



Advances and challenges in polysaccharide extraction from the woody structure of fruiting bodies of Reishi mushroom, *Ganoderma lucidum*

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ABSTRACT

Polysaccharides are one of the key bioactive compounds present in Reishi mushroom, *Ganoderma lucidum*. Therefore, it is necessary to provide an up-to-date comprehensive outline of the advances in the field of polysaccharide extraction from *G. lucidum* and to better understand the obstacles and advantages of the different methods. Thus, this review has combined conventional and modern methods developed since the year 2000 and has compared them with regard to their advantages and disadvantages. A search was conducted in Scopus, PubMed, and Google Scholar databases for articles published between the years 2000 to 2023 using the following keywords: Reishi mushroom, *G. lucidum*, polysaccharide, beta glucan (β -glucan), and extraction. The association between the obstacles of polysaccharide extraction and the special cell structure of fruiting bodies of *G. lucidum* has also been discussed. This review also demonstrates how the integration of modern extraction methods and the use of appropriate statistical evaluation methods may create the potential to improve the efficiency of polysaccharide extraction.

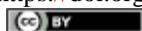
1. Introduction

Ganoderma lucidum (synonym: *G. lingzhi*) (Curtis) P. Karst (commonly known as Lingzhi, Reishi or Mannentake) is a mushroom-producing fungus belonging to Aphyllophorales (Basidiomycota) with known mycological and taxonomical characteristics [1]. The extraordinary importance of *G. lucidum* in strengthening the immune system and curing or prevention of a number of diseases and disorders (such as heart diseases, diabetes, cancer, viral infection, etc.) has been documented in the literature [2-6]. The most prominent compounds that confer these therapeutic properties to *G. lucidum* include polysaccharides (particularly β -glucans), terpenoids, and proteins [7-9]. Among these compounds, *G. lucidum* polysaccharides (known as GLPs) are the most abundant and important group of bioactive compounds with biological properties such as antioxidant, anti-fat,

anti-cancer, anti-inflammatory, strengthening the immune system and antimicrobial activity. In addition, polysaccharides (including GLPs) together with terpenoids are considered the most important index of pharmacological effects of bioactive compounds based on Chinese Pharmacopeia (edition 2015), which makes them valuable in the pharmaceutical industry [10]. Therefore, GLPs have been used in various industries such as pharmaceuticals, food, and cosmetics [2,3,6,10]. In addition to industrial applications, GLPs can also be used as tools to differentiate species and strains of a specific mushroom sample through polysaccharide fingerprint profile or HPLC [11,12]. Despite successful extraction with high efficiency in other edible and medicinal mushrooms, extraction of polysaccharides from the woody and complex-textured fruiting bodies of *G. lucidum* remains a major challenge [13]. Therefore, several recent studies have focused on optimizing the extraction methods of GLPs

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obtained from different parts of *G. lucidum* (fruiting bodies, mycelia, and spores) [10]. To the best of our knowledge, this review is the first to combine research findings on conventional, advanced and innovative extraction methods specific to GLPs of *G. lucidum* fruiting bodies and to make critical comparisons between these methods. Besides, this review for the first time attempted to explain the reasons why it is difficult to extract GLPs from fruiting bodies of this mushroom from the perspective of cell structure. In this review, we review the advances in the field of GLPs extraction from *G. lucidum* fruiting bodies, critically evaluate the advantages and limitations of each method, and also discuss their contributions to future prospects.

2. The chemical structure of the cell walls of fruiting bodies of *G. lucidum* and major challenge to polysaccharide extraction

G. lucidum has a hard woody cap with a generally smooth, shiny, colored outer surface and an elegant appearance [11,14]. The difficulty in extracting GLPs.

