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EXTRACTION OF MUSHROOM β- GLUCAN AND ITS IMMUNOMODULATORY EFFECTS

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Abstract: Mushrooms have been used as a food supplement traditionally and also known to exhibit several medicinal properties. The healing and immunomodulating property of mushrooms have been known because of some biologically active polysaccharides that mostly belong to the group of β-glucan. β-glucans are naturally occurring polysaccharides which are found as the cell wall constituents of bacteria and fungi. This substance increases host immune defense as an immunomodulating agent with involvement of specific interaction with several cell surface receptors. This review and databases were extensively searched and describes about extraction and purification of mushroom β-glucan and focuses on its immunomodulatory properties. The present information will give a new notion to upgrade research works on β-glucan as a mushroom medicine.

Keywords: Basidiomycetes, β-glucan, Extraction, Purification, Immunomodulating

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INTRODUCTION

Mushroom is one of the greatest wonder of nature with increasing demand for both of its medicinal and nutritional values. Mushrooms include 14000 to 22000 species while the real number may be higher associated with the un-description of species and the non differentiation associated with overlapping morphological characters (1). There are over 2000 species of mushrooms that are edible; however a dozen of them are commercially cultivated; a few of them are highly poisonous if consumed (2). Among medicinal mushrooms, *Ganoderma* is number one and has been considered as king of medicinal mushrooms followed by *Lentinula* and others including *Pleurotus*, the later produces oyster mushroom. The main components of the fungal cell wall are polysaccharides and glycoprotein. In a recent review Reshetnikov et. al (2001) have listed 650 species and 7 intraspecific taxa from 182 genera of higher Hetero- and Homo-basidiomycetes that contain pharmacologically active polysaccharides that can be derived from fruit bodies culture mycelium and culture broths (3). β-glucans-(1-3), (1-6) which are branched glucose polymers, derived from the cell wall of a variety of plants and microorganisms as well as barley, yeast and mushrooms (4-5).

β -glucan :

The healing and immunostimulating properties of mushrooms have been known for thousands of years. β-glucans are ingredients with biological activity, thus these biological response modifiers [6-8] mainly activate the immune system, with the possibility to even having effects as an anticarcinogen [9,10]. Glucans are a heterogenous group of glucose polymers, consisting of a backbone of β(1,3)-linked β-D-glucopyranosyl units with β(1,6)-linked side chains of varying distribution and length (11). The structure varies with the linkage degree: β-glucans with none or little β-1,6-linkages mainly have a single helix structure; while recent work describes that the triple helix structure, together with the molecular mass, affects the biological activity of the β-glucans [9,10,12,13] . Activation of macrophages, neutrophil granulocytes and Natural Killer (NK) cells by β- glucans lead to elevated phagocytic activities and production of reactive oxygen intermediates and pro- inflammatory cytokines *in vitro* and *in vivo* (14,15). Experiments have been carried out using mouse model which leads to the successful results in stimulatory effect of innate immunity by mushroom β-glucan.

β-glucan (soluble) have been found to enhance the type 1 immune response by inducing production of IFNY (16). Bacterial or fungal products can initiate the immune response mostly by binding to the innate immune receptors like lectin receptors (mannose receptor, Dectin- 1) (17). Dectin-1 (or β-glucan receptor, β-GR) was described by Brown and Gordon by using a blocking monoclonal antibody against CR3 and anti-Dectin-1 antibody (18). Moreover

experiments are being carried out on the wound healing properties of β -glucan using mouse model.

The goal of this review article is to focus on the extraction of beta glucan from mushroom as well as to discuss about its immunomodulatory effects.

Extraction of β -glucan From Mushroom

There is a broad similarity in the various methods that have been developed to extract the anti-cancer polysaccharides from mushroom fruit-bodies, mycelium and liquid media (19).

