# Chapter 1

# Chapter 1.Introduction

## 1.1. Overview of *Pleurotus* mushroom

### 1.1.1. Mushroom: A historical journey from 'culinary delicacy' to 'medicinal value'

Palaeontologists revealed the existence of fungi back in the Silurian period, around 408-438 million years ago, in the Paleozoic era. Eventually, this plethora of fungal diversity was expanded by the Pennsylvanian period. During this period, basidiomycetes and ascomycetes were the key mushrooms reported to have existence. Thereby indicating the existence of mushrooms as a part of fungal diversity back from 300 million years ago. During the ancient civilizations of Greeks, Egypt, Roman, and China, mushrooms were appreciated as a delicacy; later on, as civilization progressed, mushrooms were appraised for their nutritional and medicinal value apart from being a food source (Chang & Miles, 2004).

Gordon Wasson, the father of modern ethno-mycology, proposed a hypothesis that during 4000BC, Soma Rasa, a Vedic juice used by 'Aryans' in various rituals, which was assumed to possess divine qualities on the soul of the consumer, was based on a psychoactive fly agaric mushroom(Amanita muscaria). This hypothesis was published in 1967 and entitled 'Soma: Divine Mushroom of Immortality'(Wasson, 1971).

Today, there are 14,000 mushrooms in the universe, out of which about 3000 are edible, 700 are of medicinal value, and around 1400 are recognized as

poisonous. Most of the mushroom-derived preparations are enlisted as dietary supplements or nutraceuticals. Ganoderma is the king of medicinal mushrooms in the list of medicinal mushrooms, followed by Lentinula and *Pleurotus* genus (Chang & Miles, 2004).

### 1.1.2. *Pleurotus* mushroom

*Pleurotus* genus belongs to a distinct class of Basidiomycetes fungi of the order Agaricales and family Tricholomataceae. The fruiting bodies of this mushroom open up like an oyster shell from the stalk; therefore, *Pleurotus* species are titled oyster mushrooms. *Pleurotus* mushrooms are soft and available in a variety of colors: dark blue, white, and creamto brown, yellow, and pink. Environmental factors like light and temperature influence their color intensity. They have a fragrant odor and delicious taste. These mushrooms are habitat worldwide, from temperate to tropical regions (12- 32 ˚C). Hence they are referred to as 'mushrooms of high adaptability.' Approximately 50 species are recognized in the genus *Pleurotus*. Mostly they are parasitic, and few are saprophytic (Chang & Miles, 2004).

The Pileus of *Pleurotus* mushrooms is usually shell and tongue-shaped, which get depressed when they are older. Pileus diameter varies in size from 5- 30 cm. The Stipe is eccentric or laterally attached. The gills are decurrent,and the spores are smooth and elongated (Chang & Miles, 2004). The schematic diagram of the *Pleurotus* mushroom is shown in Figure 1.1.