from fruiting bodies of *G. lucidum* is largely related to the cell wall structure of this fungus. The cell wall of fruiting bodies of *G. lucidum* mainly consists of two parts, including a hard and rough fibrous structure composed of cellulose and chitin, and a matrix-like structure containing α -glucan, β -glucan, and glycoprotein [3]. The arrangement of these parts from the inside to the outside, respectively, include cytoplasmic membrane, mannoprotein, chitin, β -glucan, mannoprotein and glycoprotein (Scheme 1a). Fruiting bodies of *G. lucidum* also have a trimitic hyphal system, and as a result, have binding, generative and skeletal hyphae (Scheme 1b). Similar to the skeletons in vertebrates, the skeletal hyphae in *G. lucidum* acts as a hyphal framework which resemble wood fibers and provide the hard and woody characteristics of fruiting bodies [15]. The presence of both binding and skeletal hyphae in the structure of the cell wall, the special arrangement of the cell wall components, and the bond between polysaccharides and chitin have shaped very difficult special conditions for the extraction of GLPs from fruiting bodies of *G. lucidum* [1,16].

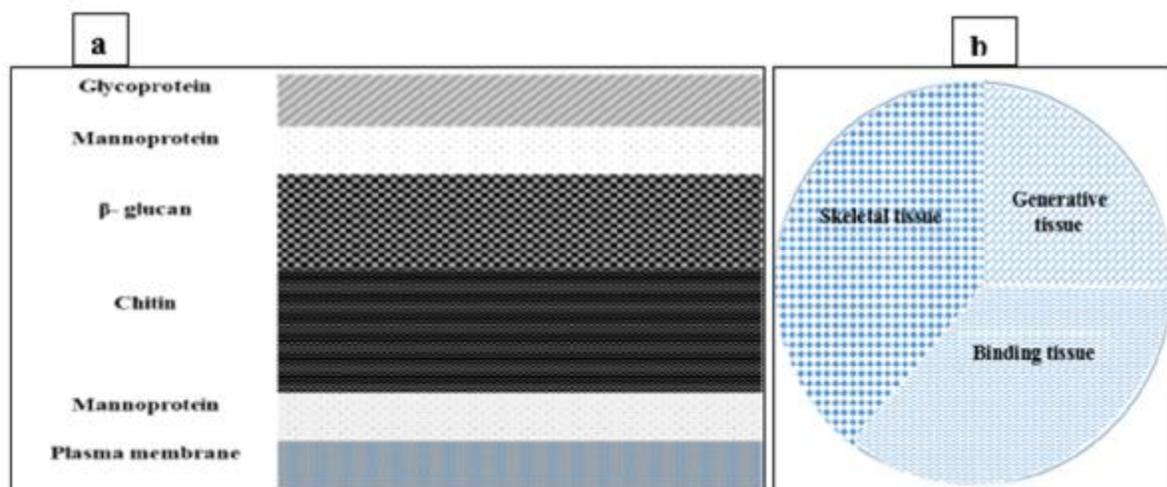


Fig. 1. Arrangement of the cell wall components of Reishi mushroom fruiting bodies (A); Tissue organization of trimitic hyphal system in fruiting bodies of *G. lucidum* (B). Figure 1-A and 1-B were re-drawn with modifications based on the original reference no. 13 and 14, respectively.

3. Glucans present in the cell wall

The cell walls of *G. lucidum* fruiting bodies are composed of (1-3), (1-6)- α/β -glucans, glycoproteins and water soluble heteropolysaccharides which are present between 10–50% in the dry fruiting bodies [6,17-20]. Among the cell wall polysaccharides, β -glucans play a key role in the medicinal values of *G. lucidum* and also in other medicinal mushrooms. The

basic subunit of mushroom β -glucans includes β -D-glucose that is linked to one another by 1→3 glycosidic chain with 1→6 glycosidic branches. β -glucans also exist with heteropolysaccharide chains of glucose (as main sugar molecule), mannose, galactose, fucose, xylose and arabinose combined with different combinations and different types of glycosidic linkages, as well as peptide bonds [21]. The biological activity of mushroom β -glucans are known to be

determined by parameters such as molecular weight, monosaccharide composition, solubility in water, degree of branching, polymer charge and spatial configuration, and even the method of extraction [22]. For example, the most biologically active mushroom β -glucans that stimulate the immune system are those with a degree of branching between 0.2 and 0.33

