

ANALYSIS OF BIOACTIVE COMPOUNDS FROM PLEUROTUS OSTREATUS AGAINST CANCER CELLS

PROJECT WORK

Submitted in partial fulfillment of the requirements for the award of
Bachelor of science degree in microbiology

By

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**SCHOOL OF BIO & CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY**

SATHYABAMA

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CATEGORY - 1 UNIVERSITY BY UGC

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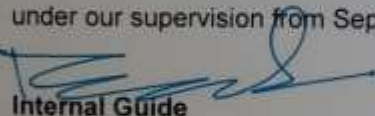
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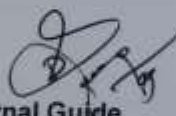
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **SUBHASHNI B (41300045)**, who carried out the project entitled "**ANALYSIS OF BIOACTIVE COMPOUNDS FROM PLEUROTUS OSTREATUS AGAINST CANCER CELLS**" under our supervision from September 2023 to April 2024.



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I would like to express my sincere and deep sense of gratitude to my Project Guide Dr. P. PRAKASH, assistant professor for his valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

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ABSTRACT

The oyster mushroom, or *Pleurotus ostreatus*, is a macro fungus that is a member of the Agaricaceae family and class Basidiomycetes. The mushroom is the second most widely grown food crop in the world. It is well-known for its pharmacological and nutritional properties, and several pure bioactive chemicals have been identified from the fruiting body with a series of solvents such as hexane, chloroform, methanol with increasing polarity. The methanol extract of *Pleurotus ostreatus* showed significant anti-cancer activity on MCF-7 cell line. Anti-microbial activity of *Pleurotus ostreatus* against gram positive bacteria such as staphylococcus aureus, gram negative bacteria such as aeromonas, klebsiella, pseudomonas, vibrio cholera, e.coli and for anti-fungal candida were studied by disk diffusion method. The thin layer chromatography was used to determine the number of compounds in a mixture. Finally the anti-oxidant potential was studied by DPPH assay.

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LIST OF ABBREVIATIONS

TLC	THIN LAYER CHROMATOGRAPHY
DPPH	2,2-DIPHENYL-1- PICRYLHYDRAZYL
IC50	HALF-MAXIMAL INHIBITORY CONCENTRATION
RSA%	RADICAL SCAVENGING ACTIVITY
μL	MICROLITER
nm	NANO METER

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CHAPTER 1

INTRODUCTION

1. CANCER

The word "cancer" actually describes over 100 different variations of the illness. The body's tissues are almost all capable of producing cancer; some can even produce multiple kinds of cancer. Every cancer also has distinct characteristics. But these various cancers seem to have very similar basic processes producing them . (Weinberg, Robert A.,in1996).A population of cells that can invade tissue and spread to distant sites, causing significant morbidity and, if left untreated, the host's death, is what is known as cancer.

1. Description of cancer

Carcinogenesis, the series of complex steps that transform a normal cell in the body into a cancer cell, is one of the many complex diseases that make up the cancer family. From a clinical perspective, cancer is a broad category of diseases that may include up to 100 different types. These diseases range in terms of prognosis, invasiveness, age at which they first manifest, rate of growth, state of cellular differentiation, diagnostic detectability, and propensity for metastasis. (Ruddon RW, in 2007 Mar 29).

Cancer often has unclear origins, while both internal and external factors such as immune system flaws and genetic predisposition as well as environmental factors such as radiation and toxins in the environment can contribute. It is obvious that one of the main contributing factors is cigarette use. . (Ruddon RW, in 2007 Mar 29).

Although cancer can occur at many age, it is usually considered a disease of aging. The average age at the time of diagnosis for cancer of all sites is 67 years, and about 76% of all cancers are diagnosed at age 55 or older. Although cancer is relatively rare in children, it is the second leading cause of death in children ages 1-14. In this age group leukemia is the most common cause of death, but other cancers such as osteosarcoma, Neuroblastoma, wilms' tumor(a kidney cancer) and lymphoma also occur. . (Ruddon RW, in 2007 Mar 29).

Cancer is a leading cause of death in the Western world. In the United States and a number of European countries, cancer is the second leading killer after cardiovascular disease. Over 1.3 million new causes of cancer occur in the United States each year, not including basal cell and squamous cell skin cancer, which add another 1 million cases annually. These skin cancers are seldom fatal, do not usually metastasize, and are curable with appropriate treatment, so they are usually considered separately. Melanoma, by contrast, is a type of skin cancer that is more dangerous and can be fatal, so it is considered with the other. The highest mortality rates are seen with lung, colorectal, breast, and prostate cancer. (Ruddon RW, in 2007 Mar 29).

.

1.1.2 Burden of cancer

An estimated 20 million new cases and 9.7 million deaths from cancer were predicted for 2022. 53.5 million people were thought to still be alive five years after being diagnosed with cancer. In their lifetime, 1 in 5 people will have cancer; 1 in 9 men and 1 in 12 women will pass away from the illness. A total of 2.5 million new cases, or 12.4% of all new cases, were lung cancer, making it the most common cancer globally. Second-placed female breast cancer (2.3 million cases, 11.6%) was followed by stomach cancer (970 000 cases, 4.9%), prostate cancer (1.5 million cases, 7.3%), and colon cancer (1.9 million cases, 9.6%). In 2020, cancer will be the primary cause of death, accounting for about 10 million deaths. The most common cancers in the US in 2020 (in terms of new cases; 1.09 million cases) were breast cancer (2.26 million cases), lung cancer (2.21 million cases), intestine and rectum cancer (1.93 million cases), prostate cancer (1.93 million cases), carcinoma (1.20 million cases), and stomach cancer (1.20 million cases). The body's oxygen deficit is the main factor contributing to cancer-related death. In 2020, around 2.3 million individuals globally were impacted by breast cancer, with 0.685 million of those cases

According to data, colon cancer (900 000 deaths, 9.3%), liver cancer (760 000 deaths, 7.8%), breast cancer (670 000 deaths, 6.9%), stomach cancer (660 000 deaths, 6.8%), and lung cancer (1.8 million deaths, 18.7% of all cancer fatalities) were the top causes of cancer-related deaths. Persistent tobacco usage in Asia is probably the reason behind lung cancer's resurgence as the most frequent malignancy. It is anticipated that there would be over 35 million new cases of cancer in 2050, a 77% increase over the projected 20 million cases in 2022. The fast increasing incidence of cancer worldwide is a result of changes in risk factor exposure, many of which are linked to socioeconomic development, as well as population expansion and aging. The rising rate of cancer is mostly caused by alcohol, tobacco use, and obesity; air pollution is still a major contributor to environmental risk factors.(Lyon *et al* in 2024).

3. Causes of cancer

Roughly one-third of cancer fatalities are caused by tobacco use, excessive body mass index, alcohol consumption, inadequate intake of fruits and vegetables, and insufficient physical exercise. One to four percent of cancer cases globally are thought to be related to pollution of the air, water, and soil. Despite the relatively low risk levels per individual, there can be a significant population effect due to the enormous number of people who are exposed—involuntarily. Drugs with immunosuppressive effects may also increase the risk of certain cancers. An increased risk of developing certain cancers can be inherited in the genetic material passed from generation to generation, accounting for up to 4 percent of all cancers worldwide

In low- and lower-middle-income nations, infections that cause cancer, like hepatitis and the human papillomavirus (HPV), account for about 30% of cancer cases. Immunosuppressive medications may potentially raise the chance of developing several malignancies. When genetic factors and outside agents interact, normal cells can become tumor cells, which is how cancer begins:

- Physical carcinogens, such as ionizing and UV radiation
- Chemical carcinogens ,such as alcohol, asbestos, and tobacco smoke

- biological carcinogens, such as parasitic, bacterial, or viral infections.(Gelband H, Sloan FA, editors in 2007)

1.1.4 Classification of cancer

Cancers are categorized according to their histological type, which refers to the kind of tissue in which the cancer begins and primary site ,the part of the body where the cancer initially appears in the body. Let's examine the main types of cancer:

➤ *CARCINOMA:*

- Between 80 and 90 percent of cancer cases are carcinomas.The epithelium that lines the body's exterior and interior surfaces is where they begin. Epithelial tissue is present in the skin, internal passageways (such as the gastrointestinal tract), and the covering and lining of organs.
- Two subtypes of carcinomas are distinguished:
 - *Adenocarcinoma:* Forms in glands or organs, such as the colon, breast, or lung.
 - *squamous cell carcinoma:*originates in Squamous epithelium, which can develop anywhere in the body.
- Often, these tumors impact glands or organs with secretory function

➤ *Sarcoma:*

- Sarcomas develop from connective and supporting tissues like muscle, fat, cartilage, and tendons.
- Typical instances consist of:
 - osteosarcoma , chondrosarcoma,leiomyosarcoma, rhabdomyosarcoma
 - ,mesothelioma, fibrosarcoma ,
 - Angiosarcoma,Liposarcoma ,
 - Glioma, myxosarcoma , mixed mesodermal tumor

➤ *Myeloma:*

- The plasma cells seen in bone marrow are the source of myeloma.
- Certain blood proteins are produced by plasma cells.

➤ *Leukemia:*

- Leukemias, sometimes referred to as "blood cancers" or "liquid cancers," develop in the bone marrow, which is the organ that produces red blood cells.

- Leukemia translates to "white blood" from Greek.
- The overproduction of immature white blood cells is linked to it.
- *Lymphoma:*
- Both non-Hodgkin and Hodgkin lymphomas are lymphomas that impact the lymphatic system.
- White blood cells known as lymphocytes are involved in these malignancies.

1.1.5 Cancer staging

The term "stage" describes the overall degree of the cancer, taking into account elements like tumor size and whether or not it has spread. Doctors can better assess the cancer's severity and choose a course of treatment when they are aware of the stage. An individual's treatment options and the severity of their cancer diagnosis are heavily influenced by their cancer's stage. Now let's examine the various phases:

1. Stage 0 (Carcinoma in situ):

- Also referred to as in situ cancer, this stage denotes the presence of aberrant cells without evidence of invasion into neighboring tissues.
- In order to stop the cancer from spreading to become invasive, treatment frequently entails eliminating the aberrant cells.

2. Stage I:

- The cancer is confined to its original tissue and is localized.
- Typically, tumors are tiny in size.
- Nodes of lymph are not impacted.
- The outlook is usually good.

3. Stage II:

- A tumor that is marginally bigger than Stage I. may affect structures or tissues nearby.
- Lymph nodes could be impacted or not.
- Prognosis differs according to type of cancer.

4. Stage III:

- The tumor has extended to tissues or lymph nodes in the vicinity, a larger tumor.

- The type of cancer and its spread determine the prognosis.

5. *stage IV (metastatic or advanced):*

- Cancer has progressed to distant organs or tissues. The prognosis is usually worse.
- Symptom management and disease progression slowing are the main goals of treatment.

1.1.6 Symptoms of cancer

The term "symptoms" refers to the feelings or views that a person has regarding modifications in health function. The perceived degree of symptoms does not last. In reaction to aggravating circumstances, they provide birth to distressing feelings that can eventually result in very unsettling unpleasant emotions. Symptoms in clinical oncology are typically not evaluated through outside observation. Patients report them in the form of medical issues. The following are some typical indications and symptoms of cancer:

- *Fatigue:* An ongoing, intense state of exhaustion that doesn't go away with rest.
- *Pain:* an unpleasant sensory and emotional experience
- *Unexpected weight changes:* Unexpected weight increase or loss of at least ten pounds without a recognized cause.
- *Eating disorders:* include trouble swallowing, abdominal pain, nausea, vomiting, or appetite loss.
- *Lumps or swelling:* Any strange lumps or swelling that develops anywhere on the body.
- *Emotional distress:* an extremely disagreeable emotional response

The severity of these symptoms varies depending on how long it has been since the commencement of treatment; typically, a few weeks after therapy begins, treatment-related symptoms peak. The term "symptom trajectory" refers to the variation in symptom kind and severity over time. Furthermore, there is a greater chance of certain symptom subgroups co-occurring. "Symptom clusters" are groups of symptoms that appear simultaneously as a result of an illness or therapy.

Depending on the kind of cancer, the course of treatment, and the timing of symptom evaluation, different symptom clusters occur.

1.2 PLEUROTUS OSTREATUS

Pleurotus ostreatus both naturally colonized on almost all hardwoods, particularly on dead and decaying tree stumps, fallen branches, agro/wood by-products, food and cellulose wastes, wheat straw bales, and sawdust with simple methods including just pasteurization to represent high-quality food having a higher yield and growth and shorter substrate colonizing time than other cultivated mushrooms. The cap (pileus) of the oyster mushroom has whitish to grey color and fleshy. Stipe cream and smooth surface. Spores are whitish to lilac grey in mass, cylindrical to oblong shape. *Pleurotus ostreatus* contains important essential nutrients for human nutrition and is a natural source used in both traditional and modern medicine. Moreover, *Pleurotus ostreatus* belongs to the “white-rot fungi” category, which produces the ligninolytic enzymes laccase and peroxidase degrading hemicellulose, cellulose and lignin. *Pleurotus ostreatus* is a culinary-medicinal mushroom because of its excellent taste, flavor, high nutritional value, and pharmacological potential supported by both in vitro and in vivo experimental studies, as well as clinical trials. Edible mushrooms have been consumed as food and medicine due to their valuable macro and micronutrients, and mycochemicals including phenolic compounds, triterpenoids, sterols, fatty acids, and proteins. They are also taken as part of a regular diet and have additional benefits such as managing chronic inflammatory illnesses, boosting immunity, managing obesity, preventing diabetes, treating cancer, and managing degenerative diseases.

KINGDOM	Fungi
SCIENTIFIC NAME	<i>Pleurotus ostreatus</i>
DIVISION	Basidiomycota
DOMAIN	Eukaryota
SUBDIVISION	Basidiomycotina
CLASS	Basidiomycetes
SUBCLASS	Holobasidiomycetidae
ORDER	Agaricales
FAMILY	Tricholomataceae
GENUS	Pleurotus



fig1.1:Oyster mushroom

CHAPTER 2

LITERATURE SURVEY

2.1 PLEUROTUS OSTREATUS

The most widely grown edible mushroom globally is *Pleurotus ostreatus*, followed by *Agaricus bisporus*. Together with therapeutic qualities, it has ecological and economic worth. With the emergence of new varieties, mushroom culture has shifted toward diversification. The ability of edible mushrooms to colonize and decompose a wide range of lignocellulosic substrates and other wastes, mostly generated by the agricultural, forest, and food processing sectors, is noteworthy. In particular, *P. ostreatus* needs less time to grow than other edible mushrooms. Sterilization is not necessary for the substrate used in their cultivation; pasteurization is, and that is less expensive. Profitability is increased when oyster mushrooms are grown because a significant portion of the substrate is converted to fruiting bodies. *P. ostreatus* is easy to grow and inexpensive to maintain. It requires little in the way of environmental controls, and pests and diseases rarely harm its fruiting bodies. When compared to other mushroom culture methods, all of these factors make *P. ostreatus* cultivation a good choice (Carmen Sánchez, 2010).

The impact of potassium humate on *Pleurotus florida* growth rate and nutritional value was examined in the current study. Different potassium humate concentrations—10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%—were used to develop *Pleurotus florida*. *Pleurotus florida* at each treatment is assessed for total biomass, protein, fiber, ash, and moisture content after incubation. Compared to the untreated control, they were found to be higher in all treated groups. In comparison to the untreated control, the greatest biomass and all nutritional contents were seen at a potassium humate concentration of 60%. (prakash.p et al, 2011).