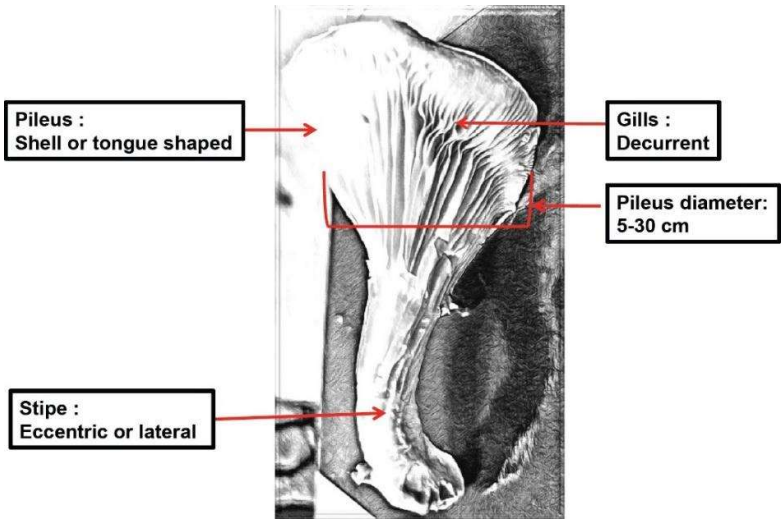


**Figure 1.1.** Schematic diagram of *Pleurotus* mushroom.

*Pleurotus* mushroom is a common type of edible mushroom. Today they represent the 2nd largest cultivated and consumable mushroom globally, preceded by button mushrooms. *Pleurotus* genera are rich in fibers, protein, and carbohydrates. The protein content range from 10 % - 30 % of dry weight and sometimes extends to 40 %. Fresh mushrooms generally contain 3 % - 28 % of the dry weight of carbohydrates and 3 % -30 % of the dry weight of fibers. The fat content is comparatively low, around 3 % - 5%, which is generally higher in the stalk than in the cap. There is a high content of minerals and vitamins. The moisture content in fresh mushrooms ranges from 90 % - 95 %, which reduces to 9 % - 10 % on drying (Agarwal et al., 2017).

Apart from nutritional value, the *Pleurotus* genus is known for producing a number of bioactive molecules. Multi- directional health-promoting and therapeutic activities are due to the interaction of these bioactive molecules. The abundance of research explicitly indicates the multidirectional therapeutic potential of *Pleurotus* mushroom in various arena, to list few of them as hyperlipidemic, antihypertensive,

antidiabetic, hepato-protective, antioxidant, anti-microbial, arthritis, anti-cancer, and anti-cataractogenic (Golak-siwulska et al., 2018).

### 1.1.3. Anti-cancer potential of *Pleurotus* mushroom

From the hub of natural products, in the last few decades, the researcher's attention has been inclined toward edible mushrooms for exploring their anti-cancer potential. Mycotherapy of cancer is a new promising field that covers anti-carcinogenic agents derived from mushrooms. In 1957, Lucas and his co-workers were pioneers in discovering anti-cancer activity of Basidiomycetes mushrooms against Sarcoma 180 tumor cells (Lucas EH, Montesano R, Pepper MS, Hafner M, 1957). Among the basidiomycetes, the genus *Pleurotus* has been intensively studied in the late 20th century due to its antitumor activity. Research articles are screened from the electronic search engines 'Science direct' and 'Pub med,' which are collectively summarized and tabularized in Table 1.1, which emphasize *in-vitro - in-vivo* anti-cancer activity on different types of *Pleurotus* species, bioactive myco-metabolites responsible for anti-cancer potential with their mechanism of action. Apart from this, the number of research on the anti-cancer potential of *Pleurotus* species in the last decades has increased rapidly, around the double fold. Among the 13 *Pleurotus* species studied for anti-cancer potential, *Pleurotus osteratus* is an intensively investigated mushroom for anti-cancer potential, followed by *Pleurotus eryngii*.

**Table 1.1.** Reported anti-cancer potential of *Pleurotus* mushroom.

| Type of *Pleurotus* Species | Extract/samples | *In-vitro* studies | *In-vivo* studies | Explored mechanistic pathway | Ref |
|---|---|---|---|---|---|
| ***Pleurotus florida*** | Gold nanoparticles | Human lung cancer cell (A549), human chronic myelogenous leukemiabone marrow cell (K562), human cervix cancer cell line (HeLa), and human breast cancer cell line (MDA-MB-231) | | | (Bhat et al., 2013) |
| | Novel isomeric molecule (3-methoxy 4-hydroxy cinnamic acid) from methanolic extract | A549 | - | | (Menaga et al., 2021) |
| | Trimetallic nanoparticle | MDA-MB-231 | | - | (Chaturvedi et al., 2020) |
| ***Pleurotus osteratus*** | Water-soluble polysaccharides | Human breast cancer cell lines (MCF-7, and T-47D) | | | (Radzki et al., 2016) |
| | Water-soluble proteoglycan from mycelium | | Sarcoma-180 tumor bearing mice | Pre-G0/G1 phase arrest. | (Sarangi et al., 2006) |
| | Alkali-extracted polysaccharide from fruiting bodies. | | Sarcoma-180 tumor-bearing mice | | (Kong et al., 2014) |
| | Ostreolysin (Oly), protein isolated. Recombinant version of this protein (rOly) produced. | | Xenografts and syngeneic colon cancer cell bearing mice | | (Nimri et al., 2017) |
| | Extract | Leukemia cell lines (KG-1, and Jurkat cells) | | Increase in BAX expression and decrease in MMP-9 expression. | (Ebrahimi et al., 2018) |
| | Ethanolic extract | | 7,12-Dimethylbenz. (a) antheracene (DMBA) induced mammary carcinogenesis in rats | | (Krishnamoorthy & Sankaran, 2016) |
| | Silver nanoparticles | MCF-7 | | | (Yehia & Al-Sheikh, 2014) |

