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A comparative study on changes in the chemical profile of glucans of the Reishi mushroom, *Ganoderma lucidum*, in response to different methods of extraction

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ABSTRACT

The beneficial biological effects of Reishi mushroom, *Ganoderma lucidum*, are primarily due to its polysaccharides containing β-glucans. However, the rigid chemical structure of fruiting bodies in *G. lucidum* makes the extraction of polysaccharides more difficult compared to other edible mushrooms. There are a number of studies reporting various methods for the extraction of β-glucans from *G. lucidum* fruiting bodies. However, there are very limited data on the evaluation of the effect of the extraction method on the chemical profile of β-glucans. The present study sought to evaluate 15 different basic chemical and analytical techniques, including ten conventional methods, three optimized conventional methods, and two advanced methods (including enzymatic and supercritical CO_2 methods) for extracting polysaccharides from *G. lucidum* fruiting bodies. The use of the conventional methods resulted in low amounts of glucans and extraction efficiency. Among the optimized conventional methods, the alkaline extraction method produced a high amount of β-glucan (9.30% dw) and a high extraction efficiency of approximately 11% dw ($p \leq 0.05$). The use of β-glucanase led to the highest total glucan (16.3% dw) and β-glucan (14.73% dw) contents ($p \leq 0.05$), with 10.98% dw extraction efficiency. The highest extraction efficiency (14.12% dw) was also obtained from the supercritical CO_2 method ($p \leq 0.05$) with 11.31% dw β-glucan. In conclusion, the present study showed that the most effective methods for extracting β-glucans from *G. lucidum* fruiting bodies include supercritical CO_2 extraction, enzymatic, and alkaline methods. These findings may provide tools for strategic decision-making regarding the choice of extraction method.

1. Introduction

Ganoderma lucidum (Curtis.) P. Karst, commonly known as Reishi, is one of the most globally known species of medicinal mushrooms and contains approximately 400 known bioactive compounds [1]. The beneficial biological effects of Reishi are mostly attributed to its polysaccharides, particularly β-glucans, which are among the key components of the fungal cell wall and play key roles in immune system regulation and have antioxidant, antimicrobial, antiaging, antiviral, anticancer, anti-inflammatory, and cholesterol-reducing activities [2]. However, the methods of extracting β-glucans from the fruiting bodies of the Reishi mushroom are not as simple as those of other edible mushrooms [3] because it has a rigid and woody cell wall structure [4]. Hence, the extraction efficiency of β-glucans largely depends on both the polysaccharide extraction efficiency and the amount of β-glucans present in the polysaccharide extract.

There are a number of studies reporting various methods for extracting polysaccharides containing β-glucans from *G. lucidum* fruiting bodies [5-7]. However, a research gap exists because each study has tested only a limited number of pre-selected extraction methods and

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has not provided further data on cost-effectiveness. Furthermore, few of these studies have mentioned the efficiency of the extraction methods, without which the final results may be misleading with regard to the selection of the optimal method. Therefore, based on the current knowledge, still it is difficult to provide an unbiased tool for selecting the optimal method for extracting polysaccharides from the fruiting bodies of *G. lucidum*.

Based on this research gap, the present study aimed to evaluate the productivity of 15 different extraction methods, including 10 basic chemical methods, three basic chemical methods optimized in the present study, and two advanced methods (i.e., enzymatic and supercritical CO_2 methods). To obtain more applicable results, the effects of these methods on the chemical profile of β-glucans were compared in terms of extraction efficiency and the amount of glucans in the extract

2. Materials & Methods

2.1 Solid-state fermentation for the production of mushrooms

Fruiting bodies (fruiting bodies) of a commercial strain of Reishi, *Ganoderma lucidum* (strain no. M9720; Mycelia Company, Deinze, Belgium), were produced in the mushroom laboratory of the Industrial Fungi Biotechnology Research Department, Academic Center for Education, Culture and Research (ACECR)-Mashhad, Iran. Freshly prepared pure mycelia of *G. lucidum* were made on solid media composed of 20 g of malt extract (Merck), 2 g of yeast extract (Merck), and 15 g of agar (Merck) topped with distilled water to 1000 mL [8]. The inoculated petri dishes were then incubated at 25±2°C in the dark for two weeks [8]. The pure mycelia were inoculated into a mixture of boiled wheat grains supplemented with 0.2% w/v $CaCO_3$ and 2% w/v $CaSO_4$, which was subsequently used to colonize a solid lignocellulosic substrate consisting of 82% dw sawdust (from white poplar trees, *Populus alba*, in northern Iran), 15% dw wheat bran (from an Iranian commercial wheat cultivar), and 3% dw gypsum [9]. Following vegetative growth of mycelia at 23°C for 18 days, the bags were placed at 16–18°C with a relative humidity of 80–90% to stimulate primordia formation. The carbon dioxide concentration was set at less than 2000 ppm. During cropping, light of 1000 lux was provided. The formation of fruiting bodies lasted for 3–4 flushes with intervals of 20–23 days. Fresh fruiting bodies were harvested and lyophilized (0.08 minibars, 48 h) as previously reported [10] until a fine and uniform powder with a particle size of 18 mesh was obtained via an 80-mesh abrasive mill.

