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Exploring extraction techniques for medicinal mushroom bioactive compounds: A comprehensive review of advantages and limitations

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Abstract
Mushrooms exhibit a robust cellular architecture featuring chitin, a structural substance found in the exoskeletons of insects and crustaceans. These resilient cell walls function as a protective enclosure for the various bioactive constituents present within the mushroom. Consequently, it is necessary to employ extraction methodologies to liberate the active substances, including beta-glucans, triterpenoids, ergosterols, peptides, and phenolic compounds, for the purpose of exploitation. Due to the distinct chemical and physical properties of these bioactive compounds, diverse extraction techniques are required. The effectiveness and precision of these techniques depend on the development of sophisticated instruments. Additionally, precise handling of the extraction process holds paramount importance in preserving the integrity and activity of these fragile bioactive components. Avoiding harsh extraction conditions that could lead to degradation or denaturation of the compounds is essential to maximize the yield and bioavailability of the extracted substances. As scientific knowledge and technological capabilities continue to evolve, the full potential of bioactive compounds extracted from medicinal mushrooms that can help in improving human health and well-being is yet to be fully realized. So in this review we evaluate the success rates, encountered challenges and scalability potential of different extraction techniques, aiming to meet the growing market demands along with their applications in the field of medicinal mushroom biotechnology.

Keywords: Extraction, bioactive compounds, medicinal mushrooms, advanced techniques, conventional techniques

1. Introduction
For centuries, medicinal mushrooms have held a revered place in traditional medicine due to their plethora of therapeutic properties and potential to yield bioactive compounds with profound health benefits. Approximately 2.2-3.8 million fungal species have been recognized globally, with 150,000 of them having been officially described. Among these, around 2,000 species are considered edible, while more than 200 species of wild mushrooms are acknowledged for their medicinal properties [1, 2]. As interest in natural remedies and alternative medicine continues to soar, researchers have intensified their efforts to unlock the full potential of these remarkable fungi, central to this pursuit is the crucial aspect of extracting valuable bioactive compounds from medicinal mushrooms.

Within this review, we delve into the realm of extraction techniques, exploring the myriad advantages and disadvantages associated with each method. With a primary focus on isolating bioactive compounds from medicinal mushrooms, researchers and scientists have devised an array of innovative extraction methods, each presenting a unique set of strengths and limitations. The extraction process plays a pivotal role in determining the yield, purity, and overall efficacy of the bioactive compounds derived from medicinal mushrooms. These bioactive compounds ascribed anticancer, antibacterial, antitumor, anti-inflammatory, hypoglycemic and immunomodulatory [3, 4]. Consequently, researchers must make well-informed choices based on their specific objectives and the desired bioactive molecules they seek to isolate. This review endeavours to shed light on the diverse extraction techniques and their contributions to the rapidly expanding field of medicinal mushroom research. For instance *Ophiocordyceps sinensis* cultured mycelia and made methanolic extracts in semi-artificial liquid media showed antibacterial properties [5].

Within the first section, we embark on an exploration of the advantages offered by various extraction methods. Prominent among these advantages are the abilities to obtain highly concentrated bioactive compounds, enhance their bioavailability, and preserve the medicinal

properties of mushrooms without compromising their potency. Moreover, certain extraction techniques align with the global demand for eco-friendly and sustainable solutions, aligning with the growing consciousness towards environmentally responsible. However, the advantages must be carefully weighed against the inherent disadvantages associated with each extraction method. Factors such as cost, complexity, and the potential for chemical alterations are significant considerations that can impact the efficiency and safety of the extraction process. Additionally, while some techniques may excel in specific scenarios, they may prove less effective when confronted with the challenge of extracting a diverse range of bioactive compounds from various medicinal mushroom species

The subsequent section delves Into the practical application of these extraction techniques for different medicinal mushroom species. From *Ganoderma lucidum* to *Cordyceps militaris*, each mushroom harbors a distinctive array of bioactive compounds, rendering the extraction process to be both dynamic and challenging. Here, we evaluate the success rates, encountered challenges and scalability potential of different extraction techniques, aiming to meet the growing market demands. However, it's essential to remember that the concentration of bioactive compounds can vary depending on the mushroom species, growing conditions, and preparation methods.

2. Bioactive compounds and their potential biological activities

Mushrooms serve as a valuable reservoir of such compounds, gaining recognition not only for their culinary appeal but also for their potential medicinal and nutritional benefits. Bioactive compounds found in mushrooms encompass a diverse array of naturally derived chemical substances with significant impacts on living organisms. These biologically active substances, including proteins, β-glucans (polysaccharides), as well as terpenoids, steroids, and phenolics like secondary metabolites, are present within the cell walls of mushrooms. The intricate composition of bioactive compounds found in mushrooms varies significantly not only among different species, but also within individual mushrooms. This diversity is influenced by various factors including concentration, developmental stage, fruiting body conditions, age, and storage environment. These variables collectively contribute to the unique profile of bioactive compounds present in mushrooms, ultimately influencing their therapeutic potential and medicinal applications [6]. Noteworthy bioactive compounds commonly found in medicinal mushrooms include.

