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ECO-FRIENDLY CAROTENOID EXTRACTION: ADVANCED PURIFICATION TECHNIQUES AND HEALTH ENHANCING BENEFITS

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Abstract
Carotenoids, such as β-carotene, lutein, lycopene, and astaxanthin, are naturally occurring compounds with wide ranging therapeutic potential especially their antioxidant, anti-inflammatory, antimicrobial, anticancer and neuro protective effects. Nevertheless, standard extraction methods involve the use of organic solvents toxic to the external and internal health. The aim of this study is to present the utilization of green technologies for obtaining and purifying carotenoids of natural origin, promoting the improvement of extraction efficiency and suppressing environmental impacts. Supercritical fluid extraction (SFE), microwave assisted extraction (MAE), and ultrasound assisted extraction (UAE) are discussed for their need of safer solvents (like water or ethanol combinations) and shortened extraction times and solvent consumption. The bioactivity of carotenoids is retained when using ecofriendly methods and the efficacy of carotenoids is evaluated for therapeutic applications using anticancer, antioxidant, anti-inflammatory, antimicrobial, and neuroprotective assays. This research underlines the possibility of green extraction technologies to facilitate a sustainable practice of carotenoid rich extracts being applicable to pharmaceutical as well as nutraceutical applications.
Keywords: *Bioactive compounds, Carotenoids, Carotenoids transport mechanism, Environmental conditions, Green extraction, Modern extraction techniques, Sustainable processing, Therapeutic agents*

INTRODUCTION

A diverse group of naturally occurring pigments collectively known as carotenoids are found in various fruits, vegetables, cereals, and even some types of algae. Carotenoids include over 600 known compounds that were first isolated from carrots in the 19th century and play significant role in plant and human nutrition (1). These compounds are important for photosynthesis, light absorption, and photoprotection in plants, and they also give various foods their vibrant yellow, orange, and red colors (2). All (carotenoids) contribute substantially to human health by their antioxidant protection and anticancer potential (3).

Carotenoid synthesis by plants responds to environmental stressors (including microbial attacks), to maintain cellular function and protect tissue. They are pigments that accumulate in the chloroplast and participate in decreasing reactive oxygen species (ROS) formed as a result of exposure to Sun's UV light, hence protecting plants from oxidative stress (4). The chemical diversity of carotenoids is large, and the structures of the various carotenoids differ by degree of saturation, degree of conjugation, and variety of functional groups. Based on their structure, they are typically categorized into two primary classes: Some carotenes lacking oxygen molecules and xanthophylls with oxygen. Each subclass has distinct chemical properties and health-promoting activities in plants and animals (5).

Carotenoids are traditionally extracted using organic solvents such as hexane, acetone, and petroleum ether. These solvents are not only poisonous, but they also pollute the environment and

endanger human health during the manufacturing, handling, and disposal processes. Furthermore, these methods frequently involve high energy consumption, extended extraction times, and large solvent waste, which undermines sustainability efforts (6). The use of toxic chemicals can reduce the quality and bioactivity of extracted carotenoids, reducing their potential for medicinal uses.

Emerging green technologies, like supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE), provide novel answers to these problems. These approaches use cleaner solvents, such as water and ethanol, to reduce solvent use and environmental effect while keeping carotenoids' bioactivity (7). Their environmentally friendly character is consistent with the growing need for sustainable and health-conscious techniques in nutraceutical and pharmaceutical manufacturing based on health benefits as shown in Fig. 1.

Carotenoids serve a major antioxidant function by means of the single electron oxidation mechanism across the hydroxyl groups. Hydroxyl groups, among them principally in A and B rings, are fundamental to the ability of carotenoids to quench. This antioxidant potential, however, can be adversely affected by methylation or glycosylation of the hydroxyl group. The inhibitory activity carotenoids have on several enzyme involved free radical production, including cyclooxygenase, lipoxygenase, and NADPH oxidase, reduce oxidative stress even further (8). That's all the effectiveness of carotenoids as natural antioxidants this multifaceted mechanism underlines. It has been demonstrated in epidemiological studies that carotenoids have a health promoting effect. Besides those, consuming a carotenoid rich diet has been associated with a lesser risk of getting many chronic conditions, such as some forms of cancer, heart disease and some diseases of the eyes occurring as a consequence of aging (9). Perhaps most notably, carbons of the carotenoid class possess properties which aid in the reduction of oxidative stress, promote cellular integrity, and support activity of the immune system (10). For example, lycopene may help lower your risk of getting prostate cancer, and lutein and zeaxanthin are important for eye health, including helping to prevent age-related eye problems such as macular degeneration (11).

