International Journal of Food Science and Nutrition
www.foodsciencejournal.com
ISSN: 2455-4898
Received: 18-05-2024, Accepted: 06-06-2024, Published: 28-06-2024
Volume 9, Issue 2, 2024, Page No. 39-46

Potential, constraint and current state of extraction techniques for polysaccharides from *Codonopsis pilosula*

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Abstract
Polysaccharides from *Codonopsis pilosula*, a valuable medicinal plant in Viet Nam and other Asian countries, are attracting huge attention regarding research and applications. And appropriate extraction methods for the recovery of these compounds has become the key in industrial development. The objective of this paper is to review recent choice of techniques for polysaccharide extraction from *C. pilosula*, including their advantages, disadvantages, and future trends of methods. The yield of polysaccharides from *C. pilosula* was reported to be efficient with all green extraction methods. The enzyme-assisted extraction is recommended due to its high capability without the need of special equipment in comparison with the other green ones. Conventional methods are unpreferable due to their issues with high cost, time consuming, and unsafe manners. In addition, the combination of advanced techniques is promising as a future choice not only for polysaccharide extraction but also for other valuable compounds from plant-based herbs.

Keywords: *Codonopsis pilosula,* Đảng sâm, polysaccharide, extraction methods

Introduction

In Asian culture, herbal remedies have long been employed as traditional treatments due to their unique qualities as a rich source of medicinal phytochemicals with less adverse effects. Hence, they have been recognized as an alternative to synthetic goods and traditional medications, which is leading to the creation of innovative drugs. Among herb species in Viet Nam and other Asian countries, *Codonopsis pilosula* (Franch.) Nannf., locally named Đảng Sâm, is a well-known traditional folk medicine for thousands of years in refreshment, health enhancement, fatigue and weakness relief (Bai *et al.*, 2020) [2]. The significant amounts of bioactive chemicals found in this priceless herb, mainly polysaccharides and others including saponins, phenolic and flavonoid compounds, together with sugars, essential amino acids, and fatty acids, are the reason for these medicinal results (Makowczyńska *et al.*, 2021) [20]. In Viet Nam, *C. pilosula* has recently been the main income of Kon Tum province, thus, promoting the extraction demand of its bioactive compounds for industrial development.

Saponin content was conventionally the main studying subject in the recovery of valuable ingredients from herbal medicine. However, it is not only the sole contributing factor for health improvement but also the possible source for poison exposure. Hence, polysaccharides, another class of potent substances in *C. pilosula*, has recently drawn huge attention due to their wide range of pharmacological impacts on human health (Jing *et al.*, 2018) [16]. Thus, numerous extraction techniques for polysaccharides have been put forth and successfully applied to a variety of herbs, producing a high output of finished goods. Therefore, selecting an appropriate one is paramount to the maximization and optimization of the extraction outcome. Most of the recent polysaccharide extraction's applications already employ advanced green techniques in favor of conventional ones. However, the nature of advantages and disadvantages of each particular method raise a huge demand to a thorough understanding that will further facilitate specific adjustments and improvements for polysaccharide recovery.

This paper reviews recent studies on advanced green techniques for polysaccharides' extraction from *C. pilosula* in comparison with conventional extraction techniques for other compounds. In addition, new trends in combination methods for the recovery of polysaccharides are also covered.

Codonopsis pilosula Nannf. As a Potential Medicinal Herb

For thousands of years, traditional folk medicine in Asian nations including Viet Nam, China, Japan, and Korea has favored *C. pilosula,* a well-known and classical homology perennial plant in the *Campanulaceae* family. Its first description was found in the masterwork of ancient medicine, "Bai Cao Jing", from the Qing Dynasty period. And before the Ming Dynasty, *C. pilosula* was not listed in any text descriptions, materia medica book, or images (Xie *et al.*, 2020) [37]. In particular, *C. pilosula* is a more well-known dish or health product and is frequently used to make food, soups, and beverages due to its evidently beneficial effects on health, non-poisonous nature, and abundance of resources (Sun *et al.*, 2019) [31]. The China Food and Drug Administration has approved nearly 200 of its health food products. As a result, according to, a significant amount of *C. pilosula* is consumed annually, roughly 40,000 tons in China (Gao *et al.*, 2018a) [12]. In Viet Nam, *C. pilosula*, or Đảng sâm, has been considered as an equal alteration to the Korean *Panax ginseng* regarding similar pharmacological capabilities and cost efficiency.