2.1 Beta-glucans

These polysaccharides possess a complex molecular structure characterised by variations in molecular weight, primary structure, degree of branching, solubility, and linkage type. Fungal glucans can exhibit insoluble or soluble properties in alkali but are notably soluble in water, recognized for their immune-boosting properties. Beta-glucans play a crucial role in activating the immune system, assisting the body in its defence against infections and diseases. Biologically active fungal β-glucans have been discovered within mushroom fruiting bodies and isolated for further exploration. For example, Lentinan, derived from *Lentinula edodes*, demonstrates antitumor and immunomodulatory effects [7]. Extract from *Agaricus subrufescens* contains β-1,3-, β-1,4-, and β-1,6-glucans, stimulating the release of proinflammatory cytokines in monocytes and human endothelial cells [8]. *Pleurotus pulmonarius* displays anti-inflammatory properties attributed to (1,3)-glucopyranosyl [9]. *Ganoderma lucidum* glucans, including ganoderan A and B, exhibit hypoglycemic effects [10], while ganopoly demonstrates hepatoprotective actions in chronic Hepatitis B patients [11]. These glucans also display immunomodulatory effects by enhancing lymphocyte proliferation and antibody production, as well as demonstrating antigenotoxic and antitumor activities [12, 13]. *Ganoderma lucidum* showcases antioxidative and scavenging effects [14], while *Pleurotus ostreatus* fruiting bodies contain a β-glucan responsible for antitumor activity against HeLa tumour cells [15, 16]. Various immune receptors, including dectin-1, the primary β-glucan receptor, innate immune receptors, and complement receptors, have shown responses to β-glucans, underscoring their antimicrobial immune properties [17]. This capability enables β-glucans to strengthen the immune system, fortify against common ailments, and promote overall well-being. Within the innate immune system, β-glucans bind to macrophages, aiding in the recognition and elimination of invaders through phagocytosis. Additionally, the thymus gland produces T lymphocytes equipped with T-cell receptors and specialized cells for pathogen eradication. B lymphocytes contribute to humoral immunity by producing antibodies, while T lymphocytes serve as natural killer cells, targeting bacteria, infected cells, tumour cells, and viruses, collectively defending the body against harmful pathogens [17, 18].

2.2 Triterpenoids

While contributing to the bitter taste of specific mushrooms, these compounds also showcase notable anti-inflammatory, antioxidant, and antitumor properties. Certain triterpenoids, such as ganoderic acid found in Reishi mushroom (*Ganoderma lucidum*), are especially intriguing for their potential therapeutic uses [16, 19]. Research suggests that mushrooms containing triterpenes offer defence against atherosclerosis and provide antiviral and antioxidative advantages [20, 21]. In one study [22], numerous neuroprotective triterpenes were isolated from *Antrodia camphorata*. Bioactive triterpenoids can mitigate inflammation by inhibiting enzymes and cytokines, potentially alleviating conditions like arthritis. Their antioxidant abilities shield the cells from damage induced by free radicals, potentially thwarting diseases like cancer. Additionally, triterpenoids enhance the immune system, assisting in the defence against pathogens. Some types even demonstrate antimicrobial properties, combating bacteria, fungi, and viruses. Moreover, specific triterpenoids exhibit promise in impeding tumour growth and inducing apoptosis in cancer cells.

2.3 Ergosterol (Provitamin D)

The presence of this compound in medicinal mushrooms has a similar role as cholesterol in the synthesis of vitamin D in the skin, ergosterol in mushrooms can be converted into vitamin D2 when exposed to sunlight. Acting as a precursor to vitamin D, ergosterol aids in maintaining bone health and enhancing calcium absorption. Additionally, it demonstrates anti-inflammatory properties, potentially easing symptoms associated with inflammatory conditions like arthritis and asthma. Furthermore, ergosterol exhibits antioxidant activity,

effectively neutralizing free radicals and shielding cells from oxidative harm, thus potentially mitigating the risk of chronic illnesses such as cancer and cardiovascular disease. Moreover, ergosterol is involved in modulating the immune system, bolstering immune function, and bolstering defenses against infections.

2.4 Peptides and Proteins

Mushrooms produce a variety of bioactive peptides and proteins with distinct functionalities. Among these, lectins lack enzymatic activity, while other proteins like fungal immunomodulatory proteins, laccases, and ribosome-inactivating proteins possess enzymatic functions. Lectin proteins have demonstrated potential antitumor and immunomodulatory effects, although excessive consumption of certain lectins may pose risks. Research conducted by Chu *et al*. [25] illustrated antifungal properties in both *Pleurotus ostreatus* and *Agrocybe cylindracea* [26]. Additionally, a peptide derived from *Russule paludosa* exhibited antiviral properties [27]. Cordymin, a low molecular weight peptide with anti-inflammatory activity, has been identified in *Cordyceps sinensis* [28, 29] and *Cordyceps militaris* [19, 30]. Ribosome-inactivating proteins, enzymes that eliminate adenosine residues from rRNA, have shown potential antitumor effects [31]. Laccases, phenol oxidases found in various fungi, play a role in decomposing lignocellulosic materials. Moreover, mushrooms are rich in essential amino acids, making them a valuable protein source, particularly for individuals following vegetarian or vegan diets.

