ABSTRACT

The present thesis entitled as "**Isolation, purification and characterization of bioactive polysaccharides from edible mushrooms and polysaccharide based nanoparticles**" consists of two sections. The first section represents the structural characterization and immunological activities of two different polysaccharides, isolated from two different edible mushrooms (*Termitomyces heimii and Lentinus fusipes)*. The second section deals with the green synthesis of silver nanoparticles (AgNPs) using aqueous solution of a hetero polysaccharide (consisting of glucose, fucose and galactose), extracted from the *Lentinus squarrosulus* (Mont.) Singer and its effectiveness as an antibacterial agent. The entire thesis is divided into five chapters.

Chapter I

Part A: Now-a-days mushrooms are popularly used as food flavouring substances due to their unique taste, flavor, high nutritive value and medicinal values. Several bioactive substances like polysaccharides, phenolic compounds, sterols, terpenes and ceramides have been identified from mushrooms; among them polysaccharides (PS) can be considered as one of the major functional component responsible for various health benefits. In recent years mushroom-derived polysaccharides have drawn the attention of chemist and immunobiologists for their immunostimulatory, anti-oxidant, antimicrobial, antiflammatory, and anti-tumour properties. These bioactive polysaccharides bind to lymphocyte, serum specific proteins which activate macrophages, T-helper, NK, and other effector cells which fuel up the host's immune system. Thereby increase the production of antibodies, interleukins (IL-1, IL-2) as well as interferon (IFN-γ). It has been observed that the chemical compositions, molecular weight, conformation, glycosidic linkage and degree of branching are significantly related with immunostimulating activities. Several mushroom polysaccharides are widely used and commercialized worldwide as anti cancer agents for therapeutic purpose. **Lentinan** (from *Lentinus edodes***, Japan**), **Schizophyllan** (from *Schizophyllum commune),* **Agarican** (from *Agaricus blazei***, USA**), and Grifron-

D (from *Grifola frondosa*, **Japan**) have been commercialized and used clinically as anti-tumor agents.

Part B: Nanoparticles are defined as "a discrete entity with at least one dimension being 100 nm or less". These materials show superior properties, e.g., enhanced catalytic, adsorption, enhanced optical properties, increased hardness, compressive strength, etc. due to their extremely small size and large surface to volume ratio. At present it is the most rapid growing area of research for versatile applications of nanoparticles (NPs) in various fields of science. Much attention has been focused on metal nanoparticles (especially Au and Ag-nanoparticles) because of their significant applications in the area of catalysis, optics, sensing, electronics, and biotechnology.

Synthesis of metal nanoparticles using green renewable entities is very effective and therefore can be used as an economic and valuable alternative for the large-scale production of metal nanoparticles. Polysaccharides represent an excellent scaffold for nanoparticle synthesis as they have hemiacetal end to reduce metal salt precursors and lot of hydroxyl group and other functionalities to stabilize the synthesized metal NPs. Polysaccharides with hydroxyl and amino groups bind tightly to the surface of the metal nanoparticles giving them a hydrophilic surface. There are series of reports where heparin, hyaluronic acid (HA), alginic acid, chitosan as well as plant polysaccharides like starch, cellulose, dextran were employed in the synthesis and stabilization of AgNPs and AuNPs.

