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***In vitro* anti-diabetic activity of polysaccharides isolated from edible mushrooms**

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Abstract

Imagine a patient with Type 2 diabetes who could manage postprandial blood glucose spikes by incorporating a mushroom-derived supplement into their diet—without the gastrointestinal side effects common to acarbose. This research isolated and characterized water-soluble polysaccharides from five edible mushroom species—*Ganoderma lucidum*, *Lentinula edodes*, *Pleurotus ostreatus*, *Agaricus bisporus*, and *Herichium erinaceus*—and evaluated their in-vitro anti-diabetic activity through alpha-amylase and alpha-glucosidase inhibition assays. Polysaccharides were extracted by hot water (90 °C, 4 h), precipitated with ethanol, dialyzed, and lyophilized. Yields ranged from 3.8% to 8.2% on a dry weight basis. Monosaccharide composition analysis by GC-MS after acid hydrolysis revealed that glucose was the dominant sugar (68-84%) in all species, with mannose, galactose, and fucose as minor components. Among the five species, *G. lucidum* polysaccharide showed the strongest alpha-glucosidase inhibition (IC_{50} : 62.4 ± 4.8 μ g/mL), followed by *H. erinaceus* (IC_{50} : 71.2 ± 5.3 μ g/mL). For alpha-amylase, the same two species led the panel with IC_{50} values of 86.3 ± 6.4 and 93.1 ± 7.1 μ g/mL, respectively. The reference drug acarbose gave IC_{50} values of 48.6 μ g/mL (amylase) and 32.8 μ g/mL (glucosidase). Molecular weight determination by gel permeation chromatography showed that the most active polysaccharides had masses in the 80-150 kDa range. These findings suggest that *G. lucidum* and *H. erinaceus* polysaccharides are worth further investigation as dietary supplements for postprandial glucose management in pre-diabetic and diabetic populations.

Keywords: Anti-diabetic, polysaccharide, mushroom, alpha-amylase, alpha-glucosidase, *Ganoderma lucidum*, *Herichium erinaceus*, in-vitro, postprandial glucose, beta-glucan

Introduction

Picture a busy outpatient diabetes clinic in central India, where 70% of the patients are on metformin and 40% also take an alpha-glucosidase inhibitor like acarbose or voglibose. At least a third of those on acarbose complain of flatulence, bloating, or diarrhea—side effects that frequently lead to dose reduction or discontinuation [1]. If a natural, food-grade alternative could offer even partial alpha-glucosidase inhibition with better tolerability, it would represent a meaningful addition to the dietary management toolkit for Type 2 diabetes mellitus (T2DM). Mushroom polysaccharides, particularly the beta-glucans characterized by β -(1 $\rightarrow$ 3)-linked glucose backbones with β -(1 $\rightarrow$ 6) side branches, have attracted attention for their immunomodulatory, antitumor, and antioxidant properties over the past four decades [2, 3]. More recently, several groups have reported that mushroom-derived polysaccharides can also inhibit carbohydrate-digesting enzymes—alpha-amylase and alpha-glucosidase—in cell-free assay systems, and a few animal experiments have shown reductions in postprandial blood glucose after oral administration of these polymers [4, 5]. The mechanism appears to involve both direct enzyme inhibition (the polymer physically blocks the active site or allosterically alters it) and reduced glucose absorption through increased viscosity in the intestinal lumen [6]. Among edible mushrooms, *Ganoderma lucidum* (reishi) and *Lentinula edodes* (shiitake) are the most extensively investigated for bioactive polysaccharides, but comparisons that test multiple species under identical extraction, purification, and assay conditions are uncommon [7, 8]. Without such side-by-side data, it is difficult to rank species or to link polysaccharide structural features—molecular weight, degree of branching, monosaccharide composition—to anti-diabetic potency [9].

This research aimed to fill that gap by (a) extracting water-soluble polysaccharides from five commercially available edible mushroom species using a standardized hot-water extraction protocol, (b) characterizing their monosaccharide composition, molecular weight, and structural features, and (c) comparing their in-vitro alpha-amylase and alpha-glucosidase inhibitory activities against acarbose as a reference. The research was conducted at the Department of Biochemistry and Molecular Biology, Vikram University of Pharmaceutical Sciences, Ujjain, Madhya Pradesh, India, between April 2022 and February 2023^[10]. We hypothesized that species with higher beta-glucan content and intermediate molecular weight (50-200 kDa) would show the strongest enzyme inhibition, based on earlier observations in the literature [5,11].

