Botanica
ISSN 2538-8657
https://doi.org/10.35513/Botlit.2025.2.3
2025, 31(2): 75–83

Original research

Determination of mannose-containing polysaccharides in the fruiting bodies of *Cantharellus cibarius* and *Craterellus cornucopioides* using lectins

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Received: 12 December 2024. Accepted: 2 June 2025. Published online: 20 June 2025.

Abstract

Panchak L.V., Antonyuk L.Ya., Antonyuk V.O., 2025: Determination of mannose-containing polysaccharides in the fruiting bodies of *Cantharellus cibarius* and *Craterellus cornucopioides* using lectins. – Botanica, 31(2): 75–83. https://doi.org/10.35513/Botlit.2025.2.3

Lectins can bind selectively to carbohydrates in polysaccharide structures without causing any chemical transformation. Studying polysaccharides using lectins can provide valuable structural information and is useful for preliminary research. The present study aimed to analyse the water-soluble, mannose-containing polysaccharides of *Cantharellus cibarius* Fr. and *Craterellus cornucopioides* (Fr.) Pers. using lectins in a haemagglutination inhibition reaction. Similar interaction profiles were observed between the lectins and the polysaccharides of *Cantharellus cibarius*, and the mannan of *Saccharomyces cerevisiae* Meyen ex E.C. Hansen was found to be similar, indicating structural similarities between the two. Similar interaction profiles were also observed for the mannose-containing polysaccharides of *Craterellus cornucopioides* and *Flammulina velutipes* (Curtis) Singer. The structures of the polysaccharides from *Saccharomyces cerevisiae* and *Flammulina velutipes* have previously been determined using modern instrumental methods. Studies of these polysaccharides using lectins confirm these structures and enable conclusions to be drawn about the *Craterellus cornucopioides* polysaccharide structure. The results of this study suggest that lectins could be used more widely to analyse the structure of water-soluble polysaccharides containing mannose.

Keywords: agglutination, fungi, glycan structure, lectin agglutination, mannofucogalactan, mushrooms.

INTRODUCTION

The fruiting bodies of basidiomycetes are mainly composed of polysaccharides. These polysaccharides form the basis of the cell wall, and some of them are also found in a dissolved state in the cells. Water-soluble fungal polysaccharides primarily consist of galactose, glucose, and mannose at varying molar ratios. These monosaccharides are connected by various glycosidic bonds and can be linear or branched (Wang et al., 2022). Other monosaccharides, such as arabinose, xylose, fucose, ribose and rhamnose, are also present in fungal polysaccharides, though less commonly. Fungal acidic polysaccharides of fungi most often con-

tain glucuronic and galacturonic acids (Maity et al., 2021; Shu et al., 2019). Water-soluble fungal polysaccharides are more often represented by heteroglycans than homoglycans. Water-soluble polysaccharides are most often extracted from raw materials using hot water. The mass of polysaccharides extracted in this way is usually small, rarely exceeding a few per cent (Wang et al., 2022). Water-extracted polysaccharides very often include protein and melanin components. Melanin also can covalently attach to polysaccharides, giving them a dark colour (Zhong et al., 2008). Purifying polysaccharides from protein and melanin components can be difficult.

Most fungal polysaccharides are found in the cell walls of fungi. The fungal cell wall consists of two main types of polysaccharides: rigid fibrils of chitin or cellulose and macrocytic alpha- and beta-glucans, as well as glycoproteins. The outer layer of the cell wall contains mannans (Gow & Lenardon, 2023; Yadav et al., 2020) or heteroglycans, which consist of a main chain of mannose, galactose, fucose and xylose in the main chain and with various monosaccharide substituents in the side chains (Tel-Çayan et al., 2020; Chun et al., 2021). Fungi fruiting bodies, when applied with 1–10% alkali, yield hemicellulose, which comprises 30–70% of the total mass of fungal biomass.

