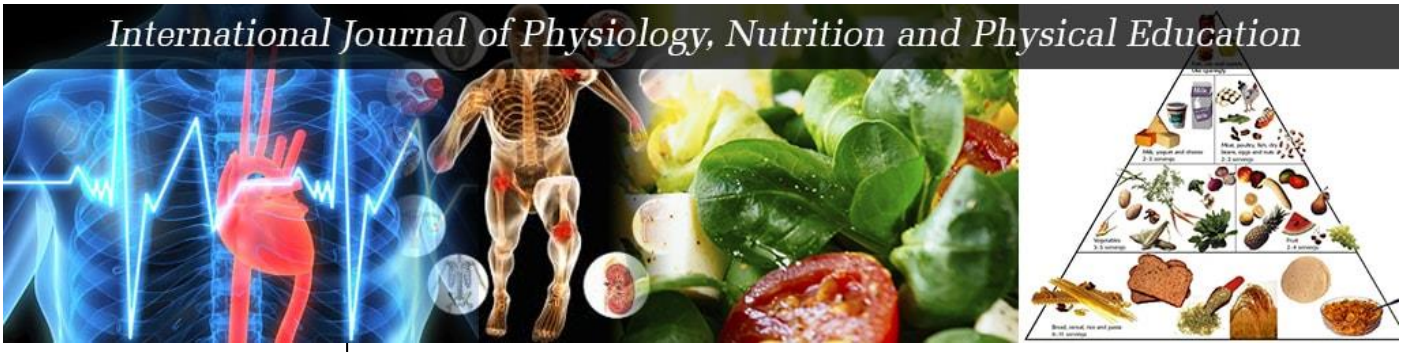

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A review on biologically active polysaccharides isolation from an edible mushroom, pleurotus Florida and its Assam culture variety

Dr. Arnab Kanti Ojha

Abstract
The number of mushrooms on earth is estimated at 140,000, yet maybe only 10% (approximately 14,000 named species) are known. Mushrooms comprise a vast and yet largely untapped source of powerful new pharmaceutical products. In particular, and most importantly for modern medicine, they represent an unlimited source of polysaccharides with antitumor and immuno stimulating properties. Many, Basidiomycetes mushrooms (if not all) contain biologically active polysaccharides in fruit bodies, cultured mycelium, culture broth. Data on mushroom polysaccharides have been collected from 651 species and 7 infra specific texa from 182 genera of higher Hetero-and Homo basidiomycetes. These polysaccharides are of different chemical composition, with most belonging to the group of β-glucans; these have β-(1,3) linkages in the main chain of the glucan and additional β-(1,6) branch points that are needed for their antitumor action. High molecular weight glucans appear to be more effective than those of low molecular weight. Chemical modification is often carried out to improve the antitumor activity of polysaccharides and their clinical qualities (mostly water solubility). *Pleurotus* species are commonly called oyster mushrooms. There are about 40 species of this mushroom [1] such as *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus citrinopileatus*, *Pleurotus cornucopiae*, *Pleurotus tuber-regium*, *Pleurotus abalones*, *P. sapidus*, *P. corticatus*, *P. columbinus*, *P. spodoleucus*, *P. ferulae*, *P. nebrodensis*, *P. eryngii*, *P. pulmonarius*, *P. tuber-regium*, *P. cystiodisus*, *P. djamor*, *P. salmoneostramineus*, and *Pleurotus florida* etc. They enjoy worldwide distribution, both in temperate and tropical parts of the world. Oyster mushrooms now rank second among the important cultivated mushrooms in the world [1]. The information presented in this review is helpful in exploring and understanding the different mushroom polysaccharides and their biological activities isolation of these edible mushrooms *Pleurotus florida* and its Assam culture variety from water extract as well as alkali extract.