A vital component of a typical human diet, the number of mushrooms consumed has increased significantly in recent years and covers a wide range of species. About 40 distinct species in the genus *Pleurotus* are collectively referred to as "oyster mushrooms." *Pleurotus ostreatus* (*P. ostreatus*) is one of the several species in this genus that is widely consumed worldwide because of its flavor, taste, high nutritional value, and therapeutic qualities. *P. ostreatus* has been shown to have antidiabetic, antibacterial, anticholesterolic, antiarthritic, antioxidant, anticancer, eye health, and antiviral properties due to the presence of multiple nutritional compositions and active components. In this review, we specifically highlight *P. ostreatus*'s excellent nutritional values in relation to their possible medical applications, suggesting that *P. ostreatus* mushrooms are the most significant functional foods that include nutraceuticals.(Deepalakshmi , K *et al*,2014)

Investigations were conducted on the cultivation of *Pleurotus ostreatus* on a variety of substrates, including rice straw, rice straw + wheat straw, rice straw + paper, sugarcane bagasse, and alder sawdust. 10% rice bran was added to all substrates, with the exception of rice straw. The supplement-free substrate was regarded as the control. Analyses were conducted on the effects of different substrates on mycelial growth, time taken for colonization, time taken for the primordial appearance, yield of mushrooms, size of the mushroom, and chemical composition. For the cultivation of mushrooms, rice straw (control) was determined to be the best substrate overall, with yield (381.85 grams) and BE(95.46%)%, followed by rice plus wheat straw and rice straw plus paper waste. The nutritional makeup of mushroom fruit cultivated on rice straw was likewise superior.(Sharma, S *et al*,2013).

2.2 ANTICANCER ACTIVITY OF PLEUROTUS OSTREATUS

The ethanolic extract inhibited the growth and proliferation of MCF-7 cells. It also induced cell shrinkage, membrane blebbing and nuclear fragmentation, resulting in apoptosis of malignant cells. The molecular docking analysis showed that ligand 15 (Linoleic acid ethyl ester), ligand 27 (Ergosta-5,7,9(11),22-tetraen-3-ol, (3. beta.,22E),

ligand 28 (Stigmasta-5,22-dien-3-ol, acetate, (3. beta.,22Z), ligand 30 (Ergosta-5,7,22-Trien-3-Ol, (3. Beta.,22E) and ligand 32 (gamma. Sitosterol) exhibited better binding affinities with EGFR, PR and NF- κ B proteins. This result provides a strong ground for confirmation of the in vitro anticancer effect of *Pleurotus ostreatus*. From the present in vitro and in silico studies, it can be concluded that *Pleurotus ostreatus* is a useful source of essential elements and reservoir of bioactive compounds which confer its significant antioxidant, antibacterial and anticancer properties. .(Mishra V et al,2022).

Beta glucan's ability to function as an immunomodulator has been suggested to provide it anticancer potential. Using 7,12 Dimethylbenz(a)anthracene (DMBA) as a cancer promoter, female rats were used in this work to examine the anticancer effect of the beta glucan found in oyster mushrooms. There were four groups of experimental rats: one group received beta glucan treatment (at two different doses of 0.25 g.kg⁻¹ and 1 g.kg⁻¹), one group received DMBA treatment, and one group was controlled. In comparison to the DMBA group, the beta glucan-treated group displayed a decreased incidence of tumor cases, indicating inhibition of carcinogenesis. The group treated with beta glucan had fewer tumor nodules overall and in terms of total volume than DMBA organization. When the histopathology of the tumor nodules was analyzed, the group that received beta glucan treatment scored lower than the group that received DMBA, indicating a less severe tumor. In conclusion, beta glucan derived from oyster mushrooms exhibits antitumor efficacy against breast cancer produced by DMBA in vivo. (Susanti, I et al,2018).

The oyster mushroom, or *Pleurotus ostreatus*, is a macrofungus that is a member of the Agaricaceae family and class Basidiomycetes. The mushroom is the second most widely grown food crop in the world. It is well-known for its pharmacological and nutritional properties, and several pure bioactive chemicals have been identified from the fruiting body and mycelium's various solvent extracts. Numerous scholarly articles have documented the numerous health benefits that these bioactive chemicals possess, including antiviral, immunomodulatory, anti-inflammatory, hypocholesterolemic, and tumor chemopreventive properties. *Pleurotus*

ostreatus's bioactive molecule with anticancer action is included in this brief review along with updated information. Upon critical analysis, it was found that *Pleurotus ostreatus* exhibited significant anticancer activity on both animal and human cell lines when compared to a control group. This review involved consultation with students and a total of 13 reported anticancer activities.(Dipan Sarma *et al*,2018)

2.1: Table for anti-cancer activity of *pleurotus ostreatus*

Sl. No	Experimental model	Supplementation	Outcome/ Result	References
1.	HCT- 116 cell line (colorectal carcinoma cell line)	Gemmotherapeutic extract of young parts of <i>P. ostreatus</i>	Exhibit antitumor activity.	Pauliuc <i>et al</i> ,2013
2.	Ehrlich Tumor (ET) and Sarcoma 180 (S180)	Polysaccharide fraction extracted from mycelium biomass of <i>Pleurotus ostreatus</i>	Inhibited the development of Ehrlich Tumor (ET) and Sarcoma 180 (S180)	Facchini JM <i>et al</i> ,2014
3.	Brine shrimp lethality bioassay	Ethyl acetate, hexane and chloroform extract of <i>Pleurotus ostreatus</i>	Potent anticancer activity	Sala Uddin GM <i>et al</i> ,2015
4.	BGC 823 human gastric cancer cell line <i>in vitro/in vivo</i>	Polysaccharides extracted from <i>Pleurotus ostreatus</i> mycelium and a	POMP2 inhibited the proliferation of BGC-823 cells <i>in vitro/ in vivo</i>	Cao XY <i>et al</i> ,2015

		compound <i>Pleurotus</i> <i>ostreatus</i> mycelium polysaccharides 2 (POMP2)		
5.	Male BALB/c (nu/nu) nude mice	Polysaccharides extracted from <i>Pleurotus</i> <i>ostreatus</i> mycelium and a compound <i>Pleurotus</i> <i>ostreatus</i> mycelium polysaccharides 2 (POMP2)	POMP2 significantly decreases the tumor weight compared with control <i>in vivo</i>	Cao XY <i>et al</i> ,2015
6.	Human Caco- 2 cell line in vitro	<i>Pleurotus</i> <i>ostreatus</i> polysaccharide extract	Down-Regulates the Expression of MMP-2 and MMP-9 on human Caco-2 cells.	Cojocaru S <i>et al</i> ,2013
7.	Benzene- induced leukemia in Wister rats	Fungal metabolites of <i>Pleurotus</i> <i>ostreatus</i>	Enhanced leukopoiesis, showed phagocytic actions and exhibits significant therapeutic potential to suppress leukemia (P<0.05)	Olufemi AE <i>et al</i> , 2012

8.	Dalton lymphoma (DL)-bearing mice, Sarcoma-180, and B16F0 melanoma tumor-bearing mice	Cibacron blue affinity purified protein (CBAEP)	CBAEP reduced Dalton lymphoma (DL) cell growth and activated immunosuppression condition in DL tumor-bearing mice.	Maiti S <i>et al</i> , 2011
9.	Hela tumor cell and human embryo kidney 293T cells in vitro	Aqueous polysaccharide POPS-1 of fruiting bodies of <i>Pleurotus ostreatus</i>	Significantly higher antitumor activity against Hela tumor cell in vitro and lower cytotoxicity to human embryo kidney 293T cells.	Tong H <i>et al</i> , 2009
10.	The Ehrlich ascitic tumor bearing female Swiss albino mice	Polysaccharide extract from <i>Pleurotus ostreatus</i>	Inhibit the development of Ehrlich ascitic tumor cells and reduced the neoplastic cells.	Wolff ERS <i>et al</i> , 2008
11.	Breast cancer (MCF-7, MDA-MB-231) and colon cancer (HT-29, HCT-116) cells	<i>Pleurotus. ostreatus</i> extracts	<i>P. ostreatus</i> prevents the proliferation of breast and colon cancer cells	Jedinak A <i>et al</i> , 2008
12.	Sarcoma 180 bearing mouse <i>in vitro</i> / <i>in vivo</i>	Water-soluble proteoglycan fractions from <i>Postreatus</i>	Sarcoma 180 bearing mice decreased the number of tumor	Sarangi I <i>et al</i> , 2006.