Dietary carotenoid intake averages 6–15 mg/day with the carotenoid intakes varying depending on the individual dietary pattern and regional food availability. This intake supports carotenoids as antioxidants in the diet (12), and exceeds that of vitamins C and E in some populations, highlighting its importance. Moreover, carotenoids' low toxicity in biological systems has been extensively researched as a potential therapeutic application, in particular, as an alternative or complement to conventional anticancer treatment. The anticancer potential of carotenoids is shown through mechanisms of cell cycle arrest and control of malignant cell growth (13).

Thus, carotenoids form an essential part of the continuing string of extensive health benefits, while also fulfilling important roles in biological, pharmacological, and antioxidative activities as supports behind disease prevention. Therefore, they have shown great promise for development in nutraceuticals and functional foods. This study aims to explore the application of green technologies for the isolation and purification of novel Carotenoids and evaluate their therapeutic activities.

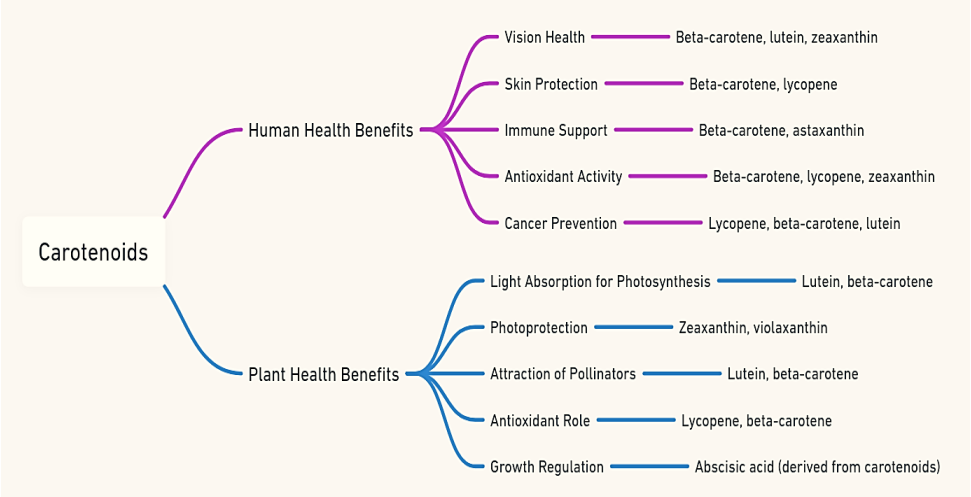

Fig. 1. The health benefits of Carotenoid in both plants and animals

COMPREHENSIVE ANALYTICAL METHODOLOGIES

ISOLATION OF CAROTENOIDS FROM MULBERRY (*MORUS ALBA L.*) LEAVES USING MACROPOROUS RESIN ADSORPTION

The leaves of *Morus Alba* L. commonly known as mulberry are widely used in traditional Asian medicines for their benefits upon the liver, eyes and controlling of blood pressure. These leaves are known to contain several bioactive compounds such as alkaloids, polysaccharides and 1-deoxynojirimycin (DNJ), a compound used for treatment and prevention of diabetes. Since these health benefits would enable these key bioactive carotenoid components from mulberry leaves to be utilized in medicinal or nutraceutical applications, an efficient and simple isolation method could be developed.

Traditional methods, such as column chromatography, are suitable for small-scale analytical work but are inefficient for larger-scale extractions due to their time and solvent needs. To effectively isolate carotenoids for therapeutic applications, a combination of Ultrasound-Assisted Extraction (UAE) and macroporous resin adsorption was utilized. UAE was chosen because of its propensity to dissolve plant cell walls, allowing carotenoids to be released into the solvent while remaining bioactive. The optimum settings included a 70:30 ratio of ethanol and water as the solvent, 30 minutes of sonication at 40 kHz, and a temperature of 30°C. The resultant extracts were filtered and further purified using macroporous resin adsorption. In order to find an optimal resin and desorption solvent, this study investigated static absorption tests at first. After that sequential absorption and desorption were carried out to determine the processing conditions optimal for carotenoid isolation (14).