In the initial step dried mushroom powder or mycelium is repeatedly heated in 80% ethanol to extract and eliminate low molecular weight substances. Crude fractions 1, 11 and 111 are obtained from the remaining ethanol extract residue by extraction with water (100oC, 3h), 1% ammonium oxalate (100oC, 6h) and 5% sodium hydroxide (80oC, 6h) in that order . Further purification of the polysaccharides are achieved by a combination of techniques including ethanol concentration, fractional precipitation, acidic precipitation with acetic acid, ion-exchange chromatography, gel filtration and affinity chromatography (Fig. 1).

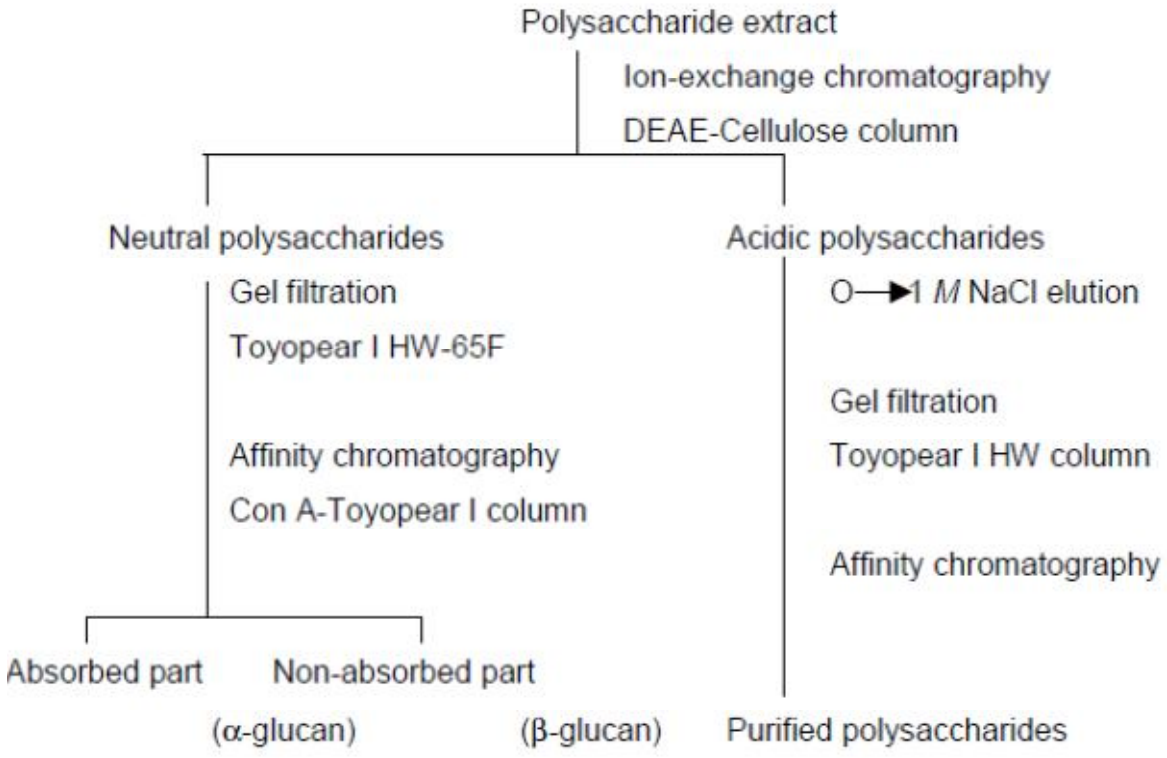


Figure: 1. Fraction purification of polysaccharides by chromatography (Mizuno 1999).

A recent study by Yap and Ng (2001) has established a more efficient procedure for the extraction of β-D-glucans from *Lentinus edodes* (Fig. 2), (20).

