

Effectiveness of Green Extraction Techniques for Fucoxanthin Recovery from Algae: A Systematic Review and Meta-Analysis

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Fucoxanthin, a key carotenoid derived from algae, exhibits various biological activities, prompting interest in sustainable extraction methods. Green extraction techniques such as ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), and deep eutectic solvents (DES) have emerged as environmentally friendly alternatives to conventional methods, which often pose efficiency and ecological concerns. This systematic review and meta-analysis aimed to quantitatively assess the effectiveness of green extraction methods in enhancing fucoxanthin yield from various algal species. A systematic search was conducted under PRISMA guidelines across Scopus, PubMed, Web of Science, and ScienceDirect up to March 31, 2025. Eligible studies included *in vitro* or *ex vivo* experiments on algal fucoxanthin extraction using green methods, with quantitative outcomes and conventional controls. Data extraction and risk of bias assessment were performed independently. Meta-analysis was conducted using RevMan 5.4, applying a random-effects model to calculate standardized mean differences (SMD) and I^2 to assess heterogeneity. Fourteen studies were included in the review, of which eight were eligible for meta-analysis. Green extraction methods showed a trend toward higher fucoxanthin yields that did not reach statistical significance (SMD = 2.21, 95% CI: -0.44 to 4.87, $p = 0.10$). Substantial heterogeneity ($I^2 = 66\%$) was noted, attributed to algal species and extraction conditions. Some individual studies, particularly those utilizing UAE and SFE, reported significant yield improvements. Although not statistically conclusive, green extraction methods show promising potential to enhance fucoxanthin recovery while offering environmental and operational benefits. Further research is needed to optimize parameters across algal species.

Keywords: Fucoxanthin, Green extraction, UAE, SFE, DES, algae, Meta-analysis

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Fucoxanthin, a xanthophyll carotenoid predominantly found in brown algae and microalgae, has garnered significant attention in recent decades due to its broad spectrum of biological activities [1]. This compound is recognized for its antioxidant, anti-inflammatory, anticancer, anti-obesity, and antidiabetic properties, making it a promising candidate for applications in the pharmaceutical, nutraceutical, and cosmetic industries. The growing demand for fucoxanthin has driven efforts to develop efficient and sustainable extraction methods aligned with the principles of green chemistry [2, 3].

Conventional extraction methods for fucoxanthin commonly rely on volatile organic solvents such as methanol, ethanol, or chloroform [4]. While effective, these methods have several drawbacks, including high energy consumption, prolonged extraction times, potential thermal degradation of fucoxanthin, and adverse environmental impacts due to toxic and non-biodegradable solvents. These limitations have spurred innovation toward developing greener and more efficient extraction techniques [5, 6].

Green extraction methods such as ultrasound-assisted extraction (UAE), supercritical CO_2 extraction (SFE), and deep eutectic solvents (DES) offer promising alternatives [7]. UAE utilizes ultrasonic waves to enhance solvent penetration and the release of bioactive compounds through cavitation effects, reducing extraction time and solvent usage. Although it requires high-pressure equipment, SFE employs tunable supercritical CO_2 as a solvent, enabling selective extraction without leaving harmful residues [8, 9]. DESs are novel solvents formed from mixtures of two or more components that create a eutectic point. They are known for their non-toxic, biodegradable nature and strong solubilizing ability for various secondary metabolites.

Although numerous individual studies have examined the effectiveness of various green extraction techniques for fucoxanthin, their findings often vary significantly. This variation may arise from differences in algal species (microalgae vs. macroalgae), experimental conditions, and specific extraction parameters. For instance, several studies have

reported that SFE and UAE can yield significantly higher fucoxanthin levels than conventional methods, particularly when optimized extraction parameters [4, 10] are used. However, a comprehensive and quantitative comparison of green versus conventional extraction methods and among different green methods remains absent.

The identified research gap lies in the lack of a systematic review and meta-analysis that quantitatively evaluates the effectiveness of green extraction techniques in enhancing fucoxanthin yield across various algal sources. Existing studies focus on specific methods or algal types, making it challenging to draw general conclusions about overall efficiency and relative advantages. Scientifically, this review is necessary to synthesize available evidence, better understand the trends and potential of green extraction methods, and identify key factors contributing to variability in results. The findings will provide evidence-based guidance for researchers and industry stakeholders in selecting the most efficient, sustainable, and cost-effective methods for large-scale fucoxanthin production.