MATERIALS

After study approval from respective Institutes, this study was conducted inter-collaboratively in diverse settings. Mulberry leaves (Morus alba L.) were provided by Shijiazhuang Pharmaceutical Company based in Shijiazhuang, China and were collected from Henan Province, China. The purchase of leaves and their registered obtained vendors were inter-collaboratively with respective institutes. The leaves were milled, ground by the milling machine, and passed through a 200 mm mesh sieve and stored in the desiccator until use. Experiments were performed in duplicate to ensure repetitive SOPs pertinency.

CAROTENOID QUANTIFICATION ANALYTICAL METHOD

Total carotenoid quantification was carried out for mulberry leaves using UV spectrophotometry. This method was chosen for its simplicity, rapid analysis, and ability to accurately measure carotenoid content in complex plant matrices. With alkali, carotenoids formed a red complex with the aluminum ions permitting spectrophotometric assay. Total carotenoid content was calculated on an equivalent carotenoid basis, using carotenoid as a reference compound. To prepare samples, 1 to 6 mL volume were taken and added to a 25 ml volumetric flask containing 80% ethanol. Sequential addition of reagents including $NaNO_2$, KOH was followed by measurement of absorbance at 500 nm. A linear regression ($A = 12.81C – 0.001060$) with $R^2 = 0.9998$ over a carotenoid concentration range of 0.00214 mg/mL to 0.0643 mg/mL was obtained, with an average recovery rate = 97.9% and RSD (n = 3) = 1.33% (15).

For comparison, SFE was used as an alternate technique to separate carotenoids. At 50°C and 300 pressure, carbon dioxide was employed as the solvent and ethanol as a co-solvent. By using less solvent, this technique maintained the carotenoids' thermal stability. Together with those derived from UAE and macroporous resin adsorption, the carotenoids extracted via SFE were examined.

CAROTENOID ANALYSIS BY HPLC

For compound-specific analysis, High-Performance Liquid Chromatography (HPLC) was employed due to its precision in identifying and quantifying individual carotenoids. We used a Lab Alliance system coupled with an Agilent TC C18 column for the HPLC analysis. The mobile phase consisted of a blend of acetonitrile, methanol, and a diluted phosphoric acid solution in a 100:10:340:0.3 volume ratio. Sample injections of 20 mL were introduced at a flow rate of 1.0 mL/min and detection was

set at 254 nm wavelength. Before analysis, the samples were filtered by 0.45 mm membranes from Chromatography Science and Technology Company, located in Tianjin, China. HPLC remains the gold standard for carotenoid analysis due to its versatility and reliability in handling complex samples.

GREEN CLOUD POINT EXTRACTION (CPE) PHENOLS AND CAROTENOIDS FROM POMEGRANATE PEEL

To investigate more environmentally friendly approaches, Microwave-Assisted Extraction (MAE) was used for the first extraction, followed by Cloud Point Extraction (CPE) for phenol and carotenoid separation. Microwave-Assisted Extraction (MAE) was chosen for its ability to heat quickly, resulting in shorter extraction periods and lower solvent usage. A microwave extractor was used to process dried pomegranate peel powder with an 80:20 mixture of methanol and water at 120°C for 10 minutes. This approach successfully extracted phenols and carotenoids from the matrix.

Cloud Point Extraction (CPE) was used on pomegranate peel samples to test more environmentally friendly extraction processes. CPE was chosen for its low solvent usage and compatibility with non-toxic surfactants like Triton X-114, which aligned with the study's dedication to green chemistry principles. This approach effectively separates bioactive substances by leveraging phase transitions caused by surfactant micelles, minimizing the need for toxic organic solvents. The improved CPE approach optimized pH, surfactant content, and temperature to maximize carotenoid recovery. A Response Surface Design technique was used to systematically improve the extraction process, resulting in great reproducibility and efficiency.

