ORIGINAL ARTICLE

Study on Nutritional and Therapeutic Properties of *Pleurotus ostreatus*

Krishna Keerthika Oruganti[1], Monya Sree Kommineni[1], Sarvani Gadiraju[1], Sreedhar Bodiga[2], Vijaya Lakshmi Bodiga[3], Suryanarayana Veeravilli[4], Sivaram Prasad Ch. V[4], Praveen Kumar Vemuri[1]*

[1]Department of Biotechnology, Koneru Lakshmaih Education Foundation, Guntur, Andhra Pradesh, India, [2]Laboratory of Biochemistry, Department of Basic Sciences, Forest College and Research Institute Hyderabad, Mulugu, Telangana, India, [3]Department of Biochemistry and Molecular Biology, Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad, Telangana, India, [4]Department of Humanities and Basic Sciences, Aditya Engineering College, JNTUK, Surampalem, Andhra Pradesh, India

Abstract

Background: *Pleurotus ostreatus*, the oyster mushroom, is primarily an edible mushroom with high nutritional value and biomedical relevance, as it contains numerous bioactive components that generate the development of therapeutic effects. **Aim:** The purpose of this study is to determine the phytopharmacological effects of *P. ostreatus*. **Material and Methods:** To depict the medicinal properties of oyster mushroom, FTIR, HPLC, GC-MS, anticancer and antimicrobial activities were conducted. **Results:** The presence of 12 bioactive compounds was revealed using gas chromatography-mass spectrometry, and components in the extract were identified and quantified using high pressure liquid chromatography. The extract's anticancer efficacy against HeLa and MCF-7 at 25 µg/ml and exhibits strong cytotoxicity activity of 6.8 µg/mL and 17.5 µg/mL, respectively. Antimicrobial efficacy of *P. ostreatus* aqueous extract against *Bacillus megaterium* and *Bacillus amyloliquefaciens* seems promising. A significant inhibitory zone was discovered against *B. megaterium* at 90 µg/ml of sample. **Conclusion:** Our primary data from this research work can help in the detection of bioactive component's, the determination of their efficacy through in vivo research, and the demonstration of their safety and efficacy in clinical trials.

Key words: *Pleurotus ostreatus*, *Bacillus amyloliquefaciens*, *Bacillus megaterium*, Anti-cancerous activity, High pressure liquid chromatography, Fourier transform-infrared spectroscopy, Gas chromatography-mass spectrometry

INTRODUCTION

Pleurotus ostreatus is a nutritious mushroom with biomedical significance since it includes a large number of bioactive components that cause development the development of therapeutic actions.[1] *P. ostreatus* species were regarded as therapeutic food. *P. ostreatus*, sometimes known as the oyster mushroom, has a high protein content, vitamins, minerals, fiber, and other antioxidants such as selenium protect bodily cells from harm caused by free radicals that may assist to prevent chronic diseases and enhance the immune system.[2] Phytochemical analysis of oyster mushroom extract discovered the presence of Carbohydrates, Tannins, Saponin, Phenol, volatile oil, Terpenes, Anthraquinones, in addition of its vital.[3-6] Studies have also revealed antinociceptive, hypercholesterolemia, antioxidant, and antitumor effects, as well as antidiabetic action in various solvents.[7,8] The oyster mushroom, *P. ostreatus*, is gradually being recognized as having a key function in human health and nutrition, and its probiotic characteristics are well documented.[9] Fourier transform-infrared spectroscopy (FT-IR) and GCMS are used to evaluate various functional groups and chemicals *P. ostreatus* was examined for antimicrobial and anticancer activities to see if it had the functional properties needed to be used in the development of novel medicines.[10] As a result, the aim of this research was to assess diverse bioactive compounds and their bioactivity.