**Table 1.1.** Continued

| Type of *Pleurotus* Species | Extract/samples | *In-vitro* studies | *In-vivo* studies | Explored mechanistic pathway | Ref |
|---|---|---|---|---|---|
| ***Pleurotus osteratus*** | Cibacron blue affinity-purified protein | | DL, Sarcoma-180, and B16F0 melanoma tumor-bearing mice | | (Swatilekha maiti et al., 2011) |
| | Aqueous extract | Human colon cancer cell line (COLO-205) | | Increased level of caspase 9, caspase 3, and BAX expression, decreased level of BCL2 expression, and G0/G1 phase arrest. | (Arora & Tandon, 2015) |
| | Selenium polysaccharides | | | Apoptosis induction, disrupt BAX/BCL2 proteinratio, and inhibit epithelial to mesenchymal transition. | (Y. Zhang et al., 2020) |
| | Aqueous polysaccharides | Human colon cancer cell line (HT-29) | | Up-regulation of cytsolic cytochrome-c, and BAX. | (Lavi et al., 2006) |
| | Aqueous polysaccharides | Human prostate cancer cell line (PC-3) | | | (Gu & Sivam, 2006) |
| | Hot water extraction | MCF-7 | | | (Martin & Brophy, 2010) |
| | Carboxymethylated α(1→3) poly saccharides | HeLa | - | | (Wiater et al., 2011) |
| | Polysaccharides | | Sarcoma-180 tumor bearing mice | | (Wisbeck et al., 2017) |
| | Methanolic extract | MCF-7, MDA-MB 231, HT-29, and human colon cancer cell line (HCT-116) | | G0/G1 cell arrest, upregulated expression of p21, and inhibit phosphorylation of Rb. | (Jedinak & Sliva, 2008) |
| ***Pleurotus eryngii*** | Partially methylated mannogalactan from cold aqueous extract | Murine melanoma cancer cell line (B16F10) | B16F10 melanoma-bearing C57BL/6 mice | | (Biscaia et al., 2017) |
| | Hetero polysaccharides | Human hepatoblastoma cell line (HepG2) | | S-phase arrest and production of intracellular reactive oxygen species(ROS). | (Ren et al., 2016) |

