International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; 9(1): 726-729
www.biochemjournal.com
Received: 15-11-2024
Accepted: 18-12-2024

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Ultrasound-assisted extraction of anthocyanins from butterfly pea flower (*Clitoria ternatea* L.): A sustainable approach

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DOI: https://doi.org/10.33545/26174693.2025.v9.i1i.3616

Abstract
The Butterfly Pea Flower (*Clitoria ternatea* L.), rich in anthocyanins, offers significant health advantages and applications in food, pharmaceuticals, and cosmetics. Traditional extraction methods often face challenges such as high energy and solvent use. Ultrasound-assisted extraction (UAE) emerges as a sustainable solution, offering faster processing, lower energy demands, and higher yields. The purpose of this study is to extract anthocyanins from butterfly pea flower (*Clitoria ternatea* L.) using ultrasound-assisted extraction (UAE). Different solvents (distilled water, 70% ethanol-water, phosphate buffer) were tested for their efficiency, and the effects of ultrasound parameters (ultrasonic power (200–250 W), sonication time (15–35 minutes), and solid-solvent ratios (1:20–1:40 g/ml)) on total anthocyanin content (TAC) were evaluated. The highest TAC was observed with UAE using phosphate buffer. Results indicated significant variations in total anthocyanin content, ranging from 14.19±0.168 to 49.26±0.2 mg CE/g of dry weight, based on the experimental conditions. The highest yield (49.26±0.2 mg CE/g) was achieved at 225 W, 35 minutes, and a 1:30 g/ml ratio. Beyond optimal conditions, efficiency declined due to dilution and degradation. Statistical analysis confirmed the significance of the studied variables. UAE proved to be an efficient and sustainable technique for anthocyanin extraction, offering higher yields and reduced processing times compared to conventional methods.

Keywords: Anthocyanins, butterfly pea flower, ultrasound-assisted extraction, total anthocyanin content, statistical analysis

Introduction

Natural pigments have emerged as crucial components in the food industry, offering both functional and aesthetic benefits. Derived from sources like plants, algae, and microorganisms, these pigments, including carotenoids, anthocyanins, betalains, and chlorophylls, are widely considered as natural pigments in food products (Cortez *et al*., 2017) [1]. Beyond their coloring properties, natural pigments exhibit bioactive properties like antimicrobial, antioxidant, and anti-inflammatory activities, which contribute to the health-promoting attributes of food (Vinha *et al*., 2018) [2]. Their application extends to functional foods, beverages, and confectioneries, enhancing both visual appeal and nutritional quality. Additionally, natural pigments are integral to developing intelligent and active packaging systems, where they act as indicators of freshness, spoilage, or contamination by undergoing specific color changes under varying conditions (Luo *et al*., 2022) [3]. This dual role of natural pigments as colorants and bioactive agents underscores their importance in addressing consumer demand for safe, sustainable, and health-focused food products.

Anthocyanins are naturally occurring flavonoid pigments that give plants their red, blue, and purple hues. These compounds exhibit remarkable antioxidant, anti-inflammatory, and anti-cancer properties, making them invaluable in the food, pharmaceutical, and cosmetic industries (Khoo *et al*., 2017; Giusti and Wrolstad, 2001) [4, 5]. Anthocyanins also act as natural colorants, providing an eco-friendly alternative to synthetic dyes. The butterfly pea flower (*Clitoria ternatea* L.), native to Southeast Asia, is high in anthocyanins, particularly delphinidin derivatives. Traditionally used in herbal remedies and as a natural food colorant, it has gained popularity due to its antioxidant properties and health benefits (Goh *et al*., 2021; Wijaya *et al.*, 2021) [6, 7]. Conventional extraction methods like maceration, soxhlet extraction, etc. for anthocyanins are often time-consuming and inefficient, involving high solvent consumption and thermal degradation of bioactive compounds. Ultrasound-assisted extraction (UAE) has emerged as a sustainable alternative, utilizing ultrasonic cavitation to enhance cell disintegration and mass transfer, resulting in higher yields and reduced processing times (Chemat *et al*., 2017; Vinatoru *et al*., 2017; Jeyaraj *et al*., 2021) [8, 9, 10]. Recent advancements demonstrate the synergy between UAE and green solvents, such as natural deep eutectic solvents, to achieve sustainable extractions (Thakur *et al*., 2022). This study aims to optimize UAE parameters to maximize anthocyanin extraction from butterfly pea flowers and evaluate the influence of solvent choice and operating conditions on extraction efficiency.