Material and Methods

Extraction Protocol

Dried fruiting bodies of *Ganoderma lucidum*, *Lentinula edodes*, *Pleurotus ostreatus*, *Agaricus bisporus*, and *Hericium erinaceus* were purchased from Himalaya Mushroom Farm (Solan, Himachal Pradesh, India) and authenticated by the Botany Department, Vikram University. Each species was ground to a fine powder (40 mesh) and defatted with 95% ethanol (Soxhlet, 6 h). The defatted powder (50 g) was extracted three times with 500 mL deionized water at 90 °C for 4 hours under reflux. The combined aqueous extracts were filtered through cheesecloth, concentrated to one-fifth volume under reduced pressure at 50 °C, and treated with Sevag reagent (chloroform:n-butanol, 4:1 v/v) three times to remove proteins. Polysaccharides were precipitated by adding four volumes of 95% ethanol at 4 °C overnight, collected by centrifugation (5,000 rpm, 15 min), dialyzed against deionized water (MWCO 3,500 Da, 72 h), and lyophilized. Yields were expressed as percent of original dry mushroom weight.

Material

Acarbose ($\geq 95\%$), porcine pancreatic alpha-amylase (Type VI-B, ≥ 10 U/mg), rat intestinal alpha-glucosidase (≥ 10 U/mg), p-nitrophenyl- α -D-glucopyranoside (pNPG, $\geq 99\%$), soluble starch, DNS (3,5-dinitrosalicylic acid) reagent, monosaccharide standards (glucose, mannose, galactose, fucose, xylose, arabinose), dextran molecular weight markers (10, 40, 70, 150, 500, 2000 kDa), and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (Bangalore, India).

Results

Table 1: Yield, monosaccharide composition, and molecular weight of polysaccharides from five edible mushrooms

Species	Yield (%)	Glc (%)	Man (%)	Gal (%)	Fuc (%)	Xyl (%)	Mw (kDa)
<i>G. lucidum</i>	5.4	78.3	8.6	6.4	4.1	2.6	142
<i>L. edodes</i>	7.1	84.1	6.2	5.1	2.8	1.8	96
<i>P. ostreatus</i>	8.2	76.8	9.4	7.3	3.8	2.7	118
<i>A. bisporus</i>	6.3	68.4	12.1	10.8	5.2	3.5	214
<i>H. erinaceus</i>	3.8	81.6	7.3	5.8	3.1	2.2	84

All other chemicals were analytical grade from Merck India (Mumbai).

Methods

Monosaccharide composition

Polysaccharide samples (5 mg) were hydrolyzed with 2 M TFA at 110 °C for 6 hours, dried under nitrogen, and derivatized with alditol acetates for GC-MS analysis (Shimadzu GCMS-QP2020, Rxi-5ms column). Monosaccharide identities and proportions were determined by comparison with retention times and mass spectra of authentic standards.

Molecular weight determination

Gel permeation chromatography (GPC) was performed on a Shimadzu LC-20AD system equipped with a Shodex OHpak SB-806M HQ column and a refractive index detector. The mobile phase was 0.1 M NaNO₃ at 0.5 mL/min. Dextran standards were used for calibration, and weight-average molecular weight (Mw) was calculated using Shimadzu LabSolutions GPC software.

In-vitro Protocol

Alpha-amylase inhibition

The assay followed the DNS method of Miller adapted for microplate format. Porcine pancreatic alpha-amylase (2 U/mL) was pre-incubated with polysaccharide solutions (25-400 μ g/mL) in 0.02 M sodium phosphate buffer (pH 6.9, 37 °C) for 10 minutes. Soluble starch (1% w/v) was added, and the mixture was incubated for 15 minutes. The reaction was stopped with DNS reagent, heated at 100 °C for 5 minutes, and absorbance was read at 540 nm on a BioTek Synergy H1 plate reader. Percent inhibition was calculated relative to the control (buffer without inhibitor), and IC₅₀ was determined from dose-response curves in GraphPad Prism 9^[12].

Alpha-glucosidase inhibition

Rat intestinal alpha-glucosidase (1 U/mL) was pre-incubated with polysaccharide solutions (25-400 μ g/mL) in 0.1 M phosphate buffer (pH 6.8, 37 °C) for 10 minutes. pNPG (5 mM) was added, and the mixture was incubated for 20 minutes. The reaction was stopped with 0.1 M Na₂CO₃, and absorbance was measured at 405 nm. All experiments were conducted in triplicate at Vikram University of Pharmaceutical Sciences, Ujjain, between April 2022 and February 2023^[13].