Address for correspondence:
Praveen Kumar Vemuri, Department of Biotechnology, Koneru Lakshmaih Education Foundation, Guntur, Andhra Pradesh, India.
E-mail: vemuripraveen@gmail.com

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MATERIALS AND METHODS

Extraction and purification of oyster mushroom

Oyster mushrooms are procured from local market, Hyderabad, Telangana, India and washed with distilled water, made into an aqueous extract as smoothie with use of motor and pestle. In a 250 ml conical flask 100ml distilled water and 10 g of mushroom was mixed. The extract is mixed with the help of magnetic stirrer for about 24 h. Later the extract is autoclaved to avoid unwanted contamination and filtered with muslin cloth. This lysate is subjected for dialysis against phosphate-buffered saline (PBS) buffer for 4 h to remove impurities and made as smoothie.[11]

Preliminary phytochemical screening

The aqueous extract of *P. ostreatus* was screened for phytochemical constituents using standard procedures as described by Harborne 1998.[12]

FT-IR analysis of oyster mushroom extract

Place the sample in the space between the two windows. The sample of each concentrate was stacked an FT-IR spectroscope, with a scan range from 400 to 4000 cml with a goal of 4 $cm^{-1.}$ FT-IR spectrometer (Perkin-Elmer), interfaced to Spectrum operating system, was used during FT-IR spectra acquisition.[13]

Gas chromatography-mass spectroscopy (GC-MS)

The research was carried out with the help an Agilent 7890 N, equipped with an Eclipse Plus column (60 m × 0.25 mm × 0.25 µm). Helium gas is supplied at a constant flow rate of 1.0 mL/min. The injector port was heated to 250°C, using the spit less injection mode. The initial temperature of the oven was maintained at 40°C for 3 min, then raised to 150°C at a rate of 5°C/min and held for 1 min, and finally raised to 220°C at a rate of 10°C/min and maintained for 2 min.[14]

High pressure liquid chromatography (HPLC)

P. ostreatus aqueous extract and fructose standard was injected in HPLC with mobile phase of acetonitrile and water. Mobile phase is prepared with HPLC grade acetonitrile and HPLC grade water (Merck) in combination of 120:80 ratio. 27 mg of fructose is diluted in 1ml of distilled water is prepared as standard. 900 µl of mobile phase and 100 µl of fructose were injected to HPLC machine, UV absorbance at 210 nm and Flow rate of 0.5 ml/min is used with C18 column (5 µm, 250 × 4.6 mm) for 30 min.[15]

Anti-cancerous activity of oyster mushroom

MCF-7 cells (1×10^6) and HeLa cells (1×10^6) were seeded and after 24 h treated with extract (1.0 mg/ml) for the indicated period (0–48 h). Cells were harvested by trypsinization, washed with Dulbecco's PBS, and resuspended in propidium iodide (50 µg/ml). Cell cycle analysis was performed on a Flow cytometer (Beckman Coulter Inc.).[16]

Antibacterial activity (agar well diffusion method)

The nutrient agar media were employed in an agar well diffusion approach to test the antibacterial activity of *P. ostreatus* aqueous extracts against *Bacillus megaterium*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Bacillus licheniformis*. Using a sterile cotton swab, bacterial strains were swabbed over Nutrient Agar medium and wells were cutted using a sterile well cutter. Extracts of varying concentrations of 30, 60, and 90 µg/ml solvent were aseptically transferred to the wells separately and incubated at 37°C for 24 h and the diameter of the inhibition zone was recorded. Control wells were maintained with sterile distilled water.[17]

RESULTS AND DISCUSSION

Qualitative phytochemical screening

The screening of early phytochemicals of different bioactive compounds was found. Standard methods were used to conduct the screening. In this research, the qualitative analysis of the extracts revealed a strong indication of the existence of metabolites. The majority of phytoconstituents were found in the solution of *P. ostreatus*, comprising phenols, carbohydrates, tannins, terpenoids, volatile oil, Antra quinones, and saponins. The screening was performed using standard procedures and is summarized in the Table 1.

Table 1: Preliminary phytochemical screening of bioactive components present in aqueous extract of *Pleurotus ostreatus*

Phytochemical	Presence
Carbohydrates	+ve
Tannins	+ve
Saponin	+ve
Flavonoid	-ve
Phenol	+ve
Glycosides	-ve
Saponin	+ve
Volatile oil	+ve
Terpenes	+ve
Cardiac Glycoside	-ve
Anthraquinones	+ve

FT-IR

The FT-IR spectrum of mushroom extract as shown, several absorption peaks were centered at 1150.07, 1450.23, 1637.84, 2066.60, and 3476.69 cm^{-1}. The absorption peak centered at 1150.07 cm^{-1} referrers to the C-O stretch vibration, which relates to Ester linkages [Figure 1].