Materials and Methods

Raw Materials and Chemicals

Fresh butterfly pea flowers were collected from the Garden Section, GBPUAT, Pantnagar. The petals of flower were manually separated and dried using tray dryer at 40 °C. The dried petals were ground to obtain fine powder. Finally, the powder was sieved and stored in airtight amber glass jars till further processing. Analytical-grade chemicals and reagents were used for all experiments.

Selection of Solvents

Three solvents viz. distilled water, 70% ethanol-water, and phosphate buffer (pH 7) were chosen based on their extraction efficiency and compatibility with UAE. All solvents were of analytical grade.

Ultrasound-Assisted Extraction (UAE)

The powdered butterfly pea flower (5 g) was mixed with solvent and were subjected to ultrasonication. UAE was performed using a probe type ultrasonicator (frequency: 40 kHz; probe diameter: 20 mm) under varying conditions of UAE parameters (ultrasonic power, sonication time and solid-solvent ratio) as given in Table 1. After this, the extracts were filtered, centrifuged, and stored at 4 °C for TAC analysis.

Total Anthocyanin Content (TAC) determination

TAC was measured using the pH differential method, with absorbance recorded at 520 nm and 700 nm at pH levels of 1.0 and 4.5. The results were reported as milligrams of cyanidin-3-glucoside equivalents per gram (mg CE/g) of dry weight (Giusti and Wrolstad, 2001) [5].

Statistical Analysis

Experimental data were analyzed using IBM SPSS Statistics software. One-way ANOVA was employed to evaluate the effects of UAE parameters, and significant differences among means were determined using the Tukey HSD test at a significance level of $p<0.05$.

Results and Discussion

Comparison of Solvents

The efficiency of different solvents in anthocyanin extraction varied significantly. Phosphate buffer exhibited the highest TAC (34.6 mg CE/g), followed by distilled water (33.66 mg CE/g) and 70% ethanol-water (23.78 mg CE/g) as shown in Table 2. The buffering capacity of phosphate buffer likely contributed to better pigment stability and extraction efficiency (Khoo *et al*., 2017; César *et al*., 2018; Salacheep *et al*., 2020) [4, 12, 13].

Table 1: Independent parameters for ultrasound assisted extraction of anthocyanin

Independent parameters	Symbols	Levels		
Ultrasonic power (W)	X_1	200	225	250
Sonication time (min)	X_2	15	25	35
Solid-solvent ratio (g/ml)	X_3	1:20	1:30	1:40

Table 2: TAC for different solvents at centre points

Solvents	TAC (mg CE/g)
Distilled water	33.66
70% ethanol-water	23.78
Phosphate buffer	34.6

Effect of UAE Parameters on TAC

The experiments were conducted using the single-factor experiment design wherein one independent parameter was varied at a time keeping other parameters constant at centre points (ultrasonic power (225 W), sonication time (25 minutes), and solid-solvent ratio (1:30 g/ml)). Phosphate buffer was used as solvent for UAE of anthocyanin from butterfly pea flower. Table 3 shows the values of TAC obtained at different combinations of UAE parameters and the effect of each independent parameter on TAC was analysed.

Table 3: TAC of extracts at different combinations of UAE parameters

S. No.	Ultrasonic power (X_1, W)	Sonication time (X_2, min)	Solid-solvent ratio (X_3, g/ml)	TAC (mg CE/g DW)
1	225	25	1:20	14.19±0.168
2	225	25	1:40	23.88±0.176
3	200	25	1:30	32.9±0.2
4	250	25	1:30	39.5±0.2
5	225	15	1:30	36.4±0.2
6	225	35	1:30	49.26±0.243

Note: TAC values are represented as Mean ± SD.