C. pilosula is primarily used to nourish the stomach and spleen. In particular, clinical trials indicate that *C. pilosula* is frequently effective in treating spleen-lung weakness, internal heat and thirst, loose stool and lack of appetite, and palpitations associated with shortness of breath (Sun *et al.*, 2019; Zou *et al.*, 2020) [31, 50]. Recent research in pharmacology has revealed that *C. pilosula* has a wide range of effects. These include stimulating axonal growth, protecting the digestive system (Fan *et al.*, 2018) [11], improving memory and ameliorating memory impairment (Wei *et al.*, 2017) [36], controlling and boosting the body's immune response via controlling the amount of antibodies, cytokines, lymphocytes, and the neuroendocrine-immune network, which has a broad impact on humoral, cellular, non-specific, and specific immunity (Zhao *et al.*, 2013) [46], and lowering oxidation and aging (Zou *et al.*, 2020) [50].

The Importance of Polysaccharides in *Codonopsis Pilosula*

Research on medicinal plants in particular is just as substantial as the research on conventional drugs, especially when it comes to the advantageous phytochemicals found in these plants and the pharmaceutical and cosmeceutical industries' shift towards natural products. Health benefits have been proven by numerous studies showing that the root of *C. pilosula* contains a variety of bioactive substances, including saponin, organic acids, alkaloids, phenylpropanoids, triterpenoids, polyacetylenes, coumarins, flavonoids, microelements, and especially polysaccharides (Jing *et al.*, 2018, Zou *et al.*, 2020) [16, 50]. For instance, the significant impact that antioxidant activities play in human health has been linked to the high quantity of bioactive chemicals found in medicinal plants. And recently, one of the key components thought to be essential for the therapeutic effects of *C. pilosula* is polysaccharide (Jing *et al.*, 2018) [16]. *C. pilosula* polysaccharides (CPs) has been suggested to be useful as functional ingredients in food products or as agents to treat a variety of diseases due to their several pharmacological actions including immunomodulatory, antioxidant and antiaging, antitumor, and antifatigue (Zou *et al.*, 2019, Zou *et al.*, 2020) [49, 50]. Recently, a new neutral polysaccharide known as CERP-1 was isolated and obtained from the residue of *C. pilosula* with a molecular weight of 4.840×103 Da and 95.19% ± 3.58% sugar (Liu *et al.*, 2018) [5]. It is proved to have significant antihyperglycemic effects both *in vitro* and *in vivo*, especially in mice on high-fat diets and streptozotocin-induced Type 2 diabetes mellitus (T2DM). As a functional food ingredient, it is suggested to treat diabetes and its complications. Hence, the finding and characterization of new polysaccharides with potential therapeutic action has been considered potential. The majority of current research on CPs has just focused on the polysaccharides found in roots, however studies on the polysaccharides found in sections above ground are scarce (Zou *et al.*, 2020) [50]. Therefore, the opportunities for CPs' recovery and application are practically promising for both food and pharmaceutical industries.

Constraints in the Recovery Processes for Polysaccharides from *Codonopsis Pilosula*

Bioactive compounds, including polysaccharides, are sourced from a range of natural outlets and parts of plants, e.g. leaves, roots, barks, tubers, woods, gums or oleoresin, exudates, fruits, figs, flowers, rhizomes, berries, twigs, and the entirety of the plant, generate active chemicals in varying concentrations and quantities. And obtaining polysaccharides from herbal plants is the key for both research and industrial development (Akhtar *et al.*, 2019) [1]. However, the recovery processes for polysaccharides from *C. pilosula* recently face certain issues. And the outer complicated structure in *C. pilosula* or other medicinal herbs, known as the cell wall, is found to have a direct involvement. On the positive side, the cell wall stimulates cell division and differentiation and acts as a protective barrier, among other functions. Because of its mechanical strength, the cell wall is able to sustain the internal turgor pressure. It exhibits adaptability to stressors and triggers the expression of genes that code for enzymes that alter cellular structure. This has to do with how their composition has evolved and changed throughout time to accommodate varied terrestrial environments for plants (Zhang *et al.*, 2021) [39].

