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Research Article

Identification Of Bioactive Complex, Molecular Docking, And Anticancer Effect Of *Filifusus Filamentosus*

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ABSTRACT

Components from natural resources are sought as alternatives to synthetic drugs for treating fatal diseases like cancer. This investigation focuses on *F. filamentosus*, with its extracted using methanol and identified through GC-MS analyses. Molecular docking aids in understanding interactions between inhibitors and enzymes, summarizing recent advancements in in silico research for anticancer drug discovery. These reports indicate that molluscs, particularly gastropods, are valuable sources for discovering novel compounds for pharmaceutical drug development. The current study focuses on examining the GC-MS analysis, docking studies, anticancer activity, apoptosis effect, cell nuclear morphology, and DNA fragmentation of the methanolic extract of *F. filamentosus*. In this study, AutoDock 4.2 was utilized to analyze compounds from the gastropod *F. filamentosus* that can bind to the 5p21 protein, thereby inhibiting uncontrolled cell proliferation. Notably, 2-4-Di-tert-butyl-phenol was identified as having a promising association with the 5p21 protein binding site. The cytotoxic effects of *F. filamentosus* were examined using the MCF-7 cell line. While the search for chemopreventive compounds in marine organisms has been widely reported, the presence of such compounds in *F. filamentosus* has been only minimally explored. The apoptotic potential of methanolic extracts from *F. filamentosus* was examined in this work. The findings of this study indicated that chemicals with chemopreventive and anticancer characteristics were present in the methanolic extract of *F. filamentosus*. Similarly, its capacity to trigger apoptosis in MCF-7 cells accounts for its moderate cytotoxic effect. As a result, this study's findings have produced a new source of molluscs with strong apoptotic effects, this finding help to developing natural treatments for cancerous tumours in the future which would be less expensive, safer, and have less side effects.

Keywords: *F. filamentosus*, Drug Designing, Molecular docking, 2-4-Di-tert-butyl-phenol, Anticancer, MCF-7, apoptotic.

1. INTRODUCTION

Throughout history, molluscs have provided a wide range of human resources, including food, shells, dyes and medicine. In many cultures shelled gastropods and bivalves are regarded as a delicacy or healthy food and they also feature in a range of traditional natural remedies (Herbert *et al*., 2003; Prabhakar and Roy, 2009). In most cases there has been no scientific research undertaken to substantiate the health benefits of molluscs. However, there is increasing interest in the bioactivity of mollusc extracts and secondary metabolites (Cimino and Gavagnin, 2006). Currently, natural products isolated from molluscs and their structural analogues are particularly well represented in the anticancer compounds in clinical trials (Simmons *et al*., 2005). Nevertheless, it is presently unclear whether the production of bioactive secondary metabolites is ubiquitous within the phylum mollusca.

Consequently, there should be much scope for future drug discovery within this phylum. The continual discovery of novel drug leads from the enormous pool of available species requires a strategic approach, such as the investigation of traditional medicines and previously unstudied sources that are likely to have independently evolved novel pathways for secondary metabolism. Consequently, it could be predicted that distinct chemical structures will occur within molluscan groups that have evolved under different environmental and biological pressures.

Gas Chromatography - Mass Spectrometry (GC-MS) is a sensitive analytical technique that is used in a wide range of applications such as environment monitoring, flavor and fragrance analysis (Paranthaman, 2012), pesticide analysis, metabolite analysis (Reade *et al*., 2014) forensic and criminal cases etc (Chauhan, 2014). It is considered as the method of choice for detection of volatile compounds due to its high sensitivity over other analytical techniques like Liquid Chromatography - Mass Spectrometry (LC-MS) (Agilent Technologogies, 2007). With the selection of suitable column, a wide range of compounds such as eicoscanoids, essential oils, FAs, wax, esters, perfumes, terpenes can be analyzed in GC-MS (Agilent Technologogies, 2007).

In the last several decades, research has expanded from land to ocean in order to find new drug and this diversity has provided a unique source of chemical compounds with potential bioactivities that could lead to potential new drug candidates. The study of the molecular interactions between biologically active natural products and the corresponding cellular receptors is of great importance from a biological as well as medicinal point of view (Gautam, 2007). Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. This is called as induced fit. Molecular docking is an internet service that calculates the sites, the energy of small molecules and geometry of interacting protein (Schames, 2004). After docking stimulation, well - docked protein - ligand complexes are produced in experimental laboratories for testing.