**Table 1.1.** Continued

| Type of *Pleurotus* Species | Extract/samples | *In-vitro* studies | *In-vivo* studies | Explored mechanistic pathway | Ref |
|---|---|---|---|---|---|
| ***Pleurotus eryngii*** | Polypeptide from mycelium | Cervical, breast,and stomach cancer cells line | | | (Sun et al., 2017) |
| | Protein from fruiting bodies. | A549 | | | (Mariga et al., 2014) |
| | Polyphenol-rich extract | human colon cancer cell line (HCT116) | | Down regulation of cyclin B and cyclin E, and the upregulation of caspase-3 and cleaved caspase-3. | (Hu et al., 2018) |
| | Protein | Human and murine colon cancer cell (HCT116) and (MC38) | Allograft colon cancer cell-bearing mice. | Upregulation of p21,p53, c- PARP, caspase-3, and cleaved caspase-3, and downregulation of cyclin B, cyclin E and cdc-2 | (Yuan et al., 2017) |
| | Water-soluble polysaccharide | HepG2 | | | (G. Ma et al., 2014) |
| | Water-soluble polysaccharide | | Renca tumor-bearing mice | | (Yang et al., 2013) |
| ***Pleurotus djamor var. roseus*** | Silver nanoparticles | PC3 | | | (Raman et al., 2015) |
| | RNAase | HepG2, and MCF-7 | | | (Wu et al., 2010) |
| | Sterol derivatives isolated | MDA-MB-231, and murine mouse lymphoblastic lymphoma cell line (EL4) | | - | (Jagadeesh et al., 2020) |
| ***Pleurotus sajor-caju*** | N- hexane extract | HTC116 | | Increase in BAX, p53, caspase-3, and decrease BCL2 expression. Disruption of p21/p53cell cycle regulation pathway | (Finimundy et al., 2018a) |
| | Aqueous extracts | HepG2, and HeLa | | | (Finimundy et al., 2013) |
| | RNAase | HepG2, and murine blood cancer cell line (L1210) | | | (Ngai & Ng, 2004) |

**Table 1.1.** Continued

| Type of *Pleurotus* Species | Extract/samples | *In-vitro* studies | *In-vivo* studies | Explored mechanistic pathway | Ref |
|---|---|---|---|---|---|
| ***Pleurotus sajor-caju*** | Polysaccharides rich fraction | - | EAC cell line inoculated in female Swiss mice | | (Dalonso et al., 2010) |
| ***Pleurotus abalonus*** | Polysaccharides from fruiting bodies | Human colon cancer cell line (LoVo) | | S-phase arrest and increase the production of ROS. | (Ren et al., 2015) |
| | Polysaccharides fractions from fruiting bodies | MCF-7 | | Loss of membrane potential, increase in BAX/BCL2 ratio, caspase-9/3 activation, PARP degradation, intracellular ROS production, upregulation of p-53, and S-phase arrest. | (Shi et al., 2013) |
| ***Pleurotus nebrodensis*** | Polysaccharide | A549 | A549 tumor-bearing mice | | (Cui et al., 2014) |
| | Nebrodeolysin | HepG2, and HeLa | | | (Lv et al., 2009) |
| | Aqueous extract | MCF-7, MDA-MB 231, HeLa, COLO-205, HepG2, and PC-3 | | | (Cha et al., 2012) |
| ***Pleurotus citrinopileatus*** | Lectin | | Sarcoma-180 tumor-bearing mice | | (Y.R.Li et al., 2008) |
| | Glycoprotein | Human blood cancer cell line (U937 cells) | | S phase arrest, and apoptoticinduction | (Chen et al., 2009) |
| | Polysaccharides | | Murine liver cancer cell line (H22) tumor-bearingmice | S phase arrest, and apoptoticinduction | (Q. Wang et al., 2021) |
| ***Pleurotus tuber-regium*** | (1→3)-β glucans from the sclerotia were carboxymethylated | HL-60 | Sarcoma-180 tumor-bearing mice | | (M. Zhang et al., 2004) |
| ***Pleurotus cornucopiae*** | Laccase | HepG2, and L1210 | | | (Wong et al., 2010) |
| ***Pleurotus citrinopileatus*** | Monoterpenoids and sesquiterpenoids | HeLa and HepG2 | | | (S. Wang et al., 2013) |

**Table 1.1.** Continued

| Type of *Pleurotus* Species | Extract/samples | *In-vitro* studies | *In-vivo* studies | Explored mechanistic pathway | Ref |
|---|---|---|---|---|---|
| ***Pleurotus pulmonarius*** | Polysaccharide -protein complex | Human liver cancer cell line (Huh7), and (SMMC-7721) | Xenograft BALB/c nude mice | Suppression of PI3K/AKT pathway. Inhibition of autocrine VEGF-induced PI3K/AKT signaling. | (Xu et al., 2012) |
| ***Pleurotus ferulae*** | Terpenoids rich ethanol extract | B16F10, and human gastric cancer cell line (BGC-823) | Murine melanoma model | G0/G1 cell arrest, and increase in caspase-3 expression. Reduced mitochondria membrane potential. | (W. Wang et al., 2014) |
| ***Pleurotus geesteranus*** | Polysaccharides and polysaccharides-protein complexes | MCF-7 | | | (M. Zhang et al., 2011) |

