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Research Article

Investigation of Mushroom-derived Bioactive Compounds against Cancer - An *In-silico* Molecular Docking Analysis

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ABSTRACT

Oyster mushrooms (*Pleurotus ostreatus*), are included in the category of nutritious foods, due to the presence of vitamins, proteins and different kinds of chemicals which preserve good health. It exhibited several medicinal properties like anti-tumor, immune-modulatory, anti-inflammatory, anti-arthritic, hypo-cholesterolaemic, anti-diabetic, anti-oxidant, anti-hypertensive, antiviral and antimicrobial activities. The current study was aimed at the identification of phytochemicals by suitable methodology followed by molecular docking studies and the effect of test extract on different cell lines. Preliminary phytochemical testing was conducted; identification of different phytochemicals was carried out by gas chromatography–mass spectrometry (GC–MS) method. The protein 1DI8 (CDK-2) from the protein data bank was used to analyze the molecular docking investigation of the identified phytochemicals. In the present study, the methanolic extract of *P. ostreatus* contained six compounds -Propanedioic acid, phenyl-, cyclopropane carboxylic acid, 1-amino-, 2-Undecene, 4,5-dimethyl-, [R*, R*-(E Heptanoic acid, 2-methyl-2-butyl ester, acetic acid, 2-propyltetrahydropyran-3,3-tetradecene. Amongst all of these phytochemicals, propanedioic acid, and phenyl-was shown to possess the highest score with 1DI8 (CDK-2) in the molecular docking investigation. The anti-cancer effect was also evaluated; the test extract was found to possess the same activity which was evident by the IC_{50} values. It was concluded that *P. ostreatus* possessed six phytochemicals, out of which propanedioic acid and phenyl were found to have therapeutic potential in the treatment of cancers and was proved by locking mechanism through molecular docking studies. As *P. ostreatus* is known for higher biomass availability, further research is of utmost importance in the treatment of cancers.

INTRODUCTION

Mushrooms have been prized for their distinctive flavor and delicate aroma in gourmet cuisine all over the world since ancient times.[1] Many species of mushrooms possessed and generated numerous inexplicable biological properties.[2] The nutritive value of Mushrooms was found to have much importance clinically due to the presence of these bioactive compounds. They stood as a remedy for plenty of diseases and also for nourishment.[3] Though mushrooms have a history of their use in medicine, in the present time few contemporary studies have promoted them in the maintenance of good health and vivacity.[4] Globally, cancer is one of the major public health burdens that is affecting both developed and developing countries. World Health Organization (WHO) revealed that cancer was one of the leading causes of death encountered around the world and was responsible for 10 million deaths by the end of 2023.[5] Each type of cancer requires a specific treatment which in turn depends on the diagnosis. Surgery, anticancer medication therapy, radiotherapy, and systemic therapy (chemotherapy and targeted biological therapies) are only a few of the many cancer treatments available. In clinical chemotherapy, there is every chance of developing resistance to the drugs causing many side effects.[6] Major research is being approached by a number of research laboratories globally to deliver the best therapeutic effect

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and patient compliance. The numerous side effects and difficulties caused by the present anti-cancer medications in the market make an urgent need for new, efficient therapeutic modalities that are less toxic.[7] There is evidence available that patients generally depend on both complementary and alternative therapy simultaneously.[8] It is becoming more widely accepted that medicinal plants and the phytochemical constituents derived from them are effective adjunct therapies for cancer. Numerous clinical studies have documented the positive effects of herbal treatments when combined with conventional medications on cancer patients' survival rates, immune system function, and quality of life (QoL).[9] Every year, between 3000 and 4000 plants from 2000 species are evaluated for their anti-cancer properties.[10]

Additionally, apart from vital nutrients, biologically active substances were also produced by plants that are favorable in the maintenance of human health and the treatment of a number of ailments.[8] They have been employed in the food, pharmaceutical, and cosmetics sectors, and include a variety of substances including lipids, phytochemicals, pharmaceutics, flavors, and aroma. Using traditional medicines is the cornerstone of the healthcare system in underdeveloped nations like India, and the existence of phytochemical substances is the key justification for using plants as traditional medicine.[11]