Ultrasound Power

Increasing ultrasound power enhanced TAC due to improved cavitation effects, which enhance cell wall disruption and solvent penetration (Vinatoru *et al*., 2017) [9]. It was observed that TAC increased with the increasing ultrasonic power. At 250 W, TAC was observed to be 39.50±0.2 mg CE/g. However, the maximum TAC (49.26±0.2 mg CE/g) was observed at ultrasonic power of 225 W and sonication time of 35 min. The decrement in TAC at highest ultrasonic power might be because of degradation, possibly due to over-agitation and localized heating, which can break down anthocyanin molecules

(Thakur *et al*., 2022; Chemat *et al*., 2017; Wang and Weller, 2006) [11, 8, 14].

Sonication Time

TAC increased with sonication time up to 35 minutes, as prolonged exposure improved cell disruption and pigment release. However, first TAC decreased with increasing sonication time up to 25 minutes and then increased when sonication time increased upto 35 minutes. The further increase in sonication time (40-60 minutes) reduced TAC, likely due to oxidative degradation of anthocyanins under extended sonication (Yusoff *et al*., 2022; Khoo *et al*., 2017) [15, 4].

Solid-Solvent Ratio

A solid-solvent ratio of 1:30 g/mL provided optimal TAC by balancing solvent availability and mass transfer efficiency. Lower ratios (1:20) resulted in insufficient solvent contact, while higher ratios (1:40) diluted the solute concentration, reducing extraction efficiency (César *et al*., 2018; Giusti and Wrolstad, 2001) [12, 5]. Ultrasonication also enhances the stability of bioactive compounds, as noted in studies on other natural pigments (Duan *et al*., 2019; Londoño-Londoño *et al*., 2010) [16, 17].

Table 4: Comparison of means using Tukey hsd test

Solid-Solvent Ratio (g/ml)	TAC	Ultrasonic Power (W)	TAC	Sonication Time (min)	TAC
01:20	14.19±0.168^{c}	200	32.9±0.2^{c}	15	36.4±0.2^{b}
01:30	34.6±0.2^{a}	225	34.6±0.2^{b}	25	34.6±0.2^{c}
01:40	23.88±0.176^{b}	250	39.5±0.2^{a}	35	49.26±0.243^{a}

Note: Data were expressed as the mean values ± standard derivations of three replicates. Different superscript characters presented the significant differences (p<0.05) by one-way ANOVA tests.

The synergistic effects of these parameters highlight the importance of optimization in UAE. Statistical analysis (ANOVA) confirmed the all the parameters viz. ultrasound power, sonication time, and solid-solvent ratio have significant impact of on TAC (p<0.05). Table 4 shows the results of Tukey hsd test and reported that all the means are significantly different.

The results underscore the advantages of UAE over conventional methods, aligning with previous studies that reported higher yields and shorter extraction times using ultrasound techniques (Chemat *et al*., 2017; Vinatoru *et al*., 2017) [8, 9]. The role of cavitation in enhancing mass transfer and cell wall disruption is well-documented, and its application in anthocyanin extraction offers both efficiency and sustainability (Wang and Weller, 2006; Zhou *et al*., 2018) [14, 18]. However, the degradation observed at higher power and prolonged sonication emphasizes the need for careful parameter control. Future research could explore the integration of UAE with other green extraction technologies to further enhance anthocyanin recovery.

Conclusion

This study highlights the potential of UAE as an efficient and sustainable technique for anthocyanin extraction from butterfly pea flower. Phosphate buffer proved to be the most effective solvent. The highest TAC (49.26±0.2 mg CE/g) was observed at UAE parameters—225 W ultrasonic power, 35 minutes sonication time, and a 1:30 g/mL solid-solvent ratio. UAE offers a viable alternative to conventional methods, ensuring higher yields, reduced processing time, and environmental sustainability.

Acknowledgement

The authors gratefully acknowledge the Dean, College of Technology, and the Head, Department of PHPFE, GBPUA&T, Pantnagar, India, for providing the research facilities, financial support, and technical assistance necessary for the successful completion of this study.

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