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Research Note

Subcritical Water Extraction of Fucoidan from *Sargassum polycystum*: A Sustainable Approach

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Subcritical water extraction (SWE) is receiving increasing attraction as an emerging green technique for the extraction of bioactive compounds from diverse sources. The present study has been carried out to investigate the potential of SWE for extraction of fucoidan from *Sargassum polycystum.* The extraction was carried out under optimal conditions of extraction temperature (180 °C), pressure (40 bar), and a solid-to-liquid ratio of 1:20 for 60 min. The SWE process yielded 5.24% fucoidan, demonstrating the effectiveness of SWE in extracting bioactive compounds from brown seaweed. The SWE extracts exhibited fucose and sulfate contents of 21.36% and 26.9%, respectively, which are consistent with the typical composition of fucoidan derived from brown seaweeds. The uronic acid content was determined to be 3.39%. Overall, this study highlights the potential of SWE as a green alternative for fucoidan extraction, capable of preserving polysaccharide bioactivity while reducing environmental impact.

Keywords: *Sargassum polycystum,* Subcritical water extraction, fucoidan, green extraction technology,

Fucoidan, a complex sulfated polysaccharide predominantly found in brown seaweeds, has garnered significant interest due to its diverse bioactive properties, including anti-inflammatory, anti-viral, anti-coagulant, and anti-cancer effects (Bittkau, Neupane, & Alban, 2020). *S. polycystum* is known for its relatively high fucoidan content compared to other brown seaweed species (Digala et al., 2022). Traditionally, fucoidan is extracted using methods such as hot water extraction, enzymatic hydrolysis, acidic extraction etc. However, these techniques often involve prolonged processing times, high energy consumption, and the use of harsh chemicals that can degrade the structural integrity of fucoidan (Yuan & Macquarrie, 2015). Subcritical water extraction (SWE) has emerged as an efficient alternative for extracting bioactive compounds while preserving their quality (Ummat, Sivagnanam, Rajauria, O'Donnell, & Tiwari, 2021). Subcritical water extraction which is also referred to as "hot compressed water" is considered a greener method for the isolation of high value-added compounds (Lin et al., 2022).

Subcritical water refers to liquid water maintained at temperatures and pressures below its critical point. In SWE, the pressure must be maintained above the vapour pressure to keep water in its liquid state. Water in its subcritical state undergoes significant changes in its physicochemical properties in terms of polarity, viscosity, surface tension, dielectric constant etc. (Plaza & Marina, 2023). At high temperature and pressure, water dissociates into hydronium (H_3O^+) and hydroxyl (OH^-) ions creating a mildly acidic environment that is conducive to the hydrolysis of biomass components (Hans, Pattnaik, Malik, & Naik, 2023). As the temperature increases, the dielectric constant of water decreases enabling dissolution of the desired compounds (Alboofetileh et al., 2019). It is hence presumed that the reduced viscosity and enhanced diffusivity of water in this state will improve its ability to penetrate into algal cell walls, facilitating the release of bioactive components such as fucoidan, phenolics, phlorotannins etc. (Cocero et al., 2018; Cheng, Xue, Yu, Du, & Yang, 2021).

SWE offers several advantages over conventional extraction methods, such as higher extraction

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efficiency, shorter processing time, elimination of organic solvents, and improved preservation of bioactivity. Studies have shown that SWE not only enhances extraction yield but also ensures reduced environmental impact compared to acid, enzymatic or solvent-based methods (Bahrami, Nateghi, Rashidi, Nobandegani, & Ghorbanpour, 2025; Li et al., 2025). Conventional hot water or acid extraction methods often require prolonged extraction times, high energy input, and the use of harsh chemicals, which can lead to partial degradation and desulfation of fucoidan structure, as well as the generation of significant chemical waste. In contrast, SWE employs water at elevated temperature and pressure, enabling rapid extraction within minutes to hours, and often results in higher yield and purity with minimal loss of bioactivity.

The present study, hence aimed to explore the utility of SWE for the extraction of fucoidan from *S. polycystum*. The study is expected to demonstrate the ease of SWE in seaweed biovalorization, providing a scalable, solvent-free, and environmentally friendly method.

The marine brown seaweed *S. polycystum,* collected from the Mandapam coastal area of Tamil Nadu, India, was washed thoroughly to remove adhering salts, sand, and other impurities. The cleaned seaweed was then dried in an electric dryer at 50 °C for 5 h and ground into a fine powder to increase the surface area for extraction.

The powdered *S. polycystum* was soaked overnight in water at a solid-to-liquid ratio of 1:20 (w/v). The following day, the hydrated biomass was loaded into the extraction vessel of a high-pressure autoclave (Amar Equipments Ltd, India). After loading of the sample, the extraction was carried out under the following conditions: temperature of 180 °C, pressure of 40 bar, and extraction time of 1 h. These conditions ensured that water remained in the subcritical state, which is ideal for extracting fucoidan while minimizing degradation of its bioactive properties. After 1 h, the sample was cooled, and centrifuged to remove solid residues. Alginate was removed from the supernatant using the calcium chloride precipitation method (Rajauria et al., 2023). Following alginate removal, ethanol was added to the supernatant to precipitate fucoidan. The precipitated fucoidan was then separated by centrifugation and filtration, and dried in a hot air oven at a temperature of 50 °C. The dried fucoidan was then subjected to further analysis of fucose, sulfate and uronic acid contents. Fucose content was determined using the colorimetric method described by Dische (1955). Sulfate and uronic acid contents were estimated following the protocols detailed by Dodgson and Price (1962) and Ahmed and Labavitch (1978), respectively.