2.5 Phenolic compounds

Mushrooms contain a diverse array of phenolic compounds, including gallic acid, catechins, and quercetin, which contribute to their antioxidant and anti-inflammatory properties. These compounds, characterised by aromatic hydroxylated structures with hydroxyl groups and aromatic rings, encompass various types such as hydroxybenzoic acids, phenolic acids, flavonoids, lignans, tannins, oxidised polyphenols, and stilbenes [32, 33]. Research, including studies by Palacios *et al*. [34], has explored the antioxidant abilities of phenolic compounds across different mushroom species. For instance, *Craterellus cornucopioides* is rich in myricetin, while *Cantharellus cibarius* contains notable amounts of catechin and caffeic acid, both known for their antioxidant effects. Similarly, Lentinula squarrosulus, Lentinula polychrous, and L. *edodes* produce catechin as a significant phenolic compound with antioxidant properties. These compounds act as antioxidants by inhibiting free radicals, decomposing peroxides, scavenging oxygen, and deactivating metals, which is crucial for combating the damaging effects of reactive oxygen species (ROS). Additionally, certain mushroom-derived compounds, including flavonoids and phenolics, exhibit antibacterial, antioxidant, and antifungal properties. Consumption of mushrooms has been linked to a reduced risk of neurodegenerative diseases, partly due to compounds like hericenones and erinacines found in *Hericium erinaceus*, which stimulate nerve growth factor synthesis. Moreover, hispidin, a medicinal metabolite from Phellinus spp., acts as an ROS scavenger, while pyrogallol, present in various mushrooms like *Lactarius deliciosus* and A. *bisporus*, exhibits anti-inflammatory effects [35, 36, 37].

2.6 Other bioactive compounds

This include recently discovered agaric glycerides, comprising glycerol and a chlorinated ester of 4-hydroxybenzoic acid, exhibiting potent anti-inflammatory properties [38]. Similarly, linoleoyl phosphatidylethanolamine, sourced from the fruiting bodies of H. *erinaceum*, has demonstrated antioxidative effects and significant impacts on neurodegenerative diseases [39]. Additionally, termitomycesphins and termitomycamides, isolated from the dried fruiting bodies of *Termitomyces albuminosus*, are recognized for their potential in combating neurodegenerative conditions [40, 41].

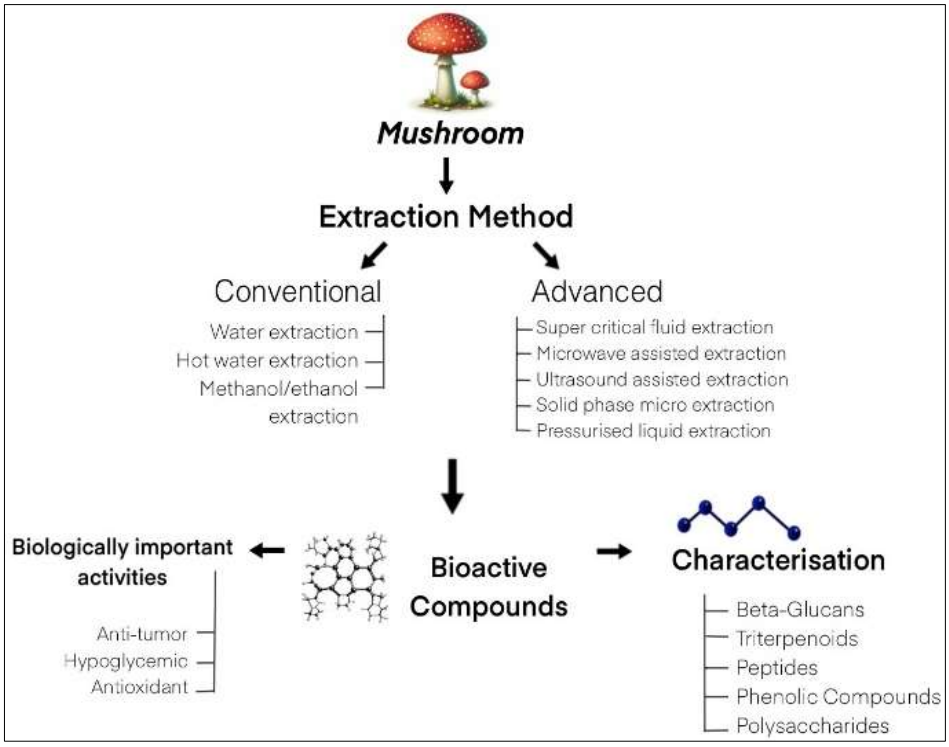

Fig 1: Schematic diagram showing steps involved in bio prospecting of medicinal mushrooms

3. Conventional Extraction Techniques

3.1 Water Extraction (Decoction): This is a straightforward method of extracting water-soluble compounds from mushrooms. In this technique, dried or fresh mushrooms are simmered in water for an extended period to allow the water-soluble components to dissolve into the liquid. The resulting mushroom-infused water can be used as a base for soups, sauces, or beverages.

3.1.1 Hot Water Extraction: This technique is similar to water extraction (decoction), but it involves using hot water to enhance the extraction process. The higher temperature can help break down the mushroom cell walls and extract a wider range of compounds. This method is often used to obtain mushroom extracts for supplement capsules or teas.

Advantages.

- **Widely accessible and cost-effective:** Water is a common solvent and readily available, making water extraction an economical choice.
- **Safe and environmentally friendly:** Water is non-toxic and does not produce harmful waste, making it an environmentally friendly option.
- **Suitable for polysaccharides:** Many bioactive polysaccharides in medicinal mushrooms can be efficiently extracted with water.

Limitations

- **Limited solvent power:** Water extraction may not be effective for extracting non-polar compounds like terpenoids.
- **Temperature-sensitive:** Some heat-sensitive bioactive compounds may degrade during high-temperature water extraction.