Keywords: Edible mushrooms, mushroom polysaccharides, *Pleurotus florida* mushroom, higher Basidiomycetes, immune modulating effect, immuno potentiators, antitumor substances

Introduction
Edible mushrooms have been recognized from long before. Mushroom, the popularly called miracle food is one of the important nutritional supplements to overcome protein energy malnutrition. From mycological point of view, the edible part of the fungus, mainly the fruit body is called basidiocarp, which is the outcome of the modification of secondary and tertiary mycelium. The basidiocarp basically comprises of pileus, stipe, and volva. The entire part of the body is basically consumed as food for the preparation of palatable dishes. Attention has been paid to scientific cultivation of mushrooms since the 17th century, and it is reported that mushroom are being used extensively in many countries for food and fodder [2-5]. Mushrooms have good flavour and taste, with high nutritive values [6]. Studies on a few species of mushrooms [7-12] have shown that in addition to the flavouring properties, the proteins of some mushrooms are equal to muscle protein in nutritive value. According to Robinson and Davidson [13], the efficiency of protein production from a given quantity of carbohydrates in mushrooms and other higher fungi is about 65% compared with about 20% for pork, 15% for milk, 5% for poultry, and 4% for beef. Nutritive values [14] of the fruit bodies of *P. florida* have been determined as 37.19% protein, 3.72% fat and 10.98% ash on a dry weight basis. Approximate composition [15] of the fresh mushroom (*Pleurotus* sp.) has also been reported as following:

Moisture	90.95
Ash	0.974
Protein	2.78
Non-protein nitrogen	0.14
Fat (ether extract)	0.165
Crude fibre	1.08

Mushroom as compared with fruits and vegetables is better source of protein, containing lysine, arginine, histidine, and threonine as amino acids in high concentrations. From the analysis of amino acids composition of the protein from the mushroom (*Pleurotus* sp.) it has been concluded that the supplementation of this mushroom with cereal diet would help to overcome lysine deficiency [15].

The biological importance of *P. florida* is known from the following points:

a. *P. florida* produces two laccase isoenzymes L1 and L2, with L2 being associated with the vegetative growth of the fungus [16].
b. The methanol extract of the oyster mushroom, *P. florida*, inhibits acute inflammation induced by carrageenan and chronic inflammation by formalin and platelet aggregation. Thus the inhibiting properties of this mushroom suggest its potential therapeutic use against vascular disorders [17].
c. The inoculation of *P. florida* increases digestibility and nutrient content of wheat straw by degradation of lignin since it is known to have lignocellulolytic enzyme activity [18].
d. This mushroom species contain essential amino acids like tryptophan, phenylalanine, leucine, and lysine [15].
e. Fresh mushroom is found to contain 0.5% total lipid in which the contents of neutral lipids, glycolipids, and phospholipids are 33.8, 19.7, and 45.6% respectively [19].
f. Ethyl acetate and methanol extracts of *P. florida* show significant lipid peroxidation inhibition activity. However, the aqueous extract does not show lipid peroxidation inhibition activity [1].
g. The ethylacetate, methanol, and aqueous extracts of *P. florida* also show significant scavenging activity of hydroxyl radical generated from Fe^{2+}-ascorbate–EDTA–H_2O_2 system. The results indicate that ethyl acetate and methanol extracts of *P. florida* possess significant antioxidant activity. The methanol extract possesses higher antioxidant activity than the other extracts. The significant antioxidant activities of *P. florida* extracts thus suggest the therapeutic value of this mushroom [1].
h. Antitumor activity of *P. florida* extract was assayed [1] using solid tumor models. The methanol extract showed significant tumor growth inhibition against the solid tumor induced by EAC cell lines in a dose-dependent manner. This suggests the antitumor property of this mushroom.

