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# IDENTIFICATION OF MUSHROOMS AS ELIXIR THROUGH A PARTIAL PRODUCT CERTIFICATION TECHNIQUE USING MEGA 11 TOOL

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## ABSTRACT

**Background and Objective:** Cancer being an abnormal group of cells which invade or spread throughout the organ/body. It is either a result of genetic mutation or gene mutation due to life style change, sometimes an involuntary exposure to environmental factors likely harmful radiations, cigarette smokes, medicines/drugs, pollution, and many more. Inherited genetic mutations play a major role in about 5-10 percent of all cancers, while the remaining 90-95 percent have their origin in environmental factors and lifestyle changes. In recent years, increased mushroom consumption has lowered the risk of cancer. Mushrooms have protective elements like folic acid, selenium, B vitamins such a niacin, riboflavin and pantothenic acid, copper, some amounts of phosphorus, zinc, potassium, antioxidants and many more bioactive compounds are used in adjuvant cancer treatments due to their anticarcinogenic or anti-tumor effects. However, are the mushrooms used in treatment safe for consumer usage?

**Methods:** Herein, we are to find if the mushrooms used are safe for usage as dietary supplements, adjuvants and their treatment process. Also, we are to use the MEGA 11 tool to identify the DNA fingerprint of the mushrooms.

**Result:** Herein, we have presented some research studies conducted in various places using the mushrooms like Ganoderma, Phellinus and Pleurotus species. This also focus on those individual bioactive compounds found in the extracts of the mushrooms and how they work together with chemotherapy to reduce cancer risk. Also, we have used the 18S cDNA sequence of our study to identify the evolutionary taxa, DNA and protein sequence of the mushrooms with the help of MEGA 11 software.

**Novelty:** The study revealed the modern adjuvant treatments used in recent years in inhibiting the cancer cell proliferation. Also, the usage of insilico tools/softwares to identify the species which are milled/dried/extracted.

**Keywords: Adjuvant cancer treatments, cancer, mutation, mushrooms, prevention, species identification**

## I. INTRODUCTION

Mushrooms have been a part of human diet for centuries, used by Roman's who considered mushrooms to be the "Food of the Gods", Greek warriors considered and used mushrooms for strength, and the Chinese have treasured these mushrooms and considered them the magic potion of life "the elixir of life". Owing to the unique flavour, the mushrooms were considered a culinary wonder. These are used as powerful ingredients in various mouth watering cuisines. Being low in calories, fat, sodium, carbohydrate and completely cholesterol-free, mushrooms have drawn the attention of health sector. Since the 13$^{th}$ century, Chinese have used mushrooms as traditional medicines, applied to overcome pain, fatigue associated with ageing, heart ailments and many more. However there are some controversies' between the use of traditional mushrooms in Chinese medicine and Western medicine, which says there are no/very less evidence supporting that mushrooms can be used in treating diseases.

Cancer being one among the five major reasons of death in India or any other country in the world, n-number of researches have been performed till date. Recent years mushroom extracts (like β-glucans, triterpenes, lectins, polysaccharide peptides) along side the mycelium and culture broth have been used in terminating the spread of cancer by intervening the signaling pathways of the tumor cells. National Cancer Institute, Memorial Sloan Kettering Cancer Center, City of Hope Medical Center are a few research centers which are actively part of

mushroom/mushroom-extract based cancer research.

The use of mushrooms in cancer treatment started at around 1998. From then on several research projects using mushrooms as single agents or adjuvants which are a combination of radiation and chemotherapeutic drugs.[1] For example: Proteoglycans were extracted from basidiomycetes Agaricus exhibited tumoricidal effect via activation of NK cells. Extracts like agaritine and ergosterol (provitamin D2) induces apoptosis of leukemia and is anti-angiogenic (inhibiting tumor blood vessel formation).[2]

Cordycepin, a bioactive compound of Cordyceps militaris inhibits the tumor metastasis thus regulating the tumor microenvironment. Cordycepin terminates many forms of cancer like leukemia, oral, liver, colon, bladder, renal, lung, breast, prostate, gliblastoma, neuroblastoma.[3] Similarly different bioactive compounds extracted from other mushroom species have become a potion in medical industry in the recent years.