4. Extraction of GLPs from *G. lucidum*

Many factors are required to obtain high-quality GLPs, but the first and most important step is effective extraction. In fact, extraction is the most important step in determining the yield and production of GLPs [24]. The influence of extraction methods on the biological activity of β -glucans present in GLPs is demonstrated by their influence on monosaccharide composition, molecular weight, structure and spatial configuration of polysaccharides [24,25]. Therefore, the choice of extraction method should be based on the properties of GLPs, such as their cell wall structure and water solubility. In this regard, the basic principle is to break the cell wall from the outer layer to the inner layer under mild-to-strong extraction conditions (such as pH and temperature) [3]. Similar to other mushrooms, the extraction of GLPs from *G. lucidum* involves three main steps, including pretreatment, extraction, and purification [3] (Table 1). However, practically, the first step to extract GLPs from *G. lucidum* is to prepare dried mushroom powder. For this purpose, freshly and young harvested fruiting bodies of *G. lucidum* are cleaned, cut, and dried (by freeze-dry method or air-dry or in a vacuum oven at 50°C) and then ground into powder using a hammer mill or grinding. The particle mesh size suitable for extraction of GLPs is typically

[3,23]. Furthermore, most of the biological activity of β -glucans is linked to their three-dimensional structure, that is, their helical conformation [6,24]. Thus, the method of extraction is one of the important factors that affects the other parameters determining the biological activity of β -glucans of *G. lucidum*.

30-40 mesh. Then, the powdered samples are ready to proceed to the next level or pre-treatment process. The most important goal of pre-treatment step is defatting (with organic solvents) and alcohol pretreatment (removal of low molecular weight impurities) [3,24]. The next step is the extraction of GLPs, which is a time-consuming process due to the complexity of the intracellular region and the structure of the cell wall for releasing polysaccharides from the complex matrix of the fungus [26-28]. The extracted crude GLPs can be further purified by ethanol precipitation, deproteinization (Sevag method, protease enzymolysis or trifluoroacetic acid method), decolorization (activated carbon, hydrogen peroxide or resin method), dialysis and fractionation (affinity, cellulose column, gel filtration or ion-exchange chromatography) [3]. The methods and the experimental conditions (such as temperature, pH, etc) used during the extraction and purification steps have a significant effect on the composition, structure and functionality of the resulted GLPs [27-34]. For example, high temperatures may cause protein coagulation, changes in polysaccharide structure, and degradation of sensitive bioactive compounds. In addition, prolonging the treatment time also leads to the release of various undesirable cell wall compounds (e.g. pectin), tissue components and cell wall residues, ultimately increasing purification costs [26].

Table 1. Schematic diagram of mushroom polysaccharides extraction and purification [modified from reference no. 3].

Pretreatment	Extraction	Purification
Defatting (with organic solvents) Alcohol pretreatment (Removal of low molecular weight impurities)	Heating (hot water) Ultrasound Microvawe. Enzymolysis Supercritical etc	Ethanol precipitation Deproteinization (Sevag method, protease enzymolysis or trifluoroacetic acid method) Decolorization (activated carbon, hydrogen peroxide or resin method) Dialysis & Fractionation (affinity, cellulose column, gel filtration or ion-exchange chromatography)

Generally, the extraction method for bioactive compounds is determinant factor for predicting the

biological activities of the selected extract [27]. Conventional methods of extraction such as hot water

extraction and acid or alkaline extraction are mostly time-consuming (up to 24h) and their operating temperature is high (50-100°C). Therefore, innovative advanced methods in extraction of GLPs to increase the extraction yield and efficiency is required. Both conventional and advanced technologies for extracting GLP are evaluated below:

4.1. Basic extraction strategies

Since GLPs are mostly soluble in water and insoluble in alcohol, the most common and basic extraction methods for extracting GLPs include hot water extraction and precipitation in alcohol or combinations of both methods. Therefore, the usual strategy includes the use of water or chemical extractants (alkaline or acidic) under high temperature or pressure [6,30,31,32].