Isolation of polysaccharide from Mushrooms

i. The isolation of pure sample is essential for a successful investigation of carbohydrates; the analytical data of carbohydrates mixed with impurities make the interpretation difficult. In Figure 1 the main steps for purification of polysaccharides are presented. The isolation of polysaccharides is initiated with hot water extraction, followed by precipitation in EtOH and centrifugation. It is then dissolved in minimum volume of water and exhaustive dialysis is carried out to remove small carbohydrates molecules using DEAE cellulose bag. The high molecular weight polysaccharides remain inside the bag and the solution is then freeze-dried.

But isolation of polysaccharide from alkali extract of edible mushroom P. Florida (Assam Var.) is quite different. The schematic diagrams are shown bellow:

PS-II and PS-III were washed several times with water followed by centrifugation. PS-II (gel) material was carefully collected from the upper layer and then the PS-III (insoluble) was further purified by washing several times with water and freeze dried.

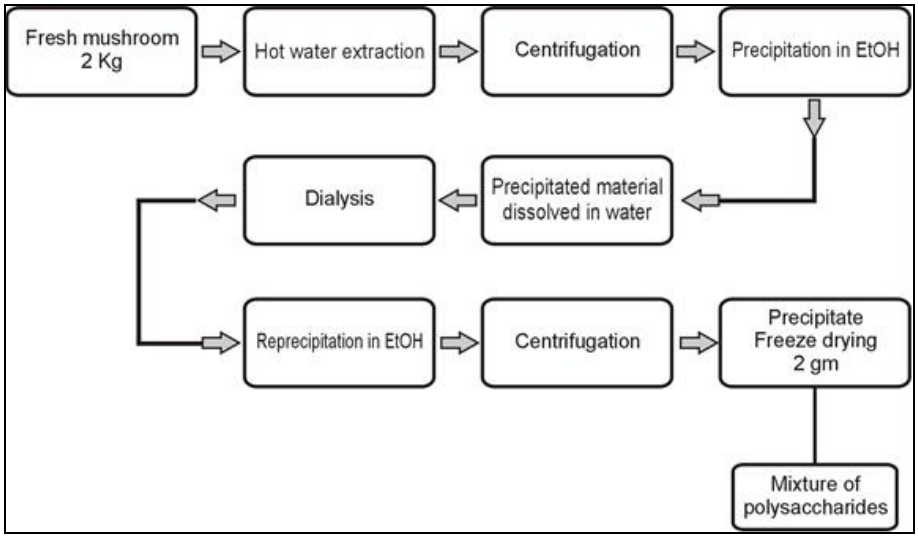
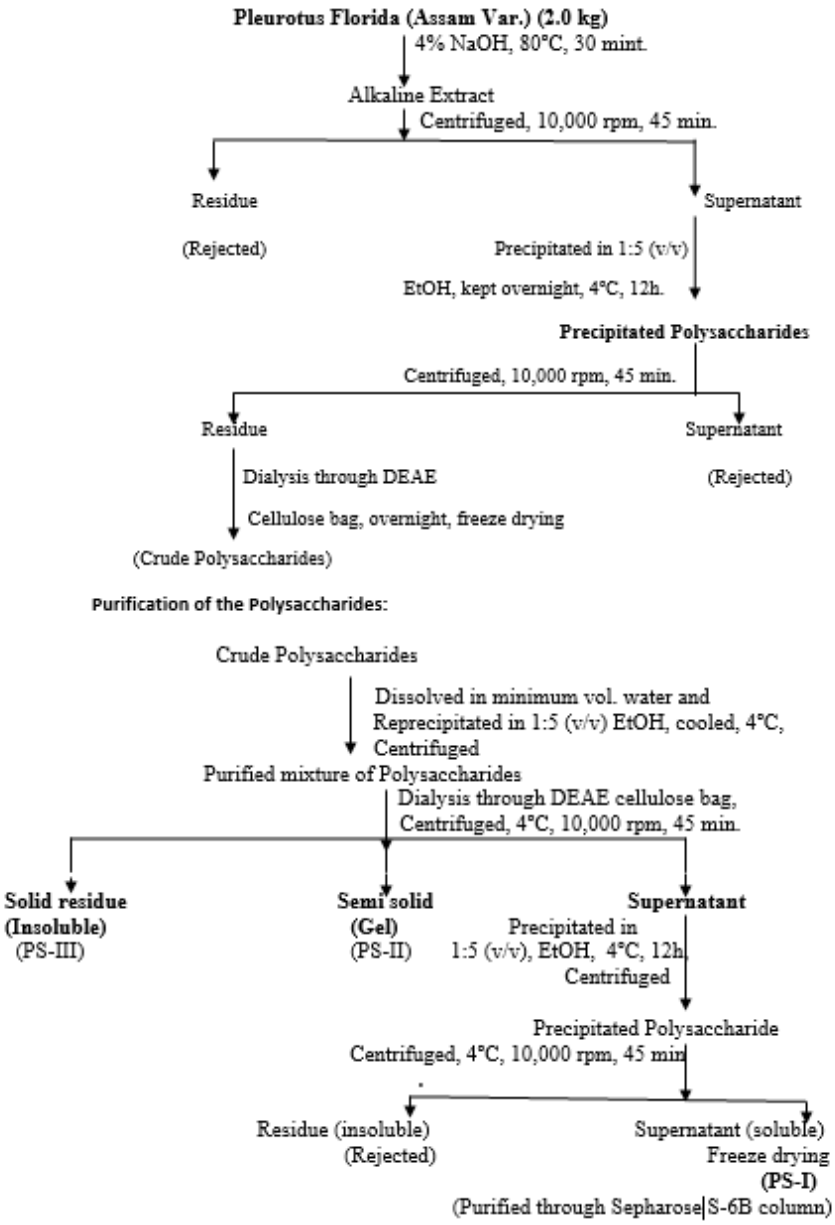