In response to this gap and urgency, the present study aims to conduct a systematic review and meta-analysis to evaluate the effectiveness of various green extraction methods in increasing fucoxanthin yield from algae. Specifically, the objectives are to: (1) identify and characterize relevant studies on fucoxanthin extraction using green methods; (2) assess the risk of bias and the methodological quality of the included studies; (3) quantitatively synthesize outcome data to compare the effectiveness of green versus conventional methods and among specific green techniques; and (4) identify factors contributing to heterogeneity and provide recommendations for future research.

METHODS

Study Design

This study was conducted as a systematic review and meta-analysis to evaluate the effectiveness of green extraction techniques in enhancing fucoxanthin yield from various algal species and to compare them with conventional extraction methods. The research protocol was developed and reported in full accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, ensuring transparency and methodological rigor.

Search Strategy

A comprehensive literature search was conducted across several major databases, including Scopus, PubMed, Web of Science, and EBSCOhost, covering all publications up to March 31, 2025. No language

restrictions were applied. Search terms included: "fucoxanthin" AND ("green extraction" OR "ultrasound-assisted extraction" OR "supercritical CO₂ extraction" OR "deep eutectic solvents") AND ("macroalgae" OR "microalgae"), using Boolean operators to maximize the retrieval of relevant studies.

Eligibility Criteria

Studies were included if they met the following criteria: (1) experimental in vitro or ex vivo designs involving algal fucoxanthin extraction; (2) application of at least one green extraction method (UAE, SFE, or DES); (3) quantitative data on fucoxanthin yield reported in standardized units (e.g., mg/g dry weight); and (4) a conventional extraction control group present for comparison. Exclusion criteria included a lack of extractable quantitative data, literature reviews, conference abstracts, unpublished studies, and studies using commercial fucoxanthin or non-algal biomass mixtures.

Data Extraction and Synthesis

Data were extracted independently by two reviewers using a piloted extraction form. The collected data included author names, publication year, algal species (macroalgae/microalgae), green extraction method, extraction parameters (e.g., temperature, duration, solvent type, solvent-to-solid ratio), fucoxanthin yield (mg/g DW), and statistical data (mean, SD, sample size). Disagreements were resolved through discussion or, if necessary, arbitration by a third reviewer to ensure accuracy and consistency.

Risk of Bias Assessment

The risk of bias was assessed using a modified version of the Cochrane Risk of Bias Tool (RoB 2.0) tailored for non-clinical experimental studies. Key domains evaluated included random sequence generation, allocation concealment, blinding (participants, personnel, outcome assessment), completeness of outcome data, and selective reporting. Two reviewers conducted assessments independently, resolving discrepancies by consensus. While certain domains, particularly randomization and allocation concealment, often showed high or unclear risk due to the nature of in vitro studies, most studies exhibited low risk regarding data completeness and reporting.

Statistical Analysis

All statistical analyses were conducted using RevMan 5.4. A random-effects model was employed to calculate pooled effect sizes due to anticipated heterogeneity. The primary effect measure was the standardized mean difference (SMD) with a 95% confidence interval (CI). Heterogeneity was evaluated using the I² statistic, interpreted as low (<25%), moderate (25–75%), or high (>75%). Funnel plots were visually examined for

potential publication bias, and further assessments were performed using Egger's regression test to support the robustness of the meta-analysis.

RESULTS AND DISCUSSION

This section presents the main findings of the systematic review and meta-analysis to evaluate the effectiveness of various green extraction methods in enhancing fucoxanthin yield from algae. The studies were identified and selected systematically according to the PRISMA guidelines, resulting in a set of eligible studies for inclusion. The selected studies displayed considerable variation in algal species, extraction techniques, measurement units, and reported fucoxanthin yields. After the screening process, 14 studies were included in the systematic review, with 8 providing complete quantitative data

further analyzed in the meta-analysis. The results are presented through study characteristics, risk of bias assessment, and statistical synthesis using forest and funnel plots.