A distinguishing feature of fungal polysaccharides is the presence of a high proportion of α or β 1 → 3 bonds between the pyranose cycles of monomers, which sets them apart from plant polysaccharides. The presence of β 1 → 3 glycans in fungal polysaccharides explains most of their pharmacological effects, including immunoregulatory, antioxidant, antitumour and hypoglycaemic properties (Hamidi et al., 2022; Wang et al., 2022).

Due to the complexity of their chemical structure, polysaccharides are analysed using various methods, including nuclear magnetic resonance spectroscopy, methylation analysis using gas chromatography-mass spectrometry, enzymatic cleavage, Smith degradation and others (Ren et al., 2019). At the same time, the use of lectins (proteins that selectively bind carbohydrates without causing their chemical transformation) can provide interesting information. The first thorough studies on using lectins to analyse polysaccharides were carried out using *Canavalia ensiformis* seed lectin (concanavalin A) in the 1960s (So & Goldstein, 1967, 1968). The precipitation reaction of polysaccharides by concanavalin A was investigated. It was found that concanavalin A readily formed insoluble complexes with glycogen, certain dextrans and fructans, and mannans with many terminal monosaccharide residues and relatively long side chains. Highly branched polysaccharides formed poorly soluble complexes with concanavalin A quickly and easily, while low-branched polysaccharides formed precipitates more slowly and were much more soluble.

These studies formed the basis for further research into the interaction between lectins, polysaccharides and glycoproteins. However, the study of polysaccharides using lectins did not become widespread, primarily due to the low selectivity of lectins for carbohydrates, which made the interpretation of results ambiguous. Another reason was the lack of data on the fine carbohydrate specificity of individual lectins at that time. Today, however, significant progress has been made in this area, making it easier to interpret the results obtained using lectins.

This study aimed to analyse mannose-containing polysaccharides using lectins obtained from *Cantharellus cibarius* and *Craterellus cornucopioides*. A key aspect of the research was identifying the potential applications of a set of lectins with different carbohydrate specificities for determining the structure of the side chains of the studied polysaccharides, establishing the presence or absence of D-galactopyranose and L-fucopyranose, identifying the type of bonds between carbohydrate structural units in the polysaccharide and determining the degree of branching of the side chains.

MATERIALS AND METHODS

Study species and raw material

The study examined the fruiting bodies of the edible fungi *Cantharellus cibarius* Fr. and *Craterellus cornucopioides* (Fr.) Pers., which are both members of the Cantharellaceae family within the Agaricomycetes class of Basidiomycota fungi. The sources of mannose-containing polysaccharides were the fruiting bodies of the edible *Flammulina velutipes* (Curtis) Singer fungus of the Physalacriaceae family and the compressed unicellular fungus *Saccharomyces cerevisiae* Meyen ex E.C. Hansen of the Saccharomycetes class.

Fig. 1. Studied fungi: (a) *Cantharellus cibarius*, (b) *Craterellus cornucopioides* and (c) *Flammulina velutipes*. Photographs by V. Antonyuk (Ukraine, Carpathian Mountains near Skole, 2022–2023).

The fruiting bodies of the *Cantharellus cibarius* and *Craterellus cornucopioides* fungi were collected in autumn near the city of Skole in Lviv Region. *Flammulina velutipes* was collected in January on the outskirts of Lviv. The material was delivered to the laboratory within 12 hours and dried in an oven at 55°C. After drying, the fruiting bodies were ground in a coffee grinder and sieved (d = 0.5 mm). The powder was treated twice with methylene chloride (1:10) to extract lipophilic substances, then dried at room temperature and extracted twice with 95% ethanol (1:10). After drying again, the defatted material was used to obtain polysaccharides.

