Summary and Conclusion

Mushrooms are cosmopolitan heterotrophic macrofungi, have been associated with human society for many centuries. The abundance and diversity of mushrooms occupy a prime position in biosphere and play a pivotal role in the dissolution of minerals, mobilization of nutrients and enhancement of carbon flow in forest ecosystem. Multidimensional studies focusing on the health and therapeutic benefits of mushroom derived bioactive substances have been increased significantly in the past few decades. The Indian sub continent is blessed with diverse eco-climatic zones that harbour a treasure trove of mushroom diversity. Though the occurrence of mushrooms is of diverse nature in India, still a large segment remains unexplored. In this context, the present thesis has focused on the diversity, nutritional and therapeutic benefits as well as antibacterial substances in mushrooms and their functioning mechanisms, which could be conducive to our better understanding of the correlations between the mushroom consumption and health promotion.

A vivid mushroom survey was conducted during May 2015 to October 2017 in Gurguripal ecoforest (22°25" - 35°8"N and 87°13" - 42°4"E) by opportunistic sampling method. In the present research work, a total number of 2031 mushroom specimens (individuals) were observed in Gurguripal ecoforest. Altogether, 67 mushroom species in 44 genera belonging to 27 families under 10 orders were noted. Among the mushroom species 34 were edible, 31 non edible and 2 reported to be poisonous. According to their habitat, 9 were parasitic (grows on living trees), 34 saprophytic (terrestrial or on humus), 24 mycorrhizal (with trees and termite nests).The mushroom flora in this area are dominated by the family Russulaceae (16.4%) followed by Tricholomataceae (11.9%) and Boletaceae (8.95%) represented by 11, 8 and 6 species respectively. The present study has explored the abundant occurrence of wild mushrooms in Gurguripal ecoforest, and in particular, the

species namely *Hypomyces chrysospermus* Tul & C.Tul, *Collybia tuberosa* (Bull) P.Kumm, *Lepista flaccida* (Sowerby) Pat., *Mycena* sp., *Tylopilus alboater* (Schwein.) Murrill, *Tylopilus violatinctus* T.J.Baroni & Both., *Scleroderma verrucosum* (Bull.) Pers, *Suillus* sp., *Lactarius piperatus* (L.) Pers., *Lentinellus cochleatus* (Pers.) P. Karst were first time reported from Paschim Medinipur, West Bengal.

Statistical analysis of collected data was done through Simpson's and Shannon diversity index formula to measure mushroom diversity indices of the study area. According to Simpson's index of diversity, the calculated value of species richness was 0.941 which indicates greater mushroom diversity of this area. According to Shannon's diversity index, the relative abundance of species was found to be 3.687. Evenness of the mushrooms was also calculated as 0.87. This result referred that all the 67 mushroom species were not evenly distributed numerically in the community indicating different microhabitats and microenvironments of the study area. An explorative ethnomycological survey was also executed in parallel during the study and the information was collected through semi-structured questionnaires and focused group discussions among the different native tribal communities of this region separately. 19 mushroom species were found to be medicinally important and effectively used in solving liver problems, curing cold and cough, lowering blood pressure and also applied against burns, itching and inflammations. The traditional knowledge regarding the medicinal uses of wild mushrooms was generally confined to elderly aged persons of the villages.

In the present investigation, nutritional compositions of nine wild edible mushroom species namely *Agaricus* sp., *Amanita bisporigera, Astraeus hygrometricus, Cantherallus* sp., *Termitomyces medius, Pleurotus ostreatus, Schizophyllum commune, Termitomyces heimii and Volvariella volvacea* were studied by standard biochemical protocols. The results showed that the protein, carbohydrate and lipid content of mushrooms ranged between 20.4 - 39.2%,