One of the most well-known docking program is a AutoDock (Morris *et al*., 2009). AutoDock is an automatic docking tool. It is designed to predict how small molecules, such as substrates, bind to a receptor of known 3D structures. A graphical user interface called AutoDock tool or ADT was utilized to generate grids, calculate the docking score and evaluate the conformers (Nurfina, 1997). Docking is most commonly used in the field of drug design, may be applied to hit identification, lead optimizations and bio- remediation. Structure - based drug design methods utilize knowledge of the three - dimensional structure of a receptor complex with a lead molecule is an attempt to optimize the bound ligand or a series of congeneric molecules. Using a model with a given structure, a medical chemist can compute an activity of a molecule (Lewis, 2005).

Cancer develops through a multistep carcinogenesis process that encompasses various cellular physiological system such as cell signalling apoptosis, thus making it a very complex disease. Most currently used anticancer drugs, which have been obtained by synthesis of novel compound or from natural sources are toxic to normal cells in addition to cancer cells and thus have first substantial harmful side effects. There is therefore a continuous search for innovation chemotherapeutic drugs that act as "Magic Bullets" specifically targeting cancer cells with minimal damage to normal cells (Saleem *et al*., 2019)

Breast cancer is cancer that starts in the breast, usually in the inner lining of the milk ducts or lobules. The various factors which influence the breast cancer are as follows age, race, alcohol intake, obesity, radiation, physical activity and adult height, reproductive, hereditary, hormonal, environmental and lifestyle factors (Rudden, 2007). Symptoms of breast cancer are the presence of lumps or thickening in the breast, swelling, dimpling, redness and soreness of skin, change in shape of nipple and nipple discharge. Detection and diagnosis of breast cancer can be by breast examination, mammography, ultrasound, breast MRI, fine needle aspiration, core needle biopsy, breast tumour pathology, and lymph node biopsy. Treatment of breast cancer includes surgery, radiation, chemotherapy, hormonal therapy, and alternative medicines.

Apoptosis serves a key function as a protective mechanism against cancer, by removing genetically damaged cells, or cells that have become cancerous. When apoptosis is triggered in response to certain physiological signals, a proteolytic cascade involving different caspases is initiated in the suicidal cells. This cascade leads to activation of nucleases that initiate the degradation of chromosomal DNA. This type of DNA fragmentation is considered hallmark of the apoptosis process (Gibbs, 2000 and Debatin, 2004). The study of apoptosis is an important field of biological inquiry since a deficiency or an excess of apoptosis is one of the causes for cancers, autoimmune disorders, diabetes, Alzheimer's organ and bone marrow transplant rejection and many others diseases (Deborah Ribble *et al*., 2005) Based on the above approach, the present study aims to design a new series of bioactive compounds structurally containing 13 components and derivatives to develop a good target for drug discovery. For this target, docking of the newly synthesized compounds was done using AutoDock 4.2. The cytotoxic effect, apoptotic property nuclear cell morphology, and DNA fragmentation of the marine gastropod *Filifusus filamentosus* was investigated.

2. MATERIALS AND METHODS

Assemblage of marine gastropode

Marine gastropods *F. filamentosus* a sea gastropod, was collected from the coasts of Tuticorin, which is located near the Gulf of Mannar. At 8.30° N and 7.40° E in Tamil Nadu, India. After being placed in a glass container, these living molluscs were cleaned by rinsing them with tap water

Preparation of extract

Methanol extract of the whole-body tissues was prepared following the slightly modified technique given by Thilaga (2005). Dried tissues were soaked in 100% A.R grade methanol for 10 days at room temperature. After filtration with Whatman No.1 paper, the methanol extract was reduced by vacuum evaporation. The extract residue was resuspended in 20ml of 100% A.R grade methanol. The methanol soluble extracts were dried and solubilized in deionized water. Different concentrations of extracts were prepared and stored at 0°C for further use.