In this context, active ingredients present in a few mushrooms have been proven to their anti-cancer capabilities, also inclined for further investigations. With the involvement of medicinal mushroom formulations commercially, there can be heightened expectations in cancer therapy, certainly clinical trials to be carried out.[7] Due to the abundant vegetation and the socioeconomic circumstances in the local population, traditional medicine is frequently used in India, particularly in the rural northeastern (NE) area.[12] A wide variety of bioactive substances found in medicinal plants have the potential to be anti-bacterial, anti-cancer, anti-inflammatory, and anti-oxidant agents. Though a huge variety of secondary metabolites identified in different plant species, only a small number of them have been investigated and represent a significant source of bioactive molecules. Suitable screening techniques are essential to maintain both quality control and hunt for novel compounds.[13] Specific drugs with a high activity profile have been delivered as a result of the extraction and characterization of these bioactive substances.[14] Fourier-transformed infrared (FTIR) and gas chromatography-mass spectrometry (GC-MS) have been widely utilized to identify functional groups and the presence of different bioactive compounds present in plant extracts.[15] GC-MS was found to be a trustworthy approach in the identification of different phytochemical constituents from plant extracts, such as alkaloids, flavonoids, organic acids, and amino acids.[16] Additionally, powerful drug discovery methods based on computer-based tools have been developed, that allow the screening of drugs from bioactive chemicals found in the medicinal plants.[17] *In-silico* pharmacology, also known as computational therapeutics and computational pharmacology, focuses on developing techniques for using software to gather, analyze, and integrate biological and medical data from a variety of sources.[17] A quick and inexpensive method for developing and testing medications is molecular docking. This method provides information on drug interaction with receptors and aids in predicting the binding of drug model to target proteins that provide a route to an authentic ligand binding at their binding sites.[18] There are over 2,70,000 plants on the planet, out of which a tiny part has been explored phytochemically. So, in order to combat cancers, plants play a crucial role in furnishing the bioactive compounds potentially for the blooming of expansion in the same platform.[18] Amongst the medicinal plants, mushrooms were found to possess approximately high amounts of carbohydrates, proteins, fiber and low levels of fat; rich in vitamins. They also contain bioactive phenolic compounds, carotenoids, and unsaturated fatty acids all of which might help to combat medical conditions via a few properties like anti-oxidant, anti-inflammatory anti-fungal, anti-bacterial, anti-hypertensive, hepatoprotective, anti-allergic, anti-diabetic, and anti-cancer. A good source of potential anti-cancer compounds can be found in mushrooms. Therefore, the goal of our research was to review all relevant literature and find mushroom extracts that demonstrated promising anticancer activity. The fungal species *Pleurotus ostreatus*, known as the oyster mushroom, was noted for being an edible variant. During World War I, it was initially grown as a survival strategy in Germany. [19] Although it may be grown on straw and other materials, one of the more popular wild mushrooms was the oyster mushroom. It smells like benzaldehyde, just same as bitter almonds. [19]

Pleurotus species grow on sawdust, wood and wet areas, require a temperature of 10 to 32°C for their growth, and possess medicinal benefits in the traditional system of medicine. Among the different varieties of mushrooms available, considering the medicinal potential of *P. ostreatus*, researchers focused and began to investigate the therapeutic efficacy. Globally, the most grown and edible species among the mushrooms is *P. ostreatus*, (Oyster mushroom). The presence of bioactive molecules confessed several therapeutic properties such as anti-oxidant and scavenging properties for free radicals, hypocholesterol and anti-atherogenic, anti-bacterial and anti-cancer, respectively.

The present study involved the investigation of the phytochemical constituents present in the methanolic extract of *P. ostreatus* using GC-MS and the anti-cancer property was corroborated by accomplishing in silico molecular docking studies.[19]

Materials and Methods

Plant Collection and Authentication

P. ostreatus were obtained from the local places of Tirupati, AP. *P. ostreatus* was authenticated by Dr. K. Madhava Chetty, M.Sc., M.Ed., M.Phil., Ph.D., PG DPD., Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh.

Procurement of Materials

All the chemicals used in the study were obtained from the SD Fine Chem Limited, Mumbai. They belonged to the Laboratory grade.

Sample Preparation and Extraction

The *P. ostreatus* fungus was thoroughly cleaned with water, evacuated and then thinly sliced. Then dried at room temperature and strained through sieve mesh 60, again dried in the oven at 450°C. A cold maceration technique was used to extract 150 g with 500 mL of methanol for 72 hours along with agitation. Using a muslin cloth, the solution was filtered, then filtered through Whatman No. 1 filter paper and obtained a stock solution of plant extract. The extract was made concentrated in a rotary evaporator that was held at a temperature of no more than 600°C. About 20 mL of the extract was collected, and dried in an oven at 450°C, leaving behind a semi-liquid. The extract thus obtained was utilised for further testing and analysis. [19]

Phytochemical Screening

The presence of various phytoconstituents like flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, and carbohydrates was carried out by standard tests in methanolic extract of *P. ostreatus* (MEPO).

Preparation of the extract for GC-MS analysis

By virtue, of Shimadzu P2010 ultra GC-MS**,** the presence of bioactive ingredients was analyzed in the methanolic extract of *P. ostreatus*. By using 1-µL of methanol a blank solvent analysis was first conducted. Then 1-µL of the reconstituted methanol extract was taken for GC-MS analysis using direct liquid injection.