4.1.1. Hot water extraction

Hot water extraction (HWE) has been introduced as the most common extraction technique to remove polysaccharides from mushroom cell walls. This strategy is based on the fact that mushroom water-soluble polysaccharides are separated using water at high temperatures (50-100°C) [28]. Thus, the most important advantages of HWE are the low cost of use and simple equipment required, while its main disadvantages are time-consuming, high-energy requirement, and high operating temperature [28,29]. Moreover, the experimental results have showed that there is still a large amount of polysaccharide residues in the water and the final product is not of high purity [29]. A high temperature may also destruct the triple-helix structure of β -glucans [34]. A high liquid-solid ratio (LSR) leads to a decrease in viscosity and ultimately increases the cost of purification [34].

4.1.2. Acidic or alkaline extraction

Acidic or alkaline extraction (AE) is more efficient than HWE [6]. In this method, alkaline solutions such as potassium hydroxide (KOH) or sodium hydroxide (NaOH), and hydrochloric acid (HCl) and ammonium oxalate ((NH₄)₂C₂O₄) are used during the extraction [35]. Acid or alkaline treatment destroys the mushroom cell wall and coarse fiber structure and the hydrolysis of the bond between protein and glucan and ultimately facilitates the release of intracellular polysaccharides [6]. The optimal experimental conditions for the alkaline extraction were found to be

60.1 °C, 77.3 min, a NaOH concentration of 5.1% and a ratio of alkaline solution to solid of 21.4 mL/g, respectively [33].

4.2. Advanced extraction methods

4.2.1. Ultrasonic-assisted extraction and Soxhlet extraction

The ultrasonic assisted-extraction (UAE) is widely considered as an alternative to conventional methods for extracting GLPs, as it needs relatively inexpensive equipment and simple operation to recover valuable compounds [36]. Therefore, compared to other extraction techniques, such as microwave-assisted extraction, UAE is more favorable [36]. UAE depends on the biological form of the fungal material and the accumulation/dispersion of solid particles in the extraction solvent. Ultrasound significantly increased the extraction of polysaccharides from mushrooms without changing their molecular weight profiles [36]. Studies have shown that UAE increases the extraction of large polysaccharide-like molecules from medicinal mushrooms compared to HWE by 52-129% at a relatively low temperature [37]. Parameters such as ultrasonic power, LSR, frequency, extraction temperature and time significantly affect the UAE method [3]. Accordingly, the optimized parameters for polysaccharide extraction from *G. lucidum* using UAE method include ultrasonic power 320 W, temperature 70 °C and 34 min [38].

Soxhlet extraction (SE) is another alternative method for extracting secondary metabolite in plants [39] and mushrooms [40]. Research has shown that SE provided a higher amounts of GLPs (10.7 %) compared to UAE, while UAE-derived extract had a higher purity (50.2 vs. 44.5 %) compared to SE [40]. Furthermore, UAE-derived extracts had higher β -(1 \rightarrow 3) / (1 \rightarrow 6)-glucans content (44.4 %) and degree of branching 0.26 which is within the most biologically active range (0.2 – 0.33) compared to HWE (25.0 %, 0.12) and SE (37.7, 0.14) [3].

4.2.2. Ultrasonic-microwave synergistic extraction

Microwave-assisted extraction has played an important role as a drying and extraction tool in the food industry over the past two decades. Microwaves are non-contact heat sources that generate thermal energy by ionic conduction between a solvent and dissolved ions, creating extreme pressure that causes cells to rupture (break down the cell wall) and release

cell contents, including polysaccharides [36,41]. Changes in the orientation of polar molecules due to changes in high frequency (generally 2450MHz) current induce vibration and rotation of molecules, which ultimately leads to an increase in the frequency of internal pressure, and finally the cell wall breaks down due to the sudden increase in temperature. Eventually, the cell wall breaks down and is destroyed, allowing dissolved substances to be released (diffused) through the cell wall [36,41].

