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Optimization of Hot-Water Extraction and Comparative Evaluation of Stirring Methods for Polysaccharide Recovery from Selected Edible Mushrooms

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Abstract

The excellent nutritional and bioactive features of mushroom-derived polysaccharides make it pertinent to opt for safer and more cost-effective extraction techniques that will improve yield, as extraction efficiency is strongly influenced by the choice of solvent and material agitation method. This study was conducted to investigate the effect of stirring mechanism (magnetic stirring, manual stirring in a water bath, and reciprocating orbital shaking water bath) on the hot-water extraction yield of polysaccharides from *Calocybe indica* (CCI-P), *Ganoderma lucidum* (GDL-P), and *Pleurotus djamor* (PRD-P) under optimized extraction parameters (solvent-to-material ratio, temperature, and time). The optimal conditions were first determined using magnetic stirring, and optimum polysaccharide yield was achieved at a duration of 8 hours, temperature of 90°C for the three mushrooms, and solvent-to-material ratio of 1:20 w/v, 1:30 w/v, and 1:50 w/v for CCI-P, GDL-P, and PRD-P, respectively. Applying these parameters, the yields were compared across the three stirring methods. Hot-water extraction with orbital shaking gave the highest yield for all the mushrooms, possibly due to homogenous temperature distribution and improved mass transfer resulting from the consistent agitation, with CCI-P having the highest mean value (23.06 ± 0.07%). The order of yield was orbital shaking > magnetic stirring > manual stirring, irrespective of the mushroom species. This research brings to the fore the important role that hydrodynamic conditions play during hot-water extraction and highlights orbital shaking as a more effective technique for maximizing polysaccharide yield. Practical insight for optimizing conventional extraction methods for bioactive components from edible fungi for functional foods and nutraceutical relevance is also provided in this research.

Keywords: edible fungi, magnetic stirring, mass transfer, mushroom polysaccharides, optimal extraction conditions, orbital shaking.

Introduction

Polysaccharides are biological macromolecules that are present in cell walls and membranes of plants, animals, bacteria, and fungi [1,2]. They are biopolymers consisting of long chains of monomeric units of carbohydrates connected by glycosidic bonds [3]. Polysaccharides are also found in the cell wall of edible mushrooms, and they contribute to the therapeutic characteristics of edible mushrooms [4]. It has been established

from several studies that mushroom polysaccharides are potent biological response modifiers as well as antidiabetic, hypocholesterolemic, anticancer, and antiviral agents [5]. These excellent characteristics of polysaccharides have led to an ever-increasing quest for their extraction and application in the food and drug industry.

Several extraction methods have been employed by various researchers. Extraction methods involving the use of hot water, and acid or alkali solutions as extraction solvents are the common conventional techniques employed in the recovery of polysaccharides from plant or mushroom matrix [6]. Some advanced enhanced extraction techniques, such as ultrasound-assisted (UAE), microwave-assisted (MAE), enzyme-assisted (EAE), and subcritical water extraction (SWE), have been recently applied in the extraction of polysaccharides in a bid to reduce the duration of processing and temperature levels, and enhance polysaccharide yields [7]. Of all these techniques, the hot water extraction technique, though having seemingly longer treatment time and high operating temperature, offers a safer, more cost-effective process, and simpler equipment requirements for the recovery of polysaccharides from mushrooms [8].

The extraction of polysaccharides from various sources is usually aimed at making them available and harnessing their functional and bioactive properties in the development of functional foods, drugs, and other culinary and industrial applications. This, therefore, highlights the need to establish optimum extraction conditions for maximized polysaccharide yield from the mushrooms. The yield of polysaccharides is not only dependent on the choice of extraction method, temperature of extraction, duration of extraction, but also on the hydrodynamic conditions of the extraction process. The impact of stirring methods has not been explored with respect to the extraction of polysaccharides from edible mushrooms and other food sources. Thus, this research is aimed at establishing optimum conditions for the extraction of polysaccharides from three edible mushrooms namely *Calocybe indica*, *Ganoderma lucidum*, and *Pleurotus djamor* in terms of temperature, raw material to solvent ratio, and time using hot water extraction (HWE) with magnetic stirring, and applying the optimum extraction parameters across three different stirring techniques: magnetic stirring, reciprocating orbital shaking, and manual stirring, to determine their impact on polysaccharide yield under uniform optimized conditions. We hope that this research will validate our proposed hypothesis that the reciprocating orbital shaking technique would have greater extraction efficiency based on its mechanism of operation.