2.2 Extraction of polysaccharides

At first step, 10 basic chemical methods for extraction of polysaccharides from *G. lucidum* fruiting bodies were used with slight modifications. These methods included hot water extraction [11], hot water extraction method followed by 30%, 70%, and 80% (v/v) ethanol precipitation [11,12], hot water extraction followed by autoclaving [12] and twice autoclaving [13], soxhlet extraction [14], pressurized ethanolic (1:1 ratio) extraction [15] and ethanolic (70:30 ratio) extraction [16], ethanol-pretreatment combined with the hot water extraction (95: 5 v/v) during 24 h [17].

Furthermore, three other methods (methods # 11-13) were developed on the basis of improvements in the basic chemical methods. Method #11 was developed on the basis of hot water extraction with ethanol pretreatment [17]. First, 100 g of dry powder from a Reishi mushroom was pretreated with 80% ethanol for 4 h (repeated 2 times) and then dried at 40°C. The resulting powder was resuspended in 100 mL of deionized water and placed in a hot water bath at 95°C for 2 h (repeated 2 times). The mixture was homogenized at intervals of 5 min using an ultraturrax (ultraturrax t25 basic ika Labortechnik) with a power of 2200 watts. Method #12 was a modified method of water–ethanol (1:1 ratio) extraction under high-pressure steaming [15]. To 100 g of dried powder of the Reishi mushroom, 40 mL of 50% ethanol was added. The extract was placed in a hot water bath at 80°C for 4 h (repeated 2 times) supplemented with ultrasonic waves (5 min on/5 min off) to homogenize the extract. After the extract was concentrated at 60°C via rotary rotation, it was centrifuged at 8000 rpm for 20 min, and the luminescent liquid after separation was dried at room temperature. This dry material was used for glucan measurement. Method # 13 was an alkaline extraction method [18], which was improved in the present study. First, 100 mL of 5% sodium hydroxide solution was added to 100 g of dried powder from the Reishi mushroom, which was subsequently placed in an ultrasonic oven. After 20 min of sonication, the alkaline extract was centrifuged at 8000 rpm for 20 min. After the alkaline pH of the extract was neutralized, the extract was concentrated with a heater at 60°C and then allowed to dry at room temperature.

Finally, two advanced extraction methods (methods # 14-15) were used, including the enzymatic [12] and supercritical CO_2 methods [19], with slight modifications. The enzymatic method was improved

with β-glucanase in the present study. First, 400 ml of distilled water was added to 20 g of dry powder of the Reishi mushroom with a particle size of 18 mesh, and the sample was placed in an autoclave (121°C, 15 min). Then, β-glucanase (1 g/100 ml) was added to the sample for enzymatic hydrolysis, and the sample was placed in a shaker (50°C, 120 revolutions) for 4 h. After pH adjustment, the hydrolyzed samples were placed in a hot water bath (90°C, 6 h), and the resulting extract was dried at room temperature. The supercritical CO_2 method was initiated with 200 g of dried powder of Reishi mushroom with a particle size of 18 mesh in a supercritical CO_2 device (Agilent Technologies 6890 N) under the following conditions: pressure of 85 bar, CO_2 flow rate of 4–10x6 m3/min and moderator flow rate of 0.15–0.5 ml/min to prevent the destruction of temperature-sensitive materials and a temperature of 50°C for 4 h. The resulting extract was dried at 60°C.

For all the aforementioned methods, the extraction efficiency was determined via the following formula:

The extraction efficiency percentage was calculated as the weight of the dried polysaccharide extract/weight of the dried fruiting bodies × 100.