Fig 1: The main steps for isolation of mushroom polysaccharides. The weight value of 2g in the figure represents the result obtained from the isolation of PS from *P. florida.*

But isolation of polysaccharide from alkali extract of edible mushroom P. Florida (Assam Var.) is quite different. The schematic diagrams are shown below



PS-II and PS-III were washed several times with water followed by centrifugation. PS-II (gel) material was carefully collected from the upper layer and then the PS-III (insoluble) was further purified by washing several times with water and freeze dried.

Review of earlier works *Pleurotus florida* Mushroom Polysaccharides

Oyester mushrooms now rank second among the important cultivated mushrooms in the world. One of the species of the genus Pleurotus is *Pleurotus florida*. P. florida is cultivated on a commercial scale in many parts of the world, including India. An antitumor glucan was isolated from the hot-water extract of an edible mushroom *Pleurotus ostreatus* [20]. Purification of the aqueous extract was accomplished by 20% NaCl solution saturated with thymol and also by precipitation with EtOH from Me_2SO solution. The glucan showed marked antitumor activity at a dose of 0.1 mg/kg in mice. It is a highly branched (1→3)-, (1→6)-β-glucan having an average structure represented by a pentasaccharide segment consisting of one nonreducing terminal, one 1,3,6-tri-*O*-substituted, and three 1,3-di-*O*-substituted β-D-glucopyranosyl residues. A heteroglycan consisting of D-glucose, D-mannose, and L-fucose, was also isolated from this mushroom. The structure of the heteroglycan [19] has (1→6)-D-galactopolymer as main chain. Another high molecular weight water-soluble polysaccharide was obtained by precipitation of 0.1N NaOH extract of the mushroom, *P. ostreatus* in EtOH [21].