Materials and Methods

Sample collection and preparation

Fresh fruiting bodies of the mushrooms: *Calocybe indica*, *Ganoderma lucidum*, and *Pleurotus*

djamor, were bought from three mushroom farms in Oshogbo and Ibadan. The identity of the mushrooms was confirmed by a botanist of the Department of Botany, Osun State University, and given Herbarium numbers: 0273, 0274, and 0275, respectively. The mushrooms were prepared as described by Ekute *et al.* [9] and stored in zip-lock polyethylene bags before pretreatment and polysaccharide extraction.

Sample pretreatment

The method described by Mfopa *et al.* [10] was followed in the pretreatment of the powdered mushroom samples. 2.0 g of the mushroom was macerated with 1:4 (w/v) n-hexane for 8 hours to remove fats. The sample was then filtered and air-dried to remove the solvent. It was then subjected to maceration in 95% ethanol (1:4 w/v) for 8 hours to remove simple sugars and pigments. The solvent was filtered off, and the residue, air-dried before the extraction process.

Polysaccharide extraction

The method described by Huang *et al.* [6] was followed in the extraction of the polysaccharides with some modifications. The extraction was conducted at varied temperatures of 75°C to 100°C at increments of 5°C, material-to-solvent ratios of 1:10 w/v, 1:20 w/v, 1:30 w/v, 1:40 w/v, 1:50 w/v, and 1:60 w/v, and extraction duration of 2, 4, 6, 8, 10, and 12 hours, respectively, for all the mushrooms. The first parameter of extraction evaluated was the varied material-to-solvent ratio at a constant temperature of 95°C and time of 8 hours (4 hours x 2). In establishing optimum extraction temperature, the optimum material-to-solvent ratio was used at a constant time of 8 hours. In establishing the optimum duration of extraction, the validated optimum material-to-solvent ratio and temperature were employed. This was done to establish optimum conditions of extraction for optimum polysaccharide yield from the three mushrooms under study.

In each case, the pretreated mushroom was extracted with hot water at the earlier stated solvent volume, extraction temperature, and extraction time ranges using a temperature-controlled SearchTech 78HW-1 constant temperature magnetic stirrer. The extraction was carried out in duplicate batches at durations of 1 hour x 2; 2 hours x 2; 3 hours x 2; 4 hours x 2; 5 hours x 2; 6 hours x 2, filtered, and the resulting supernatants pulled together and concentrated to 20% of their original volume using a RE52-2 rotary evaporator at 40°C. The crude polysaccharide was then precipitated out of solution with ice-cold absolute ethanol (1:5 v/v), and the polysaccharide-ethanol mix was left overnight in a refrigerator at 4°C. The solvent was subsequently filtered off, and the polysaccharide was collected into a clean beaker. The precipitate was purified by re-dissolution in distilled water and reprecipitation with ice-cold absolute ethanol (1:5 v/v). The purified polysaccharide was then collected after the removal of the solvent by

filtration, and vacuum oven-dried at 70°C (Shang *et al.* [11]. The polysaccharide weight was taken, and the yield calculated thus:

$$Polysaccharide\ yield\ (\%) = \frac{W_2}{W_1}\ x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$W_2 = polysaccharide\ weight\ (g)$$

$$W_1 = weight\ of\ sample\ taken\ (g)$$

The polysaccharides obtained from each of the mushrooms were labelled, CCI-P, GDL-P, and PRD-P for *Calocybe indica*, *Ganoderma lucidum*, and *Pleurotus djamor,* respectively.

Comparison of stirring methods on polysaccharide yield

Three stirring methods via different equipment, namely water bath with manual stirring (SearchTech HH-S_4 WATER BATHS), magnetic stirrer with hot plate (magnetic stirring at 150 rpm), and MRC WBT-450 Orbital shaking water bath (orbital shaking at 150 rpm), were employed to ascertain the impact of the choice of stirring techniques on the polysaccharide yield from the studied mushrooms. The established optimum extraction conditions were applied in this phase of extraction, following the procedure described above. The yields in triplicate were also recorded, and the% polysaccharide yield calculated using equation (1).

Data analysis

Origin Pro Lab 2025 Software was used to plot the graphs and perform Two-way ANOVA and post hoc Tukey tests on the results obtained, to ascertain any statistically significant difference in the polysaccharide yields across all conditions of extraction and mushroom species.