The two hundred fifty gram of dried powder was mixed with 700 mL of boiled water at 100ºC for 1 h. The sample was cooled and added equal volume of ethanol absolute. The mixture was centrifuged at 3000 rpm/min for 10 min under cooling 4ºC. The pellet was boiled in hot water for10 min and cooled, and centrifuged at 6000 rpm/min for 15 min. under cooling 4ºC. Equal volume of 95% ethanol was add to the supernatant, and left for 18 h at 4ºC, then centrifuged at 6000 rpm/min for 15 min. under cooling 4ºC. Then the pellet was recovered after centrifugation ,and dissolved in PBS buffer and dialyzed against tap water for 3 days at 4ºC with changed the distilled water every day.

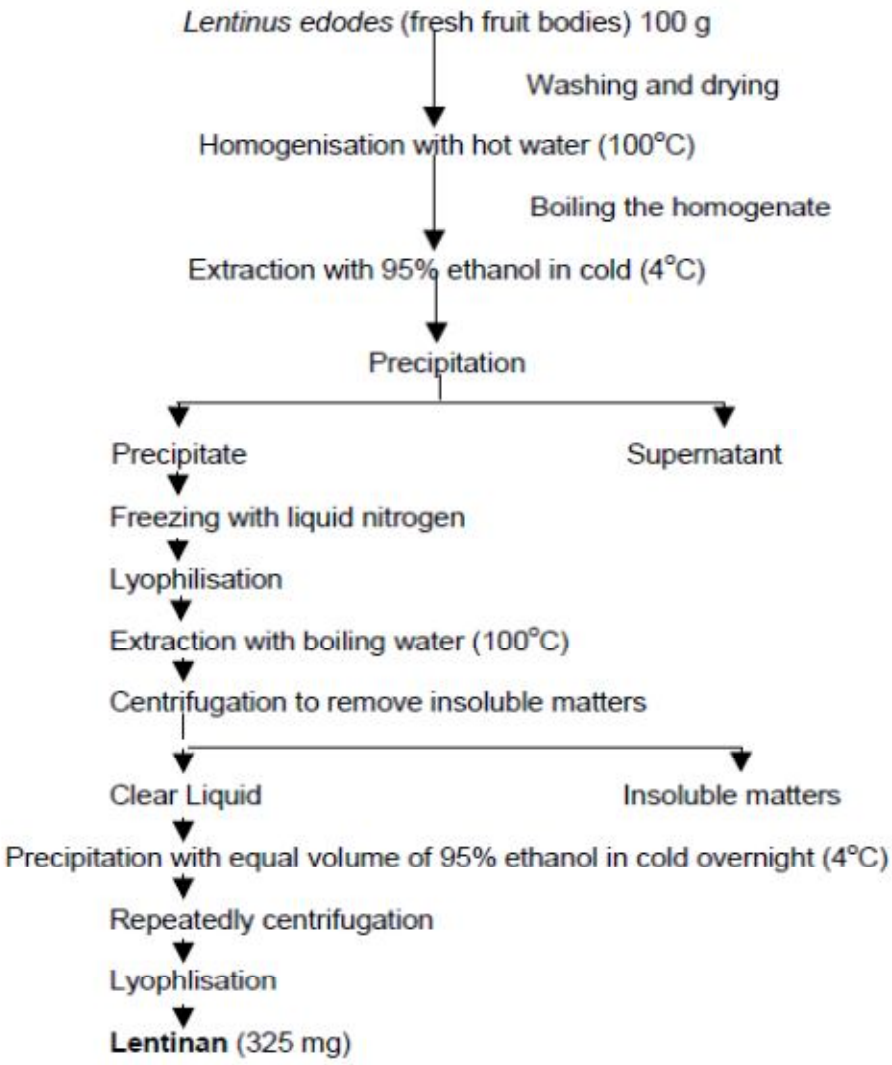
Primary purification:

The yield from previous step was taken and added equal volume of triacetic acid 20%. The suspension was filtrated by filter paper (Whitman no 1), and washed the precipitant on filter paper with ethanol 98% with three fold as filtrated solution. The solution was centrifuged at 3000 rpm/min for 10 min. Under cooling 4ºC. Then the pellet was removed and dissolved with distilled water and dialyzed (against distilled water) for 3 days with changed the distilled water every day.