2.3 Chemical analysis of β-glucan

The quantification of β-glucan present in the lyophilized extract was performed at 510 nm via a spectrophotometer based on the manufacturer's instructions for a special mushroom and yeast β-glucan assay kit (cat. no. K-YBGL, Megazyme International, Wicklow, Ireland) [10].

2.4 Statistical analysis

The data were subjected to one-way ANOVA via SPSS software version 25. The mean values were compared via Duncan's multiple range test and were reported as the means ± standard deviations. A probability of $p \leq 0.05$ was considered to indicate statistical significance. Graph prism version 9 was used to draw the graphs

3. Results

3.1 Fungal substance production

The findings revealed that the pure mycelia of *G. lucidum* completely covered the surface of the solid culture within 10 days, while spawn production lasted approximately one month. The growth quality of the mycelia in each plate and spawn had a filamentous state, which indicated the high quality of the mycelia. The total production time of *G. lucidum* fruiting bodies was approximately 120–150 days. The final yield of dry powder from *G. lucidum* fruiting bodies was 5% w/w.

3.2 Extraction efficiency and glucan contents

In the first step, 10 conventional methods for isolating polysaccharide extracts from *G. lucidum* fruiting bodies were used. As presented in Table 1, methods # 1--8 resulted in very low extraction efficiencies or glucan contents. These eight methods mostly included hot water extraction without any physical intervention or modification. Methods #9--10 included maceration with 70% ethanol and hot water extraction with 95% ethanol pretreatment, which resulted in 3.43--4.54% dw β-glucan and 9.4--9.7% dw extraction efficiency (Table 1). The amount of α-glucan obtained from methods # 1--10 was less than 1% dw.

The modified methods (methods # 11--13) generated 4.75--9.30% dw β-glucan and 11% dw extraction efficiency (Table 1). Among the modified methods, the highest content of glucans and extraction efficiency were obtained with the alkaline extraction method (method # 13), with an extraction efficiency of 11% dw and a β-glucan content of 9.30% dw. This method also generated the highest amount of α-glucan (1.67% dw) ($p \leq 0.05$). Following the alkaline extraction method, improved water–ethanol (1:1 ratio) extraction via the high-pressure steaming method generated 6.39% dw total glucan and 5.44% dw β-glucan, which were significantly different from those of methods # 1--11 ($p \leq 0.05$).

With respect to the two advanced methods of extraction used in this study, the highest extraction efficiency was observed with the supercritical CO_2 method (14.12% dw), which was significantly greater than those of the other methods ($p \leq 0.05$). Additionally, the highest amount of β-glucan (14.73% dw) was obtained from the enzymatic method (method # 14), which was significantly greater than that of the other methods ($p \leq 0.05$). The highest level of α-glucan (1.84% dw) was also obtained via the supercritical CO_2 method (Table 1).

3.3 Correlation between the extraction efficiency and β-glucan content

In accordance with the findings presented in Table 1, a linear relationship between the efficiency of extraction and the β-glucan content was determined via Pearson's correlation coefficient across the 15 tested methods (Fig. 1). The statistical results revealed an overall correlation of r = + 0.61, which was significant at a *p* value less than 0.05, even though the overall R-

squared value was low (0.38) in the regression model. As presented in Fig. 1, the lack of a strong correlation between the extraction efficiency and the amount of β-glucan was related mainly to fluctuations in the β-glucan content rather than the efficiency of extraction under different extraction methods. The most obvious fluctuations in the amount of β-glucan were observed for the modified conventional extraction methods (methods # 11--13) and the advanced extraction methods (methods # 14--15), whereas a relatively steady trend in the amount of β-glucan was observed for the conventional extraction methods, particularly methods # 1--8 (Fig. 1).

Table 1. Investigating the effects of various conventional, improved conventional, and advanced extraction methods on glucan content and extraction efficiency (n=3)