3.2 Extraction (Tincture): Alcohol extraction, also known as tincture, involves soaking mushrooms in high-proof alcohol (such as ethanol or vodka) to extract both water-soluble and alcohol-soluble compounds. The alcohol acts as a solvent, pulling out various active constituents from the mushrooms. Tinctures are commonly used in herbal medicine and can be taken orally or used topically.

3.2.1 Ethanol/Water Combination Extraction

This method involves using a combination of ethanol (alcohol) and water as the solvent to extract a broader spectrum of compounds from mushrooms. The mixture of alcohol and water provides an intermediate polarity, allowing extraction of water-soluble and alcohol-soluble components.

Advantages

- **Broad spectrum of solubility:** Ethanol can dissolve both lipophilic and hydrophilic compounds, providing a wider range of extracted bioactives.
- **High bioactive compound yield:** Ethanol extraction often yields higher concentrations of bioactive compounds compared to water extraction.
- **Long shelf life:** Extracts obtained using ethanol as the solvent generally have a longer shelf life.

Limitations

- **Higher cost:** Ethanol is more expensive than water, which can increase the overall extraction cost.
- **Potential chemical alterations:** Ethanol may cause some chemical alterations in the extracted compounds, affecting their bioactivity.

4. Advanced extraction techniques

4.1 Supercritical Fluid Extraction: This is an advanced technique that utilises supercritical fluids, typically carbon dioxide (CO_2), as a solvent to extract specific compounds from mushrooms. The supercritical CO_2 acts as a non-toxic, environmentally friendly solvent that can be adjusted to target specific compounds.

Advantages

- **High selectivity:** SFE allows the selective extraction of specific bioactive compounds, minimising the extraction of unwanted substances.
- **Mild conditions:** Supercritical CO2 extraction is carried out at lower temperatures, preserving heat-sensitive compounds.
- **Solvent-free extracts:** Supercritical CO2 leaves no solvent residues in the final extract.

Limitations

- **Costly equipment:** The initial setup cost for SFE equipment can be substantial.
- **Limited availability:** SFE equipment and expertise may not be readily available in all regions.

4.2 Microwave-Assisted Extraction (MAE): This method utilizes microwave irradiation to heat the solvent, expediting the extraction process. The energy from the microwaves induces molecular vibrations, facilitating the release of compounds from the mushroom material into the solvent. MAE proves to be a swift and effective method for extracting heat-sensitive compounds.

Advantages

- **Rapid extraction:** MAE significantly reduces the extraction time compared to traditional methods, improving productivity.
- **Energy-efficient:** Microwave-assisted extraction consumes less energy than conventional methods.
- **Enhanced extraction efficiency:** Microwave radiation enhances the penetration of the solvent into the mushroom matrix, leading to improved extraction yields.

Limitations

- **Uneven heating:** Uneven microwave distribution may lead to localised high temperatures and degradation of heat-sensitive compounds.
- **Limited scalability:** MAE may not be suitable for large-scale industrial applications.

4.3 Ultrasound-Assisted Extraction (UAE): In this method, a sonicator subjects the mushroom mixture to high-frequency ultrasound waves, inducing cavitation and improving mass transfer between the solvent and the mushroom matrix. This process increases the extraction efficiency and reduces the extraction time.

Advantages

- Increased extraction efficiency by promoting the release of compounds from the mushroom matrix to the solvent.

The cavitation phenomenon induced by ultrasound creates microbubbles that disrupt the cell walls, releasing intracellular compounds.
- It allows rapid extraction of bioactive compounds, saving time and energy compared to other conventional techniques.
- Preservation of heat-sensitive compounds.
- The UAE technique can be easily scaled up for industrial production, making it suitable for large-scale extraction processes.

Limitations
- **Equipment Cost:** The initial setup cost of ultrasound equipment can be relatively high, which may pose a barrier for small-scale or home-based applications.
- **Risk of Over Extraction:** If not controlled properly, the intense cavitation generated by ultrasound may lead to over extraction, resulting in the extraction of unwanted or undesirable compounds.
- UAE requires expertise in optimising the process parameters, such as frequency, intensity, and time, to achieve the desired results. Without proper optimization, the extraction efficiency may be compromised.

4.4 Enzyme-Assisted Extraction: It entails employing specialised enzymes to degrade the cell wall of mushrooms, thereby aiding in the liberation of intracellular compounds. The enzymes act as biological catalysts, aiding in the extraction of desired compounds and bioactive components.

Advantages
- **Enhanced extraction efficiency:** Enzymes can break down cell walls, facilitating the release of bioactive compounds from medicinal mushrooms.
- **Selectivity:** Enzymes can target specific compounds, allowing for the extraction of desired medicinal components while minimizing the extraction of unwanted substances.
- **Improved bioavailability:** Enzyme-assisted extraction can increase the bioavailability of bioactive compounds, enhancing their therapeutic effects.
- **Eco-friendly:** Enzymes are biodegradable and environmentally friendly compared to chemical solvents, reducing the environmental impact of the extraction process.

Limitations
- **Cost:** Enzymes can be expensive, which may increase the overall cost of the extraction process.
- **Specificity:** Enzymes may not be effective for all types of medicinal mushrooms or may require optimization for different species.
- **Enzyme stability:** Enzymes can be sensitive to factors such as pH, temperature, and substrate concentration, which may affect their effectiveness in extraction.
- **Time-consuming:** Enzyme-assisted extraction may require longer extraction times compared to other methods, increasing processing time.