Results and Discussion

Figures 1 – 3 show the effect of variation in temperature, extraction solvent volume (mL), and duration of extraction (hours) on the polysaccharide yield from the three mushrooms. Figure 4 shows the effect of stirring techniques on the polysaccharide yield from the three mushrooms. Tables 1 – 3 show the post hoc Tukey test for significance at $p \leq 0.05$. Based on the varied solvent volume-to-material ratio, the mean polysaccharide yield ranged from 2.37% to 21.05%, 3.41% to 6.07%, and 6.57% to 10.48% for *Calocybe indica*, *Ganoderma lucidum*, and *Pleurotus djamor,* respectively. The optimum ratio of solvent to material was 20:1 w/v for *Calocybe indica*, 30:1 w/v for *Ganoderma lucidum*, and 50:1 w/v for *Pleurotus djamor*. *Calocybe indica* had the highest polysaccharide content (21.05%), followed by PRD-P (10.48%) and GDL-P (6.07%). These variations could be due to the structural nature of the mushroom's cell wall and tissue type. *Calocybe indica* has a thick, fleshy, and compact hyphal arrangement, which translates to more storage polysaccharides in the mushroom tissue, hence the higher

polysaccharide content. *Pleurotus djamor* required a higher extraction solvent, possibly due to the spongy and porous tissue matrix attained when dried. This porosity of the cell wall tissue contributes to its absorbing more extraction solvent to achieve higher extraction efficiency for better polysaccharide yield [12]. Bains *et al.* [13] reported lower values (7.24%) for polysaccharide from *Calocybe indica* using enzyme-assisted extraction, while the polysaccharide content of *Ganoderma lucidum* recorded in this study was lower than that (13.08%) reported by Cheunsun *et al.* [14] extracted via microwave-assisted extraction. Considering the impact of extraction temperature on the yield of polysaccharides, the yields ranged from 9.83% to 21.06%, 4.62% to 6.08%, and 7.43% to 10.52% for CCI-P, GDL-P, and PRD-P, respectively. The increase in yield was relative to the rise in temperature from 75°C to 95°C. A drop in yield, though not statistically significantly different, was observed across the three mushrooms at 100°C. A similar observation was made by Du *et al.* [4]. These findings reveal the fact that high temperatures of extraction enable greater penetration of solvent through the mushroom cell wall, and of course, improve the yield of polysaccharides, while higher temperatures could lead to degradation and possible loss of the polysaccharides. Based on the post hoc test, the optimum extraction temperature is 90°C, as there was no

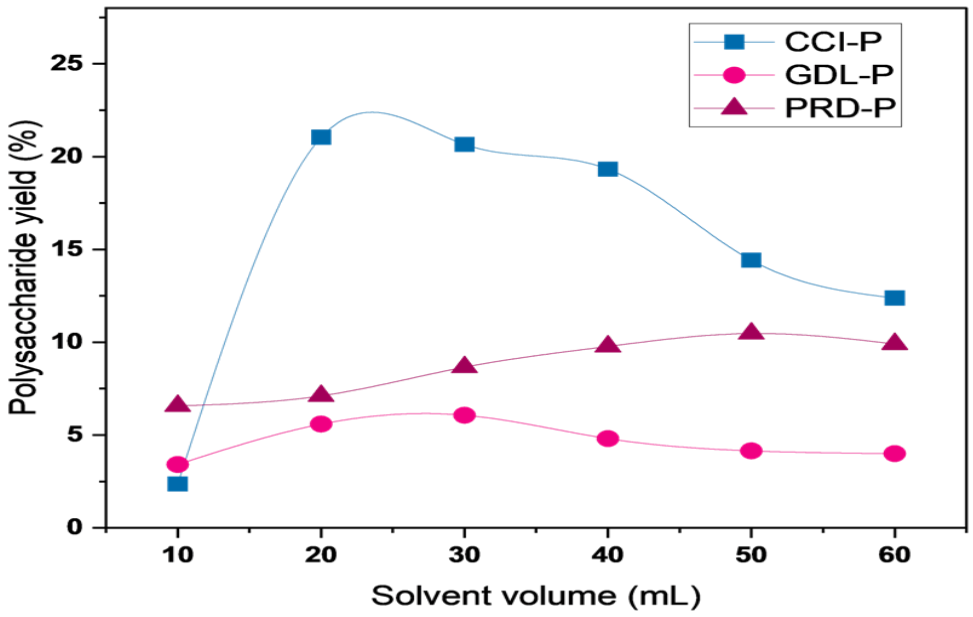

Figure 1. Effect of extraction solvent volume on polysaccharide yield from *Calocybe indica* (CCI-P), *Ganoderma lucidum* (GDL-P), and *Pleurotus djamor* (PRD-P)