		mycelia	cells in vivo.	
13.	Mice with solid Ehrlich carcinoma	DNA isolated from the fruit body of <i>Pleurotus ostreatus</i>	Increase of the life span of mice with solid Ehrlich carcinoma.	Shlyakhovenko V <i>et al</i> , 2006

2.3Anti-microbial activity

The agar well diffusion method was used to assess the antibacterial activity of the EEPO against the tested microorganisms, which included both Gram-positive and Gram-negative bacteria. The EEPO was shown to exhibit the most promising antibacterial activity against both Gram-positive and Gram-negative bacterial strains at 200 mg/mL. Table 3 presents the results of the inhibition zones of the EEPO (200 mg/mL) against *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*, which were 29.6 ± 0.2 , 27.4 ± 0.2 , 24.4 ± 0.3 , and 16.4 ± 0.2 , respectively. (Mishra V *et al*, 2022).

The antimicrobial properties against *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Agrobacterium vitis*, *Bacillus cereus*, and *Streptococcus agalactiae* were studied. By using the agar well diffusion method, the antimicrobial activities of oyster mushroom extracts against *Bacillus cereus*, *Streptococcus agalactiae*, *Agrobacterium*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Shigella dysenteriae* were investigated. The zones of inhibition varied depending on the organism, but *P.ostreatus* and *P.sajor-caju* had the highest zones of inhibition for all tested organisms, with the exception of *E. coli* and *S. dysentriae*, where *P.pulmonarius* and *P.populinus* had higher zones. While *P. pulmonarius* and *P. populinus* were shown to be significantly different ($p > 0.05$) from *B. cereus*, *E. coli*, and *S. dysenteriae*, *P. ostreatus* and *P. sajor-caju* were not statistically different against any of the examined microorganisms. The inhibition of *P. pulmonarius* and *P. populinus* against every tested microbe did not differ significantly (Okafor D. C. *et al* ,2017).

The edible fungus *Pleurotus ostreatus* has significant therapeutic significance. *P. ostreatus* was examined in this work to see if it could stop bacteria and fungi from growing. Using alcohols and water as solvents, the freeze-dried fruiting body, broth from submerged culture, and mycelial biomass of *P. ostreatus* were extracted. Next, the antibacterial activity of the extracts against the development of bacteria and fungus was evaluated. The most effective method for preventing the growth of most fungi was found to be the water extract from fruiting bodies. *Candida albicans*, *Trichosporon cutaneum*, and *Cryptococcus humicola* were the test microfungi that were most susceptible to the inhibition; *Staphylococcus aureus* and *Escherichia coli* were the test bacteria that were most sensitive. Microbiological and fungal development was only slightly inhibited by water extracts from mycelial biomass or culture broth. For the majority of test bacteria, the alcohol-based solvents from all samples exhibited much lower antimicrobial efficacy. The minimal inhibitory concentrations of this isolated chemical against the growth of bacteria and fungi were 20 µg/mL and 30 µg/mL, respectively. (Ahmed M. Younis *et al*, 2015)

2.4 Anti-oxidant activity

The DPPH assay was utilized to assess and screen *Pleurotus ostreatus* (EEPO) ethanolic extract for its ability to scavenge free radicals. Figure 4 shows the results of the EEPO's scavenging potential. According to our research, the EEPO's capacity to scavenge free radicals increased along with its concentration, peaking at 1000 µg/mL (around 50% inhibition). Nevertheless, it was also determined that the EEPO's capacity to scavenge free radicals was considerably less than that of ascorbic acid. (Mishra V *et al*, 2022). (Okafor D. C. *et al*, 2017) stated that there were notable differences in the overall antioxidant activity. *P. ostreatus* = 35.36 ± 0.01 mm, *P. sajor-caju* = 32.26 ± 0.02 mm, *P. pulmonarius* = 28.86 ± 0.01 mm, and *P. populinus* = 26.65 ± 0.01 mm were the mean total antioxidant activities.

Many ancient cultures have used mushrooms as a food and medicinal source for generations. However, due to their therapeutic properties, mushrooms remain an

important component of traditional medicine, particularly in China and South East Asia. Due to its high nutritional content, *Pleurotus* spp. is one of the many species of recognized mushrooms that is well-known for being a member of the food chain. Nevertheless, only a small number of the more than 70 species that are known are cultivated for commercial purposes, including *P. ostreatus*, *P. florida*, and *P. ajor-caju*. But because of its rapid growth and simplicity in growing on a variety of substrates, *P. ostreatus*, also known as oyster mushroom, has drawn greater interest in the food sectors. Numerous recognized medicinally valuable bioactive compounds, including those with anticancer, immunomodulatory, antiapoptotic, antihypercholesterolemic, antihyperglycemic, antibacterial, anti-inflammatory, and antiosteoporetic properties, are abundant in this mushroom. The review of the many types of bioactive chemicals found in oyster mushrooms that have antioxidant properties, including phenolics, beta carotene, lycopene, ascorbic acid, tocopherols, and ergosterols, is the main goal of this work. This study offers a thorough overview of the latest research aimed at improving the antioxidant capabilities of *P.ostreatus* by modifying the growth technique and adding certain chemicals during the process.(Shanmugaprakasham Selvamani et al,2018).(venkatakrishnan et al, 2010)Strong antioxidant activity against DPPH and ABTS radicals was shown by the ethanolic extract, with EC50 values of 0.202 ± 0.55 mg/mL and 6.42 ± 0.261 mg/mL, respectively. The antioxidant components were found in 1.82 ± 0.15 µg/mg (corresponding to quercetin) and 8.52 ± 0.6 mg/g (corresponding to catechin).

Antioxidant potential was ascertained by means of chelating activity, inhibition of lipid peroxidation, reducing power, and free radical scavenging activity (ABTS, DPPH, and superoxide radicals). Almost all of the biologically active molecules in substrates are reported to be diminished in the presence of a higher lignin component, such as beech shavings. One direct correlation between this fact and less biological activity was found. The nutritional value of the mushrooms produced will grow according to these studies, which also indicate that the mycelium's ability to break down substrate and absorb nutrients will both play a role.(Roxana Iulia Stefan et al,2015).

CAPTER 3

AIM AND OBJECTIVE

1. AIM

To study anti-cancer activity of *Pleurotus ostreatus* (oyster mushroom) and investigate its anti-microbial and anti-oxidant activity.

2. OBJECTIVES

- Cultivation and extraction of compounds from mushroom
- Antimicrobial antioxidant and anticancer activity from extraction
- Purification of extraction by chromatography
- Chemical screening and characterization of active compound

CHAPTER 4

MATERIALS AND METHODS

1. CULTIVATION AND HARVESTING

1. ***CULTIVATION OF *Pleurotus ostreatus*:***

- *Step1*

Pleurotus ostreatus grow best on straw , which have been sterilized to prevent contamination from other microorganism

- *Step2:*

Mix your *Pleurotus ostreatus* spawn or spores into the cooled substrate. Make sure the spawn is evenly distributed throughout the substrate

- *Step3:*

Pack the substrate into the plastic bags. Make some small holes and hang the bag

- *Step4:*

Place your bag, ideally with a temperature between 20-25°C

4.1.1 Harvesting of *pleurotus ostreatus*:

The right stage for picking can be judged by the shape and size of fruitbody. In young mushrooms the edge of the cap is thick and cap margin is enrolled while the cap of mature mushroom become flat and inward curling starts. After harvesting lower portion of the stalk with adhering debris should be cut using a knife

2. PREPARATION OF PLEUROTUS OSTRESTUS EXTRACT:

The harvested oyster mushroom were cut into small pieces and placed in a petri plate. Dry in the hot air oven on its lowest temperature setting at 52°C until the oyster mushrooms are brittle, not bent. The dried oyster mushroom were powdered in a mixer and the powder weighs about 60g.