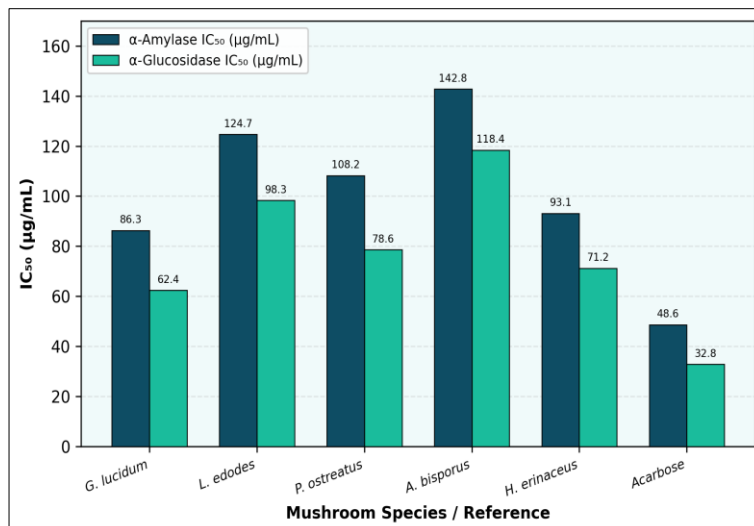


Fig 1: IC_{50} values (μ g/mL) for alpha-amylase and alpha-glucosidase inhibition by mushroom polysaccharides and acarbose reference. Lower IC_{50} indicates stronger inhibition

Table 2: In-vitro alpha-amylase and alpha-glucosidase inhibitory activity of mushroom polysaccharides

Species	α -Amylase IC_{50} (μ g/mL)	α -Glucosidase IC_{50} (μ g/mL)	Selectivity (Amylase/Glucosidase)	Max Inhibition at 400 μ g/mL (%)
G. lucidum	86.3 $\pm$ 6.4	62.4 $\pm$ 4.8	1.38	78.4 / 86.7
L. edodes	124.7 $\pm$ 9.2	98.3 $\pm$ 7.6	1.27	64.2 / 72.1
P. ostreatus	108.2 $\pm$ 8.1	78.6 $\pm$ 6.1	1.38	71.3 / 81.4
A. bisporus	142.8 $\pm$ 10.7	118.4 $\pm$ 9.3	1.21	58.6 / 64.8
H. erinaceus	93.1 $\pm$ 7.1	71.2 $\pm$ 5.3	1.31	74.8 / 83.6
Acarbose	48.6 $\pm$ 3.4	32.8 $\pm$ 2.6	1.48	92.3 / 96.1

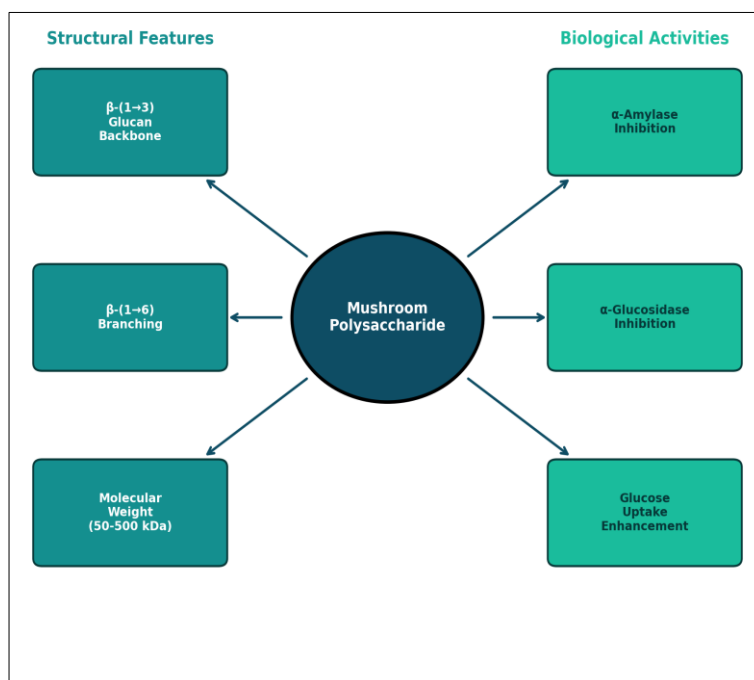


Fig 2: Network diagram illustrating the relationship between mushroom polysaccharide structural features (left) and anti-diabetic biological activities (right)