GC – MS analysis

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer System comprising a AOC 20i auto sampler and gas chromatography interfaced to a mass spectrophotometer (GC-MS) instrument employing the following conditions such as Column elite – 5MS fused silica capillary column (30 x 0.25mm ID x 0.25µm df, composed of 5% Diphenyl/95% Diphenyl Poly Siloxane), operating in electron impact mode at 70eV: Helium (99.999%) was used as a carrier gas at constant flow of 1ml/min and an injection volume 3µl (split ratio of 10:1) injector temperature 250°C. The oven temperature was programmed from 110°C (isothermal for 2min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5s and fragments from 45 to 450 a.

Identification of compounds

Interpretation of mass spectrum was conducted using the database of National Institute Of Standard Technology (NIST Ver.21), WILEY 8 and FAME having more than 62,000 patterns. The unknown components found in the body tissues of *Chicoreus ramosus* were matched with the spectrum of the known components stored in NIST, WILEY and FAME, the MS library and predicted from Duke's Ethno Botanical Database.

Auto Dock protocol

Firstly, all bound waters, ligands and cofactors were removed from the proteins. The macromolecule was checked for polar hydrogens, partial atomic Kollman charges were assigned, and then atomic salvation parameters were allotted. Torsion bonds of the inhibitors were selected and defined. Secondly, the three-dimensional grid box was created by AutoGrid algorithm to evaluate the binding energies on the macromolecule coordinates. The grid maps representing the intact ligand in the actual docking target site were calculated with AutoGrid (part of the AutoDock package). The three-dimensional grid box with 60 Å grid size (x, y, z) with a spacing of 0.300 Å, grid was created. Eventually, cubic grids encompassed the binding site where the intact ligand was embedded. Finally, AutoDock was used to calculate the binding free energy of a given inhibitor conformation in the macromolecular structure while the probable structure inaccuracies were ignored in the calculations.

Anticancer activity (Mosmann, 1983)

In vitro cytotoxicity assay (MTT assay)

To determine the cytotoxic effects of the methanol extract of experimental animals, MTT 3- (4, 5- dimethyl thiazol - 2- yl) - 2, 5 - diphenyl tetrazolium bromide assay was performed using MCF-7 (Breast carcinoma) cells. MCF-7 (Breast carcinoma) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), Penicillin (100 U/ml), Streptomycin (100 µg/ml) and amphotericin B (5µg /ml), in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt Ltd., Kolkata, India).

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4,5 dimethyl thiazole -2-yl) -2-5- diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells.

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed once and different test concentrations of test drugs were added on to the partial monolayer in microtitre plates to obtain final concentrations of 100, 200, 300, 400 µg/ml. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere and microscopic examination was carried out and observations were noted every 24h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a

wavelength of 570 nm. The untreated cells were used as the control. The effect of the samples on the proliferation of MCF-7 breast carcinoma cells was expressed as the percentage cell viability using the following formula: % of viability = Absorbance of the sample × 100 Absorbance of the control % of toxicity = 100 - % of viability

Acridine Orange/Ethidium Bromide Staining

Acridine orange/ethidium bromide (AO/EB) staining was carried out to detect morphological evidence of apoptosis. MCF-7 cells were treated with the methanolic extract *F. filamentosus* at different concentrations of 6.25 µg/mL and 12.5 µg/mL, respectively 24 h. The cells were washed with PBS (pH 7.4) and 10µL of acridine orange/ethidium bromide solution (60 µg/mL of acridine orange and 100 µg/mL of ethidium bromide in PBS) and made up to 100 µL using PBS and incubated for 5 min. The cells were then washed with PBS and examined under Nikon Eclipse Ti fluorescence microscope (Nikon Instruments Inc., NY, USA).

DAPI Staining

Cell nuclear morphology was evaluated by fluorescence microscopy following DAPI staining. MCF-7 cells were treated with the methanolic extract of *F. filamentosus* at different concentrations of 6.25 µg/mL and 12.5 µg/mL, respectively 24h. The cells were washed with PBS (pH 7.4), fixed with ice cold 70% ethanol and resuspended in DAPI, and incubated for 15min at 37°C wrapped in aluminium foil. The cells were then washed with PBS and examined under Nikon Eclipse Ti fluorescence microscope (Nikon Instruments Inc., NY, USA).