GC-MS analysis

The GC-MS analysis was conducted in the Shimadzu GCMS-QP2010 ultra GC-MS equipment with the following specifications - A Rtx-5 ms fused silica capillary column (30 x 0.25 mm ID X IEM df, made entirely of dimethyl polysiloxane) with helium (99.999%) as a carrier gas at a constant flow of 1-mL/minute was used. An injection volume of µL was used (split ratio of 10:1), with the injector temperature of 2500°C and the ion source temperature of 2800°C was maintained. The temperature in the oven was set to 1100°C (isothermal for 2 minutes), increased at a rate of 100°C/minute to 2000°C, then decreased at a rate of 50°C/minute to 2800°C, and finally held at 2800°C for a 9-minute isothermal period. The fragment sizes ranged from 40 to 550 Da, with a scan interval of 0.5 seconds, and the mass spectra were collected at 70 eV. The run lasted for about 30 minutes in total. The phytochemicals present in *P. ostreatus* were identified. As per the information obtained on the respective compounds, from the GC-MS peaks the compounds were subsequently recognized. Utilizing electron impact ionization at an energy of 70 eV, GC-MS data was examined for identification and quantification of compounds. The spectra obtained were compared using the National Institute of Standards and Technology (NIST) search to a database of known component spectra that was kept in the GC-MS library.

The average peak area was compared to the total areas to get the relative % of each component. By means of GC-MS Post Run Solution Software, the peak areas were measured and processing of data was done. [20,21]

Ligand preparation

In molecular docking, this was the initial step. The PCNP and KIs ligands of certain macromolecules were geometrically refined using the Lig Prep module, version 2.4, 2010, which was drawn using the Maestro module. A high-quality 3D structure with precise chirality was created by LigPrep's. As per MacroModel version 9.8 in 2010, by preserving the original ionization states, the tautomers and conformations were produced using the OPLS-2005 force field. Following generation, the conformers were minimized via truncated Newton conjugate gradient (TNCG) minimization up to 500 iterations. With the energy of 30 kcal/mol difference, the conformers with global energy was retained. In order to conduct conformational searches for aqueous solutions, a generalized born/solvent accessible surface (GB/SA) continuous solvation model was preferred.[22]

Protein preparation

For protein preparation, Mae stro software's protein preparation wizard was used. The protein structures were retrieved from the protein data bank,[22-27] namely 1DI8 (CDK-2). Missing hydrogens were added to the specified chains, and the bond order was correctly assigned. The sample orientations were used to optimize the H-bonds. A display of all polar hydrogens was made. Finally, the root mean square deviation (RMSD) default value of 0.30 was used to reduce the protein structure. [23]

Receptor Grid Generation

The co-crystallized ligand was extracted from the designated receptor's active site of the receptor chain. The atomic partial charge was < 0.25 defaults and the atoms had the same size as Vander Waals radii of 1.0>. The centroid of the workspace ligand served as the active site's representation of an enclosing box. This technique was followed, and the default Glide parameters were used

to create a grid centered on the ligand. The grid structure was docked with all of the ligands.[23]

Molecular Docking Process

Using the extra precision (XP) function of the Glide module, version 5.6, 2010, flexible docking was carried out on a predetermined receptor grid.[24] There were no restrictions on the defined ligand-receptor interactions. To see the results of the following docking tests using posture viewer, the output format structure was modified to pose viewer file.[24]

Generation of E-pharmacophore

KIs and PCNP were made to docked on several cancer macromolecules, an e-pharmacophore was created. Pharmacophoric sites were created automatically with improved ligands using PHASE from Schrodinger, LLC in New York (25). Several characteristics were used by PHASE, including the positive ionizable group (P), aromatic ring (R), hydrogen bond acceptor (A), hydrogen bond donor (D), negative ionizable group (N), and hydrophobe (H). Based on Glide XP descriptions, an energy value was assigned to each pharmacophoric site.[25]

Picking of the Best-scored Pose

The best docking poses for the PCNPs were assumed from the docking scores but other factors were also considered which included the values of various energies, the number of H bonds, and a visual examination of all docking poses in Maestro (Schrodinger, USA). The energy of the interaction between the protein and the ligand can be related to binding affinities. Certain criteria were established to identify the optimum docked structure for each ligand. The Glide GScore was then used directly to determine the ranks. [25,26]

Anti-Cancer Activity by *In-vitro* Cell Viability Assay

The methanolic extract of *P. ostreatus* was screened by MTT on human cells by colorimetric method. [27]

MTT Assay

3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide.

Preparation of primary stocks of test compounds

The concentrations of the test compounds with 10, 1, 0.1, 0.01, 0.001 and 0.0001, were prepared in dimethyl sulphoxide (DMSO).