Mushroom extracts used as conventional therapy for cancer patients who received treatments like chemotherapy and radiation were studied and the results of the study were published in nearly 272 e-portals including MEDLINE, EMBASE CENTRAL, Clinical trials.gov, the WHO, the Web of Science and many more. The clinical trials with mushroom extracts, mycelia or culture broth [formulated in forms of tablets, powder, capsule or other extract forms] have facilitated improvements in a) minimal side effects/reduction of side effects caused by chemotherapy/radiotherapy, b) survival rate of patients after overall immune suppression (immunomodulation), Blood count (RBC/WBC/platelets/haemoglobin/ hematocrit) and mean values of all the blood cells and heamoglobin factor, c) antitumor activity and d) quality of life. Thus providing proof of their safety, mushroom extracts are used as supplement, given to pateints who have undergone chemotherapy/radiation have recuperated well. The tumor specific antigens have become undetectable in many patients and the patients are happy and they try to incorporate the specific mushroom extracts formulated for them specifically.[4]

Dr. Shiuan Chen, 2001 from the Beckman Research Institute of City of Hope, focused on White button mushrooms formulated (freeze-dried after removing all the water content into powder) as tablets when given to patients with prostrate cancer reduced the prostrate specific antigens in patients who have undergone surgery, been through several sessions of chemotherapy. Paul

Stamets, 2009 furnished a similar study using Turkey tail mushroom formulated as capsules to help breast cancer patients suffering from compromised immune system after series of radiation.

All these studies performed so far determine that these macrofungus mushrooms have great medicinal importance and serve as an excellent alternative to hormone therapies which have more side-effects and expensive. Patients suffer from less toxicity when compared to other therapies as mycotherapy (therapy with mushrooms) have improved the psychological and physiological health.

Improved taxonomy identification of various fungal species has been identified via DNA or PCR fingerprinting techniques. There are several problems in using the morphology alone in identification.

## II. MATERIALS AND METHODS

Case Studies: Cancer studies in the past years using mushrooms

We are using a few case studies conducted in varoius parts of the world to show the positive effect of mushrooms in cancer studies. We are to see the bioactive compounds extracted from Ganoderma, Phellinus and Pleurotus species and their anti-cancer effects [Fig.1].

**Molecular Tools MEGA 11:**

Molecular Evolutionary Genetics Analysis is a sophisticated and user-friendly software suite for analyzing DNA and protein sequence data from species and populations. Herein, we have used the 18 S gene primer sequence [ExoSAP treated] of the sample C, amplified it and then the cDNA obtained was introduced in to BLAST search from the MEGA 11 software [Fig. 2]. The sequence with closest similarity was identified to be that of Pleurotus species. Also phylogeny - evolutionary taxa relationship analysis of the sample C was studied.