All lectins used in this study were produced by the "Lectinotest" Scientific Production Cooperative (Lviv). These included mannose-specific lectins obtained from: *Galanthus nivalis*, *Hippeastrum hortorum*, *Leucojum vernum*, *Allium sativum*, *Narcissus pseudonarcissus* bulbs, rhizomes of *Urtica dioica* and *Polygonatum multiflorum*; fruits of *Musa acuminata*, tubers of *Helianthus tuberosus*; seeds of *Pisum sativum*, *Lens culinaris*, *Vicia sativa*, *Canavalia ensiformis*, and fucose-specific lectins from the seeds of *Lotus tetragonolobus* seeds, and the bark of *Laburnum anagyroides*. Galactose-specific lectins were obtained from *Glycine max* seeds and *Viscum album* leaves. Lectins with specificity for complex carbohydrates were obtained from *Triticum aestivum* germ agglutinin and *Phaseolus vulgaris* seed erythroagglutinin.

Rabbit erythrocytes were obtained by puncturing an ear vein puncture in the vivarium of the Danylo Halytsky Lviv National Medical University, and human erythrocytes were obtained at the Lviv Regional Blood Services Centre (see Acknowledgements).

Reagents. The work involved the use of the following reagents: methylene chloride, ethanol (95%), acetone, diethyl ether, copper sulphate, potassium-sodium tartrate, sodium hydroxide, potassium hydroxide and hydrochloric acid (36%, Sfera Sim, Lviv). All reagents were of analytical purity.

Purification of polysaccharides. An exact weight of 25.0 g of powder from the fruiting bodies of the two fungi (*Cantharellus cibarius* and *Craterellus cornucopioides*) was extracted using methylene chloride and ethanol. The extract was then poured into 250 mL of water, placed in a boiling water bath and stirred mechanically. After two hours of heating, the extract was squeezed through a dense cloth and then centrifuged for ten minutes at 3000 g. The clarified extract was then filtered through cotton wool. Freshly prepared Fehling's reagent was added to the extracts until the polysaccharide had completely precipitated (40 mL). The precipitate formed was washed twice with distilled water in a centrifuge for 10 minutes at 500 g. The copper complex was then decomposed by washing the precipitate with 5% HCl-acidified acetone until no more copper chloride was detected in the washing liquid (10 mL). The polysaccharide precipitate was

then washed twice with pure acetone (5 ml each) and twice with diethyl ether (5 mL) before being dried in air. After drying, the precipitate was dissolved in hot distilled water. The undissolved residue was removed by centrifugation for 10 minutes at 3000 g, after which it was precipitated again with acetone and washed twice with acetone and diethyl ether. It was then dried in air and weighed. Then, 2% polysaccharide solutions were prepared in buffered saline solution (pH 7.4) for studying the interaction with lectins.

Yeast mannan purification. Purification was carried out according to the previously described method with minor changes that do not affect its essence (Liu et al., 2021). Approximately 800 g of fresh *Saccharomyces cerevisiae* (baker's yeast), purchased from a local supermarket, was extracted with 1400 mL of 7% KOH by heating in a boiling water bath for three hours with constant stirring. After cooling, the mixture was centrifuged for 10 minutes at 6000 g, and the supernatant was collected. The resulting liquid was then precipitated with two volumes of 95% ethanol. The precipitate was washed with 95% ethanol and then dissolved in 450 mL of distilled water. This solution was heated to 60°C with stirring for 30 minutes. The undissolved precipitate was then centrifuged. Fehling's reagent was then added to the clear supernatant to precipitate the mannan (approximately 110 mL). The copper complex precipitate was decomposed by washing with 5% HCl-acidified acetone until no more copper chloride was detected in the washing liquid (5 × 10 mL). The mannan precipitate was then reprecipitated from an aqueous solution with acetone, washed with diethyl ether, and dried in the air.