DNA Fragmentation Analysis

To confirm the apoptotic mode of cell death, DNA fragmentation assay was performed. The DNA fragmentation assay was carried out by following the methods of Tyagi et al., 2014. The MCF-7 cell lines and the crude extract of *F. filamentosus* were incubated for 24 hrs in 5% of CO2 incubator. After incubation the trypsin phosphate versene glucose reagent (TPVG) was added and then it was centrifuge at 15,000 rpm for 10minutes. The pellet was collected and washed with proteinase K and incubated at 55°C for 3 hrs. Then phenol, chloroform and isoamyl alcohol (in the retio of 25:24:1 v/v) was added and vortexed vigorously and incubated on ice for 5 minutes. Then it was centrifuge at 10,000 rpm for 10 minutes and the aqueous layer was transferred to a new Eppendorf tube and phenol, chloroform and isoamyl alcohol extraction was repeated. The aqueous layer was combined with 50mlof 3M sodium acetate, 2.5 ml of 100% cold ethanol and stored at -20°C overnight. Then, it was centrifuge at 15,000 rpm for 5 minutes at 4°C. The pellet was air dried for 5 -10 minutes. Next, the dried powder was resuspended in 100 µl buffer (10mM Tris/1 mM EDTA) and subjected to 1% agarose gel. The gel was stained with 1µg/ml ethidium bromide. The clear bands were visualized and photographed.

RESULTS AND DISCUSSION

GC-MS analysis

The methanolic extract *F. filamentosus* of was subjected to GCMS analysis. GC-MS analysis from experimental animal *F. filamentosus* revealed 13 compounds that could be identified as Cyclotetrasiloxane, octamethyl, Cyclooctane,1,4 - dimethyl, E-14-Hexadecanol, Cholestrol, 9-Octadecenamide, (Z) - [Oleic acid amine], Undecanal, 2-methyl-, Cyclohexanol,2-amino-,trans-, Butanal, O-methyloxime, E-2-Tetradecen-1-ol, 1,1-Cyclopropane dicarbonitrile, 2,2-dimethyl-, Cyclopentanol, 2- (aminomethyl)-, cis, Cholan-24-oic acid, 3-oxo-, methyl ester, (5á). The bioactive compound with their retention time (RT), molecular formula, molecular weight (MW) and concentration (area) are present in Table.1. The mass spectrum and structure of the compounds identified were present respectively, which could be responsible for anticancer antioxidant, antimicrobial, analgesic, anti-inflammatory, antipyretic, hepatoprotective, diuretic, antiviral activities etc.

Table 1. Activity of components identified in the methanol extract of *F. filamentosus* by GC - MS study.

Sl. No.	RT	Name of the Compound	Molecular Formula	Molecular Weight (g/mol)	Peak area %	**Activity
1	3.52	Cyclotetrasiloxane, octamethyl	$C_8H_{24}O_4Si_4$	296.616	92.61	Wound healing, Anti-ulcer, Antisoriatics, Antiseborrheics, Antioxidant, Antiarthritics, Analgesic, Antipyretic, Antiinflammatory, Antiviral, Antineoplastic, Immunomodulators
2	6.89	Cyclododecane	$C_{12}H_{24}$	168.324	84.67	Antiadthmatics, ronchodilators,