4.5 Subcritical Water Extraction: It involves using water at temperatures below its boiling point i.e. 374 °C and high pressures to extract compounds from mushrooms. This method can effectively extract both water-soluble and heat-stable compounds. Subcritical water extraction is considered an eco-friendly technique as it eliminates the need for organic solvents.

Advantages
- It doesn't require organic solvents, making it a more sustainable and environmentally friendly extraction method compared to traditional solvent-based techniques.
- It can selectively extract bioactive compounds while leaving undesirable components behind, leading to higher purity extracts.
- Since water is non-toxic and non-flammable, it eliminates the risk of solvent residues in the final extract, making it safer for consumption.
- Operating at moderate temperatures and pressures reduces energy consumption compared to other extraction methods.

Limitations
- Some compounds may have limited solubility in water, which can affect the efficiency of extraction.
- High temperatures used in SWE can lead to the degradation of thermally sensitive compounds, affecting the quality of the extract.
- Specialized equipment capable of withstanding high pressures is required for SWE, which can be expensive to acquire and maintain.

4.6 Solid-Phase Micro Extraction (SPME): It is a non-invasive extraction process that involves using a coated fiber to adsorb volatile and semi-volatile compounds from the headspace of the mushroom sample. The absorbed compounds are then desorbed and analysed using analytical instruments.

Advantages
- SPME allows for the extraction and concentration of analytes from complex matrices, such as medicinal mushroom extracts, enhancing detection sensitivity.
- It simplifies sample preparation by combining sampling, extraction, and sample introduction into a single step, reducing time and labor.
- It can be used for a wide range of analytes, including volatile and semi-volatile compounds, making it suitable for various medicinal mushroom components.
- It is a solvent-free technique or requires minimal solvent usage, reducing environmental impact and minimizing solvent-related issues such as contamination.
- It reduces matrix effects by enhancing the accuracy and precision of analytical results for medicinal mushroom samples.

Limitations
- It may have limited capacity to extract high molecular weight compounds or analytes present in low concentrations in medicinal mushroom samples.
- It may lack selectivity for certain analytes, leading to co-extraction of interfering compounds and potential challenges in data interpretation.
- The results can be influenced by sample variability, such as differences in mushroom species, growth conditions, and sample preparation methods, which may affect

method robustness.
- It requires specialized equipment (e.g., SPME fibers, holder, and injector) which may not be readily available in all laboratories.

4.7 Pressurised liquid extraction (PLE): This technique accelerates the process and uses elevated temperatures and pressures with suitable solvents to enhance extraction efficiency. Benefits of pressure-assisted extraction techniques for mushrooms may include higher extraction yields, reduced extraction times, and better preservation of thermally sensitive compounds compared to traditional extraction methods.

Advantages
- PLE can extract a wide range of compounds efficiently in a relatively short time compared to traditional methods.
- It often yields higher extraction efficiencies compared to other methods due to the enhanced solubility of compounds at high pressure and temperature.
- PLE can be automated, reducing the need for manual labor and ensuring consistent results.
- It typically requires less solvent compared to other methods, making it more environmentally friendly.
- Parameters like pressure, temperature, and solvent can be adjusted to selectively extract specific compounds.

Limitations
- PLE equipment can be expensive to purchase and maintain.
- Operating PLE equipment requires expertise, and optimizing extraction conditions can be complex.
- High pressure and temperature can sometimes lead to degradation of heat-sensitive compounds.
- Handling high-pressure systems can pose safety risks if not managed properly.

Table 1: Comparison of extraction techniques

S.No	Extraction Method	Time Consumed	Cost	Expertise required	Equipment availability	Effectiveness
1	Water Extraction	Short	Low	Low	High	Moderate
2	Hot Water Extraction	Short	Moderate	Low	High	Moderate
3	Ethanol Extraction	Moderate	Moderate	Moderate	High	High
4	Methanol Extraction	Moderate	Moderate	Moderate	Moderate	High
5	Supercritical Fluid Extraction	Moderate	High	High	Moderate	High
6	Microwave Assisted Extraction	Short	High	Moderate	Low to Moderate	High
7	Ultrasound Assisted Extraction	Short	Moderate	Low to Moderate	Low to Moderate	High
8	Solid Phase Microextraction	Short	High	High	Low	High
9	Pressurized Liquid Extraction	Moderate	High	High	Moderate	High
10	Enzyme Assisted Extraction	Moderate	Moderate	Moderate	Low to Moderate	High