The literature selection process adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework, comprising four key stages: identification, screening, eligibility assessment, and final inclusion. Studies were sourced from various databases and secondary sources, then assessed based on title, abstract, and full-text content using predefined inclusion and exclusion criteria. Most excluded studies did not meet the eligibility criteria due to reasons such as not utilizing green extraction methods, lacking quantitative data on fucoxanthin, or being narrative reviews. The complete study selection flow is depicted in Figure 1.

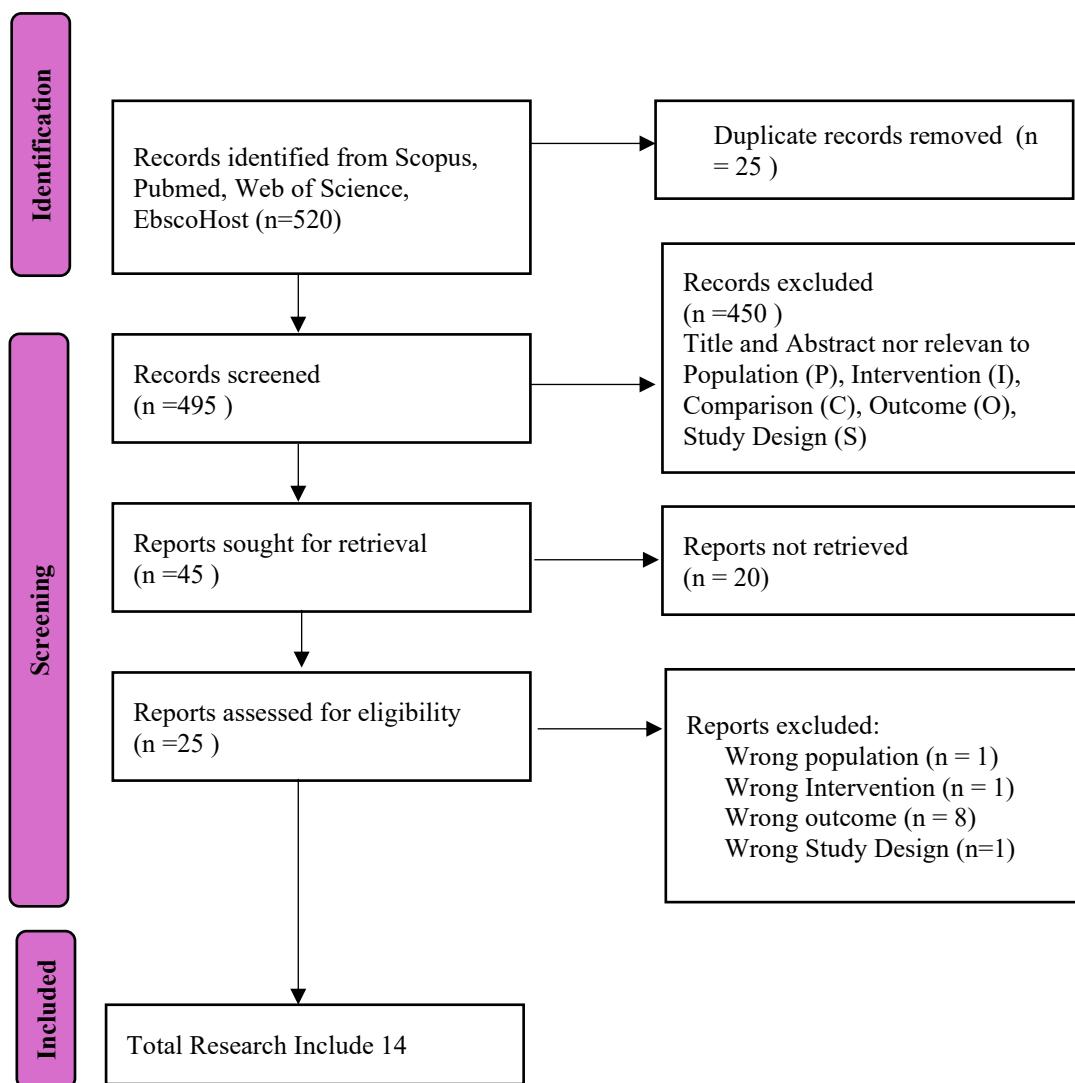


Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework.

Table 1. Summary of Study Characteristics (n = 14).