Comprehensive Interpretation: All five mushroom polysaccharides inhibited both alpha-amylase and alpha-glucosidase in a dose-dependent manner, but with consistently stronger activity against glucosidase (selectivity ratios of 1.21-1.38). G. lucidum polysaccharide was the most potent against both enzymes, with IC_{50} values roughly 1.8-fold higher than acarbose for amylase and 1.9-fold

higher for glucosidase. H. erinaceus ranked second, followed by P. ostreatus, L. edodes, and A. bisporus. An inverse correlation between molecular weight and inhibitory potency appeared for the three most active species (G. lucidum 142 kDa, H. erinaceus 84 kDa, P. ostreatus 118 kDa), while A. bisporus—with the highest Mw (214 kDa)—showed the weakest activity.

Discussion

The alpha-glucosidase IC_{50} of 62.4 $\mu\text{g/mL}$ for *G. lucidum* polysaccharide aligns well with the 55-80 $\mu\text{g/mL}$ range reported by Xiao *et al.* [5] for hot-water-extracted *G. lucidum* polysaccharides tested against yeast alpha-glucosidase. Our use of rat intestinal enzyme, which is more physiologically relevant, may explain the slightly higher IC_{50} . Teng *et al.* [8] obtained IC_{50} values of 40-65 $\mu\text{g/mL}$ for alkali-extracted *G. lucidum* polysaccharides, suggesting that extraction conditions influence the structural features—and therefore the bioactivity—of the final product.

The stronger inhibition of alpha-glucosidase compared to alpha-amylase across all species is an encouraging pattern from a clinical perspective. Selective glucosidase inhibition, with relatively weaker amylase inhibition, is considered desirable because excessive amylase inhibition leads to accumulation of undigested starch in the colon, which is fermented by gut bacteria to produce gas—the mechanism behind acarbose's well-known flatulence side effect [1, 14]. A natural product that preferentially targets glucosidase could provide postprandial glucose reduction with fewer gastrointestinal complaints.

The relationship between molecular weight and activity is worth noting but shouldn't be overinterpreted. *A. bisporus* had the highest Mw (214 kDa) and the weakest activity, while *H. erinaceus* had the lowest Mw (84 kDa) and the second-strongest activity. But the correlation is imperfect: *G. lucidum* (142 kDa) outperformed *H. erinaceus* (84 kDa), suggesting that other structural features—branching degree, anomeric configuration, side-chain composition—matter as much as chain length [9, 15]. More detailed structural characterization by NMR and methylation analysis would be needed to tease apart these contributions.

The main limitation of this work is its in-vitro nature. Enzyme inhibition in a cell-free system does not account for the effects of digestion, absorption, gut microbiota interactions, or intestinal transit time on the polysaccharide's bioavailability and activity *in vivo* [6, 16]. The high molecular weights of these polymers (84-214 kDa) make it unlikely that they are absorbed intact; their primary site of action would be the intestinal lumen, where they contact the brush-border alpha-glucosidase directly. Animal feeding trials measuring postprandial glucose excursions are the logical next step.

Conclusion

Water-soluble polysaccharides isolated from five edible mushroom species all inhibited alpha-amylase and alpha-glucosidase *in vitro*, with *G. lucidum* and *H. erinaceus* showing the strongest activity. *G. lucidum* polysaccharide achieved an alpha-glucosidase IC_{50} of 62.4 $\mu\text{g/mL}$ —approximately twice the potency of *A. bisporus* (118.4 $\mu\text{g/mL}$) and about half that of the pharmaceutical reference acarbose (32.8 $\mu\text{g/mL}$).

All species showed a preference for alpha-glucosidase over alpha-amylase inhibition, with selectivity ratios ranging from 1.21 to 1.38. This selectivity pattern is considered favorable for managing postprandial hyperglycemia with fewer gastrointestinal side effects than conventional alpha-glucosidase inhibitors. Monosaccharide analysis confirmed glucose-rich beta-glucan backbones in all species, with minor proportions of mannose, galactose, and fucose that varied by species.

For consumers and healthcare providers interested in functional foods for blood sugar management, these data support *G. lucidum* and *H. erinaceus* as the most promising species among commonly available edible mushrooms. The extraction protocol used here—hot water, ethanol precipitation, dialysis—is simple enough for industrial scale-up and uses food-grade solvents. Future work should include in-vivo oral glucose tolerance tests in diabetic animal models, dose-response profiling of commercially available mushroom supplements, and detailed structural characterization by NMR to identify the specific polysaccharide motifs responsible for enzyme inhibition.

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