting free xanthophylls. Moreover, NADES-1 exhibited lower pH, higher viscosity, and greater density than NADES-2, which may have reduced the diffusion and solubility of carotenoids during extraction [7]. Based on these findings, NADES-2 was selected as the optimal solvent for the subsequent UAE parameter optimization.

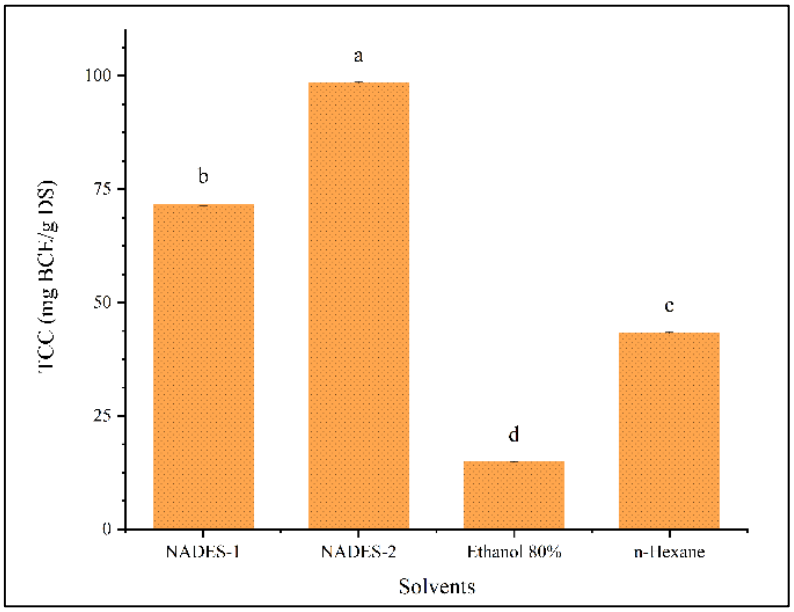


Figure 2. Total carotenoid content of each solvent. Bars represent mean values, and error bars indicate standard deviation (SD) from three independent experiments. Different letters indicate significant differences based on one way ANOVA followed LSD test ($p<0.05$)

Overall, the total carotenoid content obtained from NADES extracts was significantly higher than that extracted using conventional solvents. Although n-hexane is commonly used for carotenoid extraction due to its nonpolar nature, its yield in this study was substantially lower than that obtained with NADES systems, likely due to its limited ability to penetrate plant cell matrices and facilitate metabolite release. Similarly, the very low yield obtained with 80% ethanol suggests that highly polar solvents are less suitable for extracting predominantly lipophilic pigments such as carotenoids. This finding aligns with previous studies that have reported superior performance of NADES in extracting carotenoids from plant materials compared to conventional solvents [7], [8], [11]. The enhanced efficiency is attributed to the strong hydrogen bonding interactions in NADES, which improve penetration into plant cell structures and induce the formation of more pores and fractures through hydrogen bonding, van der Waals forces, and ionic interactions [14], [17]. Furthermore, the extraction performance is further improved when NADES are combined with Ultrasound-Assisted Extraction (UAE), due to the synergistic effects between ultrasonic cavitation and the solvent's capacity to interact with target molecules [13].

3.3. Optimization of Ultrasound-Assisted Extraction

Optimization of Ultrasound-Assisted Extraction (UAE) conditions is essential to maximize carotenoid yield from marigold flowers. In this study, extraction parameters were optimized using

Response Surface Methodology (RSM) with a Box–Behnken Design (BBD) to evaluate the influence of each variable on the total carotenoid content obtained. The selected independent variables were based on their potential effects on extraction efficiency. At this step, the hydrophobic NADES composed of menthol:lactic acid (8:1) was used as the extraction solvent due to its previously demonstrated optimal performance. Response data from the optimization experiments are presented in Table 3.

The ANOVA results for the proposed model (Table 4) showed that the quadratic model was highly significant ($p < 0.0001$), with a not-significant lack of fit ($p = 0.0602$), indicating that the model was appropriate and had a significant effect on the response. The model demonstrated a high coefficient of determination ($R^2 = 0.99$), with predicted R^2 of 0.82 and adjusted R^2 of 0.97, with a difference less than 0.2—suggesting a good agreement between experimental and predicted values. The model not only fits the current data but also shows good predictive capability for future observations. Additionally, an adequate precision value of 23.04 confirmed a sufficient signal-to-noise ratio, indicating that the model can be used to navigate the design space. The quadratic equation in codes (2) describing the effects of ultrasonic power (X_1), time (X_2), and temperature (X_3) of extraction on the total carotenoid content (TCC) of marigold flower extract, is explained as follows:

$$TCC = 254.63 + 7.64X_1 + 17.58X_2 - 0.4735X_3 + 19.53X_1X_2 + 29.25X_1X_3 - 21.80X_2X_3 - 3.07X_1^2 - 4.29X_2^2 + 6.99X_3^2 \quad \text{……..(2)}$$