1. ***Preparation of hexane extract for *pleurotus ostrestus*:***

Add 60g of *pleurotus ostrestus* powder in 300ml of hexane in 500ml conical flask, cover the conical flask with cotton plug and aluminum foil. Place the conical flask in shaker for 72hrs. Filter the extract using filter paper, with the help of funnel transfer it into a petri plate and allow it to evaporate. Add 100ml of hexane in dried

extraction which is then collected using spatula and transferred into eppendorf tube. Dry the powder separately for the next process for chloroform extraction.

4.2.2 Preparation of chloroform extract for *pleurotus ostrestus*:

Add the dried *pleurotus ostrestus* powder in 300ml of chloroform in 500ml conical flask, cover the conical flask with cotton plug and aluminum foil. Place the conical flask in shaker for 72hrs. Filter the extract using filter paper, with the help of funnel transfer it into a petri plate and allow it to evaporate. Add 100muel of chloroform in dried extraction which is then collected using spatula and transferred into eppendorf tube. Dry the powder separately for the next process for metanol extraction

4.2.3 Preparation of methanol extract for *pleurotus ostrestus*:

Add 60g of *pleurotus ostrestus* powder in 300ml of methanol in 500ml conical flask, cover the conical flask with cotton plug and aluminum foil. Place the conical flask in shaker for 72hrs. Filter the extract using filter paper, with the help of funnel transfer it into a petri plate and allow it to evaporate. Add 100muel of metanol in dried extraction which is then collected using spatula and transferred into eppendorf tube.

3. ANTI-MICROBIAL SUSCEPTIBILITY TEST/DISK DIFFUSION METHOD:

1. Materials

- Mueller-Hinton agar
- Petri Plates
- Bacterial broth culture
- swab
- Disk/well cutter
- Forceps
- Recommended organisms for quality assurance purposes are staphylococcus aureus, klebsiella ,pseudomonas, vibrio cholerae, escheria coli, aeromonas, candida

2. Procedure:

Preparation of inoculum

- Using a sterile inoculating loop, touch four or five isolated colonies of the organism to be tested.
- Suspend the organism in 1ml of Mueller-Hinton broth.
- Vortex the saline tube to create a smooth suspension.

Inoculation of the Muller-Hinton plate

- Suspend 10.56g of Mueller-Hinton agar in 200ml of distilled water and autoclave at 121°C for 45 minutes
- The plates must be poured 25ml of liquid agar and allowed to solidify at room temperature.
- Appropriately label each MH agar plate for each organism to be tested.
- Dip a sterile swab into the inoculum tube
- Rotate the swab against the side of the tube using firm pressure, to remove excess fluid.
- Inoculate the dried surface of a MH agar plate by swab three times over the entire agar surface; rotate the plate approximately 60° each time to ensure an even distribution of the inoculum.
- Rim the plate with the swab to pick up any excess liquid
- Discard the swab into an appropriate container.

Placement of the disks

- Place the appropriate disks on the surface of the agar, using forceps to dispense each disk one at a time.
- Sterilize the forceps by sowing it in flame and allow to air dry.
- Using the forceps carefully take one disk, partially remove the lid of the petri dish.
- Place the disk on the plate and gently press the disk with the forceps to ensure complete contact with the agar surface. Replace the lid to minimize exposure of the agar surface to room air.
- Do not move the disk once it has contacted the agar surface even if the disk is not in the proper location.
- Once all disks are in place, replace the lid, invert the plates, and place them in incubator at 35°C for 16-18 hours.

- After incubation, zones are observed around the discs. This denotes the antimicrobial activity results are positive. The zones are measured using scale to see the activity of *Pleurotus ostreatus* extract. If there is no zone formation the result is negative

4. ANTI-OXIDANT ASSAY/DPPH:

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a commonly employed technique to evaluate a compound's antioxidant capacity. DPPH, a persistent free radical that turns purple to yellow when reduced by an antioxidant molecule, is used in this experiment. The antioxidant's capacity for scavenging is shown by the extent of color change or absorbance reduction. The level of color change indicates the level of antioxidant activity.

1. **MATERIALS**

- 96well plate
- Pipette
- 0.00394g of DPPH
- Methanol
- *Pleurotus ostreatus* extract
- UV spectrophotometer

2. **METHODOLOGY**

- The DPPH assay should be performed in a dark room as DPPH is photosensitive.
- To perform DPPH assay, a solution of DPPH is prepared in a solvent such as methanol or ethanol. 0.00394 of DPPH is added in 100ml of methanol.
- Prepare different concentration of extract in 1ml of DMSO.
Eg: 20µL in 1ml of DMSO.
- In a 96well plate add 25µL of the sample solution.
- Add 150µL of DPPH reagent.
- 200µL of DPPH reagent as control.
- Ascorbic acid is used as control.
- Incubate in dark for 30min.

- After incubation, it is observed that the colour of the sample changes from purple to yellow or colourless
- Then run the plate in a UV spectrophotometer at a wavelength of 517nm.
- The readings are interpreted in an excel sheet. Then readings are calculated and the results are represented as a graph.

5. *Thin layer chromatography(TLC):*

A method for separating and analyzing various components of a mixture is called thin layer chromatography, or TLC. It is an easy and quick process that applies a thin coating of absorbent substance to a glass, metal, or plastic plate. Usually, this material is alumina or silica gel.

1. Principle:

The TLC plate is spotted with the sample to be analyzed at the bottom, and it is then vertically placed in a container containing a small amount of solvent. The solvent rises up the plate through capillary action. The components of the sample are carried up the plate by the solvent. The mixture's various components separate into discrete spots on the TLC plate based on their respective physical and chemical characteristics. After that, the individual components can be seen using a variety of methods, including staining with certain reagents, iodine vapour, and ultraviolet light.

Materials required

- Beaker
- TLC sheet
- Pipette
- Aluminium foil
- Toluene
- Ethyl acetate
- Formic acid

2. procedure

- Cut TLC Plate (size:2*10cm).
- Spot the extract at a position, 0.5cm above from the bottom of TLC plate.

- Place TLC in the chamber and let the solvent run up the plate. When the solvent front reaches approximately 1-0.5cm from top of the plate, remove plate from TLC chamber and note down the solvent front.
- Let TLC plate dry and then visualize in UV chamber.
- The TLC plate is stained using granules of iodine resublimed. The vapours from the granules stain the TLC plate.

4.5.3 Mobile phase preparation

- A. Hexane:ethylacetate:formicacid (7:3:0.2)
- B. Toluene:ethylacetate:formicaacid (7:2:1)

6. : CELL CULTURE

Methanol extract of oyster mushroom was used in concentration of (control 10,50,100,150,200,250,300 ug/ml. all the samples were dissolved in 0% DMEM media and used for further experiments. 1% methanol alone treated was done to blank cells. MCF-7 breast cancer cells procured from NCCS pune.

