General Introduction

'Mushroom' is a general term that mainly represents the fruiting bodies or fructifications of some macrofungi generally belong to the fungal class basidiomycotina and ascomycotina.They are the most diverse and important group of living organisms on earth, for their vital roles in ecosystem function as well as influence on humans and human related activities (Mueller et al. 2004). Mushrooms occupy diverse niches in nature, can be epigeous or hypogeous, large enough to be seen with the unaided eye and can be picked by hand (Chang and Miles 1992). They predominantly occur during the rainy season, particularly in forest ecosystem, where the dense canopy shade from trees provide a moist atmosphere and decomposing organic materials (leaf litter, plant debris). The consumption, utilisation and cultivation of mushrooms have a long history in association with human health and development. Wild mushrooms have significant impacts on the ecology and economy in forest-based areas. Wild mushrooms are the indicators of the forest health as well as its productivity (Stametes 2000). Fungi are the key organism in driving mineral cycling within an ecosystem and influencing the composition of biomass in other organisms. Nutrients present in plant and animal biomass become available to the saprotrophic community upon the death of the organism. The saprotrophic community on soil consisting largely of fungi, allows a proportion of the nutrients to become available to other organisms through the process of nutrient mineralization. Diversity, distribution and abundance of mushroom species are the functional criteria to assess the present status of a habitat that is helpful for the management of ecosystems in a forest. Several mushroom species play an important ecological role through symbiotic relationships with trees as mycorrhizae. From ancient period, mushrooms have been collected generally from forest by local people and some of them consumed while few have medicinal potentials. Native tribal people living in and around forest areas are directly concerned with wild mushrooms in order to their sociological and economical aspects. Mushrooms take part in mineral cycling and thus can be used as biofertilizer to enhance agriculture (Hawksworth 1991; Pointing and Hyde 2001). Moreover, they have important roles in medicine, food and biotechnological industries (Manoharachary et al. 2005).

Mushrooms have been valued throughout the world as food due to the rich content of protein, vitamins, minerals, fibres, trace elements and low cholesterol (Agrahar- Murugkar and Subbulakshmi 2005, Wani et al. 2010). From the beginning of civilization mushrooms have been considered as a special category of food. The Greeks thought that mushrooms provide strength for warriors during war. The Pharaohs appreciated mushroom for their delicacy, and the Romans praised mushrooms as the "Food of the Gods" and they served mushrooms in the menu only on festive occasions. Since mushrooms have the high content of proteins, usually about 20-30% on dry weight basis, considered as "poor man's protein". Moreover, due to the low fat content, they are recommended as ideal source of dietary supplement for patients with cardiac trouble or at threat with lipid. Their energy value also varies from species to species. Earlier, mushroom consumption was confined to specific areas of the world, but due to globalization, easy transportation and growing demand, the accessibility of mushrooms has spread rapidly to all areas. Wild edible mushrooms are frequently occurring in forest floor and widely appreciated as food due to their unique taste and flavour. Collection and consumption of wild edible mushrooms from nature is an imperative source of healthy nutrition for the rural population in many parts of Asia, Africa, South America and Europe (Nakalembe et al. 2009). The knowledge about their utility is passing from generation to generation by the elders of the respective area who are engaged in mushrooms collection and their consumption. Several reports have been documented on nutritional potential of wild mushrooms from Africa, Europe and Asia including India

(Barros et al. 2008; Kalač 2009; Pushpa and Purushothama 2010; Manjunathan and Kaviyarasan 2011; Singdevsachan et al. 2013). Mushrooms have been informally categorised along with the 'white vegetables' account for the huge amount of essential nutrients, associated with health benefits (Weaver and Marrs 2013).

Today, there is a growing interest in traditional medicine and an increasing demand for alternative drugs from plant resources. Researchers are searching for the development of alternative drugs from herbal products against pathogenic microbial strains. Since ancient times, mushroom nutraceuticals have been applied for the treatment of several human ailments. Their profound nutritional characteristics attracted the nutritionists, researchers, and pharmacists to explore their usage for the human welfare and included them as an important food supplements in human diet. Although mushrooms have been used as medicine in China since 100 A.D. (Gunde-Cimmerman 1999), but the basic active principles of mushrooms were investigated by the scientists since 1960. Mushrooms also have immense medicinal values as they are enriched by various bioactive substances like antibacterial, antifungal, antiviral, antiparasitic, antioxidant, anti-inflammatory, antiproliferative, anticancer, antitumor, cytotoxic, anti-HIV, hypo-cholesteromic, antidiabetic, anticoagulant, hepato-protective compounds (Wasser and Weis 1999; Ajith and Janardhanan 2001; Lindequist et al. 2005). Many varieties of naturally occurring mushrooms are found to have significant antioxidant and anticancer properties and prolong longevity (Mizuno 2000). The search for natural bioactive molecules that can serve as antimicrobial, antioxidant and anticancer agents gained interest both in industry and scientific arena. Although there are many commercial drugs in market to fight against microbes and free radical induced diseases but they are proved to be ineffective gradually (Nwachukwu and Uzoeto 2011). To overcome the disadvantages of synthetic antioxidants and antimicrobial drugs, interest has been focused on natural resources for novel bioactive agents.