Table 3. Box-Behnken Design (BBD) and Total Carotenoid Content (TCC)

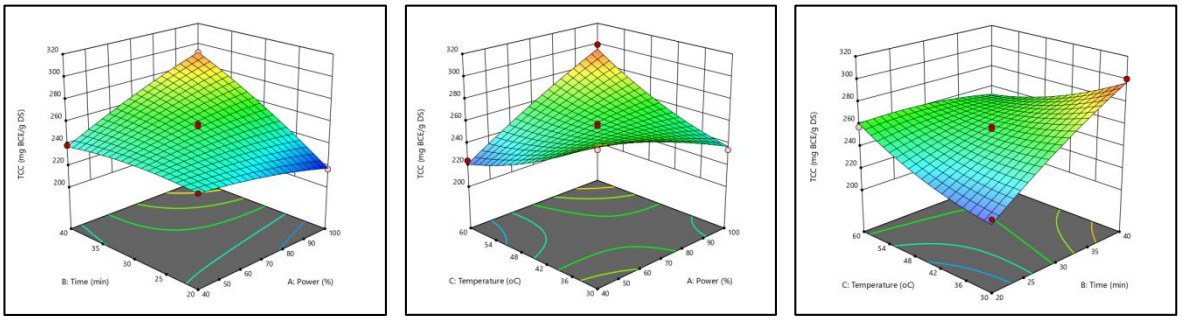
Run	X_1 Power (%)	X_2 Time (min)	X_3 Temperature (°C)	Predicted TCC (mg BCE/g DS)	Experiment TCC (mg BCE/g DS)
1	100	30	30	237.41	234.31
2	70	30	45	254.63	253.38
3	100	30	60	294.97	299.31
4	70	20	30	218.42	222.55
5	40	20	45	241.57	241.78
6	70	30	45	254.63	256.24
7	70	20	60	261.07	257.75
8	100	40	45	292.02	291.80
9	40	30	30	280.63	276.29
10	70	40	30	297.18	300.49
11	40	30	60	221.18	224.28
12	70	30	45	254.63	252.83
13	70	30	45	254.63	252.37
14	40	40	45	237.68	238.71
15	100	20	45	217.80	216.77
16	70	40	60	252.64	248.52
17	70	30	45	254.63	258.32

ANOVA also revealed that ultrasonic power (X_1) and extraction time (X_2) had statistically significant effects ($p \leq 0.05$) on total carotenoid yield. These findings are consistent with those reported by Viñas-Ospino et al. (2023b), who observed that increasing ultrasonic power and extraction time significantly improved total carotenoid content. This enhancement is attributed to the increased cavitation and mechanical effects of ultrasound, which expand the contact surface area between solid and liquid phases, thereby improving solvent penetration into the plant matrix [18]. In addition, prolonged contact time between the solvent and solid enhances diffusion, facilitating the release of carotenoids from plant tissues into the extraction medium [19]. Meanwhile, extraction temperature (X_3) showed no statistically significant effect ($p > 0.05$) on total carotenoid content. Similar results were reported by Rodrigues et al. (2020), indicating that temperature variation within the range of 30–60°C was insufficient to produce a meaningful impact on extraction efficiency.

Table 4. ANOVA for The Fitted Quadratic Polynomial Model

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	10097.85	9	1121.98	55.62	< 0.0001
X_1-Power	467.24	1	467.24	23.16	0.0019
X_2-Time	2473.33	1	2473.33	122.62	< 0.0001
X_3-Temperature	1.79	1	1.79	0.0889	0.7742
X_1X_2	1525.16	1	1525.16	75.61	< 0.0001
X_1X_3	3423.24	1	3423.24	169.71	< 0.0001
X_2X_3	1900.23	1	1900.23	94.20	< 0.0001
X_1^2	39.65	1	39.65	1.97	0.2036
X_2^2	77.51	1	77.51	3.84	0.0908
X_3^2	205.82	1	205.82	10.20	0.0152
Residual	141.20	7	20.17		
Lack of fit	115.05	3	38.35	5.87	0.0602
Pure error	26.15	4	6.54		
Cor total	10239.05	16			

Three-dimensional response surface plots were generated to illustrate the interactions between variable pairs and their effects on the response (Figure 3). The plot showing the interaction between ultrasonic power and extraction time (Figure 3a) indicated that increasing both variables led to higher total carotenoid content. Likewise, the interaction between ultrasonic power and temperature (Figure 3b) showed a similar positive trend. The response surface plot for extraction time and temperature (Figure 3c) demonstrated that longer extraction durations resulted in higher total carotenoid content, likely due to more extensive cell wall disruption induced by prolonged ultrasonic exposure [19].



