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Research Article

Pretreatment Tunes $scCO_2$ Extract Composition and Bioactivity in Three Microalgae: Chemometric and Molecular Docking Insights

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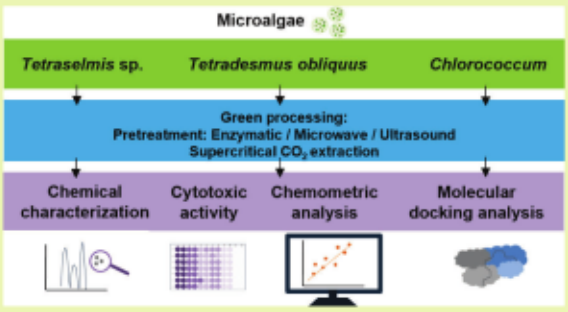
ABSTRACT: This study explores the impact of enzymatic (ENZ), microwave (MW), and ultrasound (US) pretreatments on supercritical CO_2 ($scCO_2$) extraction efficiency, chemical composition, and cytotoxic activity of *Tetraselmis* sp., *Tetradesmus obliquus*, and *Chlorococcum* sp. Pretreatments significantly enhanced extraction yields, with ENZ being most effective for *Tetraselmis* and *Chlorococcum*, and MW for *T. obliquus*. UPLC-HRMS profiling revealed species- and pretreatment-specific shifts: ENZ and US improved pigment recovery in *Tetraselmis*, while MW enriched carotenoids and chlorophyll derivatives. In *Chlorococcum*, MW boosted pigment diversity, whereas ENZ and US favored fatty acid derivatives in the extracts. Multivariate analysis confirmed significant compositional changes, particularly after ENZ and MW pretreatments. *Tetraselmis* extracts, especially those pretreated with MW, exhibited the strongest cytotoxic activity and highest selectivity indices against HeLa and MDA-MB-453 cancer cell lines. Correlation analysis identified compounds such as 2,3-dihydroxypropyl stearate, fucoxanthin, and (3β)-3-hydroxystigmast-5-en-7-one as strongly linked to cytotoxicity. Molecular docking further showed that abundant compounds in *Tetraselmis* extracts have high predicted affinities for cancer-related targets (e.g., BCL2, EGFR, PDK1). The results suggest that cytotoxic effects arise from both specific bioactive compounds and their synergistic interactions. These findings show that pretreatments can purposefully tune $scCO_2$ extracts and provide a data-driven basis for designing more sustainable microalgal extraction workflows.



KEYWORDS: *Tetraselmis, Tetradesmus, Chlorococcum, supercritical CO_2, pretreatments, cytotoxicity, UHPLC*

INTRODUCTION

Microalgae are a rich source of bioactive compounds, including proteins, lipids, pigments, polysaccharides, polyphenols, fatty acids, and sterols. Many of these compounds exhibit pharmacologically relevant activities, such as antioxidant, anti-inflammatory, and anticancer effects, supporting their potential use in functional foods, nutraceuticals, and therapeutic formulations.[1] In addition to these applications, microalgae are also employed in cosmetics, agriculture, aquaculture, and biofuel production due to their biochemical versatility.[2]

Additionally, microalgae also offer significant cultivation advantages over conventional crops. They grow rapidly, adapt to diverse environments, tolerate harsh conditions, and can be cultivated on nonarable lands without requiring fertile soil or potable water, thereby not competing with traditional agriculture.[3] These characteristics, combined with their diverse chemical profiles, make microalgae a promising resource for bioactive ingredient production. However, the efficient extraction of bioactive compounds remains a major challenge. The rigid cell wall of microalgae acts as a major obstacle, preventing the efficient recovery of valuable compounds.[4] Thus, effective cell wall disruption and extraction methods are important advantages for effective use of microalgae. Pretreatment methods not only facilitate cell wall disruption and improve extraction yields but can also alter the chemical composition of extracts, potentially influencing their biological activity. However, due to variations in cell wall structure and composition across microalgal species, no single pretreatment technique proves universally effective. Various thermal, physical, chemical, and biological approaches have been explored to optimize bioactive compound recovery.

For example, freeze/thaw cycles and ultrasound were used to enhance the release of bioactive compounds of *Tetraselmis chuii* improving bioaccessibility and antioxidant activity.[5] Dilute alkaline pretreatment of *T. suecica* facilitated enzymatic saccharification and enabled biobutanol production via

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