Abstract

Exploration of natural sources for novel bioactive compounds from mushrooms having considerable therapeutic potentials has been an emerging field of modern research over the past decades. Mushrooms are represented by 41,000 species across the globe; however, only 2% have been reported from India, despite the fact that one-third of the total global fungal diversity exists in the tropical Indian region.

The diversity study was conducted in and around Gurguripal ecoforest (22°25" - 35°8"N and 87°13" - 42°4"E) of Paschim Medinipur district in West Bengal, India. A total number of 2031 mushroom specimens (individuals) were observed, distributed among 67 mushroom species of 44 genera belonging to 27 families. Among the mushroom species 34 were edible, 31 non edible and 2 reported to be poisonous. The mushroom flora in this area are dominated by the family Russulaceae representing 11 species. The species richness of Gurguripal ecoforest was calculated as 0.941(Simpson's index) and the relative abundance of species was found to be 3.687 (Shannon's index) indicating the rich diversity and abundance of mushroom flora in this area. Low value of species evenness (calculated as 0.87) referred that all the 67 mushroom species were not evenly distributed numerically in the community indicating the existence of different microhabitats and microenvironments within Gurguripal ecoforest. Ethnomycological survey revealed that 19 mushroom species were effectively used in solving various human ailments and that precious knowledge was generally confined to traditional healers of the villages in Gurguripal.

In the present investigation, nine prime wild edible mushroom species occurring in this region were evaluated for their nutritional compositions. 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. Through overall comparative analysis it has been revealed that *Termitomyces heimii* and *Volvariella volvacea* possessed higher proximate compositions and nutritional potentials. Polyphenolic fractions of *T. heimii* and *V. volvacea* were extracted and tested for the estimation of major phenolic compounds and the results indicated that *T. heimii* was richer in phenols, flavonoids and ascorbic acids contents than *V. volvacea.* This result showed positive correlation with antioxidant activity as *T. heimii* exhibited higher radical scavenging ability through DPPH and FRAP assay. The results of gas chromatography revealed that higher quantities of unsaturated fatty acids (MUFA and PUFA) are present in *T. heimii* and the most abundant fatty acid was recorded as linoleic acid. EDAX analysis 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 antioxidant potentials *T. heimii* is preferred among the studied mushroom species as a potential dietary supplement.

The antibacterial potentials of different mushroom extracts from seven selected species were studied and it was found that methanolic extract of *T. heimii* showed the highest antibacterial activity against *Staphylococcus aureus* (18 mm ZOI) and *Shigella flexneri* (16 mm ZOI). The partially purified methanolic fraction of *T. heimii* namely F_{11} has shown highest antibacterial potential and further analysed through HPLC. The chromatogram indicated the presence of four carbohydrates and three major phenolic compounds, among which the highest peak was identified as *p*-coumaric acid (*p*-CA). Silver nanoparticles synthesized using *T. heimii* extract exhibited enhanced antibacterial activity showing 19 mm and 18 mm clear ZOI against *S. aureus* and *S. flexneri* respectively. Polysaccharide fractions from *T. heimii* namely THP-I and THP-II were purified and collected through gel permeation chromatography (GPC). Through MIC and MBC assay, THP-1 exhibited higher antibacterial

efficacy against Gram positive bacteria than the ram negative one. LC-MS analysis of THP-I indicated the abundant presence of glucose molecules. The proton magnetic resonance spectrum (^{1}H NMR) of the THP-I spotted five anomeric protons at δH 3.40, 3.42, 3.44, 3.45 and 3.94 ppm confirmed that the compound is a polysaccharide. The *in vitro* cytotoxicity of THP-I by MTT assay exhibited that the sample had significant cellular toxicity against Human Colorectal Carcinoma cell line (HCT) at a dose concentration 200 µg/ml and showed more destructive effects over a dose of 600 µg/ml.

T. heimii possess rich quantity of *p*-coumaric acid and pure form of *p*-CA has a remarkable bactericidal effect on pathogenic bacteria (for *S. aureus* and *E. coli* the MIC values were found to be 80 µg/ml and 30 µg/ml respectively). To find out the molecular mechanism of *p*-CA action, a total of 642 and 1121 trans-membrane protein sequences from *S. aureus* and *E. coli* were retrieved from microbial whole genome database IMG JGI. Those selected protein sequences from both bacteria were individually aligned using Clustal X2 and PHYLIP 3.69 software for constructing phylogenetic tree and among them 72 sequences were found to share sequential similarities. Through molecular docking study *p*-CA showed higher affinity towards 99 trans-membrane protein structures of *S. aureus*, of which 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 has explored the health benefits of mushrooms along with their bioactive potentials and can lead a new way to combat multidrug resistant bacteria.