MATERIALS AND CHEMICALS

The fresh fruits of Punicca granatum L of Bhagwa variety were obtained from Mumbai, India. The dried peels were separated carefully with tweezers, dried in a hot air oven for four days at 40°C and then finely ground into a powder. The powdered material was then further processed by passing through a mesh of 40 micrometers, followed by being stored in a freezer at temperature of -20°C. Analysis was done using reagents such as methanol, sodium carbonate, and aluminum chloride and potassium acetate, chemicals were supplied by SD Fine Chemicals (India) and Sigma Aldrich (United States).

POLYPHENOLS AND CAROTENOID EXTRACTION PROCESS THROUGH CLOUD POINT METHOD

A modified procedure based on Katsoyannos et al. (16) was used to combine pomegranate peel powder (0.5 g) with distilled water and a nonionic surfactant (Triton X-114). We vortexed the resulting mixture thoroughly, adjusted the proper pH and spun the mixture at 10,000 revolutions per minute to achieve phase separation. This was accomplished by adding sodium chloride to reduce the temperature at which cloud point formation became necessary for clear separation of phases. The aqueous and surfactant phases were then measured after additional centrifugation at 8,000 revolutions per minute and the sample placed in a water bath. To be able to get an accurate reading, the data was averaged out from six different tests as shown in Fig. 2.

EXPERIMENTAL DESIGN FOR OPTIMIZING EXTRACTION

A Response Surface Design based on a Central Composite framework was used to refine the extraction procedure. With this approach, we were able to systematically analyze how several variables influence the extraction outcomes. Surfactant concentration (volume percent), pH, temperature in Celsius and salt concentration by weight were studied as key factors. Standardized lower (-1) and upper (+1) values were adjusted to each variable. There were total of 30 tests, which include 16 trials at boundary conditions (+1 and -1), 8 trials at extreme axial points ($\pm\alpha$ = +2 and -2) and 6 repetitions at the midpoint for the sake of repeatability of the tests. The efficiency of the extraction was assessed using four main parameters: Y1 = Percentage Recovery (Y1), Y2 = Partition Coefficient (Y2), Y3 = Extraction Loss (Y3) and Y4 = Concentration Ratio (Y4).

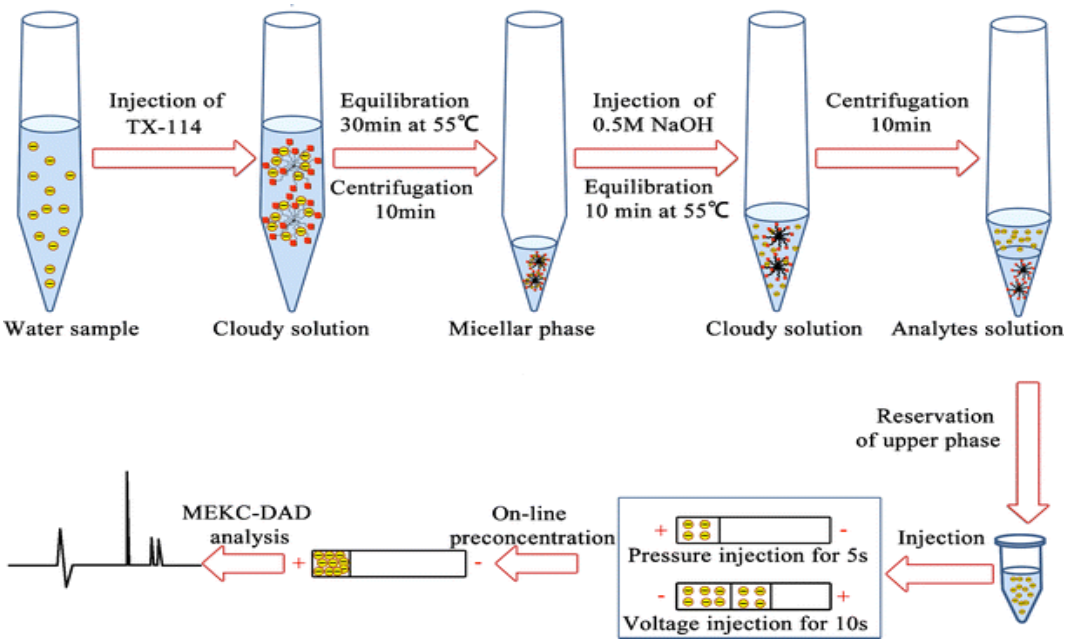

Fig. 2. Cloud point extraction (CPE)