Preparation of MTT reagent

Primary MTT stock at 5 mg/mL was produced in PBS and kept at -20°C until use.

Procedure

From the culture flask, the development medium was expelled. Using 5 mL of sterile phosphate buffered saline (PBS) cells were thoroughly washed. The PBS was then removed, and 1-mL of trypsin ethylenediaminetetraacetic acid (EDTA) was added before the cells were restricted to the flask's surface for 5 minutes at 37°C. The trypsin was inactivated by adding 5 mL of medium with FBS, the cells were mixed to form a single-cell solution, then collected in a sterile 15 mL hawk tube at 1500 rpm for 5 minutes. The supernatant was discarded, and the cell pellet was resuspended in fresh media, and cells were counted. In the sterile 96 well culture plates, cells were seeded (5000–40000 cells/well- 100 µL/well) and at 37°C they were hatched and maintained with 85 % relative humidity, 5% CO_2 and 95% O_2 for about 24 hours at night. Then, the cell culture medium was evacuated and refill was done using 100 µL of fresh culture medium with test compounds at various concentrations of 100, 10, 1, 100, 10 and 1 nM, (n = 3) and hatched for about 2 days. Then, the cells were washed with PBS and MTT reagent was used at 0.5 mg/mL and 37°C incubation was done till formazan gems were noticed (30 minutes–3 hours). After evacuation with MTT reagent, these gems were dissolved in DMSO and the plate was perused on an enzyme-linked immunosorbent assay (ELISA) plate reader at 570 nm. The %of cell viability was determined. IC_{50}, an inhibitory concentration at which there was a 50% reduction in the growth of cancer cells was determined by concentration-response curves using non-linear regression analysis. [27]

IC_{50} calculations (Percentage Viability Calculations)

IC_{50} value was evaluated as Y = a x X + b, IC_{50} = (0.5 - b)/a.

$$\% \text{ Viability} = \frac{\text{Optical Density of Test}}{\text{Optical density of Control}} \times 100$$

IC_{50} Values was calculated using Graphpad Prism 9.

Results And Discussion

The primary goal of molecular docking was to acquire a ligand-receptor complex with an optimal shape and minimal binding free energy.[28] To expose the potent drug candidates that target the molecule, substantial datasets were scrutinized using docking along with a scoring function. Molecular docking plays a crucial role in the prediction of a basic attachment of medication to that of nucleic acid.[29] In other terms, docking refers to the process of arranging two molecules in three dimensions. This is explored to establish a relation between the structure of molecules and cytotoxicity. Given the above considerations, efforts were made to explicate the underlying mechanism of anti-cancer agents by exploring the interconnection between the drugs and nucleic acids. The *in-silico* docking studies revealed whether there exists a binding of drug/compound with that of DNA/protein. Once the docking finds the interaction, the experimental procedures are made available to determine the complex's actual binding mode.[28] This enabled the discovery of

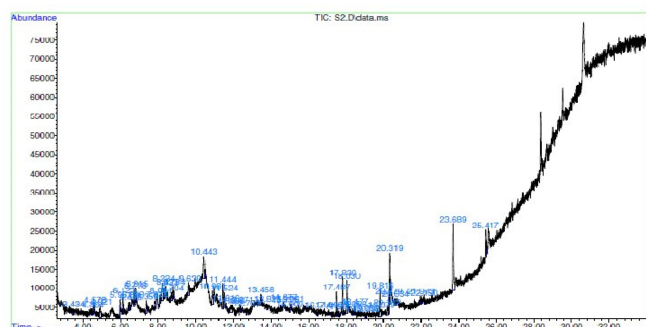

Fig. 1: GC-MS chromatogram of *P. ostreatus*

new innovative medications against cancer. Furthermore, accordingly, structural modifications that might lead to a sequence or structure-specific binding to their target can be achieved.[29] Previous studies showed that different extracts were prepared from nearly 90 varieties of mushrooms. Almost all the extracts demonstrated encouraging properties against cancers of 38 varieties. There exists a relation between cancer and free radical generation. Generation of free radicals which are produced by mitochondria and also due to metabolic activities. As reactive oxygen species (ROS) are created over a prolonged period under persistent environmental stress, the cell