Protein-containing polysaccharides extracted from fruiting bodies of a Chinese fungus *Pleurotus sajor-caju* [22] were fractionated and purified, and their antitumor activities were tested. The following active fractions are reported:

a. A protein-containing xyloglucan, MW 280,000, polysaccharide: protein= 76:24 (w/w), and the polysaccharide consisting of Man: Gal: Xyl: Glc = 2:12:42:42 (molar ratio).
b. A protein-containing mannogalactan, MW 120,000, polysaccharide: protein = 76:16 (w/w), and the polysaccharide consisting of Xyl: Man: Gal = 9:35:56 (molar ratio).
c. A protein-containing xylan, MW 200,000, protein: xylan = 62:21 (w/w).
d. A protein-containing glucoxylan, MW 90,000, protein: glucoxylan =15:71 (w/w), and the polysaccharide part consisting of Glc: Xyl = 40:44 (molar ratio).
e. A protein-containing xyloglucan, MW 70,000, polysaccharide: protein = 69:3 (w/w) and the polysaccharide consisting of Xyl: Glc=36:62 (molar

ratio).

f. A water-soluble polysaccharide, (Fr. I) isolated from the aqueous extract of *Pleurotus sajor-caju* in our laboratory was found to consist of D-glucose, D-galactose, and D-mannose in a molar ratio of 1:1:1 [23]. The experimental results revealed the presence of trisaccharide repeating unit, which is composed of (1→6)-α-D-galactose, (1→2,4)-α-D-glucose, and nonreducing end β-D-mannose.

A water-soluble polysaccharide and two water-insoluble polysaccharides were isolated from *P. citrinopileatus* mushrooms [24]. The antitumor activity of these fractions was examined in mice. The following active polysaccharides are reported:

a. A water-soluble protein containing heteropolysaccharide composed of glucose, mannose, arabinose, and galactose.
b. A water soluble glycoprotein consisting of glycan: protein = 40:60 (w/w), with the glyco-chain composed of glucose, xylose, mannose, galactose, and fucose.
c. Another glycoprotein consisting of glycan: protein = 50:50 (w/w), and the glycan moiety consisting of glucose, galactose, xylose, mannose, and fucose.
d. Two water insoluble protein-containing β-D-glucans, composed of glucan: protein = 80:20, and 68:32 (w/w), respectively. The glucan moieties of both were almost all (1→3)-β-D-glucan, and their molecular weights were 68×10^4 and 40×10^4.
e. Another two water insoluble β-D-glucans with molecular weights 190×10^4 and 120×10^4 respectively. Both are composed of glucan: protein = 87:13 (w/w). Both glucan moieties are mainly (1→3)-β-D-glucan.

The following polysaccharides from an edible mushroom, *P. cornucopiae* [25] are reported:

a. Cold water-extracted galactomannan.
b. Hot water extracted glycogen and branched (1→3)-β-glucans.

The glucan showed high inhibitory activities against the growth of mouse-implanted tumors (Sarcoma 180).

Another antitumor material, β-glucan from an edible mushroom of this genus *Pleurotus tuber-regium* [26] has been reported. Most of the clinical evidence for antitumor activity comes from the commercial polysaccharides lentinan, PSK (krestin), and Schizophyllan, but polysaccharides of some other promising medicinal mushrooms species also show good results.

A water soluble glucan was also isolated from the hot water extract of *Pleurotus florida* (cv Aasam florida) by Roy, S. K. *et al*. [27], which is biologically active towards splenocytes and thymocytes activation as well as Macrophages active. Another water soluble glucan was isolated from alkaline extract of *Pleurotus florida* (cv Aasam florida) by Ojha, A. K. *Et al*. [28], which is also biologically active towards splenocyte and thymocytes activation as well as Macrophages active.

Structures of polysaccharides

The hot water extract of the edible mushroom *P. florida* was found to consist of four different polysaccharides; a new (1→3), (1→6)-branched glucan (Fr. I) [20], a heteroglycan (Fr. II) [29] consisting of D-mannose, D-glucose and D-galactose, a (1→6)-α-glucan and a (1→3), (1→6)-β-D-glucan. A water soluble glucan was also isolated from the hot water extract of *Pleurotus florida* (cv. Aasam florida) by Roy, S. K. *et al*. [27]. From alkaline extract of this fruit bodies, three different polysaccharides were isolated; water soluble (PS-I), gel (PS-II) and water insoluble (PS-III) by Ojha, A. K. *et al*. [28]. The structural difference of the glucan (PS-I) with the previously reported one from *P. florida* (cv Aasam florida) is that the present polysaccharide contains one (1→6)-linked glucose unit less in the main chain of the polymer.