**Figure 1. Dried extracts of Ganoderma, Phellinus and Pleurotus species respectively**

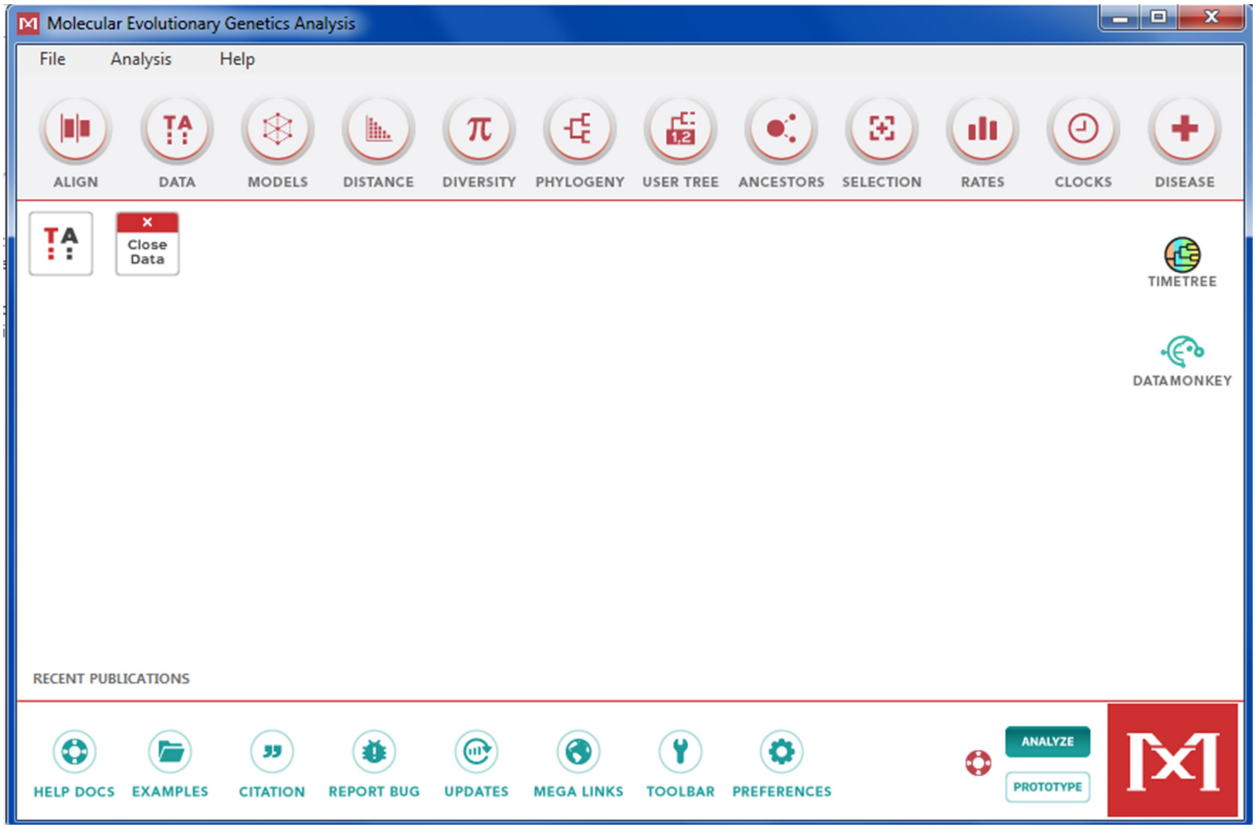



**Figure 2. Molecular Evolutionary Geentics Analysis Software with the analysis preferences**

## III. RESULTS

### Case study

***Cancer Research Using a Less Toxic Edible Macrofungi Supplement***

According to the study conducted by Penn State University, after analysing the data of 19,500 cancer patients from 1966-2020, the relationship between the low cancer risk and mushroom consumption was explored. When an individual consumes an average of 18 grams of mushrooms on daily basis, the risk of abnormally dividing cancer cells reduced by 45%.

Most of the research on mushrooms and cancers reductionist; that is, a particular mushroom type has been studied in relation to its impact on tumor regression or some other end point of cancer at a particular site in the body. The mushroom extracts such as the polysaccharides, glycoproteins, proteoglycans, polysaccharide peptide or lentinan are some of the registered adjuvant cancer therapies.

The Global Cancer Statistics 2020 India showed that breast, lip, oral cavity, cervix uteri, lung, colorectum, oesophagus, stomach, leukemia, ovary and prostate are the top 10 cancers frequently reported in both sexes. According to National Cancer Institute (NCI), there are 100 types of mushrooms in

Asia that can treat cancers but the most commonly used ones are *Ganoderma lucidum* (reishi), *Trametes versicolor* or *Coriolus versicolor* (turkey tail), *Lentinus edodes* (shiitake), and *Grifola frondosa* (maitake). In South India, *Ganoderma lucidum*, *Phellinus rimosus*, *Pleurotus florida* and *Pleurotus pulmonaris* were discovered to possess acute antitumor activities.