However, this cell wall with hard structures, e.g. cellulose, pectin, and lignin, is also, in general, the main causative agent for the limitation and reduction in the recovery of polysaccharides, as it prevents the release of valuable compounds. In addition, it also increases the treating duration, cost, and decreases the quality of the final products (Nguyen *et al.*, 2016) [24]. And the composition and structure of the plant cell walls vary depending on the species, hence, greatly increasing the scope of dealing with those obstacles. First of all, the wall's mechanical strength is a result of cellulose. Vascular terrestrial plants are characterized by cell walls that are rich in cellulose. Cellulose chains with a high degree of polymerization can have up to 25,000 glucose residues. Ordered microfibrils are formed when these chains join forces with hydrogen bonds to create regular assemblies. Hemicelluloses are the second most prevalent polysaccharide fraction. By creating a cross-link between cellulosic microfibers, these non-cellulosic polysaccharides keep microfibrils from collapsing and gliding over one another. They often form covalent or hydrogen bonds, as well as ionic and hydrophobic interactions, with other cell wall constituents (Geng *et al.*, 2019) [13]. The cell wall also contains branched heteropolysaccharides known as pectin, providing plant tissues their stiffness and integrity, keeping the water content of plant cell walls constant, taking roles in intercellular adhesion, participating in the cell wall's defense systems against infections (Łubek Nguyen *et al.*, 2022) [19].

Conventional Methods for the Extraction of Polysaccharides from Medicinal Herbs

1. Physical extraction methods

Maceration, percolation and infusion are generally chosen as physical extraction techniques for polysaccharides, in which general principles and mechanisms involved are referred to as leaching. Leaching procedures can involve a straightforward physical dissolution or solution. On the one hand, the rate at which the solvent is transported into the mass, the rate at which the solvent solubilizes the soluble constituents, and the rate at which the solution is transported out of the insoluble material all have impacts on the extraction processes (Handa, 2008) [14]. On the other hand, maceration had the longest extraction time (700 hours) when compared to other extraction methods including decoction, percolation, and Soxhlet extraction on *Cassia fistula* Linn. (Caesalpiniaceae) (Table 1). The study was conducted on the Siamese neem tree (*Azadirachta indica* A. Juss. var. *siamensis* Valeton) reported that the maceration process required 840 hours to extract, a significantly longer time than alternative approaches (Chaisawangwong and Gritsanapan, 2009) [3]. This method is the most straightforward and uncomplicated one. However, dealing with organic waste poses a challenge due to the large amount of solvents used and the necessity for proper waste management. Changes in temperature and the selection of solvents can improve the extraction process, reduce the volume required for extraction, and can be integrated into the maceration technique when such adjustments are acceptable.

Research on the physical extraction of polysaccharides has been lacking recently. It subsequently leads to the lack of applications on *C. pilosula*. The outcome from the investigation of the association between Chinese herb extraction yield and maceration technique demonstrated the effects of maceration varied depending on the type of plant (Chen *et al*., 2016) [6]. Although this research was unable to prove that maceration or other physical procedures could extract the desired chemicals in a high yield, it does show great promise for applying this kind of method to other types of herbs in the future.

Table 1. Comparison of volume of solvent, consumed time, and weight of crude extract between maceration and other extraction methods on *Cassia fistula* pods (Sakulpanich and Gritsanapan, 2008) [27].

Extraction method	Solvent	Volume of Solvent (mL)	Consumed time (hours)	Weight of crude extract (% w/w in wet pulp)
Decoction	Distilled water	900	9	48.34 ± 0.01
Maceration	70% ethanol	600	700	48.84 ± 0.02
Soxhlet		300	312	43.24 ± 0.23
Percolation		2,000	240	51.76 ± 0.15

2. Chemical extraction methods

Chemical extraction is the traditional method using only chemicals to obtain the herbal or plant extracts without any interference of heat or other forces, i.e. the ancient extraction technique known as marinated extraction uses several chemicals to extract the desired compounds at room temperature (Mohammad Azmin *et al.*, 2016) [22]. A recent study on the flavonoids extraction from *Portulaca oleracea* L. found that the marinated extraction had the lowest extraction efficiency (5.6%) and a significantly longer extraction time (2880 min) compared to other extractions such as microwave-assisted extraction (9 min, 7.1%), ultrasonic extraction (60 min, 6.7%), condensing reflux extraction (150 min, 6.8%), and Soxhlet extraction (300 min, 7.0%) (Zhu *et al.*, 2010) [47] (Table 2). Another clear-cut example of chemical extraction, the conventional liquid-liquid extraction (LLE), consists of three parts: extractant, diluent, and solute. Regarding disadvantages, in addition to being time-consuming, common problems with LLE include selecting of an appropriate extractant, emulsion formation, and the usage of significant amounts of hazardous organic solvents (Zhang and Hu, 2013) [42].