Purification of mannofucogalactan. The purification process was carried out on the fruiting bodies of *Flammulina velutipes* according to the method described by Mukumoto & Yamaguchi (1977). To achieve this, the dried, crushed fruiting bodies of the fungus were extracted with 450 mL of water in a boiling water bath, with continuous stirring. After two hours of heating, the extract was pressed through a dense fabric, centrifuged for 10 minutes at 3000 g, and then the clarified extract was filtered. The proteins in the extract were then precipitated by the addition of 65 mL of a 50% trichloroacetic acid solution. The formed precipitate was then removed by centrifugation. After neutralising the supernatant, the fraction containing mannofucogalactan was obtained by precipitating it with 40–50% acetone. The resulting precipitate was dissolved in water and purified by precipitation through a copper complex, as described above.

A study of the interaction between polysaccharides and lectins. This study employed the hemagglutination inhibition reaction previously described by Lutsik et al. (1983). The method involves determining the lowest concentration of polysaccharide that completely inhibits hemagglutination caused by lectin. To this end, an equal volume of lectin solution (15 μL) with an agglutination titre of 1:4 was added to the polysaccharide solution (15 μL) at the maximum possible concentration (in this case, 2%). After 10 minutes of incubation, the same volume of a 2% erythrocyte suspension was added and mixed. After a further 10 minutes, the mixture was centrifuged for 30 seconds at 500 g. If the polysaccharide interacts with the lectin, agglutination is not observed; if it does not interact, the erythrocytes agglutinate to form a clot that can be detached from the bottom of the microtubes by gentle shaking. Further stepwise dilution of the polysaccharide was then used to determine its minimum concentration, which completely suppresses the activity of the lectin solution with a titre of 1:4.

RESULTS

Following extraction with hot water and purification through a copper complex, 9.0 mg of polysaccharides were obtained from 25.05 g of *Cantharellus cibarius* and 27.5 g of *Craterellus cornucopioides*. We obtained 5.0 mg of polysaccharide from 85 g of *Flammulina velutipes* fruiting bodies. The resulting powders were white or greyish and dissolved in water to form viscous solutions (1%).

In parallel with the study of the interaction of lectins with polysaccharides of known structure (mannan from *Saccharomyces cerevisiae* and mannofucogalactan from fruiting bodies of *Flammulina velutipes*), the interaction of the obtained fungal polysaccharides with lectins was studied. *Saccharomyces cerevisiae* mannan is known to be a highly branched polysaccharide with oligosaccharide side chains of mannose that are interconnected by α-(1→2) and α-(1→3) bonds attached to the main α-(1→6)-α-D-mannopyranose chain (Yousefi, 2023). The *Flammulina velutipes* polysaccharide consists of a main chain of (1→6)-α-D-galactopyranose,

with every third molecule substituted by 3-O-α-D-mannopyranosyl-L-fucopyranosyl or L-fucopyranosyl residues (Mukumoto & Yamaguchi, 1977).

The results of the study investigating the inhibition of lectin agglutination by the polysaccharides of *Cantharellus cibarius* (mannose) and *Saccharomyces cerevisiae* (mannan) are presented in Table 1.

The results of the interaction between lectins and the mannose-containing polysaccharides of *Cantharellus cibarius* and *Saccharomyces cerevisiae* show that the general profile of this interaction is similar (Fig. 2). Both polysaccharides strongly inhibit the agglutination of mannose-specific lectins of monocotyledons: *Galanthus nivalis*, *Hippeastrum hortorum*, the rhizome of *Polygonatum multiflorum*, *Leucojum vernum* and *Narcissus pseudonarcissus*. They do not inhibit the agglutination of lectins with other carbohydrate specificities. The relative inhibitory power differs only for the lectins of *Musa acuminata*, *Helianthus tuberosus*, *Allium sativum* and the rhizome of *Urtica dioica*. This indicates a remarkably high structural similarity in the side-chain structure of both polysaccharides.

The polysaccharides obtained from *Flammulina velutipes* and *Craterellus cornucopioides* react similarly with lectins. However, this interaction differs from that of the mannans found in *Cantharellus cibarius* and *Saccharomyces cerevisiae* (Table 2).