Ultrasonic-microwave synergistic extraction (UMSE) is the result of integrating the vibrational cavitation of ultrasonic and high-energy action of microwave. This method was developed to extract polysaccharides from various mushroom species, including *G. lucidum*, *Inonotus obliquus*, *Morchella conica*, *Trametes orientalis*, and *Tricholoma mongolicum* Imai [31,34,41,42]. Studies agreed that UMSE achieved higher extraction yield and shorter processing time compared to HWE, UAE, and common microwave extraction methods [31,32,41].

The electromagnetic spectrum of microwave ranges between far infrared light and radio frequencies. The frequency range is situated from 300 MHz to 300 GHz, corresponding to 1 m –1 cm wavelength range. For industrial and scientific microwave heating, 915 ± 25 , 2450 ± 13 , 5800 ± 75 and $22,125 \pm 125$ MHz, are the most applied frequencies [43,44]. The advantages of this method include low solvent consumption, short process, high performance. Extraction efficiency which is abnormal increase in microwave power causes a decrease in the extraction efficiency of polysaccharides due to the destruction of molecular interaction caused by high energy [41].

4.2.3. Enzyme-assisted extraction

Enzyme-assisted extraction (EAE) provides relatively short time and low energy consumption, higher extraction efficiency, high specificity, environmental friendliness, low operating temperature, and low degradability of extracted biomolecules. All of these are important for the structure and properties of β -glucan derived from mushrooms [45]. In addition, EAE usually does not destroy the spatial or the three-dimensional structure of the polysaccharide molecule. The main structure of the cell wall of medicinal mushrooms is composed of polysaccharides, chitin (b-1,4-N-acetylglucosamine) and glucans (β -1,3 and β -1,6). Therefore, the hydrolysis and degradation of cell wall matrix can be effectively promoted by enzymes such as gluconase, chitinases, and proteases. A study

showed that the amount of GLP extracted by EAE method in the optimal conditions (cellulase/pectinase/protease enzyme mixture) at 45°C and pH=7 for 3 h) was 8.9% and the polysaccharide yield was 1.1% [43]. Also it has been demonstrated that EAE was superior to microwave-assisted extraction and HWE methods [46].

The use of EAE to extract β -glucans from fruiting bodies of *G. lucidum* has applications in both pharmaceutical and cosmeceutical industries [45]. However, the disadvantage of relatively high cost of EAE needs to be addressed in large-scale production of β -glucans [3,45].

4.2.4. Supercritical and subcritical liquid extraction

Both supercritical liquid extraction (SLE) and subcritical liquid extraction (S_b LE) have emerged as novel useful tools to replace conventional extraction methods. These methods are environmentally friendly and have potential to improve the efficiency of extracting valuable and bioactive compounds from various sources, including mushrooms compared to conventional extraction methods [43]. Difference between SLE and S_b LE is that in S_b LE (also known as hydrothermal extraction or hot-compressed water extraction) water is kept in a liquid state at temperatures and pressures below its critical point (100-374°C, 1-22.1 MPa). Subcritical conditions reduce both the dielectric constant and viscosity of water, enabling it to solubilize polar, moderately polar as well as non-polar compounds including polysaccharides of higher MW [24], whereas SLE requires temperatures higher than 374°C, and pressures higher than 22.1 Mpa [3]. Both methods result in isolating solving polar, relatively polar and even non-polar compounds, including polysaccharides of higher molecular weight [24]. S_b LE method provides simultaneous extraction/ fractionation and depolymerization of polysaccharides from mushrooms. In fact, high pressure and supercritical conditions cause the separation of the hydrogen bonds of polysaccharides, improve solubility in water and the release of acidic groups and acid hydrolysis of polysaccharides [3].

Temperature plays a vital role in S_b LE method. While low temperatures (<100°C) increase the yield of water-soluble substances (Mw<35KDa), higher temperatures (>100°C) help the extraction and separation of insoluble compounds with higher molecular weight [3,23]. However, the chemical conformation of the polysaccharide structure is sensitive to pressure and

temperature above the critical point, which might affect the biological activity of polysaccharides [34]. The optimal condition where solubility is higher to recover the maximum total amount of β -glucan from *G. lucidum* (0.44 mg/100 g dried sample) was determined to be 473 K at 10 MPa, with a water flow rate of 1 ml/min for 130 min. For batch-type and semi-continuous-type extractions, the best solubility was obtained at 473 K having efficiencies of 78.1% and 57.4%, and the highest extracted amounts of total WSOC (water-soluble organic compounds) were 328 and 241 mg WSOC/g dry sample, respectively [47].