This approach has the benefit of not requiring specialized tools or a particular setting. However, due to its numerous disadvantages, including a lengthy extraction process and a high chemical consumption, researchers are not interested in this type of extraction. Reports on the use of solvent extraction to extract polysaccharides from herbal plants, including *C. pilosula*, are scarce. Nonetheless, certain researchers have modified and combined traditional solvent extraction techniques with other contemporary techniques in an attempt to accelerate the extraction time and boost extraction yield. The combination of pressurized liquid extraction (PLE) and microwave-assisted extraction on the recovery of polysaccharides from *Fucus virsoides* and *Cystoseira barbata* reported the maximum yield of polysaccharides, along with the highest fucose, sulfate group content, and polydispersity index (Dobrinčić *et al.*, 2021) [8].

Table 2. Comparison of the extraction time and average extraction efficiency between marinated extraction and other extraction methods on flavonoids content in *Portulaca oleracea* L. (Zhu *et al.*, 2010) [47].

Extraction method	Extraction time (min)	Average extraction efficiency (mg.g^{-1})
Microwave – assisted extraction	9	7.1
Ultrasonic extraction	60	6.7
Condensing reflux extraction	150	6.8
Soxhlet extraction	300	7.0
Marinated extraction	2880	5.6

Advanced Green Methods for the Extraction of Polysaccharides from *Codonopsis Pilosula* and Other Medicinal Herbs

1. Subcritical water extraction (SWE)

Due to its efficiency, safety, and environmental protection, subcritical water extraction (SWE) has drawn increasing attention. Moreover, the molecular structure of the active substances is modified by subcritical water, which helps to enhance their biological activities (Zhang *et al.*, 2020) [2]. Subcritical water denotes heated water under adequate pressure to remain in its liquid state between 100℃ (the boiling point) and 374℃ (the critical point) at pressures ranging from 1 – 22.1 MPa. The viscosity, surface tension, and especially the dielectric constant reduced, and in the meantime, its diffusivity parameters enhanced with the increasing temperature. At elevated temperatures, sufficient pressure sustains water's liquid form. Initially, water exhibits a dielectric constant of 80 at 25℃. Interestingly, when the temperature rises to 250℃ and the pressure reaches 25 bar, the dielectric constant diminishes to 25, resembling that of methanol ($\varepsilon = 33$) and ethanol ($\varepsilon = 24$) at 25℃. Under such circumstances, water exhibits properties akin to organic solvents, capable of dissolving various medium and low polarity compounds (Hassas-Roudsari *et al.*, 2009) [15]. The energy supplied with subcritical water can disrupt the interaction between adhesive (solute-matrix) and cohesive (solute-solute) by reducing the activation energy required for desorption, while increased pressure can aid in extraction by compelling water to infiltrate the matrix (pores), a feat unattainable under normal pressure (Teo *et al.*, 2010) [32].

Recent studies have used subcritical water to extract large amounts of bioactive compounds, like polysaccharides. Water, utilized as the extraction medium in SWE, embodies an eco-friendly approach due to its non-toxic, non-flammable, and waste-free nature, making it a green solvent. Its cost-effectiveness, stemming from its abundance and favorable properties, ensures the production of high-quality extracts. Additionally, water's dielectric constant, influenced by temperature and pressure, facilitates the extraction of a wide range of chemicals. Equipment for SWE is comparatively inexpensive and enables continuous operation. However, SWE has its own drawbacks. The primary concern is the need for high temperatures, which can lead to thermal degradation for approximately 350 heat-sensitive chemicals. Additionally, SWE's potential to catalyze hydrolysis and oxidation of certain chemicals makes it more reactive and corrosive than ambient water (Teo *et al.*, 2010) [32]. Removing moisture from the extraction solution is challenging and may require additional processes like evaporation or chemical dehydration. Cleaning SWE equipment is also problematic.