Functional foods rich in natural antioxidants contain phytochemicals that combat oxidative stress in our body by maintaining a proper balance between oxidants and antioxidants. Consumption of these foods is a practical approach to minimise oxidative stress. Regular consumption of foods containing natural antioxidants can diminish the risk of chronic diseases (Oboh and Shodehinde 2009). Phenolic compounds are secondary metabolites, one of the most potent sources of antioxidants, and are naturally found in plants, honey and mushrooms (Kolayli et al. 2012). It is now established that mushrooms are an important source of chemically diverse novel secondary metabolites (terpenes, steroids and phenolic compounds) which possess a wide spectrum of biological activities (Turkoglu et al. 2007; Khatua et al. 2013). Besides these health promoting attributes such as the antioxidant, antitumor, anticholesterol and immuno-enhancing effects have also been documented earlier from many species of mushrooms (Barros et al. 2007b). Both fruiting bodies and the mycelium-contained compounds have a wide range of antioxidant and anticancer potentials (Ferreira et al. 2007). Several workers also investigated promising antioxidant properties from a number of mushrooms in India. Extracts from fruit bodies and mycelia of *Ganoderma lucidum*, *Phellinus rimosus* and several *Pleurotus* sp. from South India were found to possess antioxidant properties with high free radical scavenging activity (Jones and Janardhanan 2000; Ajith and Janardhanan 2001; Lakshmi et al. 2003). In this scenario mushroom derived secondary metabolites like phenolics, flavonoids, ascorbic acid, terpene compounds possess significant antioxidant properties and have an immense value in human diet.

Beside these compounds, polysaccharides are the main component for the bioactivities of some mushrooms. Polysaccharides present in mushrooms exhibit a limitless structural diversity that responsible for performing many biological functions. The diverse activities displayed by mushroom derived polysaccharides include antimicrobial, anticancer, anti-inflammatory, antioxidative, immunomodulatory and hepatoprotective effects (Ruthes et al. 2013; Xu et al. 2014; Ferreira et al. 2015). The mushroom's cell wall is composed of carbohydrates (with β-glucan bonds mainly), proteins and lipids (Kapteyn et al. 1995; Sanjuan et al. 1995). Mushroom derived glucans are renowned as biological response modifier and used for the treatment of various types of cancer and infectious bacterial diseases (Chan et al. 2009). Moreover, they are also functional as immunomodulator (Wasser and Weis 1999), antitumor (Wasser 2002) as well as antioxidant agents (Blokhina et al. 2003; Kozarski et al. 2011; Patra et al. 2013).The polysaccharides like glucans and heteroglycans have been reported from the various species of *Termitomyces* such as *T. eurhizus* (Mondal et al. 2004), *T. striatus* (Mondal et al. 2006), *T. robustus* (Chandra et al. 2007), *T. microcarpus* and *T. clypeatus* (Pattanayak et al. 2015). Polysaccharide composition is very much correlated with their pharmaceutical activities, and this relationship has become the topic of increasing research attention (Lo et al. 2011; Li et al. 2016). Polysaccharides from several edible mushrooms were reported to have antitumor effects against human cell lines. These polysaccharides generally belong to the glucans and exert their antitumor effects, not directly by a cytotoxic effect on cancer cells, but indirectly by stimulating the immune function, allowing a host to combat better against cancer cells.