Table 1: GC-MS analysis of phytochemicals identified in *P. ostreatus*

Peak No	*Retention time (min)*	*Area*	*Area%*	*Name of component*	*Docking score*
1	2.057	3683006	10.62	Formamide	-2.4
2	2.383	270837	0.78	1,4-Pentanediol	-4.1
3	2.643	8103665	23.36	Glycerin	-3.4
4	2.775	263521	0.76	2(5H)-Furanone, 3-methyl-	-5.1
5	2.882	1396374	4.02	3-Hexyn-2-ol	-4.4
6	3.247	210001	0.61	4-Heptanone, 3-methyl-	-5.1
7	3.507	1218161	3.51	2-Pyrrolidinone	-4.2
8	3.615	982685	2.83	Butanedioic acid, monomethyl ester	-4.6
9	3.735	966496	2.79	Cyclopropanecarboxylic acid, 1-amino-	-6.8
10	3.923	163255	0.47	Pentanal, 2,3-dimethyl-	
11	4.010	363955	1.05	Succinimide	-4.7
12	4.439	3355329	9.67	Cyclobutanone, 2-methyl-4-hydroxy-	-4.7
13	4.560	318289	0.92	Heptanoic acid, 2-methyl-2-butyl ester	-6.1
14	4.662	509553	1.47	5-Methoxypyrrolidin-2-one	-4.7
15	4.865	1948428	5.62	1, 1, 3-Trimethyl-3, 8, 9-trioxa-bicyclo [4.	
16	5.200	410027	1.21	1,4-Dioxaspiro[4,4]nonane-7-carboxy	
17	5.260	841626	2.43	Niacin	-5.4
18	5.486	339872	0.98	Propanedioic acid, phenyl-	-8.3
19	6.301	430811	1.24	2-Dodecanone	-5.8
20	6.542	291641	0.84	2-Heptanone, 5-methyl-	-5.5
21	6.717	3443004	9.92	2-Nonanol	-5.1
22	6.771	1090217	3.14	2-Undecene, 4,5-dimethyl-, [R*,R*-(E	-6.3
23	7.065	1886737	5.44	2-Butyn-1-ol, 4-methoxy	-3.8
24	7.210	314116	0.91	DL-Proline, 5-oxo-, methyl ester	-5.2
25	7.251	643852	1.86	Acetic acid, 2-propyltetrahydropyran-3	-6.1
26	7.330	181753	0.52	3-Tetradecene, (E)	-5.9
27	7.420	239801	0.69	4-Heptanone, 3-methyl	-5.2
28	7.457	380450	1.10	Niacinamide	-5.5
29	7.736	212763	0.61	Acetic acid, 2-(1-buten-3-yl)-2-nitro,	-5.5
30	9.801	225843	0.65	9-Eicosene, (E)	

Table 2: Phytochemicals identified in the methanolic extract of *P. ostreatus*

Peak No	*Retention time (min)*	*Area*	*Area%*	*Component name*	*Docking score*	*H Bonds*
1	3.735	966496	2.79	Cyclopropanecarboxylic acid, 1-amino	-6.8	2
2	4.560	318289	0.92	Heptanoic acid, 2-methyl-2-butyl ester	-6.1	1
3	5.486	339872	0.98	Propanedioic acid, phenyl-	-8.3	1
4	6.771	1090217	3.14	2-Undecene, 4,5-dimethyl-, [R*,R*-(E	-6.3	2
5	7.251	643852	1.86	Acetic acid, 2-propyltetrahydropyran-3	-6.1	1
6	7.330	181753	0.52	3-Tetradecene, (E)	-5.9	2