Currently, there is a scarcity of research on the utilization of SWE for extracting polysaccharides from *C. pilosula*. However, researchers have explored several related herbs to illustrate the effectiveness of SWE in extracting polysaccharides. Research showed that immunological activity of *Sagittaria sagittifolia* L. polysaccharides with SWE was significantly better than traditional hot water extraction with the optimal conditions were pH of 7.0, extraction temperature of 170℃, extraction time of 16 min, and a liquid to solid ratio of 30:1 (mL.g^{-1}), respectively (Zhang *et al.*, 2019) [41]. In addition, SWE demonstrated notably better extraction yields of total sugar, total protein, and phenolic components, as well as the best antioxidant properties from ginseng roots (*Panax ginseng* C.A. Mey), when compared to traditional water extraction and ethanol extraction procedures (Zhang *et al.*, 2018) [5]. Temperature had a considerable impact on both the extraction yields and antioxidant activity of these bioactive components in SWE; 180°C or 200°C was found to be the optimal temperature. According to these results, SWE is a potentially effective and ecologically benign method of extracting bioactive polysaccharides.

2. Ultrasound-assisted extraction (UAE)

The process known as ultrasound-assisted extraction (UAE) makes use of solvents and sonic radiation to improve the extraction of chemicals, particularly polysaccharides, from plant materials. The cavitation phenomenon, which is caused by the collapse of cavitation bubbles and highly localized temperature, is the generally accepted explanation for how using ultrasound enhances solvent extraction. Cell walls are broken, releasing contents into the extraction medium. As an effective and environmentally friendly process, this approach has gained popularity recently as well as become an important application of plant extraction (Song *et al.*, 2023) [30]. This facilitates the breakdown of cell walls and increases the amount of substances released from the plant matrix, including polysaccharides. The mass transfer between the plant material and the solvent is enhanced by the mechanical effects of ultrasonic vibrations. This improved mass transfer makes it easier to extract polysaccharides by improving the solvent's interaction with the target molecules (Montero-Calderon *et al.*, 2019) [23].

One of the most practical and affordable uses for polysaccharide processing is in the UAE. Frequencies of mechanical waves higher than 16 kHz are used in ultrasonication. Depending on the objective, this approach can be used in low-frequency, high-intensity or high-intensity, low-frequency settings, therefore, this approach is suitable with many types of polysaccharides which have varied degradation conditions and different sources. Ultrasound may accelerate a number of procedures and enhance the functionality of certain food products (Chemat and Khan, 2011) [4]. Ultrasound has received a lot of interest as a potential means of extracting natural products compared to conventional methods which could take hours or days to complete. With the use of ultrasound, full extractions can now be finished efficiently and reproducibly in a matter of minutes. This results in a higher-quality final product, eliminates the need for wastewater post-treatment, uses less fossil fuel than traditional extraction techniques like Soxhlet extraction, maceration, or steam distillation, and uses a fraction of the energy. Various food components, including antioxidants, fragrances, pigments, and other chemical and mineral substances, have been effectively extracted and studied from a range of matrices (Chemat & Khan, 2011) [4]. And care must be taken to ensure that the ultrasonic power used during the extraction process is not excessively high, as this might easily break the sugar chains and cause some of the polysaccharide's biological activity to be lost (Song *et al.*, 2023) [30].

A few research on the extraction of *C. pilosula* polysaccharides using UAE have been conducted recently. The yield of polysaccharides by UAE is greatly influenced by variables including ultrasonic power, the ratio of solid to liquid, extraction temperature, and extraction length (Raza *et al.*, 2017) [25]. The research showed that the UAE demonstrated the greatest polysaccharide extraction rate (8.00%), surpassing both Soxhlet extraction (7.13%) and Reflux water extraction (5.66%). A single factor experiment, and an orthogonal experiment were also used to optimize the extraction conditions of polysaccharides from *C. pilosula*. The ideal extraction procedure was determined to be a solid-liquid ratio of 1:12 (g/mL), 70% ultrasonic power, 45 minutes of extraction time, and 60ºC of extraction temperature, with a maximum yield of 13.57%. In addition, there was evidence of strong antioxidant activity in the isolated polysaccharides, with a concentration of 0.4545 ug/mL having the potential to 59.04% free radical-scavenging activity (Fan *et al.*, 2012) [10].