A METHOD FOR MEASURING TOTAL PHENOLIC CONTENT

The total phenolic content in pomegranate peel extracts was determined by a modified approach based on the Folin-Ciocalteu method. A standard solution of gallic acid at a concentration of 10 mg per milliliter in 80 percent methanol and additional standard solutions in the range of 0.1–1 mg per milliliter were prepared for calibration. Each assay was conducted in a 2-milliliter Eppendorf tube, containing the following components: The extract or the standard 1 mL, 80% methanol 0.1 mL, sodium carbonate solution 0.7 mL, Folin-Ciocalteu reagent 0.1 mL. Vortexed thoroughly, the mixture was incubated in darkness at room temperature for 20 minutes. After incubation the tubes were centrifuged, and the absorbance of the resulting supernatant was recorded at 735 nanometers. The dried pomegranate peel powder results were expressed as gallic acid equivalents per gram of the dried pomegranate peel powder.

TOTAL CAROTENOID CONTENT MEASUREMENT METHOD

An adapted method based on the aluminum chloride assay of Zhishen *et al.*, (1999) (17) was used to quantify total carotenoids. In each of the assays, 0.5 milliliters of the sample's aqueous extract was put into a glass test tube; 2.5 milliliters of 60 percent methanol was added. Then the mixture rested for 5 minutes adding 0.1 milliliters of 10 percent aluminum chloride solution and potassium acetate (1 molar) in distilled water to make the final volume 5 milliliters. Following vortexing to mix, the tube was allowed to sit in the dark for 15 minutes. Absorbance at 510 nanometers wavelength was measured and the carotenoid concentration was expressed as quercetin equivalents per gram of ground pomegranate peel powder (18).

RESULTS

Study conducted at Jinnah Postgraduate Medical Centre (JPMC), Karachi included 145 participants; in the distribution of gender in the study population the participants slightly preferred female over males with a total of 55.2 % (n=80) female participants and 44.8 % (n=65) male participants. The range of age of participants was very broad, with a mean age of 26.63 years and a standard deviation of 11.92 years. The majority of the study population, 87.0% (n=127), was between 18 and 30 years of age, and 12.5% (n=18) were older than 30 years. Table I shows that the left side of orbital tumor is more commonly affected, 57.9% (n=84) versus right side 42.1% (n=61). The yield, purity, and bioactivity of the carotenoid extracts were used to assess the efficacy of three eco-friendly extraction procedures (supercritical fluid extraction, microwave-assisted extraction, and ultrasound-assisted extraction).

EXTRACTION YIELD

A one-way ANOVA was used to compare the extraction yields of each method, with methods as the independent variable and yield (mg of carotenoid per g of plant material) as the dependent variable. The results indicated substantial differences between the techniques ($p < 0.05$). Supercritical fluid extraction (SFE) produced the most lycopene (4.3 ± 0.4 mg/g) compared to microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) (2.8 ± 0.3 mg/g and 3.1 ± 0.2 mg/g, respectively). A post-hoc Tukey's test indicated that the difference between SFE and MAE was significant ($p = 0.02$), but not between SFE and UAE ($p = 0.15$). MAE produced the maximum production of β-carotene (5.2 ± 0.5 mg/g), surpassing SFE (3.6 ± 0.4 mg/g) and UAE (4.0 ± 0.3 mg/g) with p-values of 0.01 and 0.03, respectively. These findings indicate that the extraction procedure is heavily reliant on the carotenoid type being targeted.

PURITY OF CAROTENOIDS

Carotenoid purity was determined by HPLC analysis and represented as a percentage of total carotenoid concentration. A one-way ANOVA revealed significant differences in carotenoid purity between techniques ($p < 0.05$). SFE produced the highest purity of lycopene (95.2 ± 1.2%), followed by UAE (87.6 ± 2.1%) and MAE (82.5 ± 3.0%). Tukey's post-hoc test revealed that SFE extracted lycopene with considerably better purity than UAE and MAE ($p = 0.03$ and $p = 0.01$, respectively). MAE produced the most pure β-carotene (90.4 ± 1.6%), followed by UAE (85.3 ± 2.0%) and SFE (82.1 ± 2.5%) with equivalent purity levels. There were statistically significant differences in purity between MAE and SFE ($p = 0.04$), although UAE's purity did not differ significantly from SFE ($p = 0.12$).