Table 2: Some bioactive compounds extracted with their bioactivities

S. No.	Mushroom name	Method used	Bioactive compound extracted	Bioactivities	Ref.
1.	*Ganoderma lucidum*	Water extraction	Polysaccharides	Antioxidant activity	[42]
2.	*Flammulina velutipes*	Hot water extraction	Polysaccharides	Antitumor activity	[43]
3.	*Lentinus edodes*	Hot water extraction	Lentinan	Antibacterial activity	[44]
4.	*Schizophyllum commune*	Absolute ethanol precipitation	Exopolysaccharide	Anti-inflammatory activity	[45]
5.	*Xylaria nigripes*	Gradient ethanol precipitation	Polysaccharide	Antioxidant activity	[46]
6.	*Pleurotus eryngii*	Extracted with methanol	p-Hydroxybenzoic and cinnamic acid	Anti-inflammatory Cytotoxic Antioxidant	[47]
7.	*Pleurotus ostreatoroseus*	Extracted with ethanol	Cinnamic acid	Antioxidant Anti-inflammatory Antimicrobial Hepatotoxicity	[48]
8.	*Coprinus comatus*	Microwave-assisted extraction	Polysaccharides	Antioxidant activity	[49]
9.	*Hericium erinaceum*	Ultrasound-assisted extraction	12b-hydroxyverruculogen TR-2, fumitremorgin C and methylthiogliotoxin, two hetero-spirocyclic glactam alkaloids, pseurotin A and FD-838 and cerevisterol and herierin IV	Antioxidant and antifungal activity	[50]
10.	*Agaricus bisporus*	Ultrasound-assisted extraction	Beta-glucan		[51]
11.	*Antrodia cinnamomea*	Superficial fluid extraction (SFE)	Antrodan	Anti-inflammatory activity	[52]
12.	*Pleurotus sajor-caju*	SFE	Phenolic acids: -3.4 dihydroxybenzoic acid -Chlorogenic acid -4-hydroxymethyl benzoic acid Flavonoids: -Myricetin Phenolic Aldehydes		[53]
13.	*Pleurotus sajor-caju*	Pressurised liquid extraction (PLE)	Phenolic acids: -Cinnamic acid -p-Coumaric acid Flavonoids: -Kaempferol		[53]

5. Conclusion

A spectrum of extraction methodologies presents varied advantages encompassing time efficiency, cost-effectiveness, requisite expertise, equipment accessibility, and efficacy in isolating bioactive constituents from mushrooms. Each method has its advantages and disadvantages, and researchers and industries must carefully select the appropriate technique for their specific requirements. By employing these extraction techniques effectively, we can harness the full potential of medicinal mushrooms and their bioactive compounds for various therapeutic applications. Water extraction, hot water extraction, and ethanol extraction represent prevalent techniques, yielding a plethora of compounds such as polysaccharides, glycoproteins, phenolic compounds, polyphenols, flavonoids, and triterpenoids. Methanol extraction parallels these methods, potentially enhancing efficiency in extracting similar bioactive compounds. Supercritical fluid extraction distinguishes itself by its capacity to retrieve a diverse array of compounds, including terpenes, fatty acids, and pigments. Microwave-assisted extraction and ultrasound-assisted extraction expedite extraction kinetics and enhance efficiency, facilitating the retrieval of a broad spectrum of bioactive compounds. Solid phase micro extraction targets volatile and aromatic compounds, while pressurized liquid extraction demonstrates versatility in yielding compounds contingent upon the chosen solvent. Enzyme-assisted extraction augments the selective extraction of specific compounds, notably polysaccharides. Each technique offers distinct attributes tailored to diverse extraction requirements and bioactive compound preferences.

6. References

1. Beulah H, Margret AA, Nelson J. Marvelous Medicinal Mushrooms. Int J Pharma Bio Sci. 2013;3:611-615.
2. Hyde KD. The numbers of fungi. Fungal Divers. 2022;114:1.
3. Song T, Zhang Z, Liu S, Chen J, Cai W. Effect of Cultured Substrates on the Chemical Composition and Biological Activities of Lingzhi or Reishi Medicinal Mushroom, *Ganoderma lucidum* (Agaricomycetes). Int J Med Mushrooms. 2020;22:1183-1190.
4. Elkhateeb WA, Daba GM. Medicinal mushroom: What should we know? Int J Pharm Chem Anal. 2022;9:1-9.
5. Kaushik V, Arya A, Sindhu A, Singh A. Identification, Optimization of Culture Conditions and Bioactive Potential of Chinese Caterpillar Mushroom *Ophiocordyceps sinensis* (Ascomycetes) Mycelium Isolated from Fruiting Body. Int J Med Mushrooms, 2019, 21. 10.1615/IntJMedMushrooms.2019031841.
6. Guillamón S, García-Lafuente A, Lozano M, *et al*. Edible mushrooms: Role in the prevention of cardiovascular diseases. Fitoterapia. 2010;81(7):715-723. https://doi.org/10.1016/j.fitote.2010.06.005.
7. Firenzuoli F, Gori L, Lombardo G. The medicinal