In general, ultrasound extraction can improve efficiency, require less time and temperature during the extraction process, and use less energy when compared to standard extraction methods. In the ultrasound aided extraction of polysaccharides from the roots of *C. pilosula*, the length (40–60 min) and temperature (60–90ºC) of the ultrasound treatment are often substantially shorter and lower than the traditional method (hot water extraction). As a result, the usage of UAE will be widely implemented and increased in future research to find practical and efficient uses in *C. pilosula.*

3. Microwave-assisted extraction (MAE)

The technique known as microwave-assisted extraction (MAE) makes use of microwave radiation to improve the extraction of chemicals from a variety of plant materials. With less solvent and energy used, MAE is an extraction technology that produces high-yield polysaccharides faster (Mirzadeh *et al.*, 2020) [21]. Because of its quick heating, this extraction technology has a higher extraction rate than other technologies like Soxhlet, PWE, and supercritical fluid extraction (SFE). The molecular interactions between the electric component of the microwave field and the ionic species present in the extraction mixtures (solvent-sample) and dipolar molecules account for the great ability of MAE to increase extraction yield. This approach produces volumetrically dispersed heating due to molecular friction by penetrating energy into solid materials in the form of nonionizing radiation at a spectral frequency of 300 – 300,000 MHz (Rodriguez – Jasso *et al.*, 2011) [26].

Due to its improved extraction efficiency over other traditional procedures, MAE is now frequently employed to extract polysaccharides from a variety of plant sources. Two main mechanisms work together when the polysaccharide is extracted via microwave-assisted extraction. One of these is a sudden increase in temperature, which improves the extraction rate by breaking the outer layer of plant material and reducing emulsion viscosity. Another mechanism is molecular rotation. The phenomenon causes the electrical charges around the molecules to reorganize, which enhances ion mobility and raises the extraction process's efficiency. Additionally, the yield and quality of polysaccharide generated by MAE are the same as those produced by conventional extraction (Thirugnanasambandham *et al.*, 2015) [33].

Polysaccharides from *C. pilosula* have been extracted using a microwave-assisted extraction approach (Wang *et al.*, 2012) [35]. Important factors to take into account when extracting polysaccharides from *C. pilosula* roots include microwave power, treatment temperature, extraction time, and liquid-to-solid ratio. With a recovery rate of 15.79%, the ideal parameters were a temperature of 90ºC for extraction, a duration of 6 min, a material to water ratio of 1:40, three extraction times, and 130 W of microwave power. The study also examined the overall amount of polysaccharides produced by the two methods—conventional and MAE—and found no statistically significant difference. Furthermore, results verified that there were no notable distinctions between the MAE and traditional methods about the polysaccharide structure. Thus, it is apparent that MAE is easier to use, quicker, and better suited for contemporary production (Wang *et al.*, 2012) [35]. Unlike traditional techniques, MAE can lower the temperature (70 – 90ºC) and shorten the extraction time (around 20 minutes), but it still consumes a lot of solvent. Additionally, MAE essentially did not disrupt the structural polysaccharides of *C. pilosula*. MAE is largely equivalent to conventional methods since it meets the minimal requirements for sample preparation techniques and has other benefits such as flexibility and faster processing times.

4. Enzyme-assisted extraction (EA)

Enzyme assisted extraction (EAE) is one of the green extraction techniques that is widely utilized since it is more specific in extracting desired substances from source materials and is also ecologically friendly (Song *et al.*, 2020) [29]. Enzymes function by dissolving plant cell walls and other structural elements, making it easier to remove intracellular components. The selection of enzymes is based on their capacity to hydrolyze particular plant cell wall constituents. Cell walls are multilayered, which means that distinct groups of enzymes acting on different polymers can be used to break down the wall and access the metabolites inside the cell. The most often utilized enzymes include proteases, hemicellulases, cellulases, and pectinases. Since microorganisms and plants create them in large quantities, it is possible to manufacture and acquire them for use in science or industry (Łubek-Nguyen *et al.*, 2022) [19]. Because cell structures vary widely in complexity and heterogeneity, the use of synergistically acting enzymes is frequently essential for their optimal utilization. As a result, experiments with various enzyme combinations have been conducted (Viscozyme L®, Lallzyme®, Kemzyme® Plus, Multizyme®, and Ultrazym®) (Łubek-Nguyen *et al.*, 2022) [19]. New approaches to the management of post-production wastes and natural resources have been developed through the application of EAE. It is seen as a potentially useful method for getting bigger amounts of goods or active components for the pharmaceutical, cosmetic, and food sectors as well as allied fields.

Polysaccharide extraction has been done using the EAE approach. The use of enzyme-assisted extraction minimizes the possibility of heat-induced destruction of sensitive polysaccharides by operating under mild settings. Enzymes that are specialized for particular components of cell walls can be chosen, making it possible to extract particular polysaccharides with precision. Because enzymes allow extraction to occur at lower temperatures, the extraction procedure is comparatively energy-efficient when compared to some conventional techniques. And since enzyme-assisted extraction does not require harsh chemicals, organic solvents or special equipment, it is thought to be environmentally beneficial and cost efficient against others (Zhu *et al.*, 2014) [48].