Table 1. Interaction of *Cantharellus cibarius* mannan and *Saccharomyces cerevisiae* mannan with lectins. Mc: minimum concentration of mannan required to inhibit the hemagglutination of four agglutinating units of lectin (mg/mL)

Source of lectin	Mannan		*Saccharomyces cerevisiae* mannan	
	Mc (mg/mL)	Relative inhibitory potency	Mc (mg/mL)	Relative inhibitory potency
Helianthus tuberosus	0.034	197	0.013	515
Galanthus nivalis	0.026	256	0.026	256
Musa acuminata	0.139	48	0.026	256
Hippeastrum hortorum	0.104	64	0.104	64
Polygonatum multiflorum	0.104	64	0.104	64
Narcissus pseudonarcissus	0.208	32	0.208	32
Leucojum vernum	0.42	16	0.42	16
Canavalia ensiformis	0.42	16	0.208	16
Allium sativum	0.42	16	0.83	8
Lens culinaris	6.7	1	6.7	1
Vicia sativa	6.7	1	6.7	1
Urtica dioica	– (6.7)	–	1.66	4

Notes. The relative inhibitory potency of mannans interacting with *Vicia sativa* lectins (at a concentration of 6.7 mg/mL) was taken as 1.0. The table does not include lectins from *Pisum sativum*, *Triticum aestivum* germ agglutinin, *Glycine max*, *Phaselus vulgaris* erythroagglutinin, *Lotus tetragonolobus* and *Laburnum anagyroides* bark lectin that did not interact with either polysaccharide at a concentration of 6.7 mg/mL.

Table 2. The interaction between *Flammulina velutipes* and *Craterellus cornucopioides* polysaccharides and lectins. Mc: minimum concentration of mannan that inhibits the agglutination of four agglutinating units of lectin (mg/mL)

Source of lectin	Fucogalactomannan from *Flammulina velutipes*		Fucomannan from *Craterellus cornucopioides*	
	Mc (mg/mL)	Relative inhibitory potency	Mc (mg/mL)	Relative inhibitory potency
Lotus tetragonolobus	6.7	0.25	0.41	4
Narcissus pseudonarcissus	0.83	2	0.83	2
Polygonatum multiflorum	0.21	8	0.21	8
Galanthus nivalis	0.83	2	0.83	2
Canavalia ensiformis	0.41	4	0.41	4
Leucojum vernum	1.66	1	1.66	1

Notes. The relative inhibitory potency of mannans interacting with lectins of *Leucojum vernum* (1.66 mg/mL) was taken as 1.0. The table does not include lectins from the following sources that did not interact with both polysaccharides at a concentration of 6.7 mg/mL: *Pisum sativum*, *Triticum aestivum* germ agglutinin, *Glycine max*, *Phaselus vulgaris* erythroagglutinin, *Perca fluviatilis*, *Viscum album*, *Laburnum anagyroides*, *Euonymus europaea* bark and *Urtica dioica* rhizome.