Secondary purification:

More purification process was done by Ion exchange (DEAE) to obtain as possible pure yield. The column dimension was (60×2.5) cm, the washed and eluted buffer was Tris HCl (15 mM) pH8.4.

Figure: 2. New method for extracting lentinan from *Lentinus edodes* (Yap and Ng, 2001).



Colorimetric method to quantify Glucan

A number of existing methods determine the total amount of β-1,3-glucans [21-26] but do not make any difference between their important tertiary structure. According to HANS-JOSEF et.al. a newer, fast, direct quantitative method has been established using congo red (27). Different mushroom species including *Lentinula edodes* (Shiitake*), Pleurotus ostreatus* (Oyster Mushroom), *Pleurotus pulmonarius* (Lung Oyster Mushroom), were chosen for further analysis and mycelia cultivation on a malt-yeast extract media.

A α-Helios photometer was used at a wavelength of 523 nm for the photometric determinations for the standard solution with schizophyllan and the tested samples. A direct measurement of the bathochromic shift is used. Because of the light brownish colour of some fractions a measurement of the background absorbtion at 523 nm is necessary in the range of 50 – 150 µg/ml. It should be noted that, an incorporation of congo red into the triple helix leads to a bathochromic shift.

β -glucan as an Immunomodulator :

Some polysaccharides or polysaccharide–protein complexes from mushrooms are able to stimulate the non-specific immune system and to exert antitumor activity through the stimulation of the host's defence mechanism (3,19,28,29). A variety of cell surface receptors bind β-glucan, including lectins, scavenger receptors, and integrins on monocyte/macrophages, neutrophils, and natural killer (NK) cells and various lymphocyte subpopulations (30). Engagement of these receptors by β-glucan may induce activation of leukocytes, phagocytic activity, production of inflammatory cytokines and chemokines, microbial killing, and initiate the development of adaptive immunity, all of which contribute to the anti-infective and antitumorigenic properties of β- glucan (31,32). Macrophages here play a critical role in all phases of host defense that are both innate and adaptive immuneresponses in case of an infection. The drugs activate effector cells like macrophages, T lymphocytes and NK cells to secrete cytokines like TNF-a, IFN-g, IL-1b, etc., which are antiproliferative and induce apoptosis and differentiation in tumor cells. Patients who suffer from systemic fungal infections including those caused by *Candida, Aspergillus* and *Cryptococcus* species have been described to possess high levels of circulating β-glucans in their plasma. There was established, β-glucanas a key molecular pattern recognized by neutrophils (or polymorphonuclear leukocytes (PMNs)) in response to *Candida albicans*, because antibody specific for β-glucan, a major component of yeast cell walls, blocks this response (33).

Lentinula edodes, commonly known as Shiitake mushroom has been used as medicinal food in Asian countries, especially in China and Japan and is believed to possess strong immunomodulatory properties [34]. Another wood born mushroom *Trametes versicolor*, commonly called as "turkey tail" like other medicinal mushroom, has long been esteemed in Chineese medicinal mushroom. It has a great wound healing property by strengthening the immune system, particularly by enhancing the acts of one of the most critical cells, known as T helper cells. Table 1 summarizes the most important immunomodulators from mushrooms.

Fig-3. *Pleurotus ostreatus*; Photo: Shaon Pritam Baral.

Fig- 4. *Trametes versicolor*; Photo: Shaon Pritam Baral

Table 1 . Immunomodulating drugs from mushrooms (selected) [Adapted From Ulrike L et al. 2005], (35).