GC-MS

The bioactive components contained in *P. ostreatus* aqueous extract were identified using GC-MS. GC-MS findings displaying the peak area, chemical formula, and molecular weight for the aqueous extract of *P. ostreatus* sample indicated 11 main bioactive components methyl hydroxylamine hydrochloride, pyridine, n-methyl-n-trimethylsilyl trifluoroacetamide, trimethylchlorosilane, chloroform, estrone, (Z)-o-methoxyimino-estrone, (E)-o-methoxyimino-estrone, o-TMS-(Z)-o-methoxyimino-estrone, o-TMS-(E)-o-methoxyimino-estrone, and fatty acid esters [Figure 2].

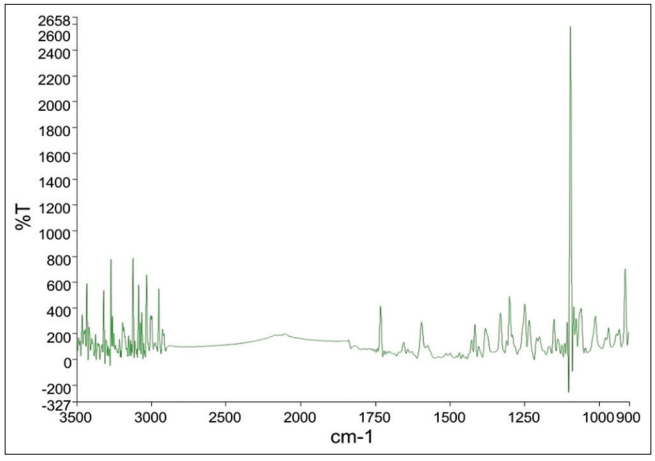

Figure 1: Fourier transform-infrared spectroscopy spectrum curve

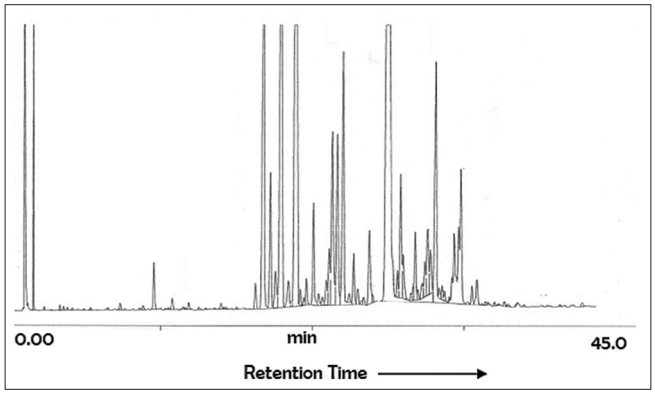

Figure 2: Gas chromatography-mass spectroscopy chromatogram of *Pleurotus ostreatus* aqueous extract

HPLC

A sample of *P. ostreatus* aqueous extract was injected into Shimadzu 3000 HPLC System. UV absorbance at 210 nm and indicated that most of the unknown substances were monosaccharaides. A substantial portion of the peptides eluted between 5% and 10% of acetonitrile (retention time 30 min) [Figure 3].

Anti-cancerous activity

In proliferation and cytotoxicity research, the MTT test is a well-known method of assessing viable cell counts. The reduction of the yellow-colored water-soluble tetrazolium dye3- [4, 5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) to formazan crystals was used to test the cytotoxic effect of the mushroom extract on human cervical cancer (HeLa) cells in this project. MTT is reduced to blue formazan product by mitochondrial dehydrogenase, which reflects proper mitochondrial function and cell viability. The cytotoxic potential results visibly demonstrate that the solution of *P. ostreatus* at 25 µg/ml exerts strong cytotoxicity against HeLa and MCF7 with an IC50 of 6.8 µg/mL and 17.5 µg/ml. The IC50 values of extract at 5 µg/ml, 10 µg/ml, and 25 µg/ml against HeLa and MCF7 are indicated in Figure 4.

Antibacterial activity

Plants have long been an important source of natural compounds for human health, particularly in natural medicines. The aqueous extract of *P. ostreatus* had *in vitro* antibacterial activity against Gram-positive and Gram-negative bacteria, according to the antimicrobial assay. The extract did not show any activity on *E. coli* and *B. licheniformis*. The results reveal that aqueous extracts of *P. ostreatus* was found to be extremely effective against *B. megaterium, B. subtilis, and B. amyloliquefaciens*. A significant inhibitory zone was discovered against *B. megaterium* at 90 µg/ml of sample [Figure 5].