						Anti-spasmodics, Anti-acne, Antioxidants, Antigout, Anaesthetics, Anticonveulsants, Antibacterial.
3	8.41	2,4-Di-tert-butyl phenol	$C_{14}H_{22}O$	206.329	95.61	Antioxident, Anticancer, Antiinflammatory, Analgesic, Antipyretics
4	9.51	E-14-Hexadecanol	$C_{22}H_{38}$	302.546	79.16	Antioxident, Anticancer, Antiviral, Antifungal
5	36.39	Cholestrol	$C_{27}H_{46}O$	386.654	89.46	Antioxident, Anticancer, Hepatopotective, Antiinflammatory, Antimicrobial, Analgesic, Antipyretic
6	16.32	9 -Octadecenamide, (Z)- [Oleic acid amine]	$C_{18}H_{35}NO$	339.564	8.80	Antiinflammatory, Antiandrogenic, Cancer preventive Dermatitigenic, Hypocholesterolemic, 5-Alpha reducatase inhibitor, Anemiagenic Insectifuge, Flavor
7	3.01	Undecanal, 2-methyl-	$C_{12}H_{24}O$	184.323	2.85	Antimicrobial
8	10.85	Cyclohexanol,2-amino-,trans-	$C_6H_{13}NO$	172.268	1.73	Antiinflammatory
9	12.59	Butanal, O-methyloxime	$C_5H_{11}NO$	101.149	7.29	Antimicrobial
10	14.76	E-2-Tetradecen-1-ol	$C_{14}H_{28}O$	210.361	1.77	Antimicrobial
11	16.89	1,1-Cyclopropane dicarbonitrile, 2,2-dimethyl-	$C_7H_8N_2$	68.119	1.18	Antimicrobial
12	17.46	Cyclopentanol, 2-(aminomethyl)-, cis-	$C_6H_{13}NO$	100.161	0.41	Antimicrobial
13	28.85	Cholan-24-oic acid, 3-oxo-, methyl ester, (5á)-	$C_{25}H_{40}O_3$	388.592	50.06	Antimicrobial Antiinflammatory, Anticancer, Antiasthma, Diuretic, Antiarthritic

Auto Docking

In the present study the AutoDock binding affinities of various structure for their potential anticancer activity was investigated. In the present study, AutoDock software were used to perform the docking simulations between the ligand 2,4-Di-tert-butyl phenol and standard drug Salirasib.

In the present study, AutoDock software were used to perform the docking simulation between the ligand 2,4-Di-tert-butyl phenol and standard drug salirasib with the protein 5p21. The results of the docking simulations are presented in Table 2. The results showed that the ligand 2,4-Di-tert-butyl phenol has a lower binding energy (- 4.67 kcal/mol) that indicated higher affinity with the protein. Also ligand has highest root mean square deviation (RMSD) score (40.37) and lowest inhibition constant (375.78 µm) when compared to the standard drug. Moreover the stability of the ligand is also high than that of standard drug and has succeeded in making strong hydrogen bond with the target protein. The distance and site of hydrogen bond is shown in the (Figure1and 2) and (Plate 1).

Table 2. Docking result of protein 5p21.

Ligand	**2,4-Di-tert-butyl phenol**	**Salirasib**
Binding energy	-4.67 kcal/mol	-4.49 kcal/mol
Ligand efficiency	-0.31	-0.18
Inhib_constant	375.78 µM	515.44 µM
H acceptor	UNL1:H	UNL1:O
H donor	A:GLU31:O	A:LYS16:NZ
Distance	1.926 Å	2.892 Å
RMSD Score	40.37	40.23