1. MTT assay

- 1×10^4 cells/well were added
- Incubated for 24hrs at 37°C in CO₂ incubator
- After incubation samples were added into the 96-well plate in triplicates
- Extract was used in concentration of (control 10,50,100,150,200,250,300 ug/ml) incubated for 24hrs at 37°C in CO₂ incubator
- 50 µl of MTT was added and incubated for 4hrs in dark conditions
- Then 100 µl of DMSO was added and kept in shaker for 20mins
- Absorbance of color yellow to purple was measured at 570nm

CHAPTER 5

RESULT AND DISCUSSION

5.1 CULTIVATION AND HARVESTING OF OYSTER MUSHROOM

The mushroom was cultivated under optimum conditions and the yield was 700g. similarly prakash et al 2011 reported that potassium humate was enhancing the mushroom yield. *Pleurotus florida* was cultivated with varying potassium humate concentrations. The maximum biomass and all the nutrient contents were recorded in 60% concentration of potassium humate than untreated control.



fig 5.1:cultivation of pleurotus ostreatus (oyster mushroom)



Fig 5.1.1:Harvesting of pleurotus ostreatus (oyster mushroom)

5.2 EXTRACTION



Fig 5.2:Dried mushroom powder

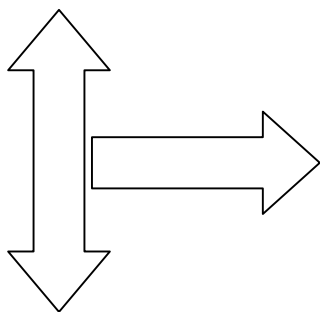


Fig 5.2.2:Hexane filtrate

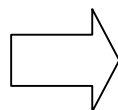


Fig 5.2.3:Hexane extract



Fig 5.2.1:Extraction using hexane



fig 5.2.4:dried mushroom powder

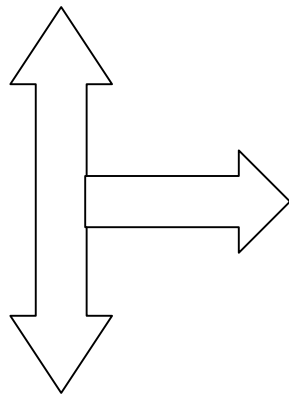
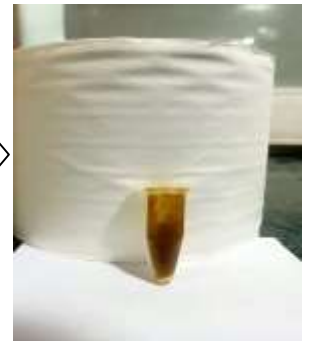
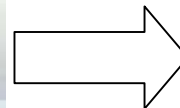


Fig 5.2.5:Extraction using chloroform



Fig 5.2.6:chloroform filtrate



5.2.7:chloroform extract



Fig 5.2.8:dried mushroom powder

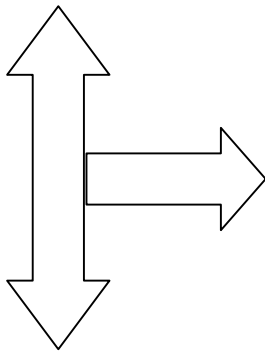


Fig 5.2.10:methanol filtrate

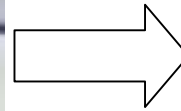


Fig 5.2.11:methanol extract



Fig 5.2.9:Extraction using methanol

5.3 ANTI-MICROBIAL ACTIVITY:

The data pertaining to the anti-microbial potential of the extract are present in table 5.1 respectively. The chloroform extract and methanol extract results increased activity against staphylococcus aureus. The methanol extract showed highest activity against vibrio cholerae. similarly, Antimicrobial activities of the extracts against *Bacillus cereus*, *Streptococcus agalactiae*, *Agrobacterium vitis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Shigella dysenteriae* were investigated. Antimicrobial activities of the oyster mushroom extracts against *Bacillus cereus*, *Streptococcus agalactiae*, *Agrobacterium*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Shigella dysenteriae* were examined by agar well diffusion method and zones of inhibition varied for different organisms but zones of inhibition were highest in *P.ostreatus* and *P.sajor-caju* for all tested organisms except in *E.coli* and *S.dysenteriae* where *P.pulmonarius* and *P.populinus* had higher zones. *P.ostreatus* and *P.sajor-caju* were not significantly different against all tested microorganisms but were found to be significantly different ($p \geq 0.05$) from *P.pulmonarius* and *P.populinus* against *B.cereus*, *E.coli* and *S.dysenteriae*. *P.pulmonarius* and *P.populinus* were not significantly different in their inhibition against all tested microorganisms (Okafor D et al., 2017)

TABLE 5.3: ANTI-MICROBIAL RESULT FOR PLEUROTUS OSTREATUS

ORGANISMS	HEXANE	CHLOROFORM	METHANOL
AEROMONAS	—	—	+
CANDIDA	—	+	+
ESCHERICHIS COLI	—	—	—
KLEBSIELA	—	—	—

PSEUDOMONAS	-	+	+
STAPHYLOCOCCUS AUREUS	-	+	+
VIBRIO CHOLERAEE	-	-	+

Present+, Absent-

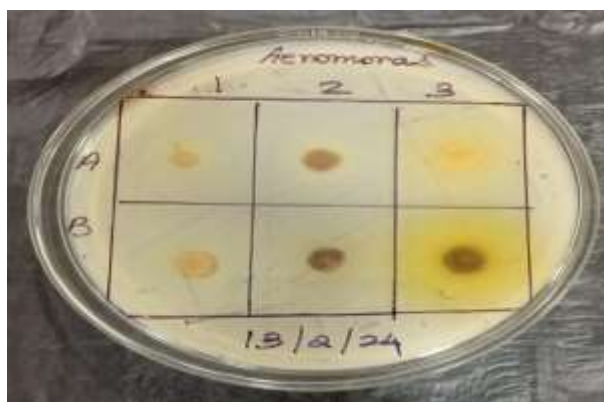


Fig 5.3:Anti-microbial activity for aeromonas
(disk diffusion method)

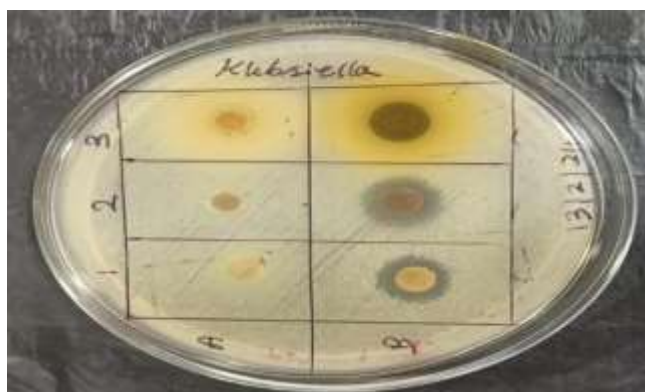


Fig 5.3.3:Anti-microbial activity for klebshiela
(disk diffusion method)

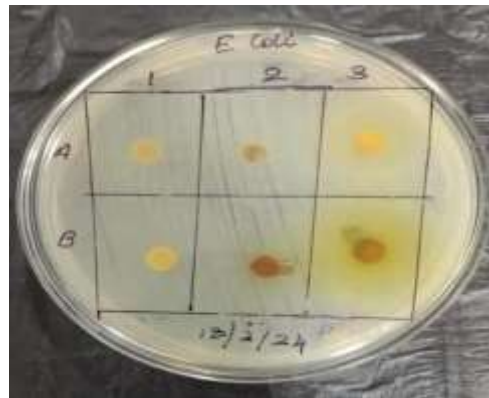


Fig 5.3.2: Anti-microbial activity for e.coli
(disk diffusion method)

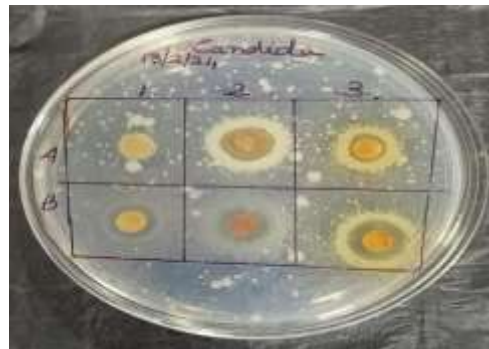


Fig 5.3.1: Anti-microbial activity for candida
(disk diffusion method)