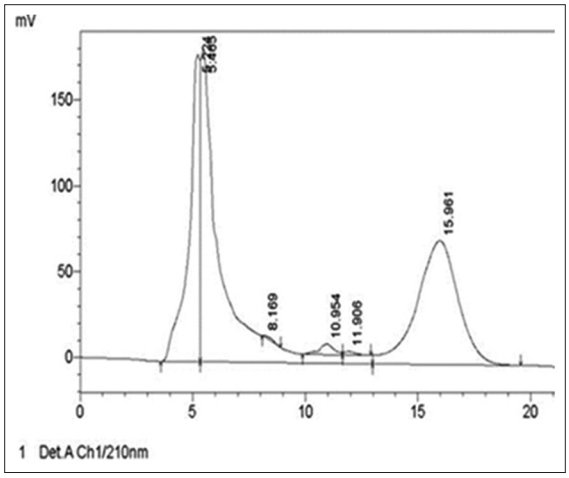

Figure 3: *Pleurotus ostreatus* extract chromatogram

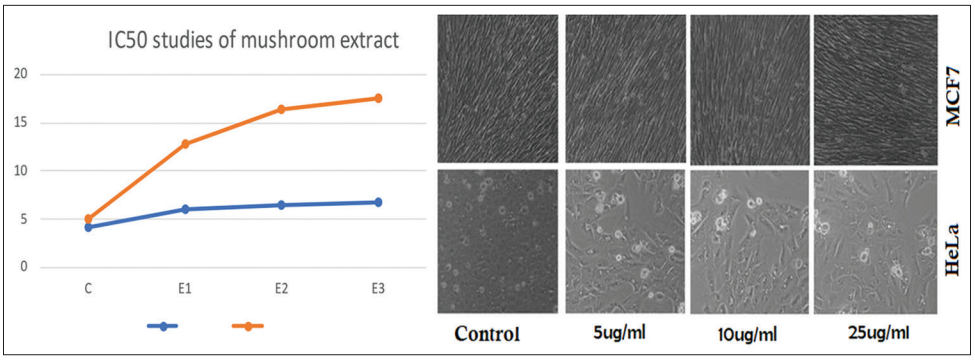

Figure 4: IC_{50} values of extract at 5 µg/ml, 10 µg/ml, and 25 µg/ml against HeLa and MCF-7

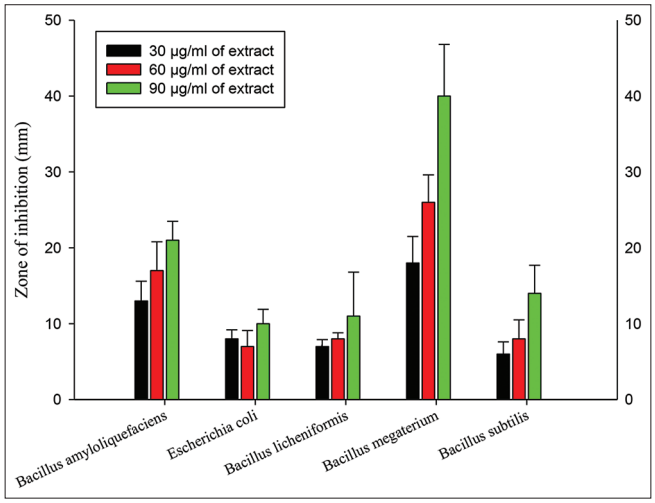

Figure 5: Antibacterial activity of aqueous extract of *Pleurotus ostreatus*

CONCLUSION

In this study, effort was done to establish numerous phytochemicals, GCMS, and FT-IR characteristics that could be useful and have a great interest in both research institutes and pharmaceutical firms for the development of new drugs. The anticancer activity of *P. ostreatus* aqueous extract toward HeLa and MCF7 cell lines has showed that it has a higher cytotoxic effect. Gram-positive and Gram-negative microorganisms were evaluated for antibacterial activity. This primary data will help in the detection of bioactive component's, the determination of their efficacy through *in vivo* research, and the demonstration of their safety and efficacy in clinical trials.

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