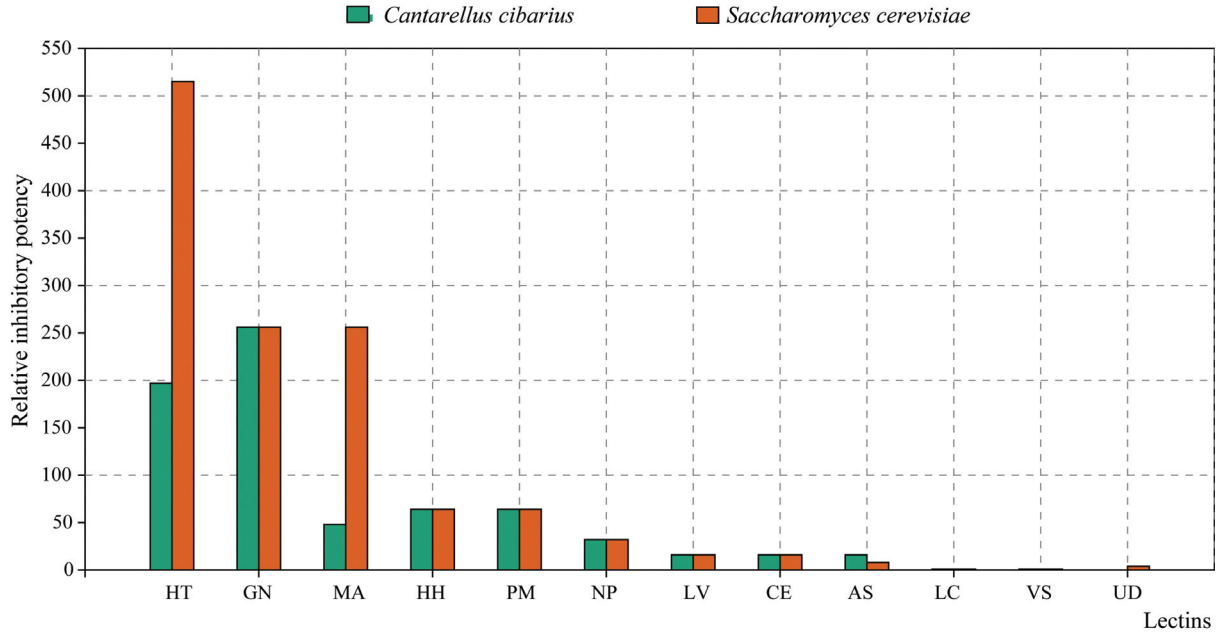

Fig. 2. The interaction between lectins and the mannose-containing polysaccharides of *Cantharellus cibarius* and *Saccharomyces cerevisiae*. Abbreviations: HT – *Helianthus tuberosus*; GN – *Galanthus nivalis*; MA – *Musa acuminata*; HH – *Hippeastrum hortorum*; PM – *Polygonatum multiflorum*; NP – *Narcissus pseudonarcissus*; LV – *Leucojum vernum*; CE – *Canavalia ensiformis*; AS – *Allium sativum*; LC – *Lens culinaris*; VS – *Vicia sativa*; UD – *Urtica dioica*.

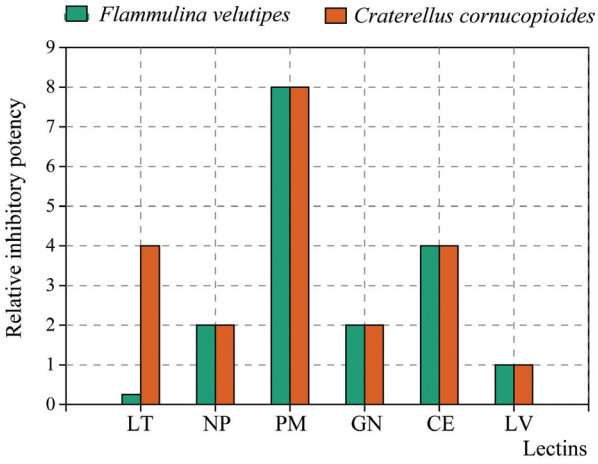

Fig. 3. Interaction of lectins with polysaccharides from *Flammulina velutipes* and *Craterellus cornucopioides.* Abbreviations: LT – *Lotus tetragonolobus*; NP – *Narcissus pseudonarcissus*; PM – *Polygonatum multiflorum*; GN – *Galanthus nivalis*; CE – *Canavalia ensiformis*; LV – *Leucojum vernum*.

The results of the interaction between lectins and the polysaccharides of *Flammulina velutipes* and *Craterellus cornucopioides* show that the overall profile of this interaction is similar (Fig. 3).

The main difference between these polysaccharides lies in how they interact with the L-fucose-specific lectin of *Lotus tetragonolobus*. This lectin interacts 16 times more strongly with the polysaccharide from *Craterellus cornucopioides* than with that from *Flammulina velutipes*. Similar interactions were observed with the other lectins tested.

