

INTRODUCTION

1.1. General introduction:

Natural resources have been a gift to people. It has supplies for all the essential needs, viz. foods, shelters, therapeutic agents, medicine etc. A number of accountable drugs have been derived based on medicinal knowledge of traditional healers (Kinsalin *et al.*, 2014). Among the uncountable diversity of natural resources, fungi hold an important share. Popularly known for the delivery of first antibiotic from fungi and also providing a good source of protein and store house to many bioactive components, some of which have been explored and rest others are yet to be explored. Fungi have fossil records from as early as Precambrian era (Heckman *et al.*, 2001).

Fungi are considered to be among the most diverse organisms on earth. Fungi include morphologically diverse groups with different structure of fruiting bodies, from unicellular yeast to hyphal forms. Diversity of fungi is not well explored and the total number of species that exists in nature is still a paradox, but nevertheless is a rich source for exploitation (Monoharachary *et al.*, 2005). The estimated diversity of fungi is about 140000 species of which about 10% is known and nearly 2000 species are known to have variable degree of edibility and about 700 species to have pharmacological properties (Chang, 1999; Wasser and Weis, 1999; Reshetnikov *et al.*, 2001). Fungal diversity is higher at mid altitude range of about 1500 m to 2500 m above mean sea level and decreases on both the extremes of referred altitude. Few fungi are found and restricted to only alpine habitat and are absent in lower altitudes. The macro-fungal diversity is affected by many factors like soil, water content, soil temperature, relative humidity, soil cover, litter depth and moss, canopy openness and density of trees (Gomez-Harnendes *et al.*, 2012).

Many fungal groups like terricolous fungi, hyphomycetes, yeasts and microcolonial fungi can be found in extreme environment like deserts. Fungi can resist stress like water deficit and desiccation by forming spores (Sterflinger *et al.*, 2012). Fungi can also be found at very high altitudes (Schmidt *et al.*, 2008). Many fungi form mycorrhizal association and help nutrient uptake and also enhance the host plant's ability to resist drought and biotic stress. They are helpful in soil stability and water retention capacity of soil (Gianinazzi *et al.*, 2010).

Fungi can be categorised on the basis of sexual reproduction into Deuteromycota where sexual reproduction is absent and four other groups where sexual reproduction is well-known which includes Oomycetes, Zygomycetes, Ascomycetes and Basidiomycetes (Guarro *et al.*, 1999).

The fruiting body of fungus are the reproductive structure, which grows above the ground and on a substrate and can be seen by a naked eye is termed Mushroom. Majority of mushrooms belong to the group Basidiomycetes and few are Ascomycetes.

The term Basidiomycetes is derived from a Greek word “basidium” and “mykes” meaning small base fungus. Basidiomycetes are most advanced group of fungi with large and conspicuous fructification which includes mushrooms, toadstools, bracket fungi and puff balls etc (Hood, 2006). They lack photosynthetic pigments and they have heterotrophic mode of nutrition, they are mainly saprophytic in nature (they derive their nutrition by decomposing cellulose and lignin). Sexual reproduction is through plasmogamy by fusion of basidiospores and monokaryotic spore or spore & hyphae or two monokaryotic hyphal cells. After dikaryophase and karyogamy, the secondary mycelia are formed that are profusely branched with septate hyphae. The septa have dolipores called clamp connection (an outgrowth on the side of septa for proper distribution of dikaryons in cell division). Karyogamy and meiosis occurs in a club shaped cell called basidia (Guarro *et al.*, 1999).

1.2. Mushrooms:

1.2.1. Importance of mushrooms:

Down the time line with the rate of population growth, three major problems, viz. food scarcity, pollution and loss of agricultural lands shall be of prime concern. A part of that can be achieved with mushrooms due to its short life cycle, a low cost of production & good quality protein diet & easy condition of growth. Mushroom can be a key link to manage solid agro waste thereby reducing pollution (Philippoussis *et al.*, 2011).

Mushrooms have important role in environment as decomposers, nutrient and element cycling, forestry, agriculture, industries, pharmaceuticals, food and human health (Xu *et al.*, 2011; Chang, 1980).