Component	Characteristic	n	Percentage (%)
Study Design	Experimental (in vitro)	14	100.0
Algae Type	Microalgae	9	64.3
	Macroalgae	5	35.7
Extraction Method	Ultrasound-assisted extraction (UAE)	6	42.9
	Deep eutectic solvents (DES)	2	14.3
	Supercritical fluid extraction (SFE)	2	14.3
	Electropermeabilization-assisted LBF system	1	7.1
	Dimethyl ether (DME) extraction	1	7.1
	Solvent extraction with chromatography	2	14.3
	Fucoxanthin yield (mg/g or %)	14	100.0
	Compared to conventional solvent extraction	9	64.3
Outcome Focus	Data available for meta-analysis comparison	11	78.6

Study Characteristics and Main Findings

The 14 studies included in this systematic review displayed considerable variation in experimental design, algal species, green extraction techniques, and fucoxanthin measurement units and outcomes. Most studies employed in vitro experimental approaches using either microalgae or brown macroalgae as sources of fucoxanthin. The most commonly used green extraction methods were ultrasound-assisted extraction (UAE), supercritical CO₂ extraction (SFE), and deep eutectic solvents (DES). Additional techniques, such as electro-assisted flotation and dimethyl ether (DME) extraction, were also explored in fewer studies. These studies were conducted in various countries and demonstrated diverse levels of effectiveness depending on the applied method. Table 1 summarizes the core characteristics of the included studies.

Table 1 provides an overview of the 14 studies included in this review. All studies used an in vitro experimental design (100%), mostly focusing on microalgae (64.3%) such as *Tisochrysis lutea*, *Phaeodactylum tricornutum*, and *Chaetoceros calcitrans*. In comparison, the remainder involved brown macroalgae such as *Undaria pinnatifida* and *Sargassum sp* (35.7%). UAE was the most frequently used green extraction method, applied in six studies (42.9%), followed by DES and SFE in two studies each (14.3%). Other approaches included electro-assisted flotation, DME extraction, and chromatography-assisted solvent extraction.

All studies (100%) reported fucoxanthin yield as the primary outcome, expressed either in mg/g or as a percentage. Most studies (64.3%) directly compared green extraction methods with conventional solvent-based techniques such as ethanol or methanol extraction. A total of 11 studies (78.6%) provided adequate quantitative data for inclusion in the meta-analysis.

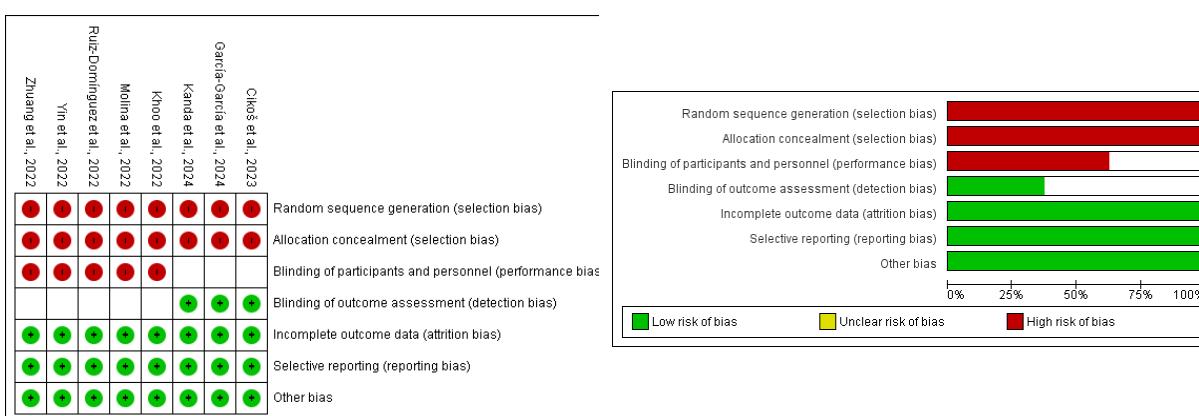
The next section outlines the key features and findings of the eight studies included in the quantitative synthesis. Despite methodological differences, most studies indicated that green extraction techniques produced comparable or higher fucoxanthin levels than conventional methods. Variations in extraction efficacy were noted depending on the technique employed, with UAE, SFE, and DES demonstrating promising results. Table 2 provides a comprehensive summary of these studies and their outcomes.