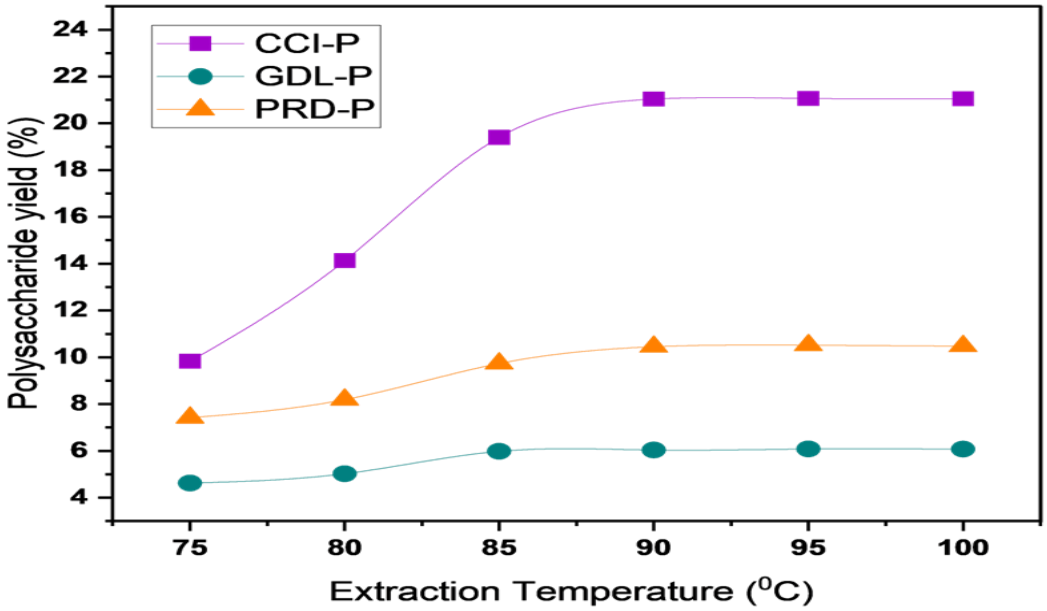

Figure 2. Effect of temperature of extraction on polysaccharide yield from *Calocybe indica* (CCI-P), *Ganoderma lucidum* (GDL-P), and *Pleurotus djamor* (PRD-P)

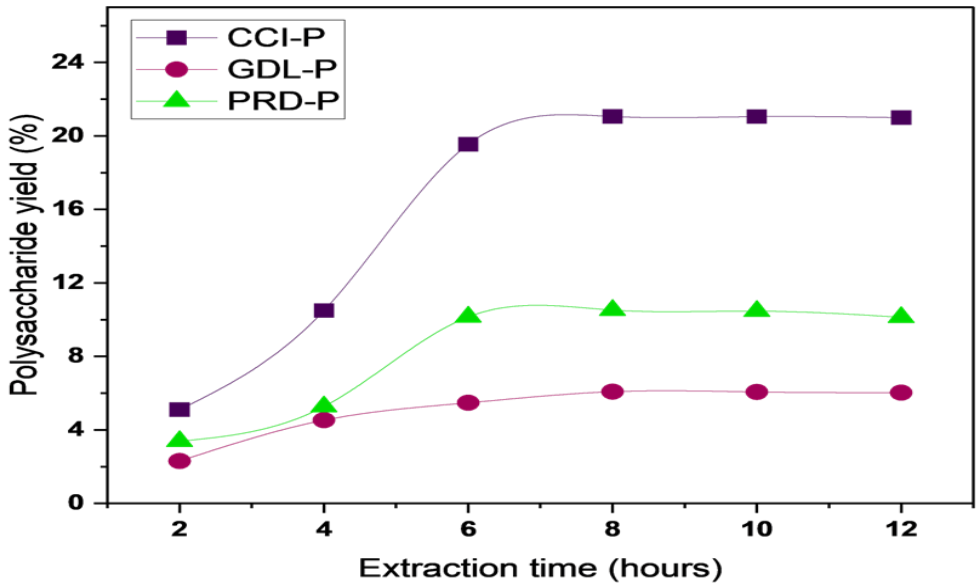

Figure 3. Effect of duration of extraction on polysaccharide yield from *Calocybe indica* (CCI-P), *Ganoderma lucidum* (GDL-P), and *Pleurotus djamor* (PRD-P)