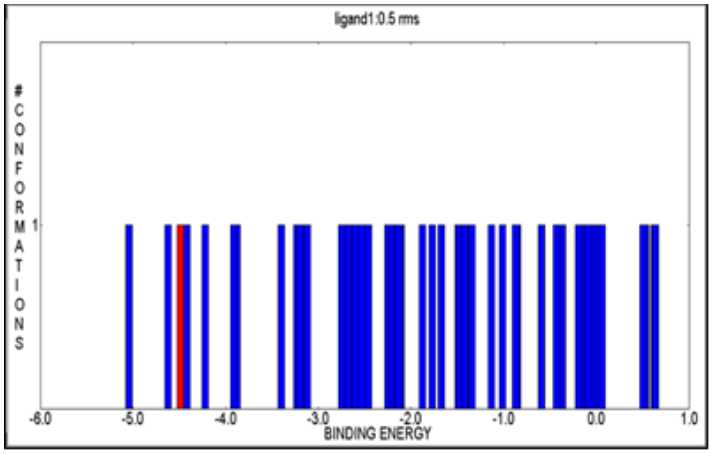

Figure 1. Docking simulation between 5p21 and Standard Salirasib

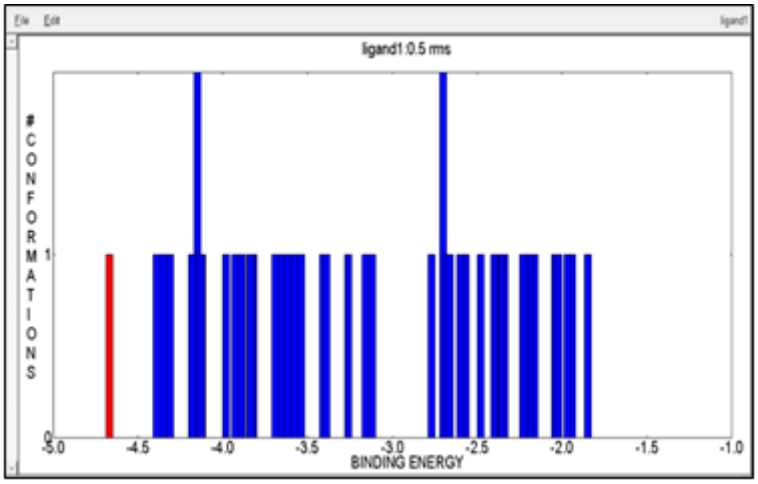

Figure 2. Docking simulation between 5p21 and 2,4-Di-tert-butyl phenol.

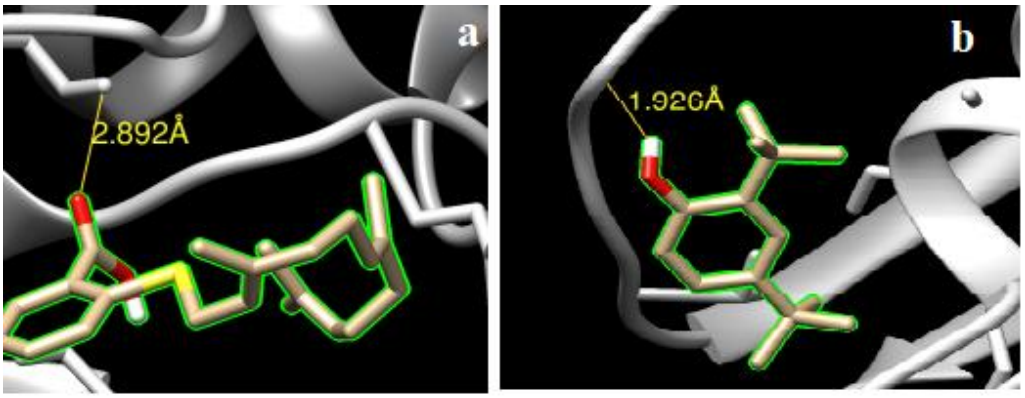

Plate 1. Visualization of interactions of ligand and standard drug with the target protein 5p21. - Green chains represents the standard drug and ligand (a. Interaction of Salirasib with 5p21, b. Interaction of 2,4-Di-tert-butyl phenol with 5p21).

Anticancer activity of methanolic extract of *F. filamentosus*

Anticancer activity of the methanolic extract of *F. filamentosus* was carried out against MCF-7 human breast cancer cell line. The results of anticancer activity of *F. filamentosus* extract on MCF-7 human breast cancer cell line were shown in Plates. (2&3) and Figure. (3) respectively. The results of the anticancer activity revealed that *F. filamentosus* showed good anticancer potential which has the IC50 of 231.50 µg/ml against MCF-7 breast cancer cells. In the present study, the cell viability was found to be increased with increase in concentration. The methanolic extract of *F. filamentosus* showed 72.92% viability at 100 µg/ml followed by 78.03%, 88.55% and 92.20% at 200, 300 and 400 µg/ml concentration respectively. The percentage toxicity was found to be 27.08% at 100µg/ml, 21.97% at 200 µg/ml, 11.48% at 300 µg/ml and 7.8% 400µg/ml at different concentrations respectively.