Meta-analysis

The methodological quality of the included studies was assessed using the Cochrane Risk of Bias tool. Each study was evaluated across key domains, including random sequence generation, allocation concealment, blinding, incomplete outcome data, and selective reporting. The assessment revealed varying levels of bias, with high or unclear risks particularly evident in domains related to randomization and blinding. Figure 2 visually presents a summary of the risk of bias for all included studies.

Table 2. Resume Findings.

No.	Author, Year	Country	Organism	Design (Methods)	Key Extraction Parameters
1.	Kholanya M et al., 2025 [11]	Netherland	<i>Saccharina latissima</i>	Experimental: Hydrophobic eutectic solvents (ES) extraction	Solid-liquid ratio (SLR) of 0.05 (g sun-dried cells/mL solvent)
2.	Zhuang et al., 2022 [12]	China	<i>Isochrysis zhangjiangensis</i>	Experimental: Ethanol-Based Green Method Coupled with Octadecylsilyl (ODS) Column Chromatography	20 mg freeze-dried microalgae powder, 8 mL absolute methanol, 2h extraction, ice bath, dimmed light
3.	Ruiz-Domínguez et al., 2022 [12]	Chile	<i>Phaeodactylum tricornutum</i>	Experimental: Supercritical fluid extraction (SFE) (Carbon dioxide and ethanol)	2.0 g freeze-dried biomass, 24 mL extraction vessel, bed density 0.787 g/mL
4.	Khoo et al., 2022 [13]	Malaysia	<i>Chaetoceros calcitrans</i>	Experimental: Electropermeabilization-assisted liquid biphasic flotation system	50 mg lyophilized biomass, 50 mL 100% (w/w) alcohol, 50 mL 250 g/L salts, 10 min air flotation at 100 cc/min
5.	García-García et al., 2024 [14]	Spain	<i>Tisochrysis lutea</i>	Experimental: Ultrasound-assisted extraction (UAE) with 2-methyl-tetrahydrofuran (2-me-THF) in ethanol	1 g freeze-dried microalgae, 20 mL methanol:chloroform (1:2 v:v) for Folch Method; 1 g microalgal biomass, 10 mL solvent for UAE
6.	Molina et al., 2022 [15]	Mexico	<i>Sargassum sp.</i>	Experimental: Shock wave-induced cavitation	100 mg sun-dried powdered alga, 4 mL ethanol-water (4:1)
7.	Flora et al., 2024 [16]	India	<i>Padina australis</i>	Experimental: Comparison of 4 methods: methanol, chloroform, methanol-chloroform mixture, ultrasonic based method	1 g dried powdered macroalgae, solid-to-solvent ratio of 1:10
8.	Pajot et al., 2023 [17]	France	<i>Tisochrysis lutea</i>	Experimental: Ultrasonic (US) probe with acetone and ethanol solvents, purification with centrifugal partition chromatography	1 g lyophilized biomass, 250 mL acetone or ethanol; 9 g absolute EtOH as solvent
9.	Cikoš et al., 2023 [18]	Slovak	<i>Fucus virsoides</i> , <i>Amphiroa rigida</i> , <i>Codium bursa</i>	Experimental: Optimization of UAE with methanol:dichloromethane (MeOH:DCM, 1:1, v/v)	Freeze-dried algae, solvent:solid ratio 20 mL g ⁻¹
10.	Xu et al., 2022 [19]	New Zealand	<i>Tisochrysis lutea</i>	Experimental: 24 different types of deep eutectic solvents (DESs) extraction	Solid to liquid ratio of 1:25 (w/v)
11.	Yin et al., 2022 [20]	China	<i>Undaria pinnatifida</i>	Experimental: Supercritical CO ₂ extraction method with entrainer ethanol	Freeze-dried alga (5g) in ethanol as solvent
12.	Lin et al., 2024 [21]	Taiwan	<i>Hyalosynedra toxoneides</i>	Experimental: Semi-continuous cultivation, ultrasonic-assisted extraction with 95% ethanol, silica gel chromatography purification	1 gram of biomass per 60 mL of 95% EtOH, 42 °C for an hour
13.	Kanda et al., 2024 [22]	Japan	<i>Chaetoceros simplex</i> var. <i>calcitrans</i>	Experimental: Liquefied DME as solvent, compared to ethanol extraction (control)	5.43 ± 0.05 g wet <i>C. simplex</i> for DME; 3.00 g dry <i>C. simplex</i> and 300 mL ethanol for control
14.	Carreira-Casais et al., 2022 [23]	Spain	<i>Sargassum muticum</i>	Experimental: Ultrasound-assisted extraction (UAE) optimized by response surface methodology (RSM)	1.050 g <i>Sargassum muticum</i> powder, 35 mL ethanol (solid to liquid ratio of 33.33 mL/g)