DISCUSSION

The interaction of lectins with oligosaccharides of different structures must be understood if conclusions about the structure of the analysed polysaccharides are to be drawn.

The weak (or absent) interaction of the mannans of *Cantharellus cibarius* and *Saccharomyces cerevisiae* with mannose-specific legume lectins (from *Pisum sativum* and *Vicia sativa* seeds) and their strong interaction with amaryllis and lily lectins (*Galanthus nivalis*, *Narcissus pseudonarcissus*, *Hippeastrum hortorum*, *Polygonatum multiflorum* and *Leucojum vernum*), are striking. However, the relative inhibitory power of the amaryllis lectins differs considerably. The polysaccharide obtained from *Saccharomyces cerevisiae* best inhibits the agglutination of *Helianthus tuberosus* lectin, and the polysaccharide obtained from *Cantharellus cibarius* inhibits it twice

as weakly. The literature indicates that *Helianthus tuberosus* lectin prefers α(1–2) linkages to α(1–3) linkages (Van Damme et al., 1999), whereas *Galanthus nivalis* lectin (Shibuya et al., 1988) exhibits a preference for binding to α(1–3) D-mannopyranosyl residues. *Narcissus pseudonarcissus* lectin preferentially interacts with Manα(1–6)Man residues (Kaku et al., 1991). The lack of interaction between chanterelle polysaccharides and *Saccharomyces cerevisiae* mannan and *Lotus tetragonolobus* indicates an absence of L-fucose in their composition.

Based on the data obtained, it can be assumed that the *Saccharomyces cerevisiae* polysaccharide has a slightly higher proportion of α(1–2) bonds between D-mannopyranosyl residues than the *Cantharellus cibarius* mannan.

Polysaccharides obtained from *Flammulina velutipes* and *Craterellus cornucopioides* also react very similarly with lectins. Still, this interaction profile differs significantly from that of the mannans of *Cantharellus cibarius* and *Saccharomyces cerevisiae.*

The *Lotus tetragonolobus* lectin selectively interacts with terminal L-fucose residues (Pereira & Kabat, 1974). Given the structure of the *Flammulina velutipes* polysaccharide, it can be assumed that the mannofucogalactan in *Craterellus cornucopioides* has significantly freer L-fucose residues in its side chains. However, in branched polysaccharides, the main-chain carbohydrates are usually unavailable for lectin interaction, meaning the lectins we used could not detect D-galactose in the polysaccharide core.

The bark lectin of *Laburnum anagyroides* requires Fucα1-2Gaβ1-4Glc trisaccharide residues as side chains for interaction (Antonyuk, 2005). However, the polysaccharide of *Craterellus cornucopioides* does not interact with this lectin, indicating the absence of such a structure in its side chains.

By comparing the mannofucogalactan of *Craterellus cornucopioides* with the mannan of *Cantharellus cibarius*, we can conclude that D-mannose is less readily available in the *Craterellus cornucopioides* polysaccharide for interaction with lectins. This may be due to the strong masking of D-mannose by other carbohydrates or to the fact that the studied polysaccharide has less branching and is more linear since the minimum concentration of this polysaccharide required to inhibit the hemagglutination reaction of mannose-specific lectins (*Canavalia ensiformis*, *Narcissus pseudonarcissus*, *Galanthus nivalis*, *Leucojum vernum* and *Polygonatum multiflorum*) is tens of times higher (Table 2, Fig. 3) than for the mannans of *Cantharellus cibarius* and *Saccharomyces cerevisiae* (Table 1, Fig. 2).

Determining the precise structure of polysaccharides is a challenging process that necessitates the use of advanced instrumental methods. However, our study shows that lectins can successfully be used for the initial characterisation of polysaccharides. Lectins are highly sensitive to the oligosaccharide side chains found on polysaccharides. Mannose-specific lectins from monocotyledons can detect not only mannose-containing oligosaccharide side chains but also infer the type of bond between them and the number or density of side chains on the main polysaccharide chain.