4.2.5. Pulsed electric field-assisted extraction

Pulsed electric field-assisted extraction (PEFAE) is a technique of electroporation that uses high voltage pulses (10-100 kV/cm) lasting 100-1000 microseconds. The cell electroporation theory is based on the concept of transmembrane potential. PEFAE increases the porosity of the cell wall and the permeability of the tissue to extract compounds from the intercellular space [29,47-49]. The most important advantages of PEFAE include its short time and low energy consumption (10–20 kJ/kg), being a non-thermal technology and its suitability for extracting temperature-sensitive bioactive compounds [28,50]. PEFAE has been applied in the extraction of polysaccharides from *Morchella esculenta* [51], although the results showed that it was less efficient than microwave-assisted extraction method for achieving total polysaccharide and triterpene in *G. lucidum* [52].

4.2.6. Supercritical CO₂ method

Supercritical fluid extraction is an effective method for separating and purifying various substances from multicomponent mixtures. Supercritical fluid extraction is used to purify a variety of products, from petrochemicals to phytochemicals. Among the various solvents used in supercritical fluid extraction, supercritical CO₂ (ScCO₂) is widely used due to its many suitable and desirable properties [53].

In recent years, ScCO₂ is widely used to extract bioactive compounds from *G. lucidum*. In this method, CO₂ gas is subjected to a temperature and pressure higher than its critical point (T_c: 31°C, P_c: 73.8 bar) [53,54]. Since a low critical temperature and non-oxidizing environment (medium) are used, the ScCO₂ method is suitable for extracting heat-sensitive or easily oxidized compounds. The most important

advantage of this technique is the ability to change the density of CO₂, control solubility, by changing the pressure or temperature. Another advantage is the possibility of removing CO₂ by reducing the pressure of the system after the extraction is complete, because CO₂ is a gas at ambient temperature. This method can save in cost and energy because no energy process is used to remove solvents. However, ScCO₂ has a limited ability to extract and is mostly used for polar compounds, so co-solvents are added in low quantities to improve extraction efficiency [55].

Factors effective in improving extraction quality include temperature, pressure, solvent-to-sample ratio, time, and in addition to using statistical models Response Surface Methodology (RSM) and Box-Behnken design (BBD) will increase the accuracy of the evaluation. Fourier-transform infrared spectroscopy (FTIR) can also be used to identify and fractionate groups [54].

To extraction of β -(1 \rightarrow 3)/(1 \rightarrow 6)-glucans from *G. lucidum* by employing SWE under the optimized conditions (158 °C, 5 MPa, 1.3 mL/min flow rate, 60 mL/g SLR and 92 min extraction time) maximum yield and β -glucans content of final product were 64.9 ± 0.8 and 61.7 ± 1.0 %, respectively. The addition of supercritical CO₂ to the pressurized hot water throughout the extraction (done at 155 °C, 1 mL/min of PHW and 40 mL/g) produced a significant improvement of the extraction yield up to 72.5% in the best tested conditions; effect primarily attributed to the acidification of the media obtained by the supercritical CO₂. Although organic or mineral acid can also be employed to reduce the pH of extracting solvent, they remain in the media and require additional purification step. Studies also reported the integration of SWE (160 °C and 4 MPa) and micronation for the semi-batch production of β -glucan from *G. lucidum* [3].

