Introduction

Fungi formed a part of human life from thousands of years. They have been utilized in several parts of the world as food, in preparing alcoholic beverages and also as medicines. A new era of fungal research actually started with the discovery of Penicillin by Fleming in 1928. Value addition to the knowledge of fungi has continuously been taking place with the urge to explore new organisms for unknown commercially exploitable, bioactive metabolites.

1.1 Medicinal mushrooms

There is always an urge to search for newer and more effective medicines around the globe. The resources could be uncountable but hidden in nature. The traditional medicinal systems all over the world could serve as templates for identifying new drugs. Fungi especially macro-fungi (regarded as mushrooms) are long known to have medicinal properties (Hobbs 1995, Denisova 2002, Smith et. al. 2002).

Mushrooms are traditionally used in the Chinese medicine and commonly used for the pharmaceutical purposes and health foods. Increasing experimental evidence indicates that mushrooms contain a large number of biologically active components that offer health benefits and protection against degenerative diseases like cancer and diabetes. Many pharmaceutical substances with potent and unique health enhancing properties have been isolated from various mushrooms (Wasser & Weis 1999, Smith et. al. 2002). For thousands of years, different communities have made use of many mushrooms as a medicine (Blanchette et. al. 1992; Vaidya 1995).

Recently, medicinal properties from various mushrooms have been explored. Especially polysaccharides isolated from mushrooms like *Ganoderma lucidum*, *Phellinus linteus*, *Coriolus versicolor*, *Lentinus edodes*, *Cordyceps militaris*, *Schizophyllum commune* etc. have been exhibited various medicinal properties (Lee et. al. 1994, Lee & Kang 1996, Kim et.al. 1999, Kim et.al. 2001, Park et.al. 1994, Park et.al. 2001).

There seems to be an undoubted relationship between diet and disease. Now a days an awareness is building towards diet that is influential for health and for controlling many chronic diseases. The concept of functional foods is thus evolved (Smith & Sullivan, 2004).
There is a large variety of edible mushrooms, which demonstrate edible and functional properties. Some of which are species of *Auricularia*, *Lentinula*, *Hericium*, *Griffola*, *Flamulina*, *Pleurotus* and *Tremella*. Some other mushrooms like *Ganoderma* and *Trametes* are non-edible because of high fibre content and coarse texture but definitely show wonderful medicinal properties.

In many countries mushrooms have long been valued nutritional supplement rather than medicine. In Japan, pushcart vendors sell medicinal mushrooms on the streets, which are regularly used in diet to maintain health and promote longevity. Some Japanese people travel hundreds of miles to collect wild mushrooms, such as Reishi (*Ganoderma* spp.), those grows only on old plum trees and are renowned for their ability to help fight cancer and degenerative diseases. For over 3,000 years, the Chinese have exploited many fungi for their health enhancing properties, especially as tonics for the immune system (Bhosle & Vaidya, 2001).

From India, studies from the region of Madhya Pradesh (India) resulted in the knowledge of ethnomycological and socio economic aspect of many medicinally important mushrooms viz., *Cyathus limbatus*, *Lycoperdon pusillum*, *Phallus impudicus*, *Ganoderma lucidum*. (Harsh et al. 1993, 1996)

There are some other medicinally important mushrooms whose properties are known to some people living only in some pockets of world. One of these mushrooms is *Phansomba*, another medicinally important mushroom from the Western Ghats of Maharashtra (Konkan region). It is used to cure teeth, tongue, throat related ailments, diarrhea and to stop excessive salivation in case of children (Vaidya & Bhor 1991, Vaidya & Rabba 1993, Vaidya & Lamrood 2000, Vaidya et al; 2005).

### 1.2 Classification and Identification of Fungi

Classification and identification are two independent but inter-related processes. Where ‘classification’ answers questions like ‘how is the fungus in front of me is related to other fungi?’ the term ‘identification’ answers questions like ‘what is the name of specimen in front of me?’ (http://www.anbg.gov.au/fungi/classification-identification.html)
Right from the era of early biologists like Linnaeus, fungi were classified as cryptogamic plants, which were in turn divided using characters like ‘lichenized’ and ‘non-lichenized’, ‘sexually’ and ‘non-sexually’ reproducing forms. This approach did not yield any studies regarding relationships amongst fungi, although it was useful in identifying fungi (Cannon & Kirk 2007). Later in mid 19th century, the discriminatin of lichenized and non-lichenized fungi was discarded.

Whittaker gave the Fifth kingdom status to fungi, in his 5-kingdom classification system, which was then accepted by biologists (Whittaker 1969). Repeated attempts are made till today trying to classify fungal community into the most approaprite manner (Ainsworth & Bisby 1950, Ainsworth 1961, Hawksworth et.al. 1995, Kirk et.al. 2001, 2008, James et.al. 2006, Hibbett et. al. 2007). The classification system for fungi evolved slowly when more and more fungi came to be described and the hierarchy was expanded to accommodate new taxa (Hawksworth et. al. 1995 b).

There is a close linkage between classification and the evolution of organisms, in the sense that a good classification system should group evolutionarily close organisms to one group. An ideal classification system should take an account of all the traits seen in fungi right from morphological characters to its genomic data. Classification sytems on these grounds seem to be still evolving with the development of newer techniques.

The concepts in fungal classification has altered continuously with the newer trends accepted amongst the taxonomists, these changes have always been reflecting in the successive editions of “Dictionary of the Fungi” till its latest edition.

The dynamically altering scenario in classification of fungi gives rise to chaos in many instances. It is worth thinking, whether confusions regarding taxonomic status of many genera are resolved or created with advancement of technology?

The most recent trend in classification is based on phylogenetic data derived from multiple gene loci. In recent years the phylogenetic studies were carried out at generic as well as specific level. The consequence of which is that some of the older literature cited maybe in conflict with the taxon it is cited under (Kirk et.al 2008).

During the development of these recent concepts in taxonomy, previously established classification systems based on taxonomic characters were considered as highly artificial. Several families in earlier classification included genera those are widely separated today on
the basis of molecular phylogenetic analysis. This is considered as a slow progress towards more naturally defined groups (Hawksworth et. al. 1995a).

Identification schemes are not classification schemes, although there may be a superficial similarity. An identification scheme for an organism can be devised only after the group has been classified (i.e. recognised as been different from other organisms). Identification of a group should be based on one or more common characteristics, which all the members of the group have and which other group donot have (Brenner et.al 2004).

The scope of this thesis is restricted to the taxonomy and identification of genus *Phellinus* Quel., based on macro and micro morphology as well as cultural characteristics upto a certain extent.

### 1.3 Origin of the topic

As said earlier, Phanasomba is used as medicine by Vaidus (local doctors) especially in the Konkan region of The Western Ghats of Maharashtra. It was found to be a common bracket mushroom causing heart rot disease of *Artocarpus heterophyllus* Lam. (vernacular name: Phanas). Phansomba is also known as Phanas-alambi, Phanas banda (Khory 1887, Dymock et.al. 1890, Andhalkar 1988, Vaidya & Bhor 1991; Vaidya & Lamrood 2000).

An extensive survey of commercially sold samples of Phansomba revealed that it mainly comprises of many specis of *Phellinus* (Vaidya & Bhor 1991) alongwith some adulteration of some species of *Ganoderma* (Bhosle 2005). It was therfore decided to study *Phellinus* spp. occurring on *Artocarpus heterophyllus* Lam. thoroughly.

The tasks during sample survey were, managing the collection of specimens, their correct identification to species level and isolation of different species into axenic cultures; the elaboration of which is given in later chapters.
1.4 About the present study

The most prior aim of the present study being, the identification and authentication of taxonomic identity of the folk medicine Phansomba it encompassed,

- Establishing taxonomic identity of Phansomba in terms of *Phellinus* spp. along with generating supportive data from culture studied.
- Opening a future line of research.

*Phellinus* Quel. is a member of Hymenochaetaceae. Taxonomic status of this family has been changing through years. Till recent past, Hymenochaetaceae was placed in order Aphyllophorales of Homobasidiomycetes ie mushroom forming fungi (Hawksworth *et al.* 1995a). Later in the beginning of 21\textsuperscript{st} century, it was included in order Hymenochaetales of subclass Agaricomycetidae of class Basidiomycetes (Kirk *et al.* 2001).

Molecular analysis with multiple gene loci, revealed a different story of phylogenetic affinities of this group of fungi making it an artificial assemblage of species (Hibbett & Donoghue 1998, McLaughlin *et al.* 2001).

Species of *Phellinus* cause heart rots and sometimes root rots of live standing trees. Being so, they part a major role in forest ecosystems as lignin decomposers. On the other hand the *Phellinus* spp. probably collectively cause more timber losses that any other genus of wood destroying fungi (Larson & Cobb-Poule 1990).

*Phellinus* in recent years has been studied for its medicinal properties. *Phellinus linteus* is much explored in Korea. Also it is regarded as medicinal in their ancient literature. *Phellinus linteus* is commonly known as Sang-Hwang in Korea. In the other countries also it is known by different names viz, Sang Gen in China and Meshimakobu in Japan. All these are herbal medicines, which are used in the treatment of palsy, gonorrhea, abdominal pain, urinary disorders, lymphatic tumour, bleeding nose, diarrhea, etc. (Mizuno 2000).

In India *Phellinus* is sold by the trade name of Phansomba in the Western Ghats region as a folk medicine.

Culture is the growth of organism or a group of organisms for the purpose of experiment or sometimes for trade (eg. mushrooms) (Kirk *et al.* 2001).

It is the most effective method for live preservation of any fungus. It is very important to study the culture of a fungus as it can then be used widely for various purposes like biomass
production, secondary metabolite analysis, molecular analysis etc. In short, studying cultures is the prerequisite for most of the experiments visualized in future studies (Vaidya 1995). In short pure culture methodology is the foundation of fundamental and applied research, as well as the commercial exploitation of fungi (McLaughlin et.al. 2001).

1.5 Objectives

1. Extensive taxonomic studies of Genus *Phellinus* s.l. from various library sources, digitization of generated data.
2. Developing a computer code for the identification of species using the digitized data.
3. Sample collection from Konkan region from *Artocarpus heterophyllus* Lam. trees and also from various trees in the campus of University of Pune, M.S., India.
4. Detailed taxonomic studies of the samples and identification using the self developed code (objective 2).
5. Comparative taxonomic studies using Parsimonial cluster analysis
6. Obtaining a fungal culture in axenic form and their maintenance- Standardization of a technique to isolate and preserve *Phellinus* cultures.
7. A systematic study of the isolated cultures on the basis of morphological, physiological and microscopic characteristics.
8. Digitization of generated data and comparative studies using Parsimonial cluster analysis.
9. Optimization of various culture parameters for the selected isolates to open a future line of work.