Lectins can also be used to analyse water-soluble glycosides. In some cases, they can be used to differentiate biocides from diglycosides. Glycosides with two monosaccharide residues can have two different structures. The two monosaccharide residues can be connected in a chain, and attached at a single point on the aglycone. In this case, they are referred to as biocides. Alternatively, the two carbohydrates can be attached to the aglycone molecule in different positions. In this case, they are referred to as diglycosides or bidesmosides. Such structures are differentiated using complex chemical methods, including hydrolysis and the methylation of the resulting sugars, followed by the analysis of the resulting derivatives by gas-liquid chromatography. At the same time, lectins can precipitate diglycosides when interacting with them, but not similar isomeric biocides. We previously investigated the interaction of lectins with hederasaponin C from *Hedera helix* leaves (Antonyuk, 2007).

Hederasaponin C is a pentasaccharide of hederagenin in which di- and trisaccharides containing L-arabinose and L-rhamnose in one chain and two D-glucosamines and L-rhamnose in the second are attached to the 3rd and 28th positions of hederagenin, respectively. As both sugar chains end with a terminal L-rhamnose, this diglycoside can precipitate lectins that interact with L-rhamnose. Indeed, when studying interactions with lectins, it was found that hederasaponin C precipitates *Glycine max* lectin, *Ricinus communis* lectin, *Caragana arborescens* bark lectin, *Amaranthus caudatus* seed lectin and

erythroagglutinin from *Phaselus vulgaris* most effectively. However, no precipitation was observed with lectins that are specific to D-mannose, N-acetyl-D-glucosamine, or L-fucose.

Despite the technique for performing the agglutination reaction being quite simple, it has several limitations. Firstly, it can only detect and identify carbohydrate and oligosaccharide structures that inhibit it. These are mainly carbohydrates present on erythrocyte membranes. For human erythrocytes, these are primarily sialic acids, L-fucose, D-galactose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, and oligosaccharide structures based on these, linked by α- and, much less often, β-bonds. Rabbit erythrocytes, on the other hand, detect various D-mannose-containing oligosaccharides very well. Although lectins can detect several carbohydrates absent from erythrocyte membranes, such as L-rhamnose, L-arabinose and uronic acids, the selectivity of these determinations is low and requires further clarification. The agglutination reaction is unaffected by β-glycans, which are prevalent in the fruiting bodies of basidiomycetes but absent from animal erythrocytes. Additionally, the agglutination inhibition reaction is semi-quantitative, involving a subjective assessment of the results. This limits the statistical processing of the results to some extent.

For the studies conducted, 9 mg of *Saccharomyces cerevisiae* mannan and *Cantharellus cibarius* mannan, as well as 5 mg of *Craterellus cornucopioides* and *Flammulina velutipes* polysaccharides, were used, along with a minimum number of reagents and a period of one week. The primary requirement is a sufficiently wide range of lectins with well-characterised carbohydrate specificity. They do not necessarily have to be of electrophoretic purity. Both purified lectins and extracts from raw materials can be used. The important thing is that they do not contain significant differences in carbohydrate specificity between isoforms.

ACKNOWLEDGEMENTS

The collection of human and animal erythrocytes was carried out following ethical, moral and legal principles (Protocol No. 10 of the 21 November 2022 meeting of the Commission on Ethics of Scientific Research, Experimental Developments and Scientific Works of Danylo Halytsky Lviv National Medical University). We want to express our deepest gratitude to the Armed Forces of Ukraine for defending our country and enabling us to conduct this research.

Author contributions. LVP performed the main volume of experimental work, focusing on the interaction of lectins with polysaccharides; LYA purified mannose-containing polysaccharides from raw materials; VOA owns the idea of the work, general guidance and design of the manuscript. All authors read and approved the final version of the article.

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