33.2 - 43.4% and 0.8 - 3.4% respectively on dry weight basis. The crude fibre and ash content varied from 2.0 - 8.6% and 2.3 - 11.5% respectively on dry weight basis. By overall analysis it has been revealed that *T. heimii* and *V. volvacea* possessed higher proximate compositions. Polyphenolic fractions of *T. heimii* and *V. volvacea* were extracted with methanol and the major phenolic compounds estimated using standard methods. *T. heimii* was rich in phenols (2365±12.34 µg/gm) followed by flavonoids (1050.33±16.73 µg/gm) and ascorbic acid (97.94±3.12 µg/gm) while *V. volvacea* showed the values as 1389±8.98 µg/gm, 987±12.23 µg/gm and 42.70±4.65 µg/gm respectively for the same. The antioxidant activity of *T. heimii* and *V. volvacea* were also measured by DPPH and FRAP assay. It has been revealed that *T. heimii* showed the lower DPPH value (IC_{50}= 43.32±3.47 mg/gm) than *V. volvacea* (IC_{50}= 48.58±3.21 mg/gm) whereas in case of FRAP analysis, the result also showed lower value in case of *T. heimii* (IC_{50}= 51.39±2.62 mg/gm) than *V. volvacea* (IC_{50}= 55.64±3.19 mg/gm). Lower values of DPPH and FRAP assay indicate higher radical scavenging ability. Gas chromatography revealed that higher quantities of unsaturated fatty acids (MUFA and PUFA) are present in *T. heimii*. The most abundant fatty acid in *T. heimii* was recorded as linoleic acid (33.53µg/ml) followed by oleic acid (25.08 µg/ml) and palmitic acid (15.60 µg/ml). In case of *V. volvacea* the most abundant fatty acid is stearic acid (87.65 µg/ml) followed by oleic acid (16.55 µg/ml), linoleic acid (9.67 µg/ml) and palmitic acid (3.43 µg/ml). EDAX analysis was performed to determine the major elements present in *T. heimii* and *V. volvacea.* The micronutrient analysis of two mushrooms showed that phosphorus (P) and potassium (K) content of *T. heimii* is significantly higher than *V. volvacea*. Altogether, the present findings suggested that due to presence of higher nutritional attributes as well as remarkable bioactive potentials *T. heimii* is preferred over all the studied mushroom species as healthy food supplement.

The antibacterial properties of hot water, acetone, ethanol and methanol extracts from seven selected mushroom species (*Astraeus hygrometricus, Auricularia auricula, Ganoderma lucidum, Pleurotus ostreatus, Schizophyllum commune, Termitomyces heimii and Volvariella volvacea*) were studied by agar well diffusion method against five Gram positive (*Micrococcus luteus* ATCC9341, *Streptococcus faecalis* MTCC5383, *Staphylococcus aureus* MTCC96, *Bacillus subtilis* MTCC441, *Bacillus cereus* MTTC3610) and five Gram negative (*Escherichia coli MTCC118, Shigella flexneri* MTCC7061, *Salmonella typhi* MTCC734, *Klebsiella pneumoniae* MTCC109, *Enterobacter aeroginosa* MTCC111, *Vibrio cholerae* MTCC3906) pathogenic bacteria. Methanolic extract of *Termitomyces heimii* showed the highest antibacterial activity against *Staphylococcus aureus* (18 mm ZOI) and *Shigella flexneri* (16 mm ZOI). Partially purified methanolic fractions of *T. heimii* were tested for their antibacterial activity and the highest active methanolic fraction (F_{11}) was further analysed through HPLC. The result showed the presence of four carbohydrates like ribose, glucose, sucrose and xylose and three major phenolic compounds namely gallic acid, *p*-coumaric acid and ferulic acid within it, among which the highest peak was identified as *p*-coumaric acid (*p*-CA). Green synthesis of silver nanoparticles using *T. heimii* extract was carried out and result of FTIR study has revealed that the absorption bands were around at 3400 cm^{-1} and 690 cm^{-1}, which are characteristics of carbohydrate rings and halogen compounds respectively. AgNPs synthesized using *T. heimii* extract exhibited increased antibacterial activity showing 19 mm and 18 mm clear ZOI against *S. aureus* and *S. flexneri* respectively.

The isolation of crude polysaccharide was done from alkaline (4% NaOH solution) extract of dried and powdered fruit bodies of *T. heimii* by standard protocol. 100 gm of dried fruit bodies of *T. heimii* yields 380 mg of water soluble crude polysaccharide. Two homogeneous polysaccharide fractions namely THP-I (20 mg) and THP-II (6 mg) were purified and collected after gel permeation chromatography (GPC). THP-I showed higher