Fr. I: →3)-α-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→

6

↑

1

α-D-Glc*p*

Fr. II α-D-Glc*p*

1

↓

4

→6)-α-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→

2

↑

1

β-D-Man*p*

The structure of the water soluble glucan, isolated from the hot water extract of *Pleurotus florida* (cv Aasam florida)

→ 6)-α-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→ 6)-β-D-Glc*p*-(1→

6

↑

1

α-D-Gal*p*

The structure of the water soluble glucan (PS-I), Gel (PS-II) and insoluble glucan (PS-III) isolated from the alkaline extract of *Pleurotus florida* (cv Aasam florida):

PS-I: → 3)-α-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→ 6)-β-D-Glc*p*-(1→

6

↑

1

α-D-Glc*p*

PS-II: → 3)-α-D-Glc*p*-(1→

PS-III: →3)-β-D-Glc*p*-(1→

Biological importance

The water-soluble glucan isolated from *Pleurotus florida* provides [30] conclusive evidence for the presence of the following repeating unit in the polysaccharide:

→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→

I. A biological parameter such as macrophage activity was studied on J $_{744.1}$ cell line (Mouse monocyte cell line) with this polysaccharide. The enhancement of the percentage of macrophages was estimated by measuring the concentration of released NO (nitric oxide). The following results shown in Table 1 indicate that the polysaccharide is active at high concentration. Macrophage stimulation of mice cell by the polysaccharide of *P. florida* by NO production assay at 550nm.

Table 1: Indicate that the polysaccharide

Sample	Concentration of polysaccharide					
Glucan	10ng/ml	100 ng/ml	1µg/ml	10µg/ml	100 µg/ml	1.0 mg/ml
	1.014	1.045	1.112	1.190	1.290	1.05

Control reading: 1.00

Increased phagocytic activity was reported after incubating mice macrophages with (1→3), (1→6)-branched glucan (Fr. I) of this polysaccharide. The most effective dose of this glucan was observed at 1mg/mL.

Table 2: Macrophage stimulation of mice cell by the polysaccharide (PS) of Pleurotus florida by NO (Nitric Oxide) production assay at 550 nm.

Sample	Concentration of the polysaccharide					
Fr. I	10 ng/mL	100 ng/mL	1 µg/mL	10 µg/mL	100 µg/mL	1.0 mg/mL
	1.0145	1.135	1.158	1.190	1.290	1.435

Control reading: 1.00

Macrophage activation of the polysaccharide isolated from hot water extract of *Pleurotus florida* (cv Aasam florida) was observed *in vitro* [27]. Upon treatment with different concentrations of the polysaccharide an enhanced production of NO was observed in a dose dependant manner with optimum production of 8.5 µM NO per 5×10^5 macrophages at 80 µg/ml of the polysaccharide (Figure 2). A similar kind of material like lentinan (a β-glucan) inhibits tumor growth by stimulating the immune system[30] through activation of macrophages, T-helper, NK and other cells.

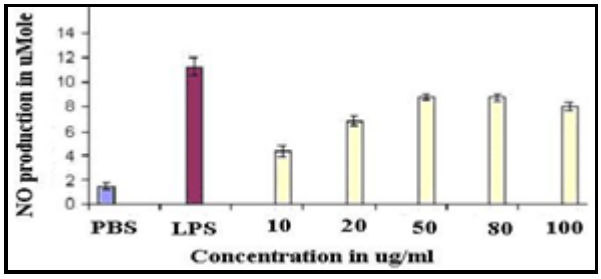


Fig 2: *In vitro* activation of peritoneal macrophage stimulated with different

Concentrations of the polysaccharide in terms of NO production.