Presently, the world is facing critical challenges in healthcare services because many antimicrobial drugs have lost their effectiveness in treating human diseases due to the gradual development of microbial resistance (Balouiri et al. 2016). Thus, the exploration of new bioactive compounds effective in treating drug resistant pathogenic microorganisms is very necessary (Freire-Moran et al. 2011). Antibiotics inhibit the growth or kill microorganisms in several ways, by interfering in metabolic processes or disrupting the organism structures (Fuchs et al. 2004). The mechanism of action is mostly related with interferences in the synthesis of the cell wall, modification of plasmatic membrane permeability, interferences in chromosome replication, or in protein synthesis (Tenover 2006). Despite the huge diversity of antibacterial compounds, bacterial resistance to first-choice antibiotics has been drastically increasing. Diseases that were easily healed are nowadays becoming a serious problem due to emergent antibiotic resistance (Peres-Bota et al. 2003). Bacteria like *Klebsiella* spp. and *Escherichia coli* synthesize broad-spectrum beta-lactamase and showed resistance to third-generation cephalosporins (Harbarth et al. 2001). Therefore, the search of new antibacterial substances effective against pathogenic microorganisms resistant to current drugs is crucial. Mushrooms are immensely rich in bioactive compounds and can be an alternative sources for new antibacterial agents. The secondary metabolites derived from mushrooms that have therapeutic effects can be isolated and purified to produce new drugs. Such mushroom based drugs are efficient and work as natural medicines for the treatment of different human ailments. This efficacy of mushrooms had proven over the long era, but the mode of action of folk medicines and related products from nature is even more complex than mechanistic clarification of a single bioactive molecule. Unfractionated or partly fractionated mushroom extracts contain mixtures of different constituents and exhibit synergistic effects most likely. Evaluation and isolation of these mixtures of the active constituents and their mode of action are the challenging tasks prior to their therapeutic trials.

Nanotechnology has been an emerging field of research and achieved growing importance in the recent past. Metal nanoparticles have gained great interest due to their distinguishing features such as electrical, optical, magnetic and catalytic properties (Bar et al. 2009). Biosynthesised silver nanoparticles (AgNPs) showed strong antibacterial activity against a few pathogenic bacteria (Muthukrishnan and Nanda 2013). In this respect nanoparticles have enhanced antibacterial activities as they can straightforwardly penetrate into the genome of bacteria and deactivating DNA and the enzymes leading to cell death. They possess a greater surface area for effective bactericidal interactions (Pal et al. 2007). Thus the use of AgNPs is considered as one of the promising approaches for overcoming antibiotic resistance of microorganisms. Chemical and physical synthesis methods of AgNPs produce hazardous by-products and require high energy consumption also. In this regard, biological methods for green synthesis of AgNPs is eco-friendly does not cause any fatal effects on human health. Due to high content of reducing and stabilising agent for synthesis of nanoparticles, biological resources such as mushrooms are now employed for this purpose (Sudhakar et al. 2014).

In recent past, there are increasing number of reports regarding phenolic compounds in many mushroom species and among them benzoic acid and cinnamic acid derivatives have been reported as highly effective. Benzoic acid derivatives like *p*-hydroxybenzoic, protocatechuic, gallic, vanillic and syringic acids were detected in different mushroom species, whereas the identification of cinnamic acid derivatives such as *p*-coumaric, *o*-coumaric, caffeic, ferulic and chlorogenic acids were also described (Mattila et al. 2001; Puttaraju et al. 2006; Kim et al. 2008; Barros et al. 2009; Heleno et al. 2011, 2012). There are a number of reports on bioactivity of different mushroom species (Barros et al. 2007b; Quereshi et al. 2010; Ozen et al. 2011), but studies with the individual compounds present in that species are scarce, except some phenolic compounds isolated and identified from plant resources (Kuete et al. 2009; Orhan et al. 2010; Lou et al. 2012). In this context Alves et al. (2013) has performed a structure-activity relationship analysis and molecular docking studies against penicillin- binding protein 2a (PBP2a) in order to provide insights into the mechanism of action of some selected phenolic compounds against drug resistant bacteria.

Molecular docking is a computer based procedure that exactly predicts the non-covalent binding of a larger molecule (receptor) with a smaller molecule (ligand) efficiently. Docking can also be used to predict the bound conformation of target binders, when the experimental holo structures are unavailable (Sousa et al. 2006). Docking studies are performed to determine the affinity and interactions of antibacterial compounds with bacterial proteins as well as their molecular mechanism of action: inhibitors of cell wall synthesis, inhibitors of nucleic acids synthesis, inhibitors of protein synthesis etc. The prediction of such interactions has an enormous practical importance because they are used to screen virtual libraries of drug-like molecules to obtain leads for future drug development.