Mushroom scientific name	Mushroom common names	Immunomodulator	Structure of immunomodulator(s)
A. brasiliensis	Royal sun Agaricus, Himematsutake	Flo-a-β FA-2-b-Md	(1-6)-β-D-glucan, heteropolysaccharides, polysaccharide–protein complex RNA–protein complex (MW 6200 daltons)
C. volvatus		H-3-B	(1-3)- β -D-glucan
F. velutipes	Winter mushroom, Enokitake	Flammulin	Protein
G. lucidum	Reishi, Ling Zhi	GLP(AI), Ganopoly, Ganoderans	β -D-glucans, heteropolysaccharides,

		Protein LZ 8	
G. frondosa	Maitake, Hen-of-the-Woods	MD-fraction Grifolan	(1-6)- β -D-glucan with (1-3)- β -D side chains (1-3)- β -D-glucan with (1-6)- β -D side chains
H. caput-medusae Syn.	Lion's Mane, Monkey's Head, Yamabushitake		Glucoxylan; Heteroxyloglucan, Glucoxylan–protein complex;
H.erinaceus			Galactoxyloglucan–protein complex
L. edodes	Shiitake, Golden Oak Mushroom	Lentinan, KS-2 LEM	(1-3)- β -D-glucan with (1-6)- β -D-glucosyl branches Complex mixture of polysaccharides and lignin
Lentinus strigellus			Polysaccharides
P. linteus			Polysaccharides
S. commune		Schizophyllan, Sonifilan, SPG	(1-3)- β -D-glucan with (1-6)- β -D-glucosyl branches
S. crispa	Cauliflower mushroom	SCG	(1-3)- β -D-glucan with (1-6)- β -D-glucosyl branches
T. versicolor	Turkey Tail, Kawaratake, Yun Zhi	Krestin (PSK), PSP	PSK and PSP: heteroglucans with a(1-4)- and β - (1-3) glycosidic linkages with a protein component; the presence of fucose in PSK and rhamnose and

			arabinose in PSP distinguishes the compounds
T. fuciformis	White Jelly fungus, Yin-erh	Tremellastin	Glucuronoxylomannans
T. lobayense			Polysaccharide–peptide complex
Tricholoma mongolicum	Mo-ku		Lectin

β –glucan Receptors

The induction of cellular responses by mushroom and other β-glucans is likely to involve their specific interactions with one or more cell surface receptors. β-glucan receptors were firstly identified on the surface of monocytes by Czop and Austen in 1985 as opsonin-independent receptors for particulate activators of the alternative complement activation pathway (36). Till now, four β-glucan receptors have been identified as candidates mediating these activities. It is namely complement receptor 3 (CR3; CD11b/CD18), lactosylceramide, selected scavenger receptors, and dectin-1 (β-GR), (11). CR3 (complement receptor 3) is a heterodimeric transmembrane glycoprotein, belonging to the β 2-integrin family, consisting of CD11b non-covalently associated with CD18. CR3 is highly expressed on Neutrophils, Monocytes, and NK cells and less present on macrophages. Dectin-1 (or β-glucan receptor, β-GR) was described by Brown and Gordon by using a blocking monoclonal antibody against CR3 and anti-Dectin1 antibody (18). Recent works are also being carried out to find the various mode of receptors-glucan interactions according to various linkage degree of β-glucans. Molecular weight, degree of branching, number of substituents, as well as ultrastructure, including the presence of single and triple helices, significantly affect the biological activities of β-glucan.

CONCLUSION

The spectrum of detected pharmacological activities of mushrooms is very broad. The current review covered and demonstrated the immunomodulatory effects of β-glucan, a polysaccharide found in mushroom and its extraction. Binding of β-glucans to specific receptors can elicit a serial cellular response through the modulating of activities of various factors including cytokines, chemokines, transcriptional factors and growth factors. Mushrooms β- glucans have short β (1,6)-linked branches coming off of the β (1,3) backbone which is variable in other fungal bodies. Extraction of mushroom beta glucan is a very sophisticated method which aims to maintain the original structure. In the opinion of Chang (38), mycelial products are the 'wave

of the future' because they ensure standardized quality and year around production. So to improve the use of mushroom β-glucan as a drug, further necessity is the establishment of suitable quality parameters and of sophisticated analytical methods to regulate these parameters.

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