Chapter II

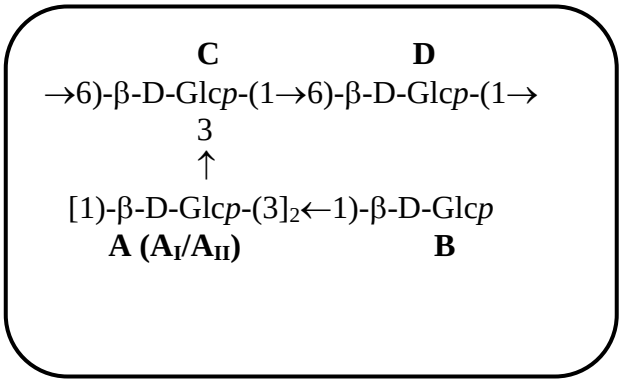
The methodologies that have been adopted to determine the structure of polysaccharides and their bioactivity studies have been discussed in this chapter. The biological activities of polysaccharides depend on the molecular weight, linking sequences of the monosaccharide residues, branching pattern etc. So, it is very important to determine the exact structure of the repeating unit of the polysaccharides isolated either from mushroom. The exact structure of the polysaccharides is determined using two types of methods: (1) **Chemical method** that includes total acid hydrolysis, methylation, periodate oxidation and smith degradation studies. (2) **Spectroscopic method** comprising of 1D (^{1}H, ^{13}C) and 2D NMR (DQF-COSY,

TOCSY, NOESY, ROESY, HSQC, HMBC etc) and Mass spectroscopic experiment (GLC-MS).

The techniques used in the synthesis and characterization of nanoparticles have also been highlighted in this chapter. Ag-NPs have been prepared by chemical synthesis method using hetero-glycan as both reducing and capping agent. Synthesized nanoparticles were characterized using UV-Visible, HR-TEM, SEM, XRD techniques. For the determination of antibacterial efficacy of AgNPs-glucan conjugates, gel electrophoresis, Fluorescence-activated cell sorting (FACS) analysis was done.

Chapter III

Two water soluble polysaccharides (PS-I & PS-II) have been isolated from the alkaline extract of the edible mushroom *Termitomyces heimii.* The detailed structural investigation and biological activities of PS-I were investigated and presented in this chapter. The average molecular weight of PS-I is ~1.48 $\times 10^5$ Da. PS-I contained (1→3), (1→6), (1→3, 6)-linked and terminal β-D-glucopyranosyl moieties in a ratio of nearly 2:1:1:1. Based on the total hydrolysis, methylation analysis, periodate oxidation, Smith degradation, partial hydrolysis and 1D/2D NMR experiments the structure of the PS-I was elucidated and it was found that the repeating unit of the PS-I consist of a backbone chain of two (1→6)-β-D-glucopyranosyl residues, one of which was branched at *O*-3 position with the side chain consisting of two (1→3)-β-D-glucopyranosyl and a terminal β-D-glucopyranosyl residue. On the basis of these experiments, the structure was established as:

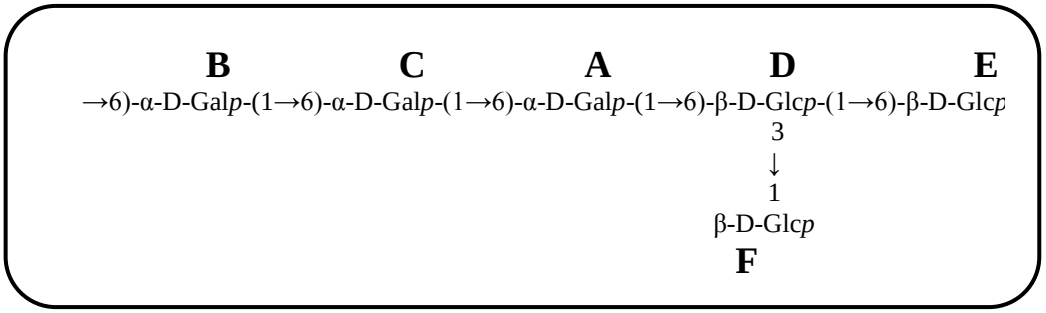


The in-vitro cellular toxicity of PS-I on human blood lymphocytes at varied concentrations ranging from 25 µg/ml to 400 µg/ml was studied by MTT assay. Cell proliferative activity was observed significantly up to 200 µg/ml but, at the dose of

400 µg/ml the PS-I showed mild toxicity. The biocompatibility was established by measuring glutathione levels (oxidised and reduced form), MDA levels on cell lysates. The reduced glutathione (GSH) level significantly increased up to 200 µg/ml, whereas at 400 µg/ml, it was moderately decrease, exhibited slight increase in GSSG level. MDA level also slight increase at 400µg/ml dose. Moreover, it also exhibited potent antioxidant activities by diminishing the ROS and NO in the nicotine stimulated lymphocytes upto 200 µg/ml. This property of PS-I was also established by FACS analysis of nicotine treated lymphocytes.