**Figure 2.** Risk of Bias Assessment Across Included Studies.

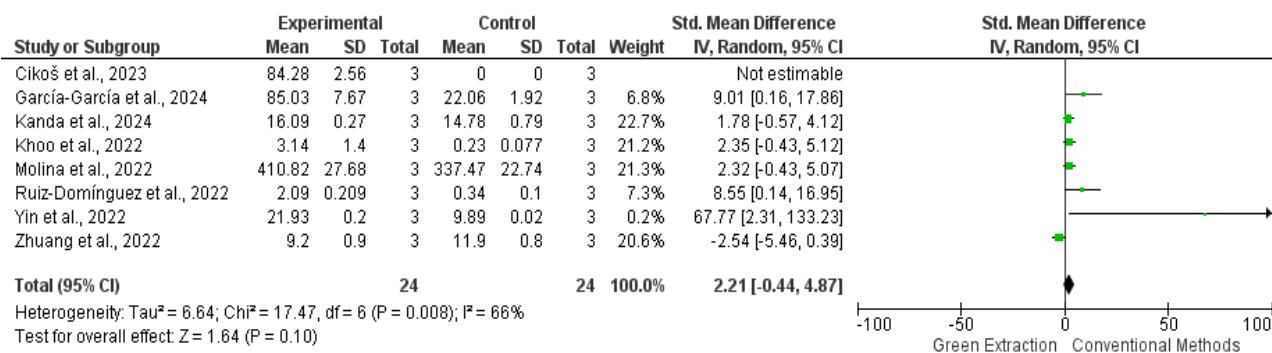


Figure 3. Forest Plot of Green vs. Conventional Extraction Methods on Fucoxanthin Yield.

The risk of bias evaluation for the eight studies in the meta-analysis revealed that the highest risk was associated with selection bias, specifically in random sequence generation and allocation concealment, where all studies were rated as high risk (indicated in red). This is expected, as all included studies were laboratory-based and did not typically incorporate randomization procedures or allocation concealment. Most studies showed an unclear risk (yellow) for performance and detection bias, reflecting insufficient reporting on participant and outcome assessor blinding, which is also common in in vitro research. Conversely, the domains of attrition bias and reporting bias generally showed a low risk (green), indicating that outcome data were reported fully and consistently with study objectives. Overall, while methodological limitations were observed in randomization and blinding, the completeness and consistency of data reporting support the reliability of these studies for quantitative synthesis.

The effect of green extraction methods on fucoxanthin yield was further analyzed through a meta-analysis of eight studies that reported comparable quantitative data. All included studies measured fucoxanthin concentrations in standardized units and compared green extraction methods with conventional solvent-based techniques. The pooled effect sizes and confidence intervals are presented in Figure 3.

The meta-analysis of the eight studies showed a trend favoring green extraction methods, with a standardized mean difference (SMD) of 2.21 (95% CI: -0.44 to 4.87; $p = 0.10$), indicating higher fucoxanthin yield than conventional methods. Although the overall effect was not statistically significant, several individual studies, particularly those using supercritical CO_2 extraction and ultrasound-assisted extraction, reported significantly improved outcomes. The heterogeneity among studies was substantial ($I^2 = 66\%$), suggesting considerable variability likely due to differences in algal species, extraction techniques, and experimental designs.

Effectiveness of Green Extraction Methods for Fucoxanthin Enhancement

This study demonstrates that green extraction methods yield higher fucoxanthin content than non-green methods, particularly ultrasound-assisted extraction (UAE) and supercritical CO_2 extraction (SFE). Although the overall effect was not statistically significant ($p=0.10$), the trend suggests a promising potential for these techniques. This indicates that while interpretation should be cautious, green extraction methods hold considerable value.