(**a**) (**b**) (**c**)

Figure 3. 3D response surface plot of the interaction effect between (a) ultrasonic power and time; (b) ultrasonic power and temperature; (c) time and temperature on total carotenoid content

According to the RSM-BBD analysis, the optimum UAE conditions for carotenoid extraction from marigold flowers were 99.96% ultrasonic power, 39.65 min extraction time, and 58.21°C, with a predicted maximum response of 303.20 mg BCE/g DS. Experimental validation, performed at 100% ultrasonic power, 40 min, and 60°C according to instrument capability, yielded 304.01 ± 1.60 mg BCE/g DS, with no significant difference from the predicted value (Table 5). Although prolonged ultrasonication may increase the risk of thermal degradation, temperature control at 60°C remained sufficient to preserve a high carotenoid yield, indicating that the enhancement of mass transfer and cell disruption outweighed possible degradation effects within the studied range [5]. These results confirm the adequacy and predictive reliability of the quadratic model for estimating total carotenoid content under the selected extraction conditions.

Table 5. Confirmation Test Results with Optimum Extraction Conditions

Parameter	Predicted value	Actual value
Ultrasonic power (%)	99.96	100
Extraction time (min)	39.65	40
Extraction temperature (°C)	58.21	60
TCC (mg BCE/g DS)	303.20[a]	304.01[a]

[a]: The same letter indicates the value is not significantly different ($p>0.05$) based on the One Sample t-Test test results.

Overall, these findings demonstrate the potential of NADES as green solvents for carotenoid extraction from marigold flowers, especially when combined with UAE. However, a major limitation of NADES-based extraction is the difficulty of obtaining a purified extract without residual NADES, due to their low volatility and limited removability by conventional evaporation. Several studies have addressed this challenge through downstream purification approaches such as chromatography, resin-based separation, and antisolvent treatment [20], [21]. In contrast, some reports have proposed the direct use of NADES extracts as ready-to-use extracts, since many NADES components are considered relatively safe and compatible with certain pharmaceutical and nutraceutical formulation systems [5]. This approach eliminates the need for extensive solvent removal steps that are typically required when conventional organic solvents are used, thereby simplifying the downstream processing and reducing the risk of residual solvent contamination. From a green pharmaceutical perspective, the direct application of NADES extracts also aligns with the principles of sustainable and environmentally responsible drug development. NADES systems are often biodegradable, exhibit low volatility, and can be formulated from renewable and food-grade components such like menthol and lactic acid in this study, which reduces environmental impact and improves safety for both operators and end users. Furthermore, minimizing solvent removal steps may decrease energy consumption, lower process costs, and reduce the generation of chemical waste, all of which are important considerations in the development of greener extraction and formulation technologies. Nevertheless, the practical implementation of this strategy requires careful evaluation of several factors, including the physicochemical compatibility of NADES with the intended dosage form, the stability of the extracted bioactive compounds within the solvent matrix, and the potential influence of NADES components on bioavailability and pharmacokinetic behavior. Therefore, the future application of NADES should consider not only extraction efficiency, but also the most appropriate post-extraction strategy, taking into account the final intended use of the extract, regulatory considerations, formulation compatibility, and broader green pharmaceutical objectives.

4. CONCLUSION

This study show that NADES are effective green solvents for the extraction of carotenoids from marigold flowers. Among the tested formulations, the hydrophobic NADES composed of menthol:lactic acid (8:1) yielded the highest total carotenoid content at 98.48 ± 0.20 mg BCE/g DS, outperforming the hydrophilic NADES (71.71 ± 0.33 mg BCE/g DS), hexane (43.35 ± 0.19 mg BCE/g DS), and 80% ethanol (14.95 ± 0.05 mg BCE/g DS). Optimization of the extraction process using Ultrasound-Assisted Extraction (UAE) resulted in optimal conditions at 100% ultrasonic power, 40 minutes extraction time, and 60°C, achieving a maximum total carotenoid content of 304.01 ± 1.60 mg BCE/g DS. These findings highlight the potential of NADES as a sustainable and efficient solvent for the extraction of carotenoids from marigold flowers.

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