Studies have indicated that wild edible mushrooms are not only important sources of food and medicine but also have profound economic importance in both developing and developed countries (Boa 2004). Only about 6.7% of the 1.5 million species of fungi estimated in the earth have been described and identified. Those are mostly occurring in temperate regions, whereas the fungal diversity of tropical regions has not been exploited enough (Hawksworth 2001). Suryanarayanan et al. (2003) opined that fungal diversity is greater in the tropics than that in temperate regions. In India the diverse climatic conditions lead to rich mushroom diversity and form a valuable non-timber forest resource for local folk. Still a large segment of tribal population depends on hunting and gathering of forest products for subsistence and survival of traditional folklore (Malhotra et al. 1992; Deb and Malhotra 1993). The different types of wild mushrooms consumed by the tribal people as food or medicinal purposes vary with locality and tribe to tribe. Ethnic tribes of India are reported to consume nearly 283 species of wild edible mushrooms (Purakayastha and Chandra 1985). Nearly 100 species of mushrooms are known to be poisonous to humans (Diaz 2005). Though ethnic tribes of our country are experienced in distinguishing poisonous from non-poisonous mushrooms, but few cases of mushroom poisonings were also reported in India (Purakayastha and Chandra 1985; Kumar and Kaviyarasan 2012; Sharma 2010). A significant number of mushroom species are traditionally sold in rural markets of India and some of them have been commercially utilised as food or medicines (Tanti et al. 2011). A wild edible mushroom *Morchella* sp. has emerge as one of the handsome sources for income generation in the tribal areas of Uttarakhand (Kumar et al. 2017). Mushrooms have been extensively studied in the western countries, while tropical countries such as India are less well explored. Several researches have been reported about traditional and ethno-medicinal knowledge of some mushrooms in some specific regions of India including the studies of Sikkim (Das 2010), Jammu & Kashmir (Kumar and Sharma 2011), Amarkantak Biosphere Reserve (Dwivedi et al. 2012), Bangalore, Karnataka (Pushpa and Purushothama 2012), Nagaland (Kumar et al. 2013), Meghalaya (Khaund and Joshi 2013), Western Ghat ranges (Thiribhuvanamala et al. 2014) and in West Bengal (Dutta and Acharya 2014).

In this context no such work was done in forest floor of Paschim Medinipur district of West Bengal, India. This district is well-known for its great regional as well as geographical diversity and experienced by a great variation in climatic characteristics. The climate of the northern and western part (adjacent to the district Bankura) of the area is being characterized by arid climate having a vicious dry heat in summer, a short winter season and moderate rainy season. While the climate of eastern and southern part (adjacent to the district Purba Medinipur) are different in nature, characterized by humid climatic condition. The average annual rainfall occurs in the area is 1550 mm which is very much variable in nature from place to place. At least 74% of the total annual rainfall occurs in monsoon period. The average annual range of temperature varies from 39 °C in summer to 10 °C in winter. The predominant soil of Paschim Medinipur district is lateritic soil which is red and yellowish in colour. The natural vegetation of the district is mainly of tropical deciduous type comprising of mixed forest trees, grasses and shrubs. In this lateritic soil, the plant species like Sal (*Shorea robusta* Roth), Mahua (*Madhuca indica* J.F.Gmel**.**), Palash (*Butea monosperma* (Lam.) Taub.), Kusum (*Schleichera oleosa* Lour.), Kurchi (*Holarrhena antidysenterica* (L.) Wall. ex A. DC), Kendu (*Diospyros melanoxylon* Roxb.), Teak (*Tectona grandis* L.f.), Neem (*Azadirachta indica* A.Juss.), Arjun (*Terminalia arjuna* (Roxb.) Wight & Arn), Siris (*Albizia lebbeck* (L.) Benth.), Gamari (*Gmelina arborea* Roxb.) etc are found. Some planted forest segments comprising *Anacardium occidentale* L., *Acacia auriculiformis* A.Cunn. ex Benth. and *Eucalyptus globulus* Labill. are noted also. In Paschim Medinipur district, total forest cover area is 171935 hectares, out of which protected area occupy 160179.30 hectares.

Gurguripal ecoforest is one of such protected forest area (Beat) controlled by Midnapore forest division of Paschim Medinipur, West Bengal. During Monsoon the dense forest of Gurguripal becomes the perfect climatic zone for the growth of macrofungi. Several wild mushrooms occurred in the forests of Gurguripal and frequently collected by local tribal people. Tribal communities live in and around Gurguripal have indigenous knowledge about food and ethnomedicinal uses of wild mushrooms occurring in this region. But no significant research or findings about diversity of mushrooms and their antibacterial potentials have been attempted so far. In this context, the present research work was designed to evaluate the diversity and antibacterial potentials of wild mushrooms in Gurguripal ecoforest, West Bengal, India.