Extraction method	Total glucan (% dw)	B-glucan (% dw)	α-glucan (% dw)	Extraction efficiency (% dw)
Method # 1	2.0±95.54^{f}	2.09±0.54^{g}	0.86±0.02^{cd}	9.5±0.54^{d}
Method # 2	2.33±0.09^{f}	0.73±0.09^{h}	0.89±0.11^{cd}	9.40±0.09^{d}
Method # 3	1.66±0.17^{f}	1.48±0.17 ^{g}	0.77±0.01^{cd}	9.7±0.17^{d}
Method # 4	2.91±0.26^{f}	1.39±0.43 ^{g}	0.70±0.07^{d}	9.5±0.43^{d}
Method # 5	1.62±0.06^{f}	1.73±0.73^{g}	0.44±0.19^{e}	9.7±0.73^{d}
Method # 6	2.56±0.03^{f}	1.56±0.54^{g}	0.10±0.02^{f}	9.75±0.54^{d}
Method # 7	2.33±0.03^{f}	1.45±0.09^{g}	0.88±0.04 ^{cd}	9.7±0.09^{d}
Method # 8	2.46±0.03 ^{f}	1.48±0.17 ^{g}	0.05^{d}±0.78	9.5±0.17^{d}
Method # 9	5.30±0.06^{e}	4.54±0.26^{e}	0.75±0.06^{d}	9.4±0.26^{d}
Method # 10	5.30±0.03^{e}	3.43±0.27 ^{f}	0.82±0.07^{cd}	9.7±0.27^{d}
Method # 11	5.14±0.81^{e}	4.75±0.78 ^{e}	0.39±0.03^{e}	11±1.63^{b}
Method # 12	6.39±0.62^{d}	5.44±0.64^{d}	0.94±0.03^{c}	11±1.01^{b}
Method # 13	10.97±0.73 ^{c}	9.30±0.61^{c}	1.67±0.20^{b}	11±0.6^{b}
Method # 14	16.30±0.2^{a}	14.73±0.27^{a}	1.57±0.21^{b}	10.98±1.36^{c}
Method # 15	13.15±0.1^{b}	11.31±0.15^{b}	1.84±0.31^{a}	14.12±1.53^{a}

Values are expressed as average ± standard deviation. Lowercase letters above each number in each column represent the statistical groups obtained via Duncan's test ($p \leq 0.05$). Methods # 1, simple hot water extraction; Method # 2, hot water extraction with 30% ethanol precipitation; Method 3, hot water extraction with 70% ethanol precipitation; Method # 4, hot water extraction with 80% ethanol precipitation; Method # 5, hot water extraction under high-pressure steaming; Method # 6, hot water extraction with two autoclaving; Method # 7, Soxhlet extraction; Method # 8, water–ethanol (1:1 ratio) extraction under high-pressure steaming; Method # 9, maceration with 70% ethanol; Method # 10, hot water extraction with 95% ethanol pretreatment; Method # 11, improved hot water extraction with 80% ethanol pretreatment; Method # 12, improved water–ethanol (1:1 ratio) extraction under high-pressure steaming; Method # 13, alkaline extraction method; Method # 14, enzymatic method; Method # 15, supercritical CO_2 extraction method.

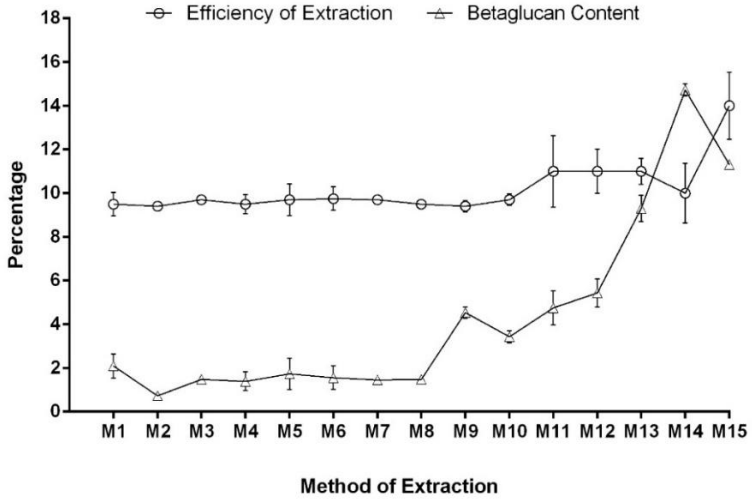

Fig. 1. Correlations between extraction efficiency (percentage, dw) and β-glucan content (percentage, dw) among 15 tested methods for the extraction of polysaccharides from *Ganoderma lucidum*. Values are expressed as average ± standard deviation. M1-M15 represent the methods explained in Tables 1 and 2.