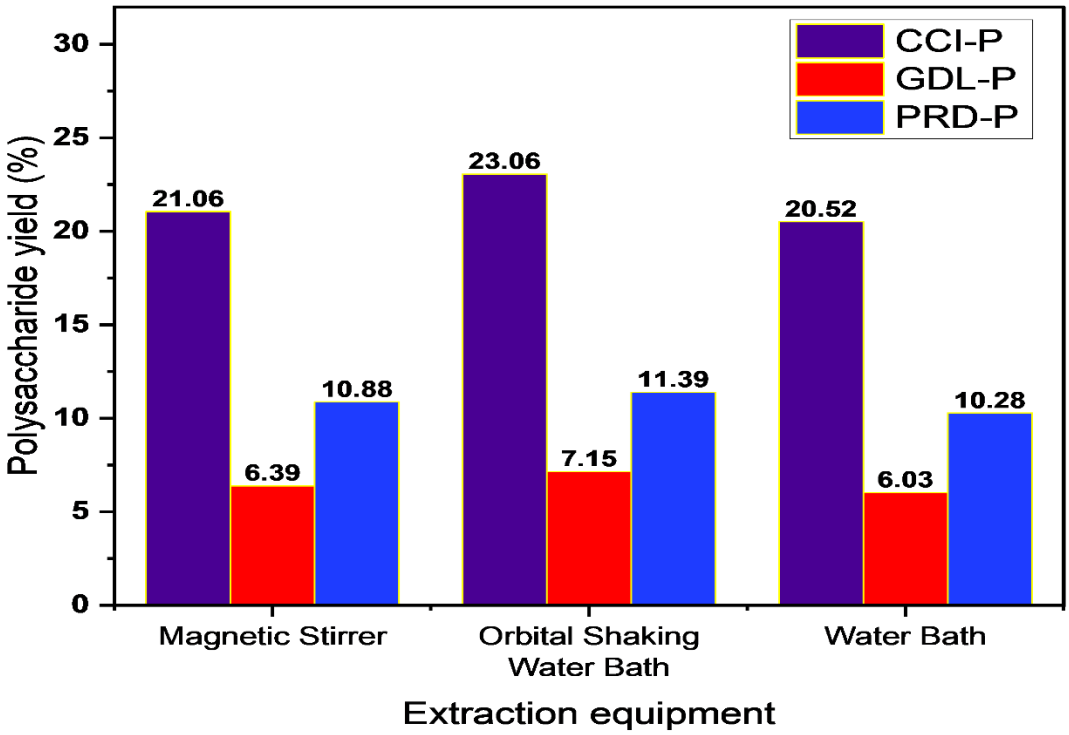

Figure 4. Effect of stirring technique (magnetic stirring, orbital shaking, and manual stirring) on polysaccharide yield from *Calocybe indica* (CCI-P), *Ganoderma lucidum* (GDL-P), and *Pleurotus djamor* (PRD-P)

Table 1. Solvent volume-to-material ratio (v/w) effect on mean polysaccharide yield (%) from *Calocybe indica* (CCI-P), *Ganoderma lucidum* (GDL-P), and *Pleurotus djamor* (PRD-P)

Solvent volume (mL)/material ratio (g)	CCI-P	GDL-P	PRD-P
10:1	2.37^{o*}	3.41^{n}	6.57^{i}
20:1	21.05^{a}	5.59^{k}	7.11^{h}
30:1	20.65^{a}	6.07^{j}	8.66^{g}
40:1	19.32^{b}	4.81^{l}	9.78^{f}
50:1	14.42^{c}	4.15^{m}	10.48^{e}
60:1	12.39^{d}	4.00^{m}	9.91^{f}

Note: $^{a-o}$ indicates post hoc Tukey test for significance

*: means that do not share a letter are significantly different

Table 2. Effect of temperature on effect on mean polysaccharide yield (%) from *Calocybe indica* (CCI-P), *Ganoderma lucidum* (GDL-P), and *Pleurotus djamor* (PRD-P)

Temperature (°C)	CCI-P	GDL-P	PRD-P
75	9.83^{e}	4.62^{i}	7.43^{g}
80	14.13^{c}	5.03^{i}	8.19^{f}
85	19.40^{b}	5.98^{h}	9.74^{e}
90	21.03^{a}	6.04^{h}	10.45^{d}
95	21.06^{a}	6.08^{h}	10.52^{d}
100	21.05^{a}	6.07^{h}	10.48^{d}