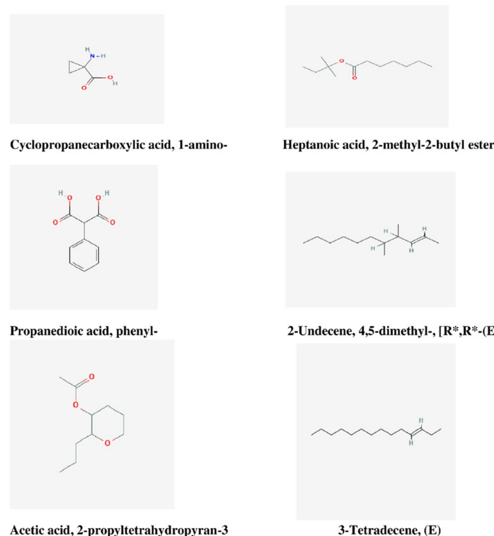

Fig. 2: Chemical structures of the compounds identified from MEPO

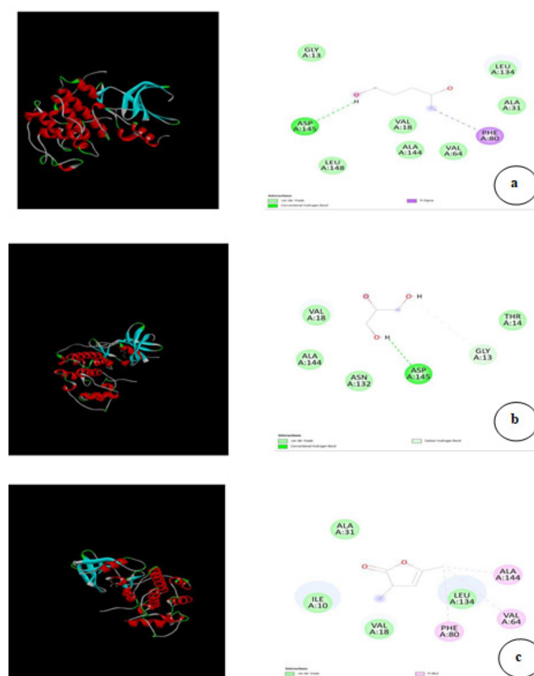

Fig. 3: Interaction of ligands with 1DI8 (CDK-2) Docking conformation of a) Propanedioic acid, phenyl-, b) Cyclopropanecarboxylic acid, c) 1-amino-, 2-Undecene, 4,5-dimethyl-,

structure and function are severely harmed, inclined to somatic mutations and neoplastic transformation.[30] Certainly, the genesis and progression of cancer are related to oxidative stress through DNA damage, genome instability and proliferation of cells.[31]

In the current study, an extraction technique was adopted as it was straightforward, quick, affordable, and requires less solvent. The GC-MS method was used to analyze the obtained extracts, also a valuable tool for the identification of the active ingredients in herbs used in food, medicine, and cosmetic items.[32] The chromatogram of GC-MS was depicted in the (Table 1 and Fig 1). Six components were recognized in the methanol extract of the adult fungus *P. ostreatus* and were evaluated further accordingly. Table 1 shows the list of the phytochemicals along with their peak number, retention time, peak area, and concentration (peak area%) and docking score (Table 2 and Figs 2, 3 and 4).

Validation of Docking Result

By docking methodology, the proper ligand poses in a protein's binding pocket with their affinities were determined. For many years, molecular docking has made a significant contribution to drug discovery. Here, the target protein sadenosyl-L-methionine decarboxylase was docked with the seven phytochemicals discovered during GC-MS research.

The validation process has two parts:

- The binding energy between the docked ligand and the protein was predicted by Discovery Studio scores [(PLP1, PLP2, JAIN, Ligand internal energy, and PMF (Potential of Mean Force Score)]. These scores were then used to conduct further investigation. The dock score obtained relies on the binding forces and energy docked on the ligand and the protein.
- The top-ranked docked pose was of hydrogen bond. The docking information for each compound was displayed in Table 2.

Ten distinct conformations for seven ligands were produced as a consequence of docking. To view the binding affinity analysis, the best-ranked docked complex's scores were copied from Discovery Studio's table browser (Table 2). PLP1, PLP2 (Steric and H-bonding intermolecular

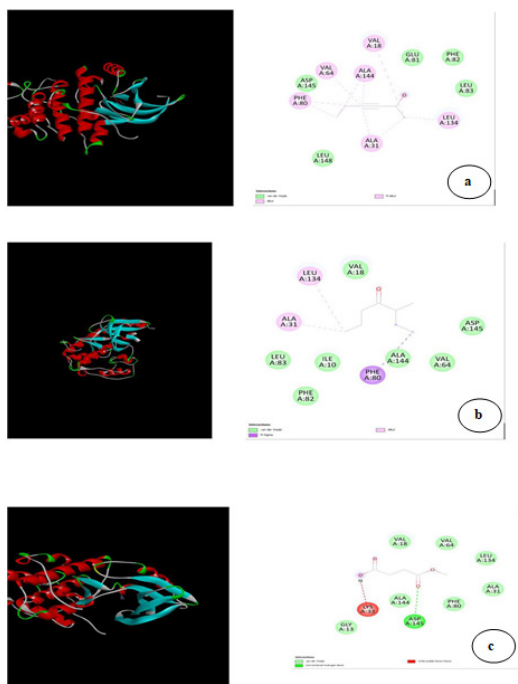

Fig. 4: Interaction of ligands with 1DI8 (CDK-2) Docking conformation of a) [R*,R*-(E Heptanoic acid, b) 2-methyl-2-butyl ester, acetic acid, c) 2-propyltetrahydropyran-3, 3-tetradecene, (E)

function), JAIN, ligand internal energy, polar attractive interactions, polar repulsive interactions, solvation of the protein and ligand, and an entropy term for the ligand were the score values included and the one with less internal energy possessed docking stability.[29] A robust receptor-ligand binding was noticed with high PLP scores. As indicated by PMF, a big score stipulated a higher affinity for receptor-ligand binding. PMF was developed keeping given the 3D structures of protein-ligand complexes, scores were determined by aggregating pairwise interaction terms across all receptor-ligand complex interatomic pairs, and the dock score function evaluated the candidate ligand poses.[29] Based on dock score the order of ligands was as follows - propanedioic acid, phenyl-> Cyclopropane carboxylic acid, 1-amino-> 2-Undecene, 4,5-dimethyl-, > [R*,R*-(E)] acetic acid, 2-propyl tetrahydropyran-3, 3-tetradecene, 2-methyl-2-butylheptanoic acid, (E). Amongst six ligands, phenyl-propanedioic acid, was exhibited with the highest PLP value. There was just a slight difference in the results for each ligand. The hydrogen bond was added as a criterion and expanded the interaction analysis. Using the H-bond monitor of Accelrys Discovery Studio software, the results were assessed. [29]