4. Discussion

The present study evaluated how different methods of extraction affect the chemical profile of glucans in the fruiting bodies of *G. lucidum.* In this context, the biochemical composition of bioactive compounds may depend on growing conditions, environmental factors, different parts of the biological source (such as plants or mushrooms), and the extraction methods used [20]. The findings of the present study revealed that the use of the β-glucanase enzyme led to the highest levels of total glucan (16.3% dw) and β-glucan (14.73% dw) in the fruiting bodies of *G. lucidum*. In agreement with these findings, the use of protease enzymes increased the amount of β-glucan in the fruiting bodies of *G. lucidum* (48.69% w/w) [4]. Compared with the use of β-glucanase, the use of protease may have led to greater amounts of β-glucan in the extract of fruiting bodies of *G. lucidum*. However, it remains unclear whether the use of proteases might provide economic benefits due to the lack of information regarding the efficiency of extraction from fruiting bodies of *G. lucidium* via proteases, while the efficiency of extraction via the enzymatic method reported in the current study was 10.98% dw. In addition, several studies have reported the use of viscozyme (which is a combination of several enzymes, including β-glucanase and cellulose) for extracting β-glucans from fruiting bodies of *G. lucidum* [12,21-23]. In the present study, however, compared with the control, the applied viscozyme did not significantly increase the amount of β-glucan.

β-1,3-glucanases is an enzyme that typically deconstructs β-glucans. The ability of β-glucanase to hydrolyze β-glucans into small oligosaccharides has implications for industrial biotechnology [24]. Therefore, the high amount of β-glucan in the β-glucanase-treated *G. lucidum* extract accompanied by the high extraction efficiency reported in this study may be economically beneficial.

The findings of the present study revealed that the highest extraction efficiency (14.12% dw) of the extract of *G. lucidum* was achieved from supercritical CO_2 extraction, which contained 11.31% dw β-glucan. Similar to these findings, supercritical CO_2 extraction is the best way to produce both high amounts of β-glucan and high yields of extraction from fruiting bodies of *G. lucidum* [19].

Following the enzymatic and supercritical CO_2 extraction methods, the alkaline extraction method resulted in high amounts of total glucan and β-glucan and a high extraction efficiency, which was not significantly different from that of the enzymatic method ($p \geq 0.05$). Since these methods were developed on the basis of conventional chemical methods (Table 1), the conventional methods successfully improved the extraction of β-glucan from *G. lucidum* fruiting bodies. In line with these results, unmodified hot water extraction typically results in very low [11,25] or moderate [26] levels of extraction efficiency in *G. lucidum*. Although the extraction of polysaccharides from mushrooms via hot water is one of the most common extraction methods because of its relatively high performance, simple equipment and easy implementation during the extraction process [27], hot water has low efficiency and prolongs the extraction time in the case of mushrooms with woody and hard textures, such as the Reishi mushroom. Thus, hot water extraction assisted by different physical techniques, such as ultrasonic, ultrasonic, and microwave methods, as well as high pressure, temperature, and pH, is required to further destroy the mushroom cell wall. Accordingly, modification of basic extraction methods results in high amounts of total glucan and β-glucan and high extraction efficiency in *G. lucidum* [5,6,7,18,28,29].

Although there was a statistically significant correlation between the extraction efficiency and the β-glucan content, there were fluctuations among some of the extraction methods tested. This finding implies that a given method of extraction of polysaccharides from *G. lucidum* fruiting bodies might result in a high extraction efficiency and low β-glucan content or vice versa. Therefore, the ultimate choice of extraction method might depend on both the extraction efficiency and the β-glucan content. However, the cost of the selected method should also be considered. On the basis of the findings of the present study, the supercritical CO_2 extraction method, as an analytical technique, resulted in the highest productivity (the highest extraction efficiency and a high β-glucan content) among the methods. Supercritical carbon dioxide is a nonpolar solvent that is frequently used in its gas-like and liquid-like properties, low critical temperature and pressure, and it has the selectivity and potentiality to extract heat-sensitive compounds. Furthermore, low polarity compounds and small molecules are easily dissolved in SC-CO_2, but large molecules and polar compounds are extracted with the addition of a co-solvent to enhance the extraction yield, which can be ethanol, methanol, or water [30].

The enzymatic method ranked second with the highest β-glucan content and a high extraction efficiency. However, the cost of the enzymatic method is apparently lower than that of the supercritical CO_2 extraction method. The third productive method presented in this study was the modified alkaline method,

which is an inexpensive chemical method that can be used where supercritical CO_2 extraction or enzymatic methods cannot be performed. In conclusion, the data presented here could be an important tool to support strategic decisions regarding the selection of extraction methods for extracting β-glucans from the fruiting bodies of the Reishi mushroom, *G. lucidum*. Further studies are warranted to investigate effects of methods of extraction on the chemical structure of the glucan types.