Note: $^{a-i}$ indicates post hoc Tukey test for significance

*: means that do not share a letter are significantly different.

significant difference between 90°C, 95°C, and 100°C, though there was a slight increase in yield at 95°C. To save energy and cost, it's best to extract polysaccharides from these mushrooms at 90°C. In a similar vein, there was an increase in polysaccharide yield with an increase in time. However, at extraction times of 10 hours and 12 hours, a decrease in yield was observed. Despite these variations in yield, there was no significant difference between polysaccharide yield at 8, 10, and 12 hours. Therefore, an extraction time of 8 hours (4 hours x 2) is recommended for polysaccharide extraction from the studied mushrooms. The mean polysaccharide yield with respect to extraction time ranged from 5.10% to 21.06% for CCI-P, 2.31% to 6.09% for GDL-P, and 3.38% to 10.52% for PRD-P. Longer extraction periods could

Table 3. Effect of extraction time on mean polysaccharide yield (%) from *Calocybe indica* (CCI-P), *Ganoderma lucidum* (GDL-P), and *Pleurotus djamor* (PRD-P)

Extraction time (Hours)	CCI-P	GDL-P	PRD-P
2	5.10^{e}	2.31^{h}	3.38^{g}
4	10.50^{c}	4.53^{f}	5.27^{e}
6	19.54^{b}	5.48^{e}	10.16^{c}
8	21.06^{a}	6.09^{d}	10.52^{c}
10	21.05^{a}	6.07^{d}	10.47^{c}
12	20.99^{a}	6.03^{d}	10.13^{c}

Note: $^{a-i}$ indicates post hoc Tukey test for significance

*: means that do not share a letter are statistically significantly different

also lead to degradation of the polysaccharide moieties present in the mushroom cell walls, thus a decrease in polysaccharide yield results. It was observed that stirring techniques employed at optimal conditions of extraction significantly impacted the polysaccharide yield. Of the three stirring techniques employed, orbital shaking gave the highest yield, followed by magnetic stirring and manual stirring. The range of mean% polysaccharide yield was 20.52 ± 0.04% to 23.06 ± 0.07%, 6.03 ± 0.03 to 7.15 ± 0.01%, and 10.28 ± 0.16% to 11.39 ± 0.05% for CCI-P, GDL-P, and PRD-P, respectively. As revealed by two-way analysis of variance, there was a significant effect of stirring method on polysaccharide yield within the same mushroom ($p \leq 0.05$); however, the effect is not species-related, as the same trend was observed across all the species studied. There is scarce literature on the yield of polysaccharides from these studied mushroom species, particularly on the varied extraction conditions, optimal extraction parameters, and choice of mechanical stirring technique. While all the stirring techniques are meant to induce mass transfer through shearing and convection, and facilitate recovery of polysaccharides from the mushroom matrix, they offer varying degrees of hydrodynamic strength, hence a difference in the yield of polysaccharides observed in this study. The orbital shaking technique gave a higher yield due to the constant rocking of the mushroom particles in the hot water, which enabled improved contact between the sample and the solvent and leaching of the polysaccharides into solution. The consistent agitation via the orbital shaking also makes the uniform distribution of temperature in all areas of the mixture in the extraction vessel possible, resulting in a higher yield. As established by some research works [15, 6], magnetic stirring, on the other hand, exerts a vortex at the center of the extraction vessel, leading to localized shear and a difference in temperature within the mixture. Manual stirring provides an inconsistent and intermittent mixing of solvent and the mushroom particles, and thus contributes to its lower yield of polysaccharides. Hot water extraction with orbital shaking competes favourably with some enhanced extraction methods like ultrasound-assisted for *Ganoderma lucidum* polysaccharide (8.1%) reported by Alzorqi *et al.* [16]. Since hot water extraction is safer and more economical, introducing orbital shaking into the extraction protocol could be strategized for large-scale extraction of polysaccharides for industrial applications.

Conclusion

We established from this study that conditions of extraction with relation to time, temperature, and extraction solvent volume play a significant role in maximizing the yield of polysaccharides. This investigation also showed that the choice of mechanical stirring impacts on yield of polysaccharides. Under identical optimal conditions, orbital shaking improves extraction

yield significantly; thus, we recommend that orbital shaking via the use of a temperature-controlled orbital shaking water bath be used as the stirring technique in carrying out hot water extraction of polysaccharides in whatever form, whether enhanced or not. This will amount to improved polysaccharide yield, and of course, more availability of these health-promoting mushroom components for food and drug formulations on both local and industrial scales.

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