Chapter-IV

Two water soluble polysaccharides (PS-I & PS-II) have been isolated from the aqueous extract of the edible mushroom *Lentinus fusipes*. The detailed structural investigation and biological activities of PS-II were investigated and presented in this chapter The average molecular weight the PS-II is ~ 60 kDa. The structural characterization of PS-II was carried out using total acid hydrolysis, methylation analyses, periodate oxidation, Smith degradation and 1D/2D NMR experiments. Total acid hydrolysis indicated the presence of D-galactose and D-glucose in a molar ratio of approximately 1:1. The chemical and NMR analyses revealed that the proposed repeating unit of the PS-II had a backbone chain consisting of three (1→6)-linked α-D-galactopyranosyl residue and two (1→6)-linked β-D-glucopyranosyl residues, one of the β-D-glucopyranosyl residue was branched at *O*-3 position with a terminal β-D-glucopyranosyl. On the basis of these experiments, the structure was established as:



The in-vitro cellular toxicity of PS-I on human blood lymphocytes at varied concentrations ranging from 20µg/ml to 320µg/ml was studied by MTT assay. Cell proliferative activity was observed significantly up to 160 µg/ml but 320 µg/ml

dosage showed mild toxicity. The biocompatibility was established by measuring glutathione levels (oxidised and reduced form), MDA levels on cell lysates. The reduced glutathione (GSH) level significantly increased up to 160 µg/ml, whereas at 320 µg/ml, it was moderately decrease, exhibited slight increase in GSSG level. MDA level also slight increase at the dose of 320 µg/ml. The PS-II exhibited significant *in vitro* splenocyte and macrophage activations with optimum dose of 20 µg/ml and 80 µg/ml respectively. Flow cytometry study revealed the protective role of the PS-II against nicotine stimulated lymphocytes at a concentration of 160 µg/ml. Moreover, the ROS scavenging property of PS-II was also established using DPPH radical scavenging assay.

Chapter-V

Silver nanoparticles (AgNPs) were synthesized using a hetero polysaccharide (PS) isolated from *Lentinus squarrosulus* (Mont.) Singer. The polysaccharide fraction (consisting of glucose, fucose and galactose) serves the role of both reducing as well as stabilizing agent. UV-vis spectroscopy showed maximum absorbance at 407 nm due to surface plasmon resonance. High resolution transmission electron microscopy (HRTEM) exhibited that the average diameter of the nanoparticles was 2.78 ± 1 nm. The selected area diffraction pattern (SAED) revealed the 'fcc'crystalline structure of Ag NPs–PS conjugates. The XRD analysis further confirmed face-centered cubic (fcc) geometry of silver nanoparticles.

Antibacterial activity of the AgNPs-PS conjugate was tested against multiple antibiotics resistant (MAR) *Escherichia coli* strain MREC33 and it was noted that killing was due to generation of Reactive oxygen species (ROS). Internalization of AgNPs-PS conjugate along with its DNA degradation capability was demonstrated using Flow cytometry. Ag NPs-PS conjugates showed only 1.017% hemolysis at LD_{50} concentration to human RBCs. This LD_{50} dosage of AgNPs-PS conjugates in combination with each of the four antibiotics (ampicillin, azithromycin, kanamycin and netilmicin) to which *E. coli* MREC33 was resistant, showed synergistic effect to inhibit complete bacterial growth. This opens a new possibility to use antibiotics in combination with reduce dosage of nanoparticles to combat MAR bacteria.