Anti-cancer Activity by MTT Assay

In the evaluation of the effect of test extract against cancer, an *in-vitro* method called MTT assay was used on 4 different cancer cell lines. The results of each cancer cell line were depicted in the following tables -

Two cancer cell lines were selected – HCT-15 and SW-620, which were colorectal cancer cell lines. The results in Tables 3 and 4 showed the %viability of cells (Fig 5) as per the concentration selected, also the IC_{50} values of MEPO were found to be 38.12 for HCT – 15 and 43.64 for SW-620, respectively.

Two more cancer cell lines were selected - MDA-mb-231 and A498, which were breast and renal cancer cell lines. The results in Tables 5 and 6 showed the %viability of cells (Fig 6) as per the concentration selected, also the IC_{50} values of MEPO were found to be 26.51 for MDA-mb-231 and 10.01 for A498 cells, respectively. Amongst all the four cancer cell lines, the test extract MEPO showed high potency against A498 renal cell lines as compared to the

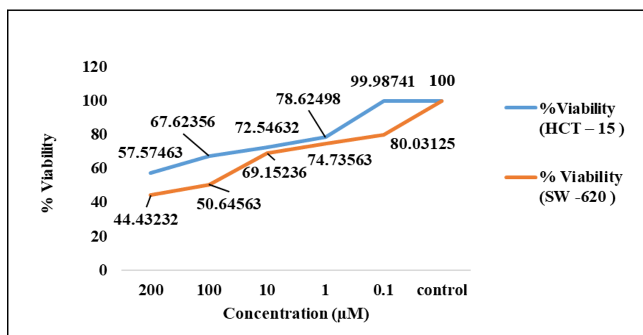

Fig. 5: Effect of test extract on HCT – 15 and SW-620 cancer cell lines

Table 3: Effect of test extract on HCT – 15 cancer cell lines with IC_{50} values

HCT 15 cancer cells			*Methanolic extract of P. ostreatus*			
Concentration (µM)	*Optical density (W1)*	*Optical density (W2)*	*Optical density (W3)*	*Average optical density*	*%Viability*	*Log IC_{50} (µM)*
200	0.1	0.12	0.3	0.173333	57.57463	38.12
100	0.65	0.92	0.98	0.85	67.62356	
10	0.3	0.8	1.2	0.766667	72.54632	
1	0.9	0.96	1.1	0.986667	78.62498	
0.1	0.98	0.93	1	0.97	99.98741	
Control	1.21	1.3	1.35	1.286667	100	

Table 4: Effect of test extract on SW-620 cancer cell lines with IC_{50} values

SW-620			*Methanolic extract of P. ostreatus*			
Concentration (µM)	*Optical density (W1)*	*Optical density (W2)*	*Optical density (W3)*	*Average optical density*	*%Viability*	*Log IC_{50} (µM)*
200	0.32	0.25	0.34	0.303333	44.43232	
100	0.9	0.87	0.9	0.89	50.64563	
10	0.85	0.96	0.92	0.91	69.15236	43.64
1	0.98	0.85	1.1	0.976667	74.73563	
0.1	1.38	1.19	1.5	1.356667	80.03125	
control	1.34	1.2	1.35	1.296667	100	

Table 5: Effect of test extract on MDA-mb-231 cancer cell lines with IC_{50} Values

MDA-mb-231			*Methanolic extract of P. ostreatus*			
Concentration (µM)	*Optical density (W1)*	*Optical density (W2)*	*Optical density (W3)*	*Average optical density*	*%Viability*	*Log IC_{50} (µM)*
200	0.2	0.12	0.26	0.193333	38.83265	
100	0.85	0.75	0.8	0.8	47.69865	
10	0.78	0.86	0.73	0.79	57.47656	26.51
1	0.86	0.62	0.93	0.803333	66.00121	
0.1	0.93	0.99	1.1	1.006667	72.52145	
Control	1.2	0.9	0.93	1.01	100	