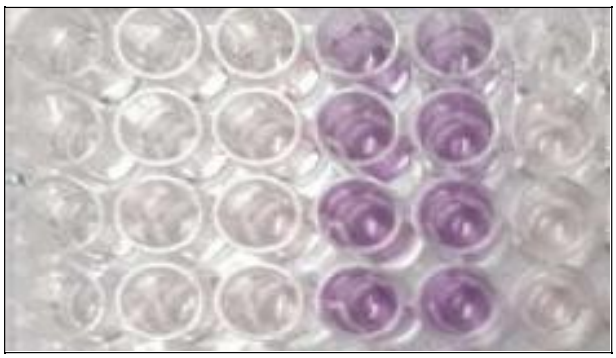

Plate 2. MTT assay

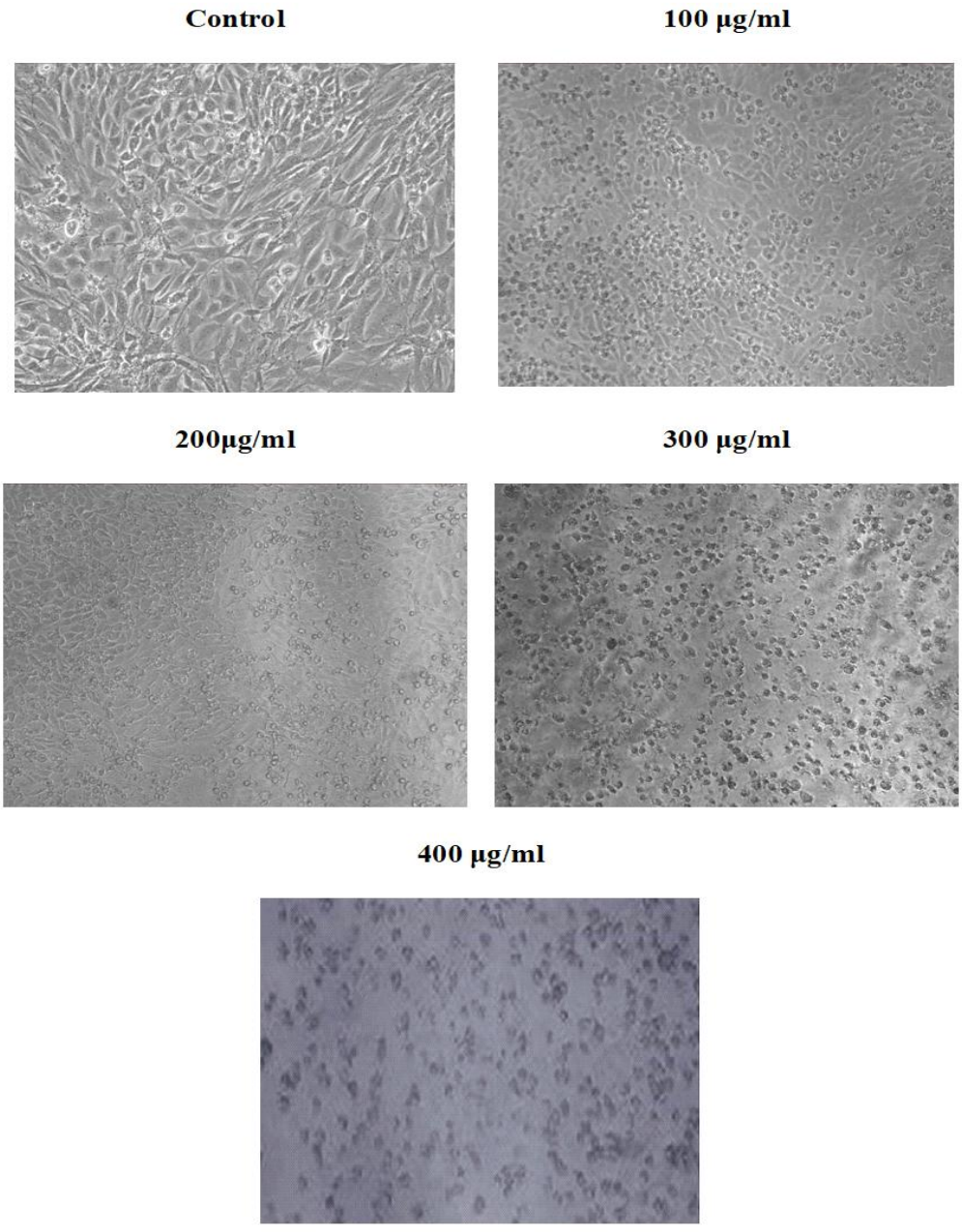

Plate 3. Anticancer activity of methanolic extract of *F. filamentosus*

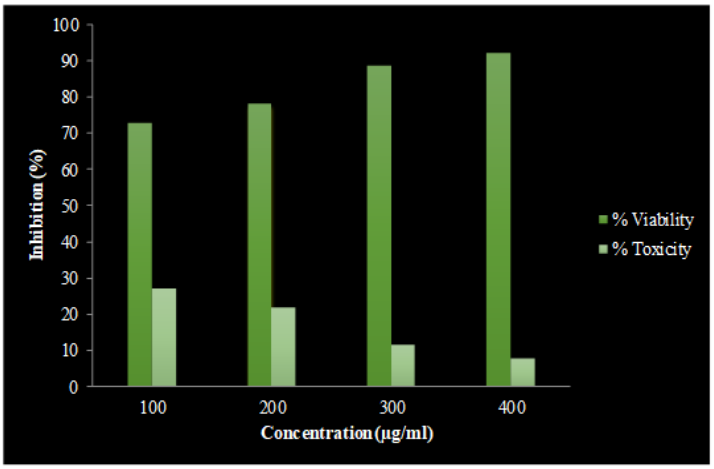

Figure 3. Anticancer activity of methanolic extract of *F. filamentosus* in MCF-7 cell line

Apoptotic effect of *F. filamentosus* extract in MCF-7cells

Acridine orange and ethidium bromide staining were performed to evaluate the cellular morphological changes in MCF-7 cells treated with the methanolic extract of *F. filamentosus*. Usually, acridine orange will enter the nucleus and stain live cells as green colour and ethidium bromide will penetrate the nucleus of dead cell due to loss of membrane integrity and stain as red colour. In the