The splenocyte and thymocytes activation test were carried out in mouse cell culture medium with the polysaccharide by the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] method [31]. Proliferation of splenocytes and thymocytes is an indicator of immunoactivation. The polysaccharide was tested to proliferate splenocytes and thymocytes as shown in Figure 3(A) and 3(B). At 20 µg/ml of the polysaccharide, splenocyte proliferation index was maximum as compared to other concentrations. 20 µg/ml of the polysaccharide can be considered as efficient splenocyte proliferators whereas 20 µg/ml of that sample shows maximum effect on thymocyte proliferation. The splenocyte proliferation index (SPI) as compared to PBS (Phosphate Buffer Solution) control was close to 1 or below that indicates low stimulatory effect on immune system.

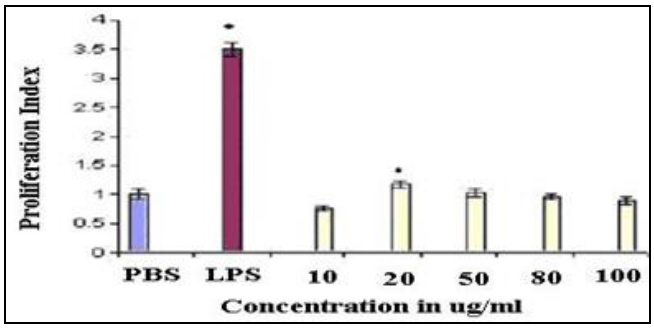


Fig 3A:

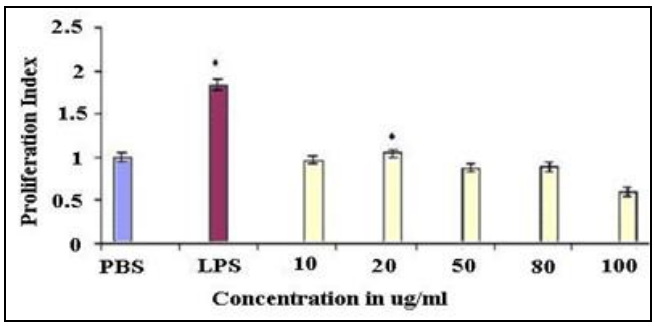


Fig 3B:

Fig 3A/3B: Effect of different concentrations of the polysaccharide on splenocyte (**A**) and thymocyte (**B**) proliferation. (*Significant compared to the PBS control).

Similarly, macrophage activation as well as the splenocyte and thymocytes activation test were carried out in mouse cell culture medium with the polysaccharide isolated from alkaline extract *Pleurotus florida* (cv Assam florida) [28] of by the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] method. And it was noticed that this polysaccharide will also active.

Nano synthesis

Recently, Green synthesize of gold nanoparticles from water soluble glucan isolated from the hot water extract of *Pleurotus florida* (cv Aasam florida) by Sen I. *et al.* [31].