1.3. Health benefits of Mushrooms:

1.3.1. Nutritional quality:

Mushrooms are considered as health food from ancient times. Greeks considered mushrooms to provide “strength for warriors”, as “Food of Gods” by Romans, “a gift from God Osiris” by Egyptians and Chinese considered mushrooms as “the elixir of life” (Velvarde *et al.*, 2015). Mushrooms are rich in many beneficial components, viz. protein and considerable amount of carbohydrate. Mushrooms are also known for low starch, low in fat and even the minimum content is dominated by unsaturated fatty acids like linoleic acid and oleic acid (Ribero *et al.*, 2009). Mushrooms are predominantly rich in minerals and vitamins, however the nutritional content varies across the genus and species. The nutritional content is also dependent on the substrate, stages of growth and the specific part of fruiting body (Eguchi *et al.*, 2015). They are also rich in many bioactive components targeting varieties of therapeutic activities. Mushrooms are endowed with all the essential amino acids & secondary metabolites like phenols & flavonoid with good antioxidant properties preventing oxidative stress and many degenerative diseases (Kozarski *et al.*, 2015).

1.3.2. Medicinal properties:

It has been associated with humans from thousands of years, they have been used by traditional healers (Geng *et al.*, 2014). The uses of mushrooms as medicines are thought to be originated from Far East (Hobbs, 1995; 2000).

Many mushrooms have been identified to possess medicinal properties that include *Auricularia auricularia*, *Tremella fuciformis*, *Tremella mesenterica*, *Schizophyllum commune*, *Grifola frondosa*, *Tremetes versicolor*, *Hericium erinaceus*, *Ganoderma lucidium*, *Lentinula edodes*, *Flammulina velutipes* etc (Wasser, 2002). They are known to have potential activities like anti-inflammatory, anti-tumour, anti-viral, antibacterial, immunomodulating, hepatoprotective, cholesterol reducing, anti-hypertensive, joint pain, anti-allergic etc (Yip *et al.*, 1987; Stamets, 2011; Hobbs, 2000; Venkatachalaputhi & Paulsamy, 2016; Punitha & Rajasekaran, 2016). The main component includes polysaccharides, proteoglycans, terpenes, alcohols, sterols etc (Velvarde *et al.*, 2015).

1.4. Taxonomy:

Mushrooms have always fascinated with its diverse shape, size and colour. The practice of collection of wild mushrooms and mycophagy (consumption of mushrooms) dates back to thousands of years (Valverde *et al.*, 2015). The traditional knowledge about its

edibility is noteworthy. Many tribes have been collecting the wild mushrooms without proper scientific knowledge about its identification. Their knowledge of differentiating edible and poisonous mushrooms has been passed through generations by trial and error method (Huffman, 2008).

Taxonomy of mushrooms has been a cumbersome work. Closely grouped mushrooms share many overlapping characters marked by fine details like spore shape, spore morphology & colour of spore print, hyphal arrangement, types of cystidia, types of cells or their arrangements etc. Sometimes overlooking any of these details often leads to misidentification resulting in poisoning sometimes with serious fatal consequences up to death. Thus, classical taxonomy alone is not appropriate to delimit the species. Classical taxonomy mainly takes into account its morphological characters that include size, colour, shape of pileus and stipe (Kumari, 2011). Microscopical characters like basidia, basidiospores, pleurocystidia, cheilocystidia, caulocystidia, types of hyphae, cell types and orientation of hyphae in pileus and stipe (Menolli *et al.*, 2010). Thus classical taxonomy coupled with modern taxonomy is important to correctly place a species in correct taxon (Kumari *et al.*, 2011). The molecular taxonomy includes DNA sequences from conserved regions which are conserved or very less variable. In mushrooms, the important markers includes ITS and LSU regions which are choice of taxonomist for taxonomical studies (Schoch *et al.*, 2012).

1.5. Problem and gap in studies:

North East India falls under Eastern Himalayan biodiversity hot spot (Chatterjee *et al.*, 2006). The climatic conditions favour the existence, propagation and rich distribution of many biotic communities some of which are endemic to this region including different species of mushrooms. Kokrajhar lies in the foot hills of Bhutan having rich diversity of mushrooms. The preliminary survey suggests that no detail study on nutritional evaluation and molecular identification have been done to the extent of distinctly identifying and characterization up to species level. Only after establishing proper identity, chemical properties and antimicrobial activity, their virtue in term of mycophagy and therapeutic dimensions can be commercialized, potentially explored and utilized.

1.6. Aim and objectives:

Understanding the wide application of macro-fungi and also considering the potential gap in available literature regarding the taxonomy, biochemical profiling, nutritional and

antimicrobial study in the mentioned area, the following objectives are anticipated in the present study:

1. To carry out detail taxonomic work.
2. To evaluate the nutritional properties.
3. To study the extract specific antimicrobial activity of candidate mushroom species.
4. To evaluate the bioactive components of identified mushrooms.
5. To perform *in silico* studies pertaining to evaluation of bioactive molecules against microbial proteins by docking.