Chapter 1

Collection and identification of Tricholoma giganteum Massee
Introduction

The fleshy fungi of class Ascomycetes and Basidiomycetes are generally termed as mushroom. These fungi had attained the status of a regular crop in France and China by 17th and 19th centuries, respectively, spreading gradually to the other countries in few years. To date, about 1,200 species of fungi belonging to the order Agaricales, Russulales and Boletales are described in comparison to about 14,000 species of mushrooms reported worldwide that contributes 10 percent of the global mushroom flora. Globally, there is an estimate of 2000 species of mushrooms considered to be edible. Among them about 283 are reported from India (Thiribhuvanamala et al., 2011). These data on the occurrence of the mushrooms reveals the richness of mycoprotein in the country. The Indian sub continent is blessed with diverse agroclimatic zones that harbour a treasure trove of fungal diversity. In India, mushroom is a unique non-traditional cash crop grown indoors, both as a seasonal crop and round-the-year under the controlled environmental conditions. Climatic conditions in India are favourable for natural occurrence of mushrooms and some of them are regularly collected and used as food by the natives particularly those belonging to tribes. The collections of mushrooms began in India four decades ago (Kamat et al., 1971; Sathe and Rahalkar, 1976; Purkyayastha and Chandra, 1976). Efforts need to be made to identify and exploit these mushroom floras for utility as their biodiversity and conservation strengthen the food security of a country (Lakhanpal, 1994).

The practice of eating mushroom probably began during the hunting and gathering period, in our prehistory. They were collected along with fruits and berries, as well as other plant material that could be consumed. Also, like plants the gatherers learned which ones were edible and which were poisonous and if there were other uses for mushrooms, i.e., medicinal or religious uses. However, unlike plants, mushrooms must have been shrouded in mysteries since unlike the plants; there was not an obvious way in which they could be reproduced. Even much later, during the dark ages, mushrooms became more firmly embedded in the mythology of the supernatural. Many myths arose because of their seemingly supernatural characteristics and strange habitat. Their growth was rapid and they seem to suddenly appear overnight as if from nowhere. Thus, their origin appeared to be magical. They became part of the lives of fairies, elves and witches. Mushrooms
have been used as food and medicine by the ancient Egyptian, Greek, Roman and Chinese civilizations. The fact that ‘some mushrooms could be eaten’ was established thousands of years ago. The archaeological record reveals edible species associated with people living 13000 years back in Chile (Rojas and Mansur, 1995) but it is in China where the eating of wild mushroom was first reliably noted, several hundred years before the birth of Christ (Aaronson, 2000). Edible mushroom were collected from forests in ancient Greek and Roman times and highly valued, though more by high-ranking people than by peasants (Buller, 1914). Today, mushrooms are liked all over the world due to their delicate taste, flavor, texture and health giving properties. In the last few decades, there has been increasing worldwide attention on the use of wild edible mushrooms (FAO, 2004) and biochemical investigations have put forward their merit as a food item. Mushrooms contain good quality protein, unsaturated fatty acids, minerals and vitamins (Wahid et al., 1988). These are low in fat, carbohydrates, salts and rich source of dietary fiber (Genders, 1990). Moreover, nucleic acid content in mushrooms is very low and hence, these are considered an ideal food for patients suffering from hypertension, diabetes and obesity (Anonymous, 2003). Not only in terms of edibility, there lie enormous applications of these mushrooms for bioremediation, biodegradation, biopesticidal and pharmacological values that could be exploited. India has a special position in macrofungal map of the world (Manoharachary et al., 2005). But, only a fraction of total fungal wealth has been subjected to scientific scrutiny (Swapna et al., 2008).

From an ecological and also an eventual cultivable perspective, mushrooms can be considered in three distinctive modes of growth, viz. as saprophytes, parasites or in a mycorrhizal association (Stamets, 1993). According to R. Gordon Wasson, the father of ethnomycology, how readily a person will collect and consume wild mushroom is dependent upon the culture in which they were raised. Mushroom diversity is abundant in nature, especially in forests. Mushroom collectors flock the areas during the growing season chiefly in rainy season to hunt for prized and delicious ones. However, it is the experience and knowledge of the people about these mushrooms that comes handy in choosing from the diverse types. Correct taxonomic identification and established facts about edibility of mushrooms should be followed while collecting mushrooms from the wild for food and medicinal purpose. In the 18th century, presumably Linnaeus was the first person who
identified a few mushrooms from India. However, a thorough systematic study of Indian fungi was initiated by Dr. E. J. Butler (1931). Kirtikar (1918) appears to be the first Indian Mycologist who collected and identified some of the local mushrooms. During the last few decades some fragmentary reports on edible mushroom have been published by different Indian workers. Some Mycologists of this country have prepared excellent monographs on certain groups of fungi. Only a comprehensive account of the Indian species of edible fleshy fungi is available so far by Purkyayastha and Chandra, 1985. The study of taxonomy regarding Indian mushrooms has been accounted to be declining in recent times (Hyde, 2003). In West Bengal, some inventories during the last ten years have been of assistance to make known the affluence of an assortment of mushrooms present in the state. This is mostly attributed to the location of various ecological domains within the state. The elevation of land of the state ranges from coastal to subalpine. Variable blend of climatic and edaphic aspects are also responsible for this affluence.