antibacterial activity (16 mm ZOI) against the pathogenic bacteria *Staphylococcus aureus*. According to MIC and MBC values, THP-1 inhibited the growth of *Staphylococcus aureus* at a concentration of 62.5 µg/ml and exhibited bactericidal efficacy at 125 µg/ml whereas it showed 125 µg/ml and 250 µg/ml as MIC and MBC respectively against *Shigella flexneri*, exhibiting its higher antibacterial efficacy against Gram positive bacteria than Gram negative one. LC-MS analysis of THP-I showed abundant presence of glucose molecules, the monomeric sugar units forms β-glucans which is a polymer of carbohydrates. The proton magnetic resonance spectrum (^{1}H NMR) of the THP-I fraction gave five anomeric protons at chemical shifts (δH) 3.40, 3.42, 3.44, 3.45 and 3.94 ppm revealed that the compound is a polysaccharide. The *in vitro* cytotoxicity of THP-I (at different concentrations ranging from 25 µg/ml to 800 µg/ml) was studied by MTT assay on Vero (non cancer) and Human Colorectal Carcinoma cell line (HCT). The results revealed that the sample had no considerable cytotoxic effects on Vero cell line but it showed significant cellular toxicity against HCT cell lines at a dose concentration 200 µg/ml and showed more destructive effects over a dose of 600 µg/ml.

As per the findings in previous sections of the thesis it was evident that *p*-coumaric acid is one of the major phenolic compound found in *T. heimii* and through standard literature it was established that the content of free *p*-CA is highest in *T. heimii* (3700 mg/kg DM) than any other mushrooms (Puttaraju et al. 2006). The antibacterial activity of *p*-CA in pure form was studied against two human pathogens *Staphylococcus aureus* MTCC96 and *Escherichia coli* MTCC118 through MIC assay. For *S. aureus* and *E. coli* the MIC values were found to be 80 µg/ml and 30 µg/ml respectively indicating that *p*-CA has a remarkable bactericidal effect against those pathogens. An *in silico* study has been designed to predict the actual mechanism of action of *p*-CA at the molecular interaction level. Initially the trans-membrane proteins of *S. aureus and E. coli* were targeted for the present investigation.

A total of 642 trans-membrane protein sequences of *Staphylococcus aureus* were retrieved from whole genome (ID: 04-02981) present in IMG JGI microbial whole genome database, among them 330 sequences were selected, while in *Escherichia coli* (ID: ATCC 25922) 614 sequences were finally selected from 1121 retrieved sequences on the basis of single amino acid change and function of the proteins. In case of *S. aureus* the selected 330 trans-membrane protein sequences were subjected to homology modelling against available multiple X-ray crystallographic structures present in the structure database. Sequences of selected trans-membrane proteins from *S. aureus* were subjected to tertiary structure prediction using Phyre2 server. All the predicted structures were subjected to to ProFunc server (https://www.ebi.ac.uk/thornton-srv/databases/profunc/) for quality assessment through Ramachandran plot. Next, 3D molecular structure of *p*-CA acid was downloaded from PubChem and docked with all the selected and predicted tertiary structures using Patchdock server. Molecular docking analysis revealed that *p*-CA has multi-dimensional inhibition properties. Based on Atomic Contact Energy (ACE) value calculated through Patchdock, *p*-CA showed higher affinity towards 99 trans-membrane protein structures of *Staphylococcus aureus* and among them 62 proteins were found to be transport proteins. *p*-CA also showed affinity towards different amino acids, peptides and carbohydrate permease proteins and interaction and inhibition of those proteins may lead to nutrition depletion to the cells.

The 614 selected trans-membrane protein sequences of *E. coli* were individually aligned with 330 selected trans-membrane protein sequences of *S. aureus* using Clustal X2 and PHYLIP 3.69 software. According to phylogenetic trees 72 sequences were found to share sequential similarities and among them 59 sequences showed functional similarities whereas 13 sequences showed sequential similarities but functional dissimilarities. Through molecular docking study, *p*-CA showed higher affinity towards 99 trans-membrane protein

structures of *S. aureus*, amongst 62 proteins were found to be transport proteins. On the basis of ACE values the proper channel blocking by *p*-CA was best observed for CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase, a bacterial membrane bound enzyme which plays an important role in conversion of 1,2- diacylglycerol (DAG) to phosphatidylglycerol (PG) which is an very important integral membrane protein of bacteria. In this regard, binding and inactivation of CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase by *p*-CA will influence the accumulation of lethal DAG within bacterial cell causing membrane lysis.

The present research work highlighted the knowledge concerning the health benefits of mushrooms supplementation have become an intriguing interest in the food nutrition area, and their bioactive potentials could exploit a new way to combat multidrug resistant bacteria.