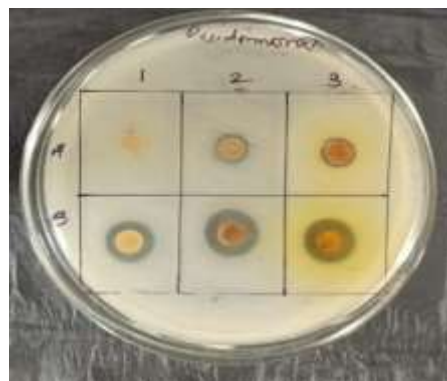


Fig 5.3.4: Anti-microbial activity for pseudomonas
(disk diffusion method)

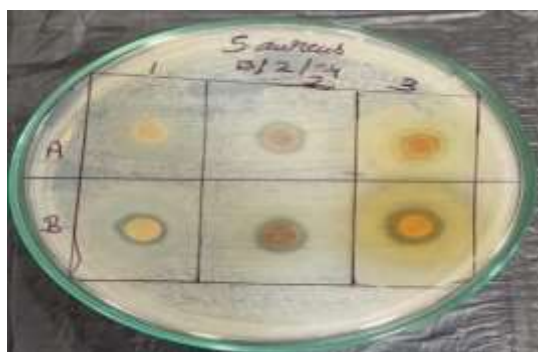


Fig 5.3.5:Anti-microbial activity for staphylococcus aureus

(disk diffusion method)

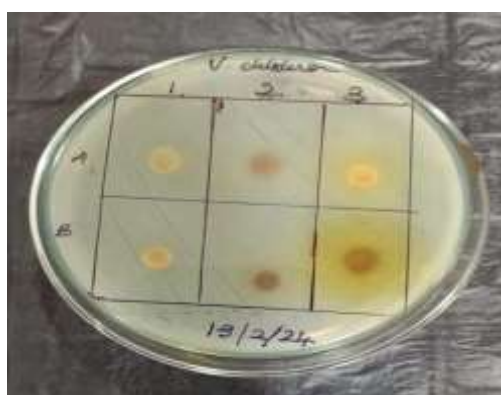


Fig 5.3.6:Anti-microbial activity for vibrio cholera

(disk diffusion method)

5.4 Antioxidant activity:

Prepare different concentration of extract in 1ml DMSO. Overall results showed methanol extract was found to be best extract for radical scavenging activity potential compared to hexane and chloroform extract. Similarly, the extracts were analyzed for their antioxidant activities using the DPPH (2,2-diphenyl-1-picrylhydrazylhydrate) scavenging activity method. The results revealed that the water extracts exhibited the highest % DPPH scavenging activity in comparison to all other solvent extracts. The highest value was obtained from the water extract of *P. sajor-caju* (78.1%), and the

lowest one was from the hexane extract of *A. bisporus* (0.8%). In general, extracts from non-polar solvents exhibited much lower antioxidant activities than those from polarsolvents.(Eman Nasr Elbatrawy, *et al*,2015).



Fig 5.4:DPPH ASSAY



Fig 5.4.1:UV Spectroscopy

TABLE FOR DPPH 5.4: DPPH ASSAY STANDARD

CONCENTRTION	CONTROL	SANDARD OD	%RSA
10	0.63	0.076	87.93650
20	0.63	0.065	89.68254
30	0.63	0.064	89.84127
40	0.63	0.066	89.52381
50	0.63	0.061	90.31746

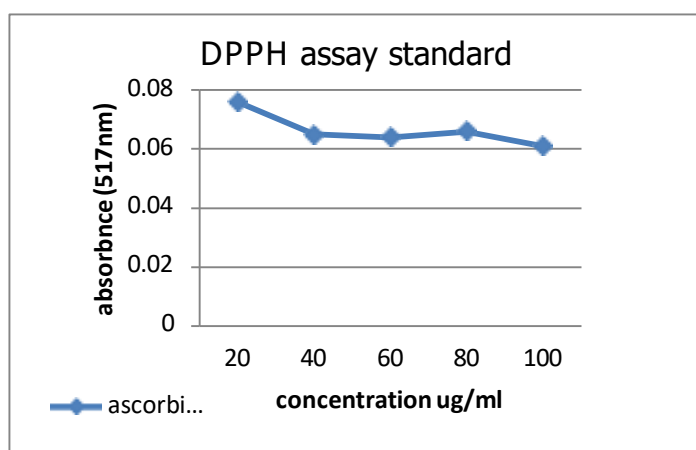


fig 5.4.2 Graphical representation of RSA%(standard)

TABLE FOR DPPH 5.4.1: PLEUROTUS OSTREATUS HEXANE EXTRACT

CONCENTRTION	CONTROL	STANDARD OD	%RSA	IC50
10	0.63	0.221	64.92063	9.29946
20	0.63	0.253	59.84127	7.41323
30	0.63	0.105	83.33333	5.52701
40	0.63	0.043	93.1746	3.64079
50	0.63	0.159	74.7619	1.75456

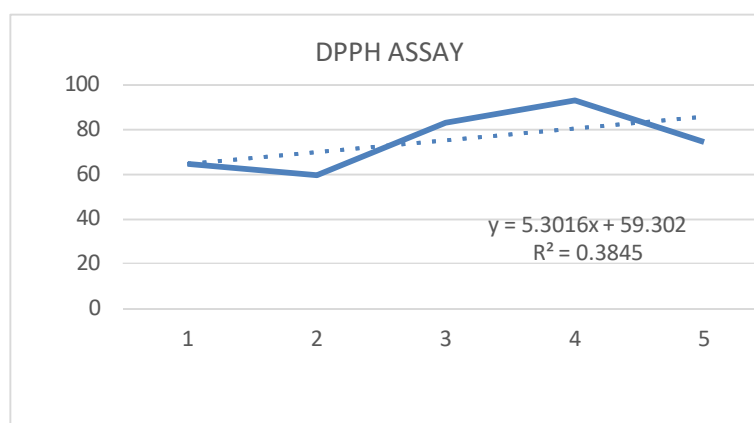


fig 5.4.3 Graphical representation of RSA%(hexane extract)

FORMULA

%RSA = [(absorbance of control - absorbance of sample) / absorbance of control] * 100

ic50

$y = mx + b$

$x = (y - b) / m$

y = concentration

m = slope, 5.301

b = intercept, 59.302

TABLE FOR DPPH 5.4.2: PLEUROTUS OSTREATUS CHLOROFORM EXTRACT

CONCENTRATION	CONTROL	STANDARD OD	%RSA	IC50
10	0.63	0.386	38.73016	3.67621
20	0.63	0,337	46.50794	1.43421
30	0.63	0.587	6.825397	0.807793
40	0.63	0.346	45.07937	3.049795
50	0.63	0.241	61.74603	5.291797

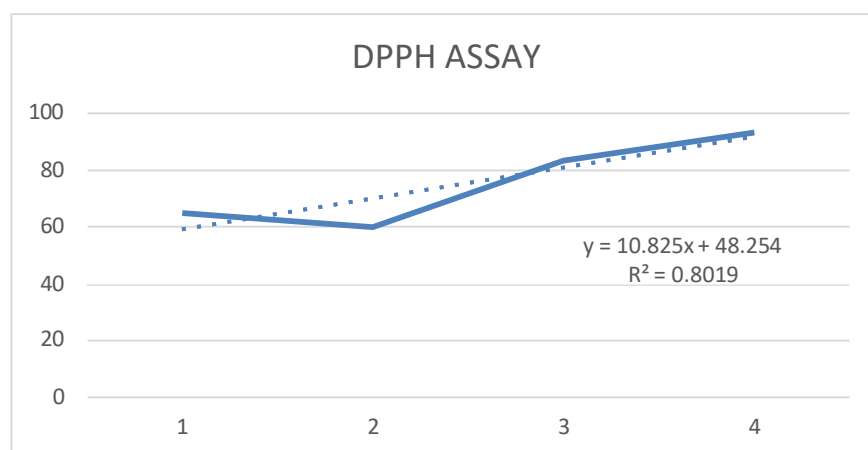


fig 5.4.4 Graphical representation of RSA%(chloroform extract)