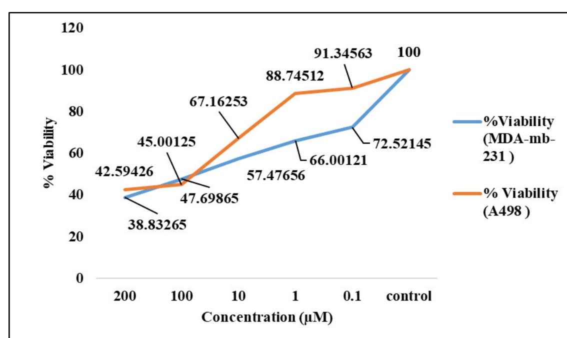

Fig. 6: Effect of test extract on MDA-mb-231 and A498 cancer cell lines

other three, and also followed by moderate potency against MDA-mb-231 and low potency for HCT – 15 and SW – 620 cell lines. Table 7 and Fig. 7 represented the IC_{50} values of test extract for different cell lines used in the present study. Based on these values the potency of the test extract was explored.

In the previous studies, phenyl-propanedioic acid was investigated on the proliferation rate of cultured human fetal skin fibroblasts and was found to possess fibroblast proliferation. Also there was an enhanced proliferation when cultured in the medium.[33] In animal studies, it induced mitochondrial dysfunction and the development of reactive oxygen species that lead to apoptosis.[34]

Table 6: Effect of test extract on A498 cancer cell lines with IC_{50} Values

A498			*Methanolic extract of P. ostreatus*			
Concentration (µM)	*Optical density (W1)*	*Optical density (W2)*	*Optical density (W3)*	*Average optical density*	*%Viability*	*Log IC_{50} (µM)*
200	0.26	0.32	0.28	0.286667	42.59426	
100	0.78	0.82	0.8	0.8	45.00125	
10	0.75	0.82	0.83	0.8	67.16253	10.01
1	0.85	0.75	0.9	0.833333	88.74512	
0.1	1.11	0.99	1.2	1.1	91.34563	
control	0.96	0.863	1.12	0.981	100	

Table 7: Effect of test extract on different cancer cell lines using MTT Assay

Type of cancer cell lines	*Name of cell line*	*IC_{50} values*
Colorectal	HCT 15	38.12
	SW – 620	43.64
Breast	MDA – MB – 231	26.51
Renal	A 498	10.12

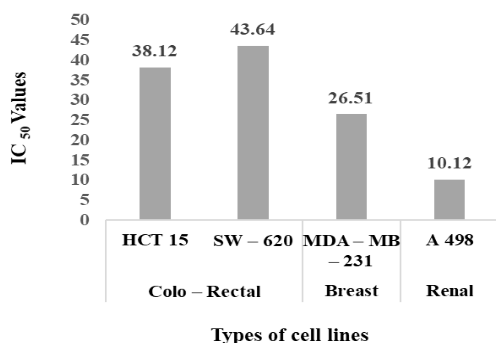

Fig. 7: Effect of test extract on different cancer cell lines in MTT assay

A study was conducted on the antiproliferative effect of *P. ostreatus* against Colo-205 and MCF7 and was proved to exhibit the same against the proliferation.

The results in the present investigation were analyzed and it was found that the methanolic extract of *P. ostreatus* showed an anti-cancer effect, also proved by the obtained IC_{50} values. The results were consistent with the previous studies conducted with the same *Pleurotus* species without alteration in the normal cells and also suppressed the proliferation of cancer cells in the breast and colon.[34] In this regard, there is a vital point that has been noticed that mushrooms possess edibility at an elevated degree, a thorough investigation to be done to prove the efficacy clinically. Moreover, the presence of bioactive compounds has been found to interfere with different types of cancers. The anti-tumor effect was due to the flavonoids present, which ultimately interact with the generation of free radicals. Each compound identified has its biological importance in the methanolic extract of *P. ostreatus*, and the molecular docking study revealed the locking mechanism that was recognized (Ligand) with the protein propanedioic acid, phenyl-, found in cancers. Additionally, the presence of phenolic compounds was responsible for inhibitory effects on cancer cells along with the anti-oxidant property. More elaborative studies are required in connection with the above compounds to combat carcinogenic diseases.

Conclusion

In conclusion, the data suggested that *P. ostreatus* extract was identified with six phytochemical constituents, out of which propanedioic acid, phenyl- was possessed with maximum locking in the molecular docking process. Additionally, the test extract produced inhibitory action on cancer cell lines like HCT 15, SW – 620, MDA – MB – 231, and A 498 at certain values, out of which the test extract produced high potency when A 498 cell lines were used. In the future, this study needs an extension of screening anti-cancer activity using suitable in vivo methods. A commercial preparation can be planned at a cheaper rate in the treatment of cancer so that the common man can afford and get the best therapy for cancer.

Author's Contribution

All authors contributed to the study. D.B., designed and was involved in the readiness of the manuscript. L.C., conducted the experiment, analyzed and compiled the results and prepared the manuscript.

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