***Bioactive Compounds of Various Mushrooms in Cancer Therapy***

*Ganoderma lucidum*

*Ganoderma lucidum* also known as red mushrooms or reishi flourishing in the Southern part of India is a medicinal mushroom. The extracts of the mushroom are capable of resisting and reducing the cancer cells. As an adjuvant drug administered to patients undergoing radiations, chemotherapy, it protects the healthy cells from the impact of radiation.

An alcohol extract of Ganoderma lucidum inhibited the rapidly multiplying breast cancer cells by immediate cell-cycle arrest at the G1 phase of the cell cycle and also induced cell death of breast cancer cells, which was mediated through the upregulation of expression of proapoptotic Bax protein.[5] Furthermore, both spores and fruitbody of red mushrooms downregulated the expression of urokinase-type plasminogen activator (uPA) and its receptor uPAR as well as secretion of uPA, resulting in the inhibition of cell motility of breast and prostate cancer cells.[6] Thus, controlling the excessive degradation of extracellular matrix components and rapid invasion of the cancer cells

Gao *et al.* stated that Ganapoly, an over the table drug formulated from Reishi extracts when given to advanced lung cancer patients, elevated the levels of immune response i.e.; increased lymphocyte count and Natural Killer (NK) cell activity.[7]

The extracts from G. Lucidum mycelia (MAK) where administered to 123 patients with benign colorectal cancer for 12 months after which the consecutive colonoscopy performed on them proved a reduction in the tumor size. This case study was compared with the other 123 patients who were deprived from the administration of MAK.[8]

*Phellinus rimosus*

*Phellinus rimosus* also known as cracked cap polypore mainly found in tribal society of Kerala has been of great medicinal use in treating mumps, has antibacterial properties, and the protective effect of *Ph. rimosus* prevents damage to liver, kidney and brain against the alcohol-induced lipid peroxidation. It also exhibits profound

antioxidant, insulinogenic, antihepatotoxic, anti-inflammatory and anti-mutagenic agents. Polysaccharide protein complex from *Ph. Rimosus* inhibited the cell viability, controlled proliferation and caused the cell death of human colon cancer cell line HCT116.[9]

Also the general weakness of a body's antioxidant status caused due to the radiation therapy for cancer patients compromising the healthy cell can be avoided by the protective effect of the polysaccharide protein complex from the mushroom. Thus, relieving the healthy cells from oxidative damage/stress, ensuring the intact molecular structure of the species.[10]

*Pleurotus species*

*Pleurotus species* also known as oyster mushrooms has been used as environmental bio-mediators transforming toxics into clean energy to be utilized by living organisms for centuries. It has been huge health benefits when it comes to supporting the immune system for cholesterol, cardiovascular health, blood pressure and many more health issues. Apart from proteins, carbs, essential minerals like copper, folic acid and zinc, vitamins like B1, B2, anitoxidant, carotenoids and other compounds like ergosterol which transforms into Vitamin D when exposed to sunlight. It has β-glucans and branched poly saccharides throughout the gills and mycelia. These are the immunomodulating compounds that helps the WBC cells recognize the tumor cells and elicit immune response. L-ergothioneine is an antioxidant extracted from mushrooms and is at it highest percent in oyster mushrooms. L-ergothionine (EGT) suppress the tumor growth hence a study on adding EGT with Toll-like receptor 2 (TLR2) indeed created an an efficient vaccine immunotherapy.[11]

*Pleurotus florida* is a variant of *P. ostreatus* has biocompounds like polysaccharides which is a stimulant of immune response. The methanolic extracts of the Pleurotus mushroom prevents proliferation of cervical cancer cell (SIHA) causing cell death.[12] Cytotoxic study against the T24 urinary baldder cancer cell line proved that P. florida evoked anti-cancer properties.[13]