4.3. Integrated extraction strategies

One suitable strategy to increase the efficiency of GLP extraction is a combination of extraction methods. Several strategies based on the combination of two or more methods for extracting mushroom polysaccharides, including GLPs, have also been developed (such as enzymes/ultrasonic, ultrasonic/microwave, enzymes-microwave-ultrasound assisted extraction) [3]. The extraction of polysaccharides (as the response) under different experimental conditions (as variables such as pH, temperature, time, ultrasonic force, etc.) of these integrated methods have been optimized using

response surface methodology (RSM) [31]. Studies showed that the combination of ultrasonic and microwave methods (ultrasonic/microwave-assisted extraction, UMAE) to extract GLPs resulted in 115.56% and 27.7% higher polysaccharides yield than conventional HWE and UAE methods, respectively [31]. Also, the combination of enzyme method with ultrasonic waves (ultrasonic-assisted enzyme extraction method, UAEE) generated a high content of polysaccharides in the extract of *G. lucidum* as much as 32.08 mg/g under the optimum experimental parameters [56].

4.4. Innovative technologies

In addition to the commonly used conventional or advanced methods for extracting GLPs, there are innovative technologies that could be used to extract GLPs. These methods use new technologies to increase service performance and operational efficiency. In fact, these methods offer industrial applicability or "professional services") such as homogenous extraction, vacuum extraction, the nanoparticle technology, water oxidizing electrolysis solvent, and two-phase extraction strategy. For example, nanotechnology is an innovative method for investigating and utilizing particles with large surface areas of approximately 1-100 nm (nanoparticle). Therefore, nanoparticles are one of the most important materials with distinguished physical and chemical properties and diverse applications [57], which can also be used for the extraction of bioactive compounds [3]. A good example of nanoparticle-assisted extraction was used to overcome the difficulties in extracting β -glucans from *Sparassis crispa* and *Phellinus linteus* mushrooms [58]. In this method, insoluble tungsten carbide nanoparticle powder was used to provide a high yielding β -glucan extract from the mushrooms [58]. After preparation of blended fruiting bodies (100 mesh), alkaline extraction was performed (pH=10) for 2 h followed by high-pressure emulsification with 20% nanoparticle at 30°C for 40 min. Finally, HWE was performed at 90°C for 30 h. This method provided high yields of 70.2% and 65.2% of β -glucan with an average particle size of 150 nm and 390 nm, for *S. crispa* and *P. linteus*, respectively. The extracted β -glucans also showed a high water solubility of 90%. Another advantage of this method is that the extract particles obtained from β -glucan are monodisperse and narrowly distributed [58]. Therefore, nanoparticle-assisted extraction could

be used to produce β -glucans from mushrooms for the food, cosmetic, and pharmaceutical industries.

Homogenate extraction is another innovative method for extracting polysaccharides from mushrooms. In this method, biological components are extracted using high-speed mechanical shearing, while cutting, smashing, and mixing replace heating and pressure. The notable advantages of this method include high extraction yield, short time processing and low extraction temperature [59]. Although this method has been developed for Shiitake mushroom (*Lentinula edodes*) [59], it can also be evaluated for extracting polysaccharides from *G. lucidum*.

The use of NaCl-based electrolyzed oxidizing water could be considered an innovative method to extract GLPs from *G. lucidum* [60]. Electrolyzed oxidizing water (EOW) has applications in food processing, such as sterilization, enzymatic inactivation and extraction of active substrate. The advantages of this method include high extraction yield, low solvent consumption, and environmental friendliness. Based on the research on this method, the optimal extraction time, temperature, pH and water-to-solid ratio were 4 h, 100°C, pH 2.5 and 20 mL/g, respectively, which yielded 78.47 ± 2.95 mg/g of GLP [60].

5. Conclusion and future prospects

Reishi, *G. lucidum*, is mushroom rich in bioactive compounds such as polysaccharides, triterpenoids, and proteins, which gives this mushroom exceptional medicinal properties and economic value [6,11]. Therefore, similar to other medicinal mushrooms, intensive research has been conducted on the extraction of bioactive compounds of *G. lucidum* [61]. This is a prerequisite for the identification, isolation, and classification of important bioactive compounds present in *G. lucidum*. However, extraction methods for cell wall polysaccharides of woody fruiting bodies of this mushroom need to be improved to enable the use of these bioactive compounds on an industrial scale. Moreover, different extraction methods result in extracts with different biological activities [62]. Thus, this review compared various methods for extracting polysaccharides containing β -glucans, some of which have been used directly for *G. lucidum*, and some for other mushrooms (including potential use in *G. lucidum*). This evaluation shows that methods for extracting polysaccharides from *G. lucidum* can be classified into three major types: old methods, more advanced methods, and complex and innovative methods (Table 2). There are significant differences

Table 2: Comparison of different methods for extracting polysaccharides from Reishi mushroom, *Ganoderma lucidum*.