Several individual studies reported statistically significant improvements, especially those using optimized SFE and UAE protocols. Due to high pressure and temperature, SFE enables efficient extraction of lipophilic compounds, which enhances fucoxanthin solubility and diffusion from the cell matrix. Conversely, the UAE employs cavitation effects that rapidly increase solvent penetration and release bioactive compounds [24–26].

Differences in outcomes among studies may stem from variation in green solvent types, extraction time, and processing temperature. Although not all results reached statistical significance, green extraction methods still offer ecological and practical advantages that should not be overlooked. A more systematic approach to parameter optimization is needed to fully explore their potential.

Yield Variability Based on Algal Type and Extraction Method

The high heterogeneity observed ($I^2= 66\%$) suggests substantial variability in extraction efficiency across studies. One major contributing factor is the type of algae used. For example, brown algae such as *Fucus virsoides* have been shown to produce higher fucoxanthin content than red or green algae [18].

Cell wall structure differences between algal types affect solvent penetration and compound release. Microalgae like *Nannochloropsis oculata* also respond differently depending on the extraction method and solvent composition, including ethanol and natural eutectic mixtures [27]. Other contributing factors include differences in extraction temperature, solvent-to-solid ratio, and duration, which complicate cross-study comparisons. Future research should rigorously report and control these variables to improve methodological comparability.

Comparative Performance of Specific Green Extraction Methods

UAE emerged as the most commonly used method due to its simplicity and time efficiency. The cavitation effect of ultrasonic waves facilitates the release of target compounds without requiring large volumes of solvent. UAE showed significant results in several studies, especially when combined with natural deep eutectic solvents [28].

SFE offers advantages in extracting nonpolar compounds like fucoxanthin and eliminates residual solvent concerns. However, the high cost and pressure requirements present practical limitations. SFE efficiency is highly dependent on precise temperature and pressure control to avoid degradation of active compounds [29].

DES offers a highly flexible and environmentally friendly approach. These solvents efficiently extract polar and semi-polar compounds, although high viscosity may impede mass transfer. The combination of DES and UAE has yielded strong results in various studies, although additional purification steps are frequently necessary to achieve high-purity extracts [12].

Statistical Significance and Interpretation of Non-Significant Results

The meta-analysis found that green extraction methods did not demonstrate statistically significant improvements in fucoxanthin yield compared to conventional methods ($SMD = 2.21$, 95% CI: -0.44 to 4.87 , $p = 0.10$). While this result does not meet conventional thresholds for significance, it should not be interpreted as evidence of no effect. Instead, it reflects limited statistical power due to small sample sizes and variability among studies.

Several well-designed studies with optimized parameters demonstrated meaningful improvements using UAE or SFE; however, these effects may have been diluted in aggregate analysis due to methodological heterogeneity. Therefore, the absence of statistical significance should be considered a limitation of the current data rather than a definitive conclusion about efficacy.

The significant heterogeneity ($I^2 = 66\%$) supports this perspective. Factors such as algal species, extraction technique, and processing conditions likely contributed to the variability in results. Even among studies utilizing the same method (e.g., UAE), differences in operational settings like temperature, time, and solvent ratio led to inconsistency. Future studies could benefit from meta-regression or subgroup analysis to identify systematic factors that influence extraction effectiveness.

From a practical standpoint, the lack of statistical significance should not deter the use of green extraction techniques. Instead, their environmental sustainability, energy efficiency, and safety benefits support their ongoing development. Green methods continue to be a relevant and valuable part of modern bioextraction strategies, especially in industrial contexts that prioritize ecological impact.

CONCLUSION

Based on this systematic review and meta-analysis, green extraction techniques—although not achieving overall statistical significance ($SMD = 2.21$, 95% CI: -0.44 to 4.87 , $p = 0.10$)—tend to yield higher fucoxanthin amounts than conventional methods. The observed heterogeneity ($I^2 = 66\%$) underscores the impact of algal species, extraction parameters, and specific techniques used. Individual studies, particularly those employing supercritical CO_2 and ultrasound-assisted extraction, reported promising results under optimized conditions. While additional research with standardized methods and larger sample sizes is needed, the environmental and operational advantages of green extraction make it a promising and sustainable approach for future fucoxanthin production.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted without any commercial or financial relationships that could potentially create a conflict of interest.

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