mushroom *Agaricus blazei* Murrill: review of literature and pharmaco-toxicological problems. Evid Based Complement Alternat Med. 2007;5(1):3-15. https://doi.org/10.1093/ecam/nem007.
8. Bernardshaw S, Johnson E, Hetland G. An extract of the mushroom *Agaricus blazei* Murill administered orally protects against systemic *Streptococcus pneumoniae* infection in mice. Scand J Immunol. 2005;62(4):393-398. https://doi.org/10.1111/j.1365-3083.2005.01667.x.
9. Lavi I, Nimri L, Levinson D, *et al*. Glucans from the edible mushroom *Pleurotus pulmonarius* inhibit colitis-associated colon carcinogenesis in mice. J Gastroenterol. 2012;47(5):504-518. https://doi.org/10.1007/s00535-011-0514-7.
10. Moro C, Palacios I, Lozano M, *et al*. Anti-inflammatory activity of methanolic extracts from edible mushrooms in LPS activated RAW 264.7 macrophages. Food Chem. 2012;130(2):350-355. https://doi.org/10.1016/j.foodchem.2011.07.049.
11. Ahmad MF, Hassan AU, Abdullah N, *et al*. *Ganoderma lucidum*: Novel Insight into Hepatoprotective Potential with Mechanisms of Action. Nutrients. 2023;15(8):1874. https://doi.org/10.3390/nu15081874
12. Wasser SP. Medicinal mushroom as a source of antitumor and immunomodulating polysaccharides. Appl Microbiol Biotechnol. 2002;60:258-274.
13. Bao X, Liu C, Fang J, *et al*. Structural and immunological studies of a major polysaccharide from spores of *Ganoderma lucidum* (Fr.) Karst. Carbohydr Res. 2001;332:67-74.
14. Rathee S, Rathee D, Rathee D, *et al*. Mushrooms as therapeutic agents. Braz J Pharmacog. 2012;22(2):459-474.
15. Tong H, Xia F, Feng K, *et al*. Structural characterization and *in vitro* antitumor activity of a novel polysaccharide isolated from the fruiting bodies of *Pleurotus ostreatus*. Bioresour Technol. 2009;100:1682-1686. https://doi.org/10.1016/j.biortech.2008.09.004.
16. Holliday J. Cordyceps. In: Coates PM, ed. Encyclopaedia of Dietary Supplements. Marcel Dekker, 2005, 4.
17. Chan GC, Chan WK, Sze DM. The effects of β-glucan on human immune and cancer cells. J Hematol Oncol. 2009;2:25-35. https://doi.org/10.1186/1756-8722-2-25.
18. Legentil L, Paris F, Ballet C, *et al*. Molecular interactions of β (1! 3)-glucans with their receptors. Molecules. 2015;20(6):9745-9766. https://doi.org/10.3390/molecules20069745.
19. Akihisa T, Tagata M, Ukiya M, *et al*. Oxygenated lanostane-type Triterpenoids from the fungus *Ganoderma lucidum*. J Nat Prod. 2005;68(4):559-563. https://doi.org/10.1021/np040230h.
20. Rathee S, Rathee D, Rathee D, *et al*. Mushrooms as therapeutic agents. Braz J Pharmacog. 2012;22(2):459-474.
21. Morigiwa A, Kitabatake K, Fujimoto Y, *et al*. Angiotensin converting enzyme inhibitory triterpenes from *Ganoderma lucidum*. Chem Pharm Bull. 1986;34:3025-3028.
22. Chen CC, Shiao YJ, Lin RD, *et al*. Neuroprotective diterpenes from the fruiting body of Antrodia Camphorata. J Nat Prod. 2006;69:689-691.
23. Tripkovic L, *et al*. Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. Am J Clin Nutr. 2012;95(6):1357-1364.
24. Barros PP, *et al*. Isolation, purification and anti-inflammatory activities of fungal metabolites. Molecules. 2019;24(7):1309.
25. Chu KT, Xia LX, Ng TB. Pleurostrin, an antifungal peptide from the oyster mushroom. Peptides. 2005;26(11):2098-2103.
26. Ngai PH, Zhao Z, Ng TB. Agrocybin, an antifungal peptide from the edible mushroom *Agrocybe cylindracea*. Peptides. 2005;26(2):191-196. https://doi.org/10.1016/j.peptides.2004.09.011.
27. Wang JB, Wang HX, Ng TB. A peptide with HIV-1 reverse transcriptase inhibitory activity from the medicinal mushroom *Russula paludosa*. Peptides. 2007;28(3):560-565. https://doi.org/10.1016/j.Peptides.2006.10.004.
28. Qian GM, Pan GF, Guo JY. Anti-inflammatory and antinociceptive effects of cordymin, a peptide purified from the medicinal mushroom *Cordyceps sinensis.* Nat Prod Res. 2011;26(24):2358-2362. https://doi.Org/10.1080/14786419.2012.658800.
29. Wang J, Liu YM, Cao W, *et al*. Anti-inflammatory and antioxidant effect of cordymin, a peptide purified from the medicinal mushroom *Cordyceps sinensis*, in middle cerebral artery occlusion-induced focal cerebral ischemia in rats. Metab Brain Dis. 2012;27(2):159-165. https://doi.org/10.1007/S11011-012-9282-1.
30. Wang ZM, Peng X, Lee KL, *et al*. Structural characterisation and immunomodulatory property of an acidic polysaccharide from mycelial culture of Cordyceps sinensis fungus Cs-HK1. Food Chemistry. 2011;125(2):637-643.
31. Wong JH, Wang HX, Ng TB. Marmorin, a new ribosome inactivating protein with antiproliferative and HIV-1 reverse transcriptase inhibitory activities from the mushroom Hypsizygus Marmoreus. Appl Microbiol Biotechnol. 2008;81(4):669-674.
32. Cote J, Caillet S, Doyon G. Bioactive compounds in cranberries and their biological properties. Crit Rev Food Sci Nutr. 2010;50(7):666-679. https://doi.org/10.1080/10408390903044107.
33. D'Archivio M, Filesi C, Vari R, *et al*. Bioavailability of the polyphenols: Status and controversies. Int J Mol Sci. 2010;11:1321-1342. https://doi.org/10.3390/ijms11041321.
34. Palacios I, Lozano M, Moro C, *et al*. Antioxidant properties of phenolic compounds occurring in edible mushrooms. Food Chem. 2011;128(3):674-678. https://doi.org/10.1016/j.foodchem.2011.03.085.
35. Dugler B, Gonuz A, Gucin F. Antimicrobial activity of the macrofungus *Cantharellus cibarius*. J Biol Sci. 2004;7(9):1535-1539.
36. Holliday J. Cordyceps. In: Coates PM, ed. Encyclopaedia of Dietary Supplements. Marcel Dekker, 2005, 4.
37. Witkowska MA, Zujko ME, Mironczuk-Chodakowska I. Comparative study of wild edible mushrooms as sources of antioxidants. Int J Med Mushrooms. 2011;13(4):335-341. https://doi.org/10.1615/IntJMedMushr. V13.i4.30.
38. Han C, Cui B. Pharmacological and pharmacokinetic studies with agario glycerides, extracted from Grifolafrondosa, in animal models of pain and inflammation. Inflammation. 2012;35(4):1269-1275.