### 1.1.4. Anti-cancer potential bioactive myco-metabolites from *Pleurotus* mushroom

The bioactive myco-metabolites responsible for health-promoting and therapeutic potential in the *Pleurotus* mushrooms range from higher molecular weight myco-metabolites to low molecular weight myco-metabolites. As per the data summarized in Table 1.1, higher molecular weight myco-metabolites are the most explored molecules for anti-cancer activity compared to lower molecular weight myco-metabolites like terpenoids, sterol, statin, and phenolic compounds are the potential classes of myco-metabolites that are yet to be explored for anti-cancer activity.

#### 1.1.4.1. Higher molecular weight bioactive myco-metabolites

The most bioactive myco-metabolites explored for anti-cancer potential is polysaccharides. The polysaccharides present in the cell wall of the mushroom are β-glucans and α-glucans. These are chemically structured as glucopyranose molecules linked with glycosidic bonds of the types (1→3)-β-, (1→6)-β- or (1→3)-α-. Among these, β-glucans, and polysaccharides are well explored for anti-cancer potential. These β -glucans differ in structure, solubility, size, and molecular weight. Their physicochemical properties are modified by changing the degree of their branching or by adding substituent groups. This influences their therapeutic potential. *Pleurotus* mushrooms are known for their abundance of β-glucans. Different *Pleurotus* mushrooms have different concentrations of total glucans, β -glucans, and α -glucans. α(1→3) glucans are lesser investigated polysaccharides compared to β-glucans. This is found in the deepest layer of the mushroom cell wall. The bioactivity is greatly affected by the solubility, structure, and

concentration of α(1→3). Chemical modification can increase bioactivity. The aqueous extraction of fruiting bodies and mycelia of *Pleurotus* mushrooms extract and isolate an army of polysaccharides. From the above-screened research articles on anti-cancer potential of 13 different species of *Pleurotus* mushroom (Table 1.1.), about 20 research articles concentrated on polysaccharides as bioactive myco-metabolites contributing to anti-cancer activity. Following polysaccharides, proteins and peptides are other classes of higher molecular weight bioactive myco-metabolites for anti-cancer activity are explored. Nebrodeolysin and osteolysin are the best examples of proteins isolated and evaluated for anti-cancer potential. Other higher molecular-weight bioactive molecules include enzymes and lectins. Among the enzymes, RNAase and lacasse have gained the limelight in the mitigation of cancer. Lectins are polysaccharides-protein complexes, and polysaccharides-peptides complexes. A list of publications supporting anti-cancer potential of isolated proteins, polypeptides, enzymes, and lectins is enlisted in Table 1.1. About 14 research articles exploited protein and its analogs as bioactive myco-metabolites responsible for anti-cancer potential.

### 1.1.4.2. Lower molecular weight bioactive myco-metabolites

Sterol derivatives (Figure 1.2.a.), phenolic compounds (Figure 1.2.b.), and novel terpenoids (Figure 1.2.c.) are fewer lower molecular weight myco-metabolites explored for the anti-cancer potential of *Pleurotus* mushroom till date, as evident from Table 1.1. The structure of explored lower molecular weight myco-metabolites for anti-cancer potential of *Pleurotus* mushroom are shown in Figure 1.2.