FORMULA

$\%RSA = [(absorbance\ of\ control - absorbance\ of\ sample) / absorbance\ of\ control] * 100$

ic50

$y = mx + b$

$x = (y - b) / m$

y = concentration

m = slop, 5.301

b = intercept , 59.302

TABLE FOR DPPH 5.4.3: PLEUROTUS OSTREATUS METHANOL EXTRACT

CONCENTRATION	CONTROL	STANDRD OD	%RSA	IC50
10	0.63	0.221	64.92063	9.29946
20	0.63	0.253	59.84127	7.41323
30	0.63	0.105	83.33333	5.52701
40	0.63	0.043	93.1746	3.64079
50	0.63	0.159	74.7619	1.75456

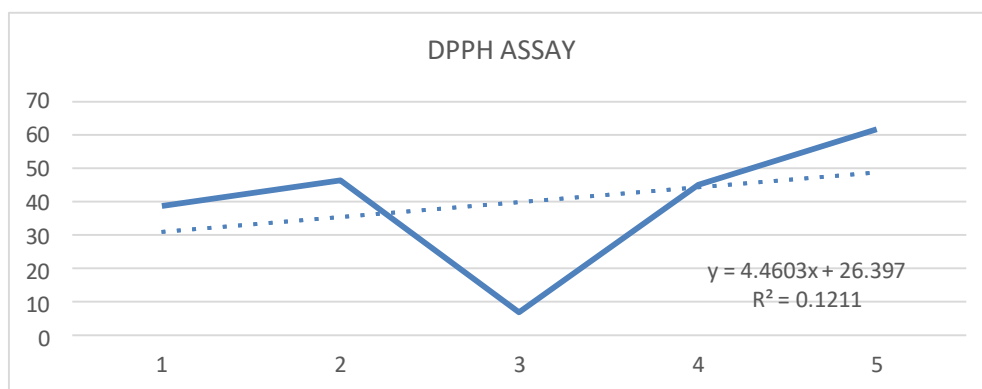


fig 5.4.4.5 Graphical representation of RSA%(methanol extract)

FORMULA

$\%RSA = \frac{(\text{absorbance of control} - \text{absorbance of sample})}{\text{absorbance of control}} \times 100$

ic50

$y = mx + b$

$x = \frac{y - b}{m}$

y = concentration

m = slope, 10.82

b = intercept, 48.25

5.5 THIN LAYER CHROMATOGRAPHY(TLC):

TLC profile showed the presence of increased number of compounds in pleurotus ostreatus of chloroform extract and methanol extract

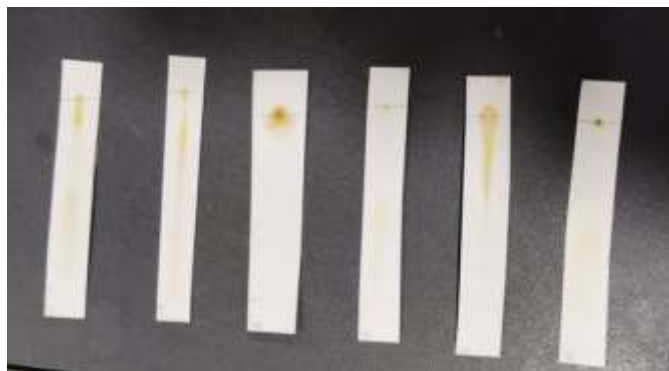


Fig 5.5:Thin layer chromatography(TLC)

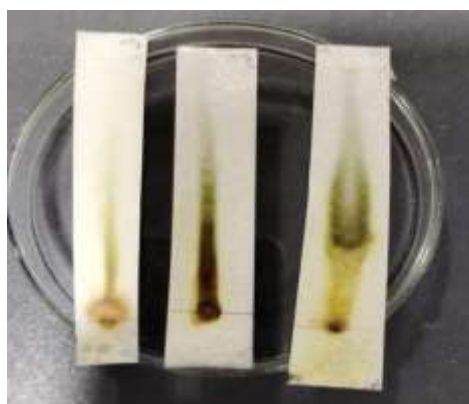


Fig 5.5.1:Iodine vapour stain

5.6ANTI-CANCER ACTIVITY

.To observe whether methanolic extraction obtained from pleurotus ostreatus affects the growth and proliferation of MCF-7 Cells, we treated the cells with various concentrations of extract and examined the cell viability using MTT reagent as MTT assay is the most prevalent test to determine the in vitro cytotoxicity of any potent drug. The methanolic extract found to inhibit the cellular growth and proliferation in a dose dependent manner with minimum 50% inhibitory concentration of 133.8 µg/ml.

TABLE 5.6: ANTI-CANCER ACTIVITY FOR MCF7 CELL LINE PLEUROTUS OSTREATUS METHANOL EXTRACT

Ug/ml	OD1	OD2
CONTROL	0.087	0.098
10	0.231	0.187
50	0.456	0.543
100	0.676	0.776
150	0.887	0.985
200	0.921	0.988
250	1.132	1.154
300	1.266	1.298

Table 5.6.1: MTT assay against MCF-7 cell line

	OM extract
10	95.4162537
50	89.6432111
100	75.2477701
150	64.0237859
200	53.617443
250	52.4529237
300	43.3597621
	36.4717542

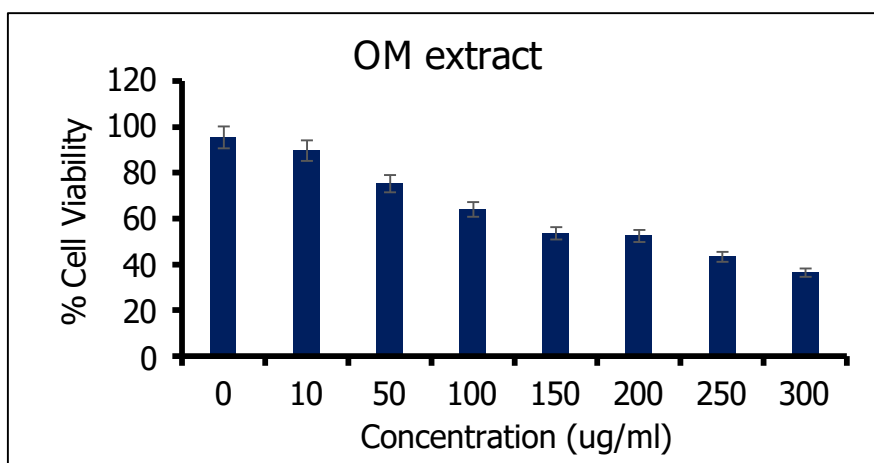


Fig 5.6 :Graphical representation of cell viability (pleurotus ostreatus)

CHAPTER 6

SUMMARY AND CONCLUSION

Cancer affects quality of life and is a global health concern. Treatment for cancer frequently has negative effects and It is critically necessary to identify an efficient therapeutic medicine for the prevention and treatment of bacterial and fungal infections. Several pure bioactive compounds have been isolated from different solvent extracts of *PLEUROTUS OSTREATUS*. The study aimed to evaluate the anti-cancer, anti-microbial and anti-oxidant of *of PLEUROTUS OSTREATUS*. Increasingly polar solvents such as hexane, chloroform, and methanol were used in the successive extraction process. The growth and proliferation of MCF-7 cells were reduced by the methanolic extract of *Pleurotus ostreatus* was shown. The antimicrobial properties of *Pleurotus ostreatus* were investigated using the disk diffusion method against gram positive bacteria like *staphylococcus aureus*, gram negative bacteria such *aeromonas*, *klebsiella*, *pseudomonas*, *vibrio cholera*, *e. coli*, and against fungal *candida* was shown. . The thin layer chromatography was used to determine the number of compounds in a mixture. Finally the anti-oxidant potential were shown in DPPH assay.

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