Type	Method	Time	Cost	Scale up	Temperature (°C)	Efficiency percentage (% dw)	Reference
Old methods	HWE	Long	Low	Easy	50-100	0.4-7.5	[66,67]
	AE	Very long	Low	Easy	60	1.41-8.21	[26]
More advanced methods	UAE	Short to moderate	High	moderate	40-70	2.07-8.1	[26,38,39]
	MAE	Short	Moderate	moderate	180	10.2	[23]
	EAE	Moderate to long	High	moderate	45	1.1	[68]
	SWE	Short to moderate	High	moderate	180	10.5	[23,69]
Complex and innovative methods	PEFAE	Short to moderate	High	moderate	room temperature	nd	[49]
	ScCo ₂	Short	High	moderate	155	61.7-72.5	[70]
	MAE + UAE (UMAE)	Short to moderate	High	moderate	45	3.2	[31]
	SFE-CO ₂	Short to moderate	High	moderate	40	37	[71]
	UAEE (EAE+ UAE)	Short to moderate	High	moderate	varied	32	[53]
	AE + nanoparticle (Nano-knife method)	Short to moderate	High	moderate	100	78.47	[55]
	SWE + ScCo ₂	Short to moderate	High	moderate	150-180	64.9	[70]

HWE, Hot water extraction; AE, acid or alkaline extraction; UAE, Ultrasonic-assisted extraction; MAE, Microwave-assisted extraction; EAE, Enzyme-assisted extraction; SWE, Subcritical liquid extraction; PEFAE, Pulsed electric field-assisted extraction; ScCO₂, Supercritical CO₂

between methods in terms of experimental conditions used, cost, efficiency, etc. In addition to comparisons between each type of methods, comparisons could be made between methods within each type. This evaluation ultimately might help researchers to select appropriate extraction methods (Table 2). In conclusion, this review shows that integrating conventional and advanced extraction methods accompanied with appropriate statistical evaluation methods such as RSM has the potential to improve the efficiency of polysaccharide extraction. The selected methods should also be economical from the standpoint of the laboratory where the extraction is performed. Furthermore, the methods of choice are expected to yield high efficiency of extraction as well as high amounts of β -glucans in the extract, since the production of high amounts of β -glucans accompanied by low efficiency of extraction is not sufficient. For example, ultrasonic-assisted enzymatic extraction (UAEE) could be used for polysaccharide extraction

from *G. lucidum* so that a higher content of polysaccharide can be achieved in shorter extraction time.

Further studies are warranted to develop more innovative approaches to extract GLPs from fruiting bodies of *G. lucidum*. Some of these innovative methods such as nanoparticle-assisted extraction [58] and homogenate extraction [59] have previously been reported to extract polysaccharides from other medicinal mushrooms. Thus, this could be a subject for further research to determine how these methods can be applied to *G. lucidum* and the extraction of GLPs from the fruiting bodies of this mushroom.

Further investigations are also required to increase the production yield of bioactive compounds (including polysaccharides) in *G. lucidum* via optimizing the growth conditions [63] and standardizing liquid medium for large-scale cultivation [11]. Solid-state fermentation of *G. lucidum* to produce fruiting bodies is time-consuming and the level of bioactive

compounds such as cell wall polysaccharides within the fungus are difficult to control. In this regard, submerged fermentation has been used as an alternate method for the efficient production of polysaccharides [64].

In addition, future studies should be performed on the traceability and authenticity of mushroom species particularly regarding compounds and extracts obtained from Reishi, *G. lucidum*. Clarity in the taxonomy of *G. lucidum* is essential to ensure that the extracted bioactive compounds are pure, of comparable quality and in similar quantities [65].

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