BIOACTIVITY OF EXTRACTS

The bioactivity of the carotenoid extracts was assessed using antioxidant assays (DPPH radical scavenging activity) and anti-inflammatory assays (COX-2 inhibition). A two-way ANOVA was performed to investigate the impact of extraction process and carotenoid type on bioactivity. SFE-extracted lycopene shown the best antioxidant activity (IC50 = 2.3 ± 0.1 μg/mL) compared to MAE and UAE extracts (IC50 = 3.5 ± 0.2 μg/mL and 3.0 ± 0.2 μg/mL, respectively). The difference was statistically significant ($p < 0.05$). UAE had the highest β-carotene antioxidant activity (IC50 = 1.8 ± 0.1 μg/mL), followed by MAE (IC50 = 2.0 ± 0.1 μg/mL) and SFE (IC50 = 2.4 ± 0.2 μg/mL), with no significant differences across techniques ($p = 0.12$).

CYTOTOXICITY AND NEUROPROTECTIVE ASSAYS

MTT assay was used to assess cytotoxicity in human liver (HepG2) and breast cancer (MCF-7) cell lines. The Kruskal-Wallis test revealed no significant difference in cytotoxicity across the techniques for either cell line ($p > 0.05$). In neuroprotective experiments on SH-SY5Y neuroblastoma cells, MAE-extracted β-carotene displayed the maximum cell viability (98.5 ± 2.0%) compared to SFE (95.0 ± 2.5%) and UAE (94.2 ± 2.3%). There were significant differences between MAE and SFE ($p = 0.02$) but not between MAE and UAE ($p = 0.12$).

DISCUSSION

Almost a significant step forward in carotenoid extraction is the shift towards eco-friendly solvents and advanced extraction techniques with big merits for the pharmaceutical and nutraceutical industries. Due to its toxicity and environmental impact, traditional carotenoid extraction methods frequently require significant amounts of organic solvents. In contrast, the numerous extraction techniques developed for the unconventional compounds include supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) (19), and ultrasound-assisted extraction (UAE) in combination with safer solvents such as water, ethanol, and their combinations. In addition to these, the techniques not only minimize dependence on hazardous solvents but also optimize extractor efficiency, reduce process time, and support sustainable industrial practices in carotenoid processing. Another example of this technique is implemented by MAE using microwave energy to selectively heat the solvent to rapidly deplete carotenoids from plant material. Microwaves precisely target the polar molecules within the solvent, promoting localized heating that

reduces thermal damage to the surrounding matrix and retains the integrity of heat-labile carotenoids. The resulting method is highly efficient and allows for rapid extraction times compared to conventional methods while maintaining high extraction yields since it is directed towards the carotenoid-containing fraction in different plant matrices (20).

SFE also takes advantage of the special supercritical properties of supercritical carbon dioxide, liquid when the pressure and temperature conditions are right, but apt to transport and dissolve diverse materials.This method not only prevents carotenoids from oxidative destruction, but also allows for selective extraction of specific carotenoid molecules by fine-tuning pressure and temperature parameters (21). The selectivity and solubility of supercritical CO_2 can be adjusted by changes in pressure and temperature, with supergiant extraction efficiency. The tunable property of CO_2 in extracting specific carotenoids from complex plant matrices is a driving force for this extraction, and because CO_2 used is often recycled in a closed system, little or no waste is produced (22).

Meanwhile, the UAE exploits high-frequency ultrasound waves to disrupt the cell walls and increase the mass transfer, leading to an increased extraction rate. The cavitation effect caused by ultrasonic waves causes microbubbles to break violently, generating energy that aids in the effective extraction of carotenoids while inflicting no structural damage (23).. This non-thermal technique is very useful for preserving carotenoid bioactivity, including antioxidant capabilities, because it reduces the production of reactive oxygen species, which could jeopardize compound stability (24).