Under optimal conditions, single-enzymatic hydrolysis was used in enzyme-assisted polysaccharide extraction method as follows: papain and cellulase had yields of 23.7% and 28.2%, respectively, depending on the enzyme amount and enzymolysis time (60U/g dry powder, 90 min, pH 5.0, and 40ºC for papain, and 120U/g dry powder, 150 min, pH 4.0, and 40ºC for cellulase) (Zhang *et al.*, 2008) [40]. Cellulase treatment provided a better yield of assistance when obtaining polysaccharides from *Condonopsis* sp. than papain did (Zou *et al.*, 2020) [50]. Another study demonstrated the beneficial extraction impact of a combination of pectinase and cellulase on the polysaccharides content from *C. pilosula* compared to traditional extractions. The findings showed that there were notable differences in the extraction yields: 16.07% for hot water extraction and 25.23% for enzyme-hydrolysis extraction (Gao *et al.*, 2018a) [12].

However, there are some drawbacks to using EAE to extract polysaccharides from *C. pilosula* roots, including the high cost and challenge of scaling up to the industrial scale of EAE because the process is dependent on a number of variables that are difficult to control consistently, including temperature, nutrient availability, and dissolved oxygen (Liu *et al.*, 2018) [5]. With the help of enzymes, extraction yields could be increased, solvent usage could be decreased, and polysaccharide quality could be enhanced at lower working temperatures.

Combined Methods for the Extraction of Polysaccharides from *Codonopsis Pilosula* and Other Medicinal Herbs

1. Ultrasonic-microwave-assisted extraction (UMAE)

This approach combines ultrasound-assisted extraction with microwave-assisted extraction, creating a synergistic method known as UMAE. UMAE harnesses the strengths of both techniques, resulting in reduced extraction duration and enhanced antioxidant properties in its polysaccharides (Zhang *et al.*, 2023) [40]. UMAE exhibits superior extraction efficiency, partly due to the impact of microwave radiation, which effectively breaks down tissue and cell walls, facilitating the release of compounds into the solvent. This process enhances the interaction between solid and liquid phases, facilitating solvent penetration for valuable components. Additionally, ultrasound-induced cavitation disrupts cell walls without altering the structure or molecular properties of the polysaccharide, thereby enhancing exposure to the solvent for efficient extraction (Ying *et al.*, 2011) [38].

The extraction study on *Dictyophora indusiata* reported that polysaccharides yields varied across four methods, with the order being UMAE > UAE > MAE > Hot water extraction (HWE) (Zhang *et al.*, 2023) [40]. Post UMAE treatment, surface characteristics exhibited a partial honeycomb shape with more noticeable collapse and folding compared to other methods, facilitating cell dissolution. Furthermore, UMAE demonstrated superior scavenging abilities for DPPH, superoxide anion, and hydroxyl radicals at both high and low polysaccharide concentrations (except for DPPH scavenging ability at 1 $mg.mL^{-1}$), indicating the highest antioxidant activity among the extracts. This enhanced activity may be attributed to increased group exposure and lower average molecular weights of polysaccharides extracted via UMAE and MAE methods, as studies suggest that antioxidant activity rises with decreasing polysaccharide molecular weight.

The study on refinery of the extraction process for polysaccharides from *Inonotus obliquus* revealed the most effective conditions for UMAE involved using a microwave power of 90 W, ultrasonic power of 50 W, with an ultrasonic frequency of 40 kHz, and a solid-to-water ratio of 1:20 (W/V), with an extraction time of 19 minutes (Chen *et al.*, 2010) [7]. Under these optimized parameters, the polysaccharide yield and purity reached 3.25% and 73.16%, respectively, surpassing those obtained through traditional hot water extraction and closely matching the predicted values (3.07% and 72.54%, respectively). These results highlighted the superior potential and efficiency of ultrasonic-microwave-assisted extraction (UMAE) compared to conventional hot water extraction methods.