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References

[1] K. Janardhanan, K. Ravikumar, and S. Karuppayil, Medicinal mushroom bioactives: potential sources for anticancer drug development, International Journal of Applied Pharmaceutics,12 (2020) 40-45.

[2] I. Mirończuk-Chodakowska, K. Kujawowicz, and A. M. Witkowska, Beta-glucans from fungi: Biological and health-promoting potential in the COVID-19 pandemic era, Nutrients, 13 (2021) 3960.

[3] F.R. Smiderle, D. Morales, A. Gil-Ramírez, L.I. de Jesus, B. Gilbert-López, M. Iacomini and C. Soler-Rivas, Evaluation of microwave-assisted and pressurized liquid extractions to obtain β-d-glucans from mushrooms. Carbohydr Polym, 156 (2017) 165–174.

[4] P. Vaithanomsat, N. Boonlum, W. Chaiyana, S. Tima, S. Anuchapreeda, C. Trakunjae, W. Apiwatanapiwat, P. Janchai, A. Boondaeng and H. Nimitkeatkai, Mushroom β-Glucan Recovered from Antler-Type Fruiting Body of Ganoderma lucidum by Enzymatic Process and Its Potential Biological Activities for Cosmeceutical Applications. Polymers (Basel), 14 (2022) 4202.

[5] C. Ma, M. Feng, X. Zhai, M. Hu, L. You, W. Luo and M. Zhao, Optimization for the extraction of polysaccharides from Ganoderma lucidum and their antioxidant and antiproliferative activities. J Taiwan Inst Chem Eng, 44 (2013) 886–894.

[6] S. Huang and Z. Ning, Extraction of polysaccharide from Ganoderma lucidum and its immune enhancement activity. Int J Biol Macromol, 47 (2010) 336–341.

[7] S. Zheng, W. Zhang, and S. Liu, Optimization of ultrasonic-assisted extraction of polysaccharides and triterpenoids from the medicinal mushroom Ganoderma lucidum and evaluation of their in vitro antioxidant capacities. PLoS One, 15 (2020) e0244749.

[8] M.A. Amoozegar, S.H. Nasr, S.S. Zabihi, S.H. Rezaeian, and K.H. Malekzadeh, A preliminary study on domestication of wild-growing medicinal mushrooms collected from Northern Iran. Curr Res Environ Appl Mycol J Fungal Biol, 8 (2018) 606–623.

[9] S. Rezaeian and H.R. Pourianfar, A comparative study on bioconversion of different agro wastes by wild and cultivated strains of Flammulina velutipes. Waste Biomass Valorization, 8 (2017) 2631–2642.

[10] S. Rezaeian, H.R. Pourianfar, and S.A. Dowom, Comparative assessment of β-glucan composition and structural characterization in wild and commercial Enoki mushrooms, Flammulina velutipes. Journal of Food Composition and Analysis, 120 (2023) 105286.

[11] Y.W. Chang and T.J. Lu, Molecular characterization of polysaccharides in hot-water extracts of Ganoderma lucidum fruiting bodies. J Food Drug Anal, 12 (2004) 59-67.

[12] I.W. Hwang, B.M. Kim, Y.C. Kim, S.H. Lee, and S.K. Chung, Improvement in β-glucan extraction from Ganoderma lucidum with high-pressure steaming and enzymatic pretreatment. Appl Biol Chem, 61 (2018) 235–242.

[13] Y.Liu, and G.Huang, Extraction and derivatization of active polysaccharidesJ. Enzyme Inhib. Med. Chem., 34(2019)1690-1696.

[14] I. Alzorqi, S. Sudheer, T.J. Lu, and S. Manickam, Ultrasonically extracted β-d-glucan from artificially cultivated mushroom, characteristic properties and antioxidant activity. Ultrason Sonochem, 35 (2017) 531–540.

[15] B. del M. Torres-Martínez, R.D. Vargas-Sánchez, F.J. Ibarra-Arias, E.V. Ibarra-Torres, G.R. Torrescano-Urrutia, and A. Sánchez-Escalante, Effect of extraction solvent on chemical composition, physicochemical and biological properties of edible mushrooms extracts. TIP. Revista especializada en ciencias químico-biológicas, 24 (2021) 1-10.