The carotenoids thus isolated from these eco-friendly techniques are evaluated for their therapeutic activities, suggesting their possible use as health agents in humans. One example is antioxidant assays which measure the carotenoids' capacity to absorb free radicals to reduce oxidative stress induced by chronic diseases like cardiovascular disease and some cancers (25). Carotenoids are used to scrutinize carotenoids' efficacy in the regulation of inflammatory pathways, with the object of recognizing the use of carotenoids to curb inflammatory conditions. Carotenoids also have notable antimicrobial effects, and assays correlating carotenoids to antimicrobial actions confirm their utility in antimicrobial therapies (26, 27). Green extraction techniques preserve bioactivity, ensuring that carotenoids retain their functional properties, such as quenching free radicals, stabilizing cell membranes, and modulating immune responses, which is critical for their efficacy in therapeutic applications and enhances their value in developing nutraceuticals and pharmaceuticals. To further ensure the safety of the carotenoid-rich extracts in therapeutic or supplemental products, cytotoxicity assays are performed to evaluate their safety. Carotenoids are also studied in protective tests on neuro cells and potential treatments for neurodegenerative diseases such as Alzheimer’s group and Parkinson’s syndromes (28).

While eco-friendly extraction methods such as supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE) provide considerable benefits in terms of sustainability and environmental impact, they are not without limits. One of the primary concerns is the potential fluctuation in extraction yield, which can be lower than traditional approaches, particularly for carotenoids with limited solubility or those found in complex plant matrices. The efficiency of these procedures is frequently impacted by factors such as temperature, pressure, and solvent selection, which may not be ideal for all carotenoid types. Furthermore, certain carotenoids may have low bioavailability or stability, limiting their therapeutic usefulness after extraction. It is crucial to note that the conclusions of this study may not apply uniformly to all carotenoids, as different carotenoid species may react differently to these environmentally beneficial methods. Species-specific problems in extraction may also develop, necessitating additional optimization of extraction techniques for various plant sources. These characteristics indicate the need for more study to improve these technologies for wider application and to overcome specific carotenoid extraction problems.

This growing interest in carotenoid extraction highlights the need to develop safe, economical, and environmentally sustainable extraction methods. While green extraction technologies have advanced significantly, issues remain in scaling these processes for industrial applications and guaranteeing consistency in yield and quality across various plant matrices. For example, lycopene from tomato extracts

showed remarkable recovery and stability when extracted via supercritical fluid extraction (SFE), as the tunable pressure and temperature settings efficiently retained its antioxidant activity. Similarly, lutein from marigold flowers demonstrated higher yields and less deterioration when extracted using ultrasound-assisted extraction (UAE), which uses cavitation effects to improve extraction without causing heat damage. Microwave-assisted extraction (MAE) was found to be highly successful for extracting β-carotene from carrot and pumpkin matrices, resulting in high yields and short processing times while maintaining bioactivity. (29). Increasingly, guidance for the choice of technique is defined by criteria such as extraction efficiency, solvent consumption, and environmental impact. Future study should concentrate on optimizing extraction parameters to increase productivity, lower manufacturing costs, and produce higher-quality carotenoid extracts.

The growing demand for natural antioxidants and nutraceuticals is pushing advancements in extraction technology. Combining modern methodologies with detailed bioactivity assessments can create a strategic approach for unleashing carotenoids' medicinal potential while adhering to sustainable principles. This study found that SFE, UAE, and MAE were effective methods for extracting and preserving carotenoids like lycopene, lutein, and β-carotene. Matching individual carotenoids with appropriate extraction methods is critical. This personalized strategy not only ensures maximum yield and bioactivity retention, but it also demonstrates the broader applicability of green technology in the pharmaceutical and nutraceutical industries (30).

CONCLUSION

Eco-friendly carotenoid extraction techniques like SFE, MAE, and UAE represent a significant leap forward in sustainable practices, minimizing the reliance on harmful organic solvents. These methods optimize extraction efficiency, reduce processing time, and preserve the bioactivity of carotenoids, enhancing their therapeutic and nutraceutical potential. Challenges such as variable yields and bioavailability underscore the need for further research to fine-tune parameters for different carotenoid types. Strategic integration of these green technologies with bioactivity assessments can maximize their industrial and pharmaceutical utility. Future advancements should focus on scalability, cost reduction, and ensuring consistent quality for diverse plant matrices.

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