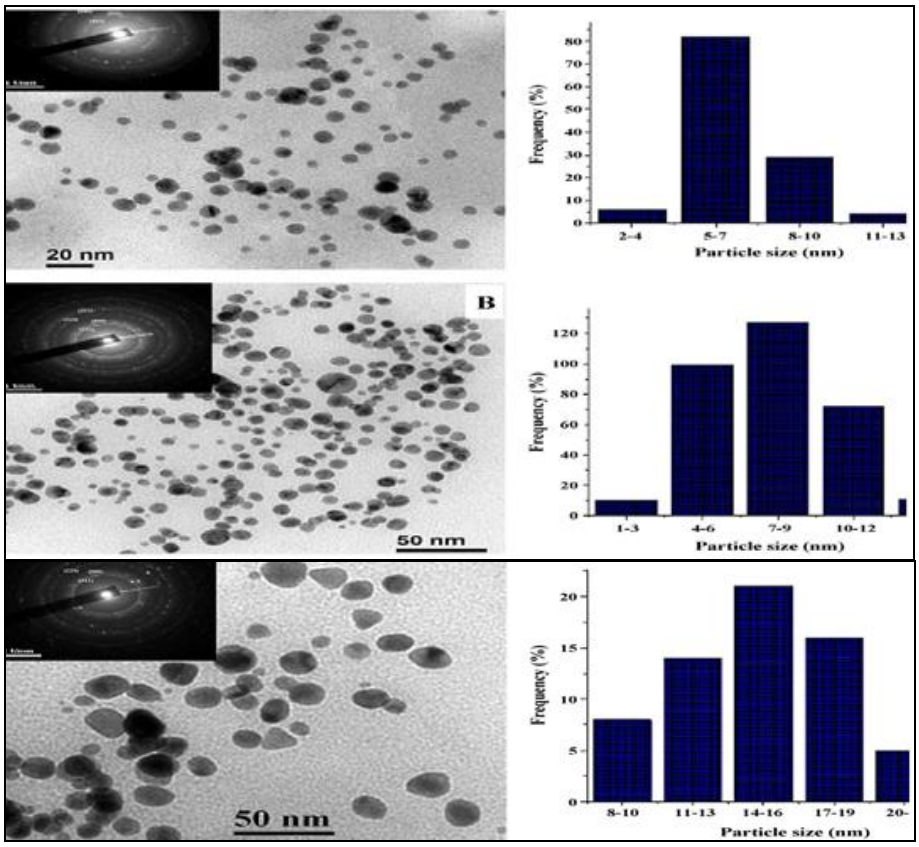


Fig 4: TEM images and the corresponding selected area electron diffraction (SAED) patterns (inset) of Au NPs prepared with 0.05% (w/v) glucan and 0.5 mM HAuCl4 (A), 1.0 mM HAuCl4 (B), and 1.5 mM HAuCl4 (C). Particle size distribution histograms of Au NPs prepared with 0.05% (w/v) glucan and 0.5 mM HAuCl4 (a), 1.0 mM HAuCl4 (b), and 1.5 mM HAuCl4 (c). HR-TEM images of Au NPs prepared with 0.5 mM HAuCl4 (d), 1.0 mM HAuCl4 (e), and 1.5 mM HAuCl4 (f) showing clear lattice fringes reveal that the growth of Au NPs occurred preferentially on the (1 1 1) plane.

Conclusion

The number of mushrooms with known pharmacological qualities is much lower still. Never the less, the species studied so far represent a vast source of anticancer and immuno stimulating polysaccharides. The following purposes: (1) Prevention of oncogenes is by oral consumption of mushrooms or their preparations; (2) direct antitumor activity against various allogeneic and syngeneic tumors; (3) immuno potentiation activity against tumor in conjunction with chemotherapy; (4) preventive effects on tumor metastasis.

Pleurotus florida is cultivated on a commercial scale in many parts of the world, including India. It is a delicious edible mushroom and used as nutritionally functional food with valuable therapeutic use. That's why these edible mushrooms are very useful as well as nutritional and medicinal purpose.

For a healthy future of nanotechnology, green synthetic strategy should be adopted for nanoparticles synthesis using environmentally benign and renewable molecules to get rid of the hazards arising out of the use of chemical reducing agents and organic solvents. Here researcher are reported the green synthesis of AuNPs and AgNPs using a glucan, isolated from water extract of an edible mushroom P. florida, cultivar Assam Florida, and P. florida blue variant respectively which act as both reducing and stabilizing agent. Three Glucans isolated from the alkaline extract of this edible mushroom should be reducing agent of the Green synthesis of metallic nanoparticles. Hence, there might have a possibility for the large scale production of metallic nanoparticles by using these glucans and application of the catalytic properties as well as active as antibacterial.

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