Subcritical extraction of *S. polycystum,* carried out under the specified conditions of 180 °C and 40 bar for duration of one-hour resulted in a fucoidan yield of 5.24% (Fig. 1). Fucoidan is a sulfated polysaccharide located within the algal cell wall; therefore, extraction of fucoidan requires disruption of the cell wall using physical, chemical, or enzymatic methods. In SWE, high temperature and pressure conditions are employed to enhance extraction efficiency. As the temperature increases, the density and viscosity of subcritical water decrease, thereby enhancing its diffusivity, which facilitates rapid solvent penetration into the algal matrix. This might aid in improving the mass transfer rates and consequently achieving higher fucoidan yields. Furthermore, the high temperature and pressure conditions employed during SWE may have effectively disrupted the algal cell wall, resulting in increased solubilization of fucoidan through effective solvent penetration leading to dissolution of the target compounds. Husni et al. (2022) reported fucoidan yields in the range of 0.68 to 2.46% using solvent assisted extraction methods. Hans et al. (2023) conducted a comparative assessment of various extraction techniques, including aqueous, chemical, ultrasound-assisted, enzyme-assisted, and subcritical water extraction, and reported that SWE outperformed all other extraction methods in recovering the highest fucoidan content.

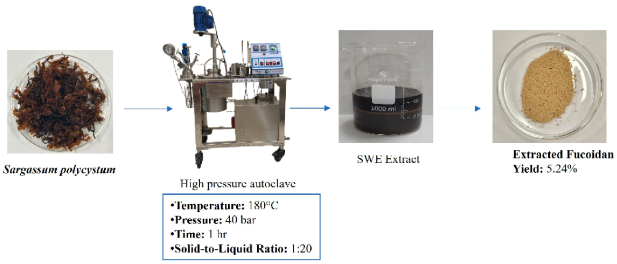

Fig. 1. Subcritical water extraction of fucoidan from *Sargassum polycystum*

The compositional analysis of the extracted fucoidan revealed the presence of fucose, sulfate, and uronic acid, as summarized in Table 1. The fucose content

was found to be 21.36% (w/w). The presence of fucose is critical to the bioactivity and functional properties of fucoidan. A high fucose content has been reported to enhance the biological activities of fucoidan, including anticancer, anti-inflammatory, antioxidant, and antiviral effects, making it very useful in the development of nutraceuticals and therapeutics. Hot water extraction of fucoidan from *S. elegans* was reported to yield a comparatively lower yield of 15% (Mabate, Daub, Malgas, & Pletschke, 2025). However, Alboofetileh et al. (2019) reported a higher fucose content of 34.13% from *Nizamuddinia zanardinii* when SWE was performed at 150 °C with a sample-to-solvent ratio of 21 g/mL.

Table 1. Fucose, sulfate and uronic acid content present in extracted fucoidan

Treatment	Fucose (%)	Sulfate (%)	Uronic acid (%)
SWE	21.36±0.12	26.9±0.24	3.39±0.38

Sulfate plays an important role in the biological activity of fucoidan, and its content has been found to be 26.9% in the SWE-derived fucoidan. Sulfate groups carry negative charges, allowing fucoidan to interact with positively charged molecules such as proteins and enzymes, thereby impacting various biological pathways (Torres et al., 2021). Furthermore, the degree of sulfation strongly influences the antioxidant activity of fucoidan, contributing to free radical neutralization and reduction of oxidative stress. It has been reported that extraction methods can significantly influence the sulfate content of fucoidan. Hans et al. (2023) demonstrated that SWE based methods are more effective in enhancing sulfate content compared to enzymatic and ultrasound assisted extraction methods. The estimated levels are consistent with sulfate levels typically observed in fucoidan extracted from brown seaweeds (Palanisamy, Vinosha, Marudhupandi, Rajasekar, & Prabhu, 2017). However, slightly higher sulfate levels (28.64%) were reported for *Saccharina japonica* when extraction was carried out at 127.01 °C and 80 bar (Saravana et al., 2018).

The uronic acid content was observed to be 3.39%, indicating the presence of acidic sugars that contribute to the structural characteristics of fucoidan. Uronic acids, such as glucuronic acid and galacturonic acid, contribute to the polyanionic nature of fucoidan, thereby enhancing its ability to interact with a range of biomolecules, particularly proteins and metal ions (Borazjani, Tabarsa, You, & Rezaei, 2018). These interactions are crucial for fucoidan's ability to regulate biological processes, including free radical scavenging, protection against oxidative stress, and modulating enzyme activity. Variations in fucoidan yield and chemical composition from *S. polycystum* may be attributed to differences in extraction conditions, harvest season, and the degree of algal maturation (Somasundaram, Shanmugam, Subramanian, & Jaganathan, 2016). Alboofetileh et al. (2019) reported a slightly lower uronic content of 2.07% in fucoidan extracted using subcritical water extraction.

Subcritical water extraction offers a promising alternative to conventional fucoidan extraction methods. In the present study, extraction conducted at 180 °C, 40 bar for a duration of one hour yielded 5.24% fucoidan from *S. polycystum*. The extracted fucoidan contained substantial levels of fucose, sulfate, and uronic acid. The results underpin the potential of subcritical water extraction as an adaptable approach for the sustainable production of fucoidan, paving the way for its enhanced utilization in various industries and applications in nutraceuticals, pharmaceuticals and cosmetics. However, this is a preliminary study, suggesting that the extraction conditions need to be fine-tuned to recover maximum bioactives from seaweed in short duration. The fine tuning has to be done by taking into consideration the important process variables such as pressure, temperature, sample to solvent ratio, stirring speed etc.

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