*Pleurotus pulmonarius* commonly known as lung Oyster/Indian Oyster is cultivated in Kerala, Odissa, and Nagaland. There are n-number of medicinal benefits of the mushroom as they prove to be an effective analgesic, antihyperglycemic, hay fever, etc. The extracts of the mushrooms have both antimicrobial and antioxidant properties. Extracts like protein polysaccharides; galectin-3 from P. pulmonaris slows down the rate of cancer cell proliferation and it also

has anti-adhesive agents which blocks the attachment and aggregation of cancer cells.[14]

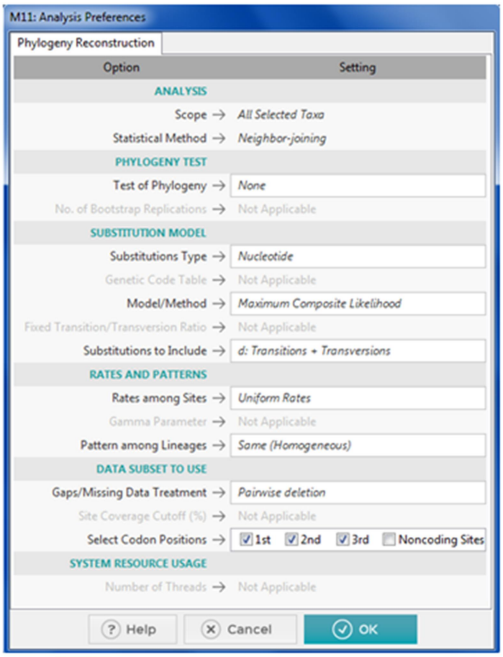
Cisplastin is a medication of chemotherapy used in a number of cancer treatments be it testicular, ovarian, cervical, breast, bladder, head and neck cancer, oesophageal, lung, brain tumor, liver cancer and many more. Chemotherapy resistance occurs when cancers that have been responding to a therapy suddenly begin to grow. So drug sensitivity must be enhanced in patients and this was possible with P. pulmonaris (oyster mushrooms). One of the studies by Wenwen Xu and his collegues exhibited the invitro liver cancer cell proliferation and invasion controlled, reduced upon administration of *P. pulmonarius*.[15]

**M11 Analysis**

Phylogeny Reconstruction

**Evolutionary relationships of taxa**

The evolutionary history was inferred using the Neighbor-Joining method [Fig. 3].[16] The optimal tree is shown. (next to the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site.[17] This analysis involved 12 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 2531 positions in the final dataset. Evolutionary analyses were conducted in MEGA11.[18]



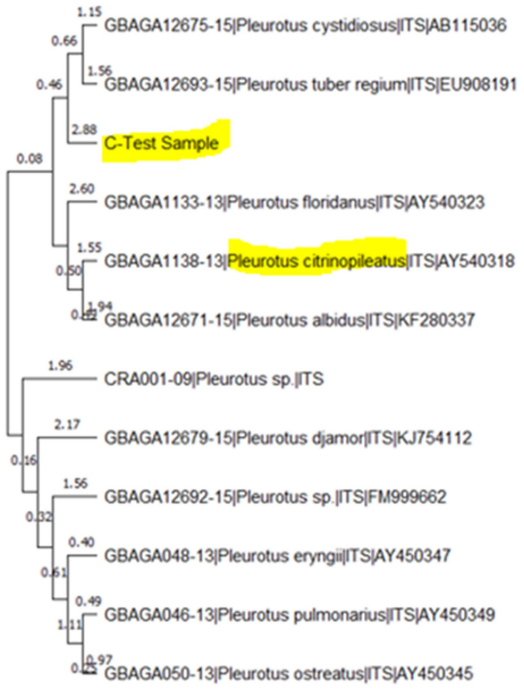




**Figure 3. Phylogeny construction using Neighbor-Joining method**

## IV. CONCLUSION

From our study we have concluded that for the extracts used in cancer therapy authentication is important and hence molecular tools like MEGA 11 softwares comes handy which which comes with various analysis prefences.

**Conflict of interest:**

The authors declare no conflict of interest

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