https://doi.org/10.1007/S10753-012-9438-5.
39. Nagai K, Chiba A, Nishino T, *et al*. Dilinoleoyl Phosphatidylethanolamine from *Hericium erinaceus* protects against ER Stress-dependent neuro-2a cell death viaprotein kinase C pathway. J Nutr Biochem. 2006;17:525-530. https://doi.org/10.1016/j.jnutbio.2005.09.007.
40. Choi JH, Maeda K, Nagai K, *et al*. Termitomycamides A to E, fatty acid amides isolated from the mushroom *Termitomyces titanicus*, suppress endoplasmic reticulum stress. Org Lett. 2010;12(21):5012-5015. https://doi.Org/10.1021/ol102186p.
41. Kawagishi H, Ishiyama D, Mori H, *et al*. Dictyophorines A and B, two stimulators of NGF-synthesis from the mushroom Dictyophora Indusiata. Phytochemistry. 1997;45(6):1203-1205. https://doi.org/10.1016/S0031-9422(97)00144-1.
42. Zhang J, Meng G, Zhai G, Yang Y, Zhao H, Jia L. Extraction, characterization and antioxidant activity of polysaccharides of spent mushroom compost of *Ganoderma lucidum*. Int J Biol Macromol. 2016;82:432-439. https://doi.org/10.1016/J.IJBIOMAC.2015.10.016.
43. Pang X, Yao W, Yang X, *et al*. Purification, characterization and biological activity on hepatocytes of a polysaccharide from *Flammulina velutipes* Mycelium. Carbohydr Polym. 2007;70:291-297. https://doi.org/10.1016/J.CARBPOL.2007.04.010.
44. Zhu H, Sheng K, Yan E, Qiao J, Lv F. Extraction, purification and antibacterial activities of a polysaccharide from spent mushroom substrate. Int J Biol Macromol. 2012;50:840-843. https://doi.org/10.1016/J.IJBIOMAC.2011.11.016.
45. Du B, Yang Y, Bian Z, Xu B. Characterization and Anti-Inflammatory Potential of an Exopolysaccharide from Submerged Mycelial Culture of *Schizophyllum* commune. Front Pharmacol. 2017;8:252. https://doi.org/10.3389/fphar.2017.00252.
46. Chang CK, Ho WJ, Chang SL, *et al*. Fractionation, characterization and antioxidant activity of exopolysaccharide from fermentation broth of a *Xylaria nigripes*. Bioact Carbohydr Diet Fibre. 2018;16:37-42. https://doi.org/10.1016/J.BCDF.2018.02.005.
47. Souilem F, Fernandes Â, Calhelha RC, *et al*. Wild mushrooms and their mycelia as sources of bioactive compounds: Antioxidant, anti-inflammatory and cytotoxic properties. Food Chem. 2017;230:40-48. https://doi.org/10.1016/J.FOODCHEM.2017.03.026.
48. Corrêa RCG, Souza AHP de, Calhelha RC, *et al*. Bioactive formulations prepared from fruiting bodies and submerged culture mycelia of the Brazilian edible mushroom *Pleurotus ostreatoroseus* Singer. Food Funct. 2015;6:2155-2164. https://doi.org/10.1039/c5fo00465a.
49. Yu J, Cui PJ, Zeng WL, *et al*. Protective effect of selenium-polysaccharides from the mycelia of *Coprinus comatus* on alloxan-induced oxidative stress in mice. Food Chem. 2009;117:42-47. https://doi.org/10.1016/J.FOODCHEM.2009.03.073.
50. Lu QQ, Tian JM, Wei J, Gao JM. Bioactive metabolites from the mycelia of the basidiomycete *Hericium erinaceum*. Nat Prod Res. 2014;28:1288-1292. https://doi.org/10.1080/14786419.2014.898145.
51. Aguiló-Aguayo I, Walton J, Viñas I, Tiwari BK. Ultrasound assisted extraction of polysaccharides from mushroom by-products. LWT. 2017;77:92-99. https://doi.org/10.1016/J.LWT.2016.11.043.
52. Chiu CH, Peng CC, Ker YB, *et al*. Physicochemical Characteristics and Anti-Inflammatory Activities of Antrodan, a Novel Glycoprotein Isolated from *Antrodia cinnamomea* Mycelia. Mol. 2014;19:22-40. https://doi.org/10.3390/molecules19010022.
53. Krüger A. Cordyceps Chapter. In Marcel Dekker; c2005. p. 4.