UMAE emerged as the most efficient method for extracting of flavonoids, terpenoids, and phenolics from *C. pilosula* in the accessment of effectiveness of microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE), and ultrasonic-microwave-assisted extraction (UMAE) using NADES (Vo *et al.*, 2024) [34]. Under the optimized parameters of NADES-based UMAE, the levels of phenolics, flavonoids, and terpenoids were found to be 30.98 ± 1.1 mg $GAE.g^{-1}$ db, 24.36 ± 0.48 mg $RE.g^{-1}$ db, and 185.47 ± 4.39 mg $UA.g^{-1}$ db, respectively. The investigation into utilizing combined extraction techniques for extracting polysaccharides from *C. pilosula* hasn't been conducted yet. However, the three instances mentioned above suggest that extracting polysaccharides from *C. pilosula* using UMAE holds significant promise for the foreseeable future.

2. Enzyme – ultrasonic – assisted extraction (EUA)

Among the four extraction techniques (hot water extraction, ultrasonic-assisted extraction, enzyme–assisted extraction) in the extraction of polysaccharides from *Astragalus cicer* L., EA and EUA extractions produced polysaccharides with higher yields and greater uronic acid concentrations (Shang *et al.*, 2018) [9]. This could be attributed to enzymatic breakdown facilitated by the enzyme complex (consisting of cellulose, papain, and pectinase), as well as the collaborative effect between the enzyme complex and ultrasonic treatment, which enhances the extraction of active polysaccharides into the solvent.

Within the same year, another research about polysaccharides extraction applied on comfrey (*Symphytum officinale* L.) root also showed the result that UA and EUA extraction methods yielded higher extraction yields and uronic acid contents compared to HW and EA methods, suggesting their effectiveness in polysaccharide extraction (Duan *et al.*, 2018) [9]. However, there was no significant difference in polysaccharide contents among the four extraction methods. UA-CPs and EUA-CPs had smaller molecular weights compared to EA-CPs and HW-CPs, likely due to the effects of enzyme complex and ultrasound, which could break down polysaccharides into smaller molecules. It is noted that polysaccharides with smaller molecular weights may exhibit more significant biological activities as they can easily pass through biological membranes without immune system interference (Li *et al.*, 2016) [17].

The research on comfrey (*Symphytum officinale* L.) polysaccharides (CRP) showed that the extraction yields of the four CRPs were observed as follows: HW-CRPs (18.18%) < UA-CRPs (20.37%) < EA-CRPs (22.67%) < EUA-CRPs (24.51%) (Chen *et al.*, 2018) [5]. This indicates that the utilization of ultrasonic assistance, enzyme assistance, particularly enzyme-ultrasonic assistance extraction technologies, can notably enhance the extraction yields of CRPs. The polysaccharide contents varied in the order of HW-CRPs (84.28%) < UA-CRPs (84.48%) < EA-CRPs (84.55%) < EUA-CRPs (85.78%) ($P > 0.05$). Although the exploration of employing combined extraction methods for extracting polysaccharides from *C. pilosula* has not been undertaken, the three examples highlighted earlier indicate that the extraction of polysaccharides from *C. pilosula* through EUA extraction shows considerable potential in the near future.

Conclusion

The biological activities of *C. pilosula*'s polysaccharides have recently drawn the attention of numerous researchers. This review updates and provides the effectiveness and drawback of multiple green extraction techniques, e.g. subcritical water extraction, ultrasound-assisted extraction, microwave-assisted extraction, and enzyme-assisted extraction, as well as the combination between these methods of extraction such as ultrasonic-microwave-assisted extraction and enzyme – ultrasonic – assisted extraction. These green techniques may have varying effects, yet still showed significant potential in polysaccharide yield, especially the enzyme method. And modern extractions have several advantages over conventional ones, e.g. rapid extraction time, high yields, etc.

Research on exploiting polysaccharides from *C. pilosula*, is extremely potential, but yet, still relatively minor in Viet Nam. Future studies should further examine the impact of various extraction techniques not only on the physicochemical characteristics and bioactivity of *C. pilosula* polysaccharides, but also on large-scale recovery of *C. pilosula* polysaccharide for industrial applications.

Competing Interests

The authors declare no conflicts of interest.

Author Contributions

Phu H. Le conceived the idea, provided support, and critically revised the manuscript. Phuc N.T. Le structured the contents. Phuc N.T. Le and Uyen P. Le wrote the manuscript. Nhi T.Y. Nguyen contributed to proofreading. All authors read and approved the final manuscript.

Acknowledgement

No external funding was received. The authors would like to express sincere gratitude to the School of Biotechnology, International University – Vietnam National University of HCMC for administrative support.

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