present study, viable cells appeared as green fluorescence with highly organised nuclei. Early apoptotic cells appeared as green-orange colour nuclei with condensed or fragmented chromatin and late apoptotic cells appeared as orange to red colour with highly condensed or fragmented chromatin and apoptotic bodies. The methanolic extract of *F. filamentosus* treated cell showed typical apoptotic morphological features such as condensed nuclei, membrane blebbing and formation of apoptotic bodies in a concentration-dependent manner, which were clearly observed under the fluorescence microscope and quantitated (Plate. 4) and (Fig. 4).

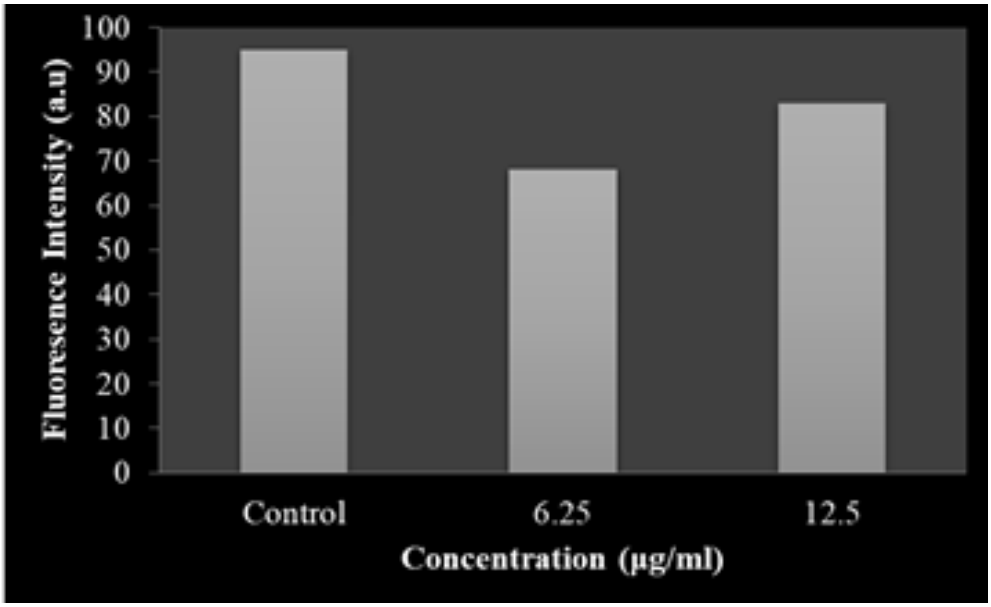

Figure 4. Acridine orange and ethidium bromide staining assay of *F. filamentosus*