Here, in the present chapter an attempt has been made to collect, characterize and identify the mushroom specimen, *Tricholoma giganteum*.

**Materials and methods**

**Collection and Description:**

*Tricholoma giganteum* was collected mainly during the monsoon season from village areas of Gangetic plane and coastal region, West Bengal, India, when the condition for the fruiting of the fleshy basidiocarps is favourable. Edibility of the mushroom was determined by interaction local and ethnic people and by observing the mushroom that was being sold in the market. The documented information was verified by cross checking with relevant literature and key informants like village elders. The collection sites were located by through surveys. The specimen was at first photographed and given proper field codes, then fresh and healthy specimens of the species were collected by digging (not pulling) them up as not to damage their bases (Hawksworth, 1974).

During and after collection, mushroom along with their surroundings were carefully observed and the salient features were noted which facilitated the identification process. In this regard, collection number (field code number), place of
collection, latitude, longitude and altitude of the location, date of collection, type of habitat, frequency of occurrence, name of the associated host, forest type, name of the collector, features and colour of cap, gills and stipe etc. about the specimen was recorded in the field notebook (http://www.for.gov.bc.ca/ric, 1999). The fungus was wrapped in aluminum foil or wax paper bags to block the moisture flow (Das and Sharma, 2005; Buyck et al., 2010).

Identification of the specimen:

The specimens were brought to the laboratory and microscopic features were determined by the use of Carl Zeiss AX10 Imager A1 phase contrast microscope. Microscopic studies were carried out on dry and wet samples, mounted in 5% KOH and Congo red. For spore measurement, 30 spores from six mature collections (n=30) were studied. Characters of the specimens in hand were systematically matched with a mushroom species already known to science; follow the sequence of family, genus and then the species (Largent and Thiers, 1973; Stuntz, 1973). Identification process of the specimen was carried out through consultation with specialized literatures resources and identification guides (Ramsbottom, 1965; Pegler, 1977, 1983, 1986; Purkayastha and Chandra, 1985; Singer, 1986; Pegler et al., 1998).

The voucher specimens have been deposited with the accession code AMFH in the mycological herbarium of the University of Calcutta, Kolkata, West Bengal, India.
Results and Discussion

Observation:

Taxonomy


Position in classification:

Kingdom: Fungi
Phylum: Basidiomycota
Subphylum: Agaricomycotina
Class: Agaricomycetes
Subclass: Agaricomycetidae
Order: Agaricales
Family: Tricholomataceae
Genus: Tricholoma
Species: Tricholoma giganteum

Source:
http://www.mycobank.org/BioloMICS.aspx?Link=T&TableKey=1468261600000067&Rec=339196&Fields=All

Macroscopic characters:

Pileus 30–35 cm diameter, conico-convex then expanding; surface initially white, soon gray with a glaucous tint, paler towards the margin, glabrous and silky smooth but cracking on drying; margin slightly incurved, scurfy, often cracking (Figure 2.1a). Lamellae emarginate, sinuate, straw yellow, ventricose, densely crowded, with lamellae of four lengths (Figure 2.1b). Stipe 15–18 × 6 cm, cylindrical, often elongate, solid to finally fistulose; surface concolorous with pileus, fibrillose-striate. Context up to 3 cm thick at disc, gradually thinner towards margin, white, firm. Spore print white.

Microscopic characters:

Basidiospores 6.69–8.66 × 4.72–6.30 μm [Q=1.37–1.41, Qav = 1.39, n = 30 spores, s = 3 specimens examined], ovoid to short ellipsoid, hyaline, inamyloid, thin walled (Figure 2.1c). Basidia (25.6–) 27.58–30.73 (−31.5) × 7.88–8.27 μm, narrowly

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clavate to subcylindrical, tetrasterigmatic; with basal clamp connection (Figure 2.1c). Lamellar edge fertile. Cheilocystidia absent. Hymenophoral trama regular, parallel, consisting of 2–5 μm diam., thin walled hyphae. Subhymenial layer narrow, 5–9 μm wide, interwoven. Pileipellis a compact, repent cutis of interwoven hyphae, 7.88–15.76 μm diam. Context consists of 2–8 μm diam, inflated to 25 μm diam, thin-walled hyphae, with clamp connections.

Figure 2.1: Photographs of Tricholoma giganteum. a. Fruit body. b. Mature fruit body showing gills. c. Basidium (inset Basidiospores).

Distribution and habitat:

T. giganteum is widely distributed throughout the tropical and subtropical of Asia and Africa. It has been reported to grow on farms and along the roads in south of Gunmaken, Japan (Zhuang and Mizuno, 1995). Recently, T. giganteum was found in Yunnan Province, China (Yuan-zhong et al., 2004). In India, it is reported growing widely in summer in Indo-gangetic plains of Howrah district, Hooghly in India (Chakravarty and Sarkar, 1982). Growing terrestrial, gregarious; saprophytic. It has also been reported growing at the base of Borassus flabellifer, Phoenix
dactylifera. It is most conspicuous during monsoon season. They are popular among the people because of their being a gastronomic and nutritional delicacy in the gangetic planes and coastal regions of West Bengal, where it is known by the name of “boro dudh chhatu”.

Specimen examined:

INDIA, West Bengal: South 24 Parganas, Gosaba block, Kachukhali, on soil, 08 August 2008, Soumya Chatterjee, AMFH 399.

Remarks:

*T. giganteum* is being characterized by its white colored conico-convex cap with slightly incurved glabrous margin and sinuate emarginated lamellae. Ovoid to short ellipsoid hyaline spore with an average Q value (Q$_{av}$=1.39) and the absence of pleurocystidia and cheilocystidia distinguishes this species from the other species of *Tricholoma*. Morphologically this specimen closely resembles with another pure white colored mushroom *Calocybe indica*, but differs due to absence of siderophilous granulation in mature basidia (Pegler *et al.*, 1998).

Edibility and other uses:

This is an edible species of *Tricholoma* and is considered to be highly nutritive. It contains essential macro, and micronutrients, in addition to rich amount of carbohydrate, protein, fibre contents. Taste of the mushroom is excellent and has a good odor and aroma (Prakasm *et al.*, 2011). The amino acid composition of *T. giganteum* includes alanine, glutamic acid, aspartic acid, serine and glycine and is contained in large amounts. This mushroom showed especially high content of aspartic and glutamic acids which closely relate to its taste (Fujita *et al.*, 1990a). Organic acids such as Oxalic and succinic acids were found to be predominant in the mushroom when determined by high performance liquid chromatographic method (HPLC) (Fujita *et al.*, 1990b). From this point of view, it is expected that *T. giganteum*, might be useful as a food of an excellent taste. The fruiting body of *T. giganteum* has many pharmaceutical uses and has long been utilized as a home remedy in Asia (Lee *et al.*, 2004). However, few studies were done on the pharmaceutical effects of *T. giganteum*. Previous works regarding *T. giganteum* revealed antitumor activity of water soluble and water insoluble fraction against
Sarcoma 180 solid tumor implanted in mice (Mizuno et al., 1995, 1996). Apart from this, angiotensin I-converting enzyme (ACE) inhibitory peptide isolated from *T. giganteum* showed a clear antihypertensive effect in spontaneously hypertensive rats. Moreover, the ACE inhibitor from *T. giganteum* was also demonstrated to be nontoxic and therefore, will be very useful in the preparation of antihypertensive drugs and functional foods (Lee et al., 2004). In a study, an antifungal efficacy of a protein isolated from the mushroom *T. giganteum*, Trichogin was evaluated. It exhibited antifungal activity against *Fusarium oxysporum*, *Mycosphaerella arachidicola* and *Physalospora piricola*. Trichogin also inhibited HIV-1 reverse transcriptase (Guo et al., 2005). In another study, a novel low-molecular-mass laccase purified from the mushroom *T. giganteum* was found to inhibit the retroviral reverse transcriptase (Wang and Ng, 2004).

In the present thesis, the mechanisms on chemopreventive potential of *T. giganteum* on Ehrlich’s ascites carcinoma cells, forestomach and lung carcinogenesis in mice were delineated in the forthcoming chapters.
References


FAO (Food and Agricultural Organization) (2004). Wild edible fungi, a global overview of their use and importance to people. Non-wood forest products. Food and Agriculture Organization, United Nations Rome, Italy.