[16] E.E. Bach, E.M.B. Hi, A.M.C. Martins, P.A.M. Nascimento, and N.S.Y. Wadt, Hypoglicemic and Hypolipedimic Effects of Ganoderma lucidum in Streptozotocin-Induced Diabetic Rats. Medicines, 5 (2018) 78.

[17] S. Sudheer, I. Alzorqi, S. Manickam, and A. Ali, Bioactive compounds of the wonder medicinal mushroom 'Ganoderma lucidum. Bioactive Molecules in Food, (2019) 1863–1893.

[18] S.Q. Huang, J.W. Li, Z. Wang, H.X. Pan, J.X. Chen, and Z.X. Ning, Optimization of Alkaline Extraction of Polysaccharides from Ganoderma lucidum and Their Effect on Immune Function in Mice. Molecules, 15 (2010) 3694–3708.

[19] Ó. Benito-Román, E. Alonso, M.J. Cocero, and M. Goto, β-Glucan recovery from Ganoderma lucidum by means of pressurized hot water and supercritical CO2. Food and Bioproducts Processing, 98 (2016) 21–28.

[20] S. Majedi, A.O. Yassen, and S.Y. Issa, Assessing the combination of three plant species: Thyme (Thymus vulgaris), Damask Rose (Rosa damascena), and Stachys lavandulifolia vahl, to determine their synergistic effects on antimicrobial properties. Chemical Review and Letters, 7 (2024) 294–310.

[21] C.A.O. Jing, X.L. Wang, L.I.U. Gao-Qiang, and G.Y. ZHOU, Extraction of β-glucan from Ganoderma lingzhi using ethanol-enzyme system pretreatment combined with typical water extraction method. Mycosystema, 35 (2016) 104–113.

[22] D.T.Do, D.H. Lam, T. Nguyen, T.T. Phuong Mai, L.T.M. Phan, H.T. Vuong, D.V. Nguyen, N.T.T. Linh, M.N. Hoang, T.P. Mai and H.H. Nguyen, Utilization of Response Surface Methodology in Optimization of Polysaccharides Extraction from Vietnamese Red Ganoderma lucidum by Ultrasound-Assisted Enzymatic Method and Examination of Bioactivities of the Extract. The Scientific World Journal, 2021 (2021) 7594092.

[23] L.W. Listriyani, S. Hudiyono, R. Rudiyono, S. Zulaeha, and A. Wibisana, Optimization of enzyme-microwave assisted extraction, characterization and antioxidant activity of polysaccharide from Ganoderma lucidum. Jurnal Bioteknologi & Biosains Indonesia (JBBI), 10 (2023) 43–58.

[24] C. Caseiro, J.N.R. Dias, C.M.G. de Andrade Fontes, and P. Bule, From Cancer Therapy to Winemaking: The Molecular Structure and Applications of β-Glucans and β-1,3-Glucanases. Int J Mol Sci, 23 (2022) 3156.

[25] X. Pang, Z. Chen, X. Gao, W. Liu, M. Slavin, W. Yao and L.L. Yu, Potential of a novel polysaccharide preparation (GLPP) from Anhui-Grown Ganoderma lucidum in tumor treatment and immunostimulation. J Food Sci, 72 (2007) 435–442.

[26] Q. Yang, S. Wang, Y. Xie, J. Sun, and J. Wang, HPLC analysis of Ganoderma lucidum polysaccharides and its effect on antioxidant enzymes activity and Bax, Bcl-2 expression. Int J Biol Macromol, 46 (2010) 167–172.

[27] W. Udchumpisai and E. Bangyeekhun, Purification, structural characterization, and biological activity of polysaccharides from Lentinus velutinus. Mycobiology, 48 (2020) 51–57.

[28] J. Chen, J. Zhou, L. Zhang, Y. Nakamura, and T. Norisuye, Chemical Structure of the Water-Insoluble Polysaccharide Isolated from the Fruiting Body of Ganoderma lucidum. Polym J, 30 (1998) 838–842.

[29] H. Daksha, M. Glanish, O. Sneha, K. Arunkumar, M. Praveen, A Simple Strategy for Obtaining Bis-Indole Derivatives under Solvent Free Conditions. J. Chem. Lett, 5 (2024) 139-143

[30] P. Uwineza, P. Aimee, and A. Waśkiewicz, Recent advances in supercritical fluid extraction of natural bioactive compounds from natural plant materials. Molecules 25 (2020): 3847.