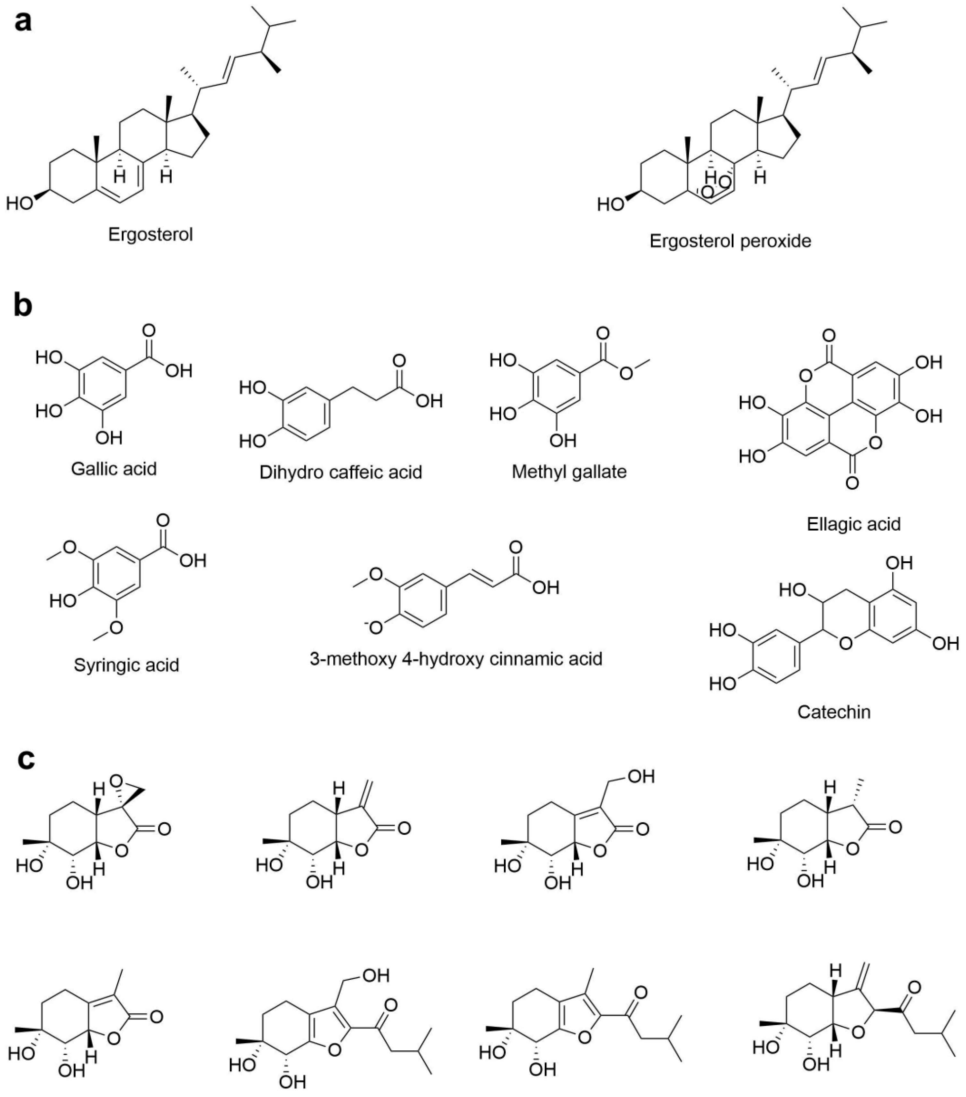


**Figure 1.2.** Explored lower molecular weight myco-metabolites for anti-cancer potential of *Pleurotus* mushroom.

### 1.1.5. Explored mechanistic pathway for anti-cancer potential of *Pleurotus* mushroom

The explored mechanistic path for anti-cancer potential of *Pleurotus* mushroom includes inhibition of autocrine VEGF induced PI3K/Akt signalling for angiogenesis, induction of intrinsic mitochondrial pathway leading to apoptosis, up-regulation of apoptosis-related signalling molecules, and down- regulation of cell cycle-related signalling molecules (Figure 1.3.).

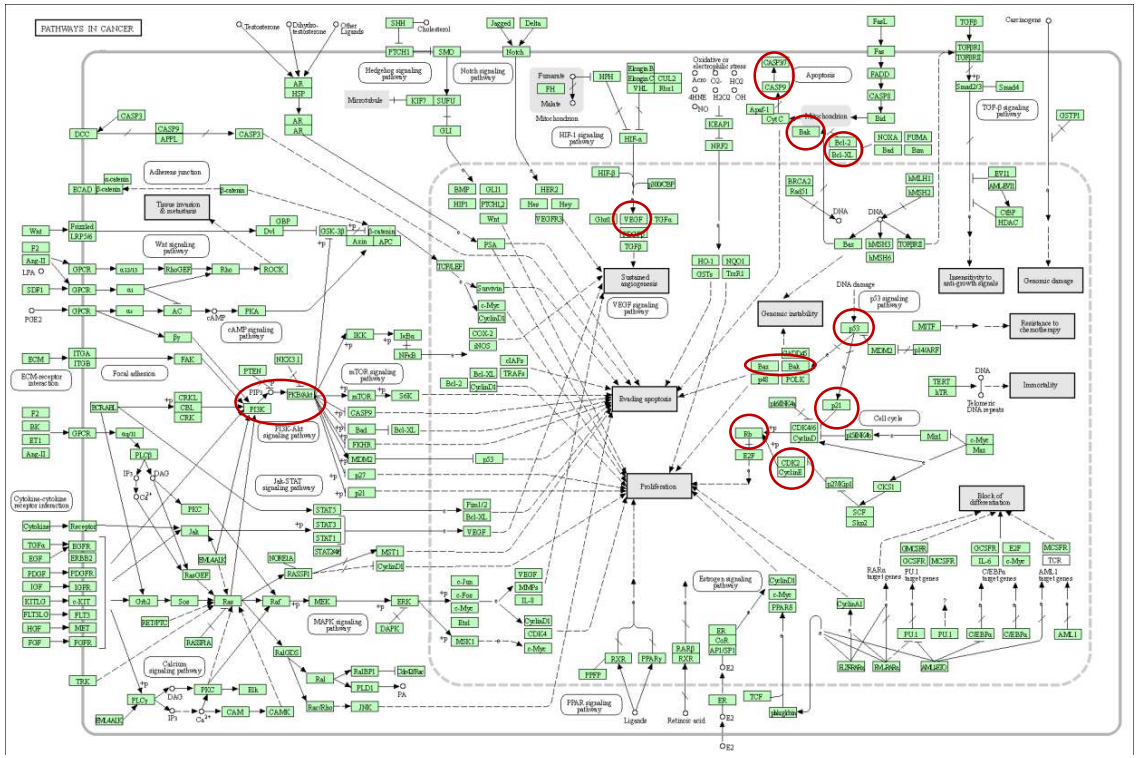


**Figure 1.3.** Explored mechanistic pathway involved in the anti-cancer potential of *Pleurotus* mushroom. Red circles are the explored mechanism. Modified image of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of cancer.

## 1.2. Overview of chemometric

Natural products-based metabolomic studies generate complicated high-dimensional data sets that are tough to analyze and interpret by visual inspection or any conventional univariate statistical analyses. Multivariate data analysis, a mathematical modeling approach, is used to extract relevant information from these huge data sets. The models generated by these chemometric techniques are ideally designed to handle the analysis of confounding and covariance patterns (both within and across variables), which are typically beyond the limits of conventional univariate statistical methods. Chemometric analysis of the complicated high-dimensional metabolomic data is executed in supervised and unsupervised ways. With the unsupervised model, frequently referred to as 'descriptive models,' the emphasis is on the data's inherent structure, relationships, and interconnections. While the supervised model attempts to convert the multivariate data from metabolite profiles into a biologically relevant representation, thus referred to as 'predictive models'(Tugizimana et al., 2013).

## 1.3. Overview of network pharmacology

Adaptive resistance to cancer therapies urges the concept of rational combinatorial targeted therapies, wherein complementary combination targeting a multitude of key pathways involved in cancer may result in superior therapeutic benefits (Holohan et al., 2013). Such an approach is executed in the field of network pharmacology, which combines system biology and network analysis, thereby exploiting a multitude of key and redundant target pathways (Hopkins, 2008). Information regarding signalling pathways can be explored to build multi-target tactics for inhibiting redundant pathways or mutant targets besides targeting multiple cancer hallmarks. Apart from reduction in drug resistance, this modality of amalgamation enhances efficacy and demotes the

adverse effect as compared to monotherapy, owing to the synergistic or additive approach. For these combinations, multiple therapeutic agents or natural products with multiple components can be explored (Yip & Papa, 2021).

Network pharmacology, an integrative *in-silico* approach, establishes a 'compound-targets/disease- targets' network and identifies underlying mechanisms for synergistic therapeutic intervention. This evolution has shifted the paradigm from 'one target-one drug' mode to 'network target-multiple components-therapeutics' mode. In the current epoch of large data, by simply comprehending the combinatorial characteristic of natural product and their mechanisms of action, it is possible to re-engineer natural products. Network pharmacology not only explores the molecular complexity of natural products but offers the possibility to systematically explore the association of natural products with complicated diseases. Thus, network-based methodologies are becoming popular research tools for exploiting the complexity of natural products in the mitigation of complicated diseases (Noor et al., 2022).

Natural products are acclaimed for their structural and functional diversity and complexity. Predominately being secondary metabolites or signalling molecules, they inherently target biologically relevant space and also have the ability to modulate undruggable targets. Along with the potential of exploring new targets, these approaches can also help to cut off the cost of the development of new therapies since these molecules already exist in nature (Harvey et al., 2015). Mostly, natural products are employed in 'complementary medicine,' wherein synergies with cancer drugs are investigated. To predict the synergistic between multiple components of natural products, various approaches of computational methods, including graph theory and differential gene expression analysis, were employed (Sun et al., 2015).

## 1.4. Rationale & Objectives

*Pleurotus* mushroom has been intensively studied for its anti-cancer activity, attributed to higher molecular weight myco-metabolites such as polysaccharides, polypeptides, protein, etc. Conversely, little is known about the anti-cancer bioactivities associated with lower molecular weight myco-metabolites, to list a few statin, terpenoids, ergosterol, and polyphenolic compounds claimed as lower molecular weight biomarkers responsible for anti-cancer activities of *Pleurotus* mushroom. Beyond this, the comprehensive myco-metabolite profiling and correlation between myco-metabolites and their bioactivities and underlying mechanistic are still illusive. The present study was planned and designed for exploring differential bioactivity-based screening of preferential extracted higher molecular weight and lower molecular weight myco-metabolites, detailed myco-metabolite profiling and correlating with bioactivity, and tracing mechanistic pathway involved in the anti-cancer intervention of potential one.

For the execution of the designed study, a few objectives were aimed:

- To screen **different species of *Pleurotus* mushroom** based on *in-vitro* cytotoxic activity.
- To carry out bioassay-guided fractionation of **screened species**, with comparative myco-metabolite profiling of and correlating with *in-vitro* cytotoxic activity.
- To study the anti-cancer mechanism of **potential fractionate** by the integrative approach of network pharmacology and experimental studies.

Each *objective* of the study is framed in the form of a **chapter** of the study.

**Chapter 1.** Bioactivity-based screening of different species of *Pleurotus* mushroom with their myco-chemical profiling.

**Chapter 2.** Chemometric-based analysis of myco-metabolite of a bioactive enriched fraction of ***Pleurotus osteratus*** (*screened species*) and its correlation with *in-vitro* cytotoxic activity.

**Chapter 3.** Tracing the anti-cancer mechanism of **HFPO1** (*potential fractionate*) by the integrative approach of network pharmacology and experimental studies.

**Chapter 4.** Tracing the anti-cancer mechanism of **EFPO1** (*potential fractionate*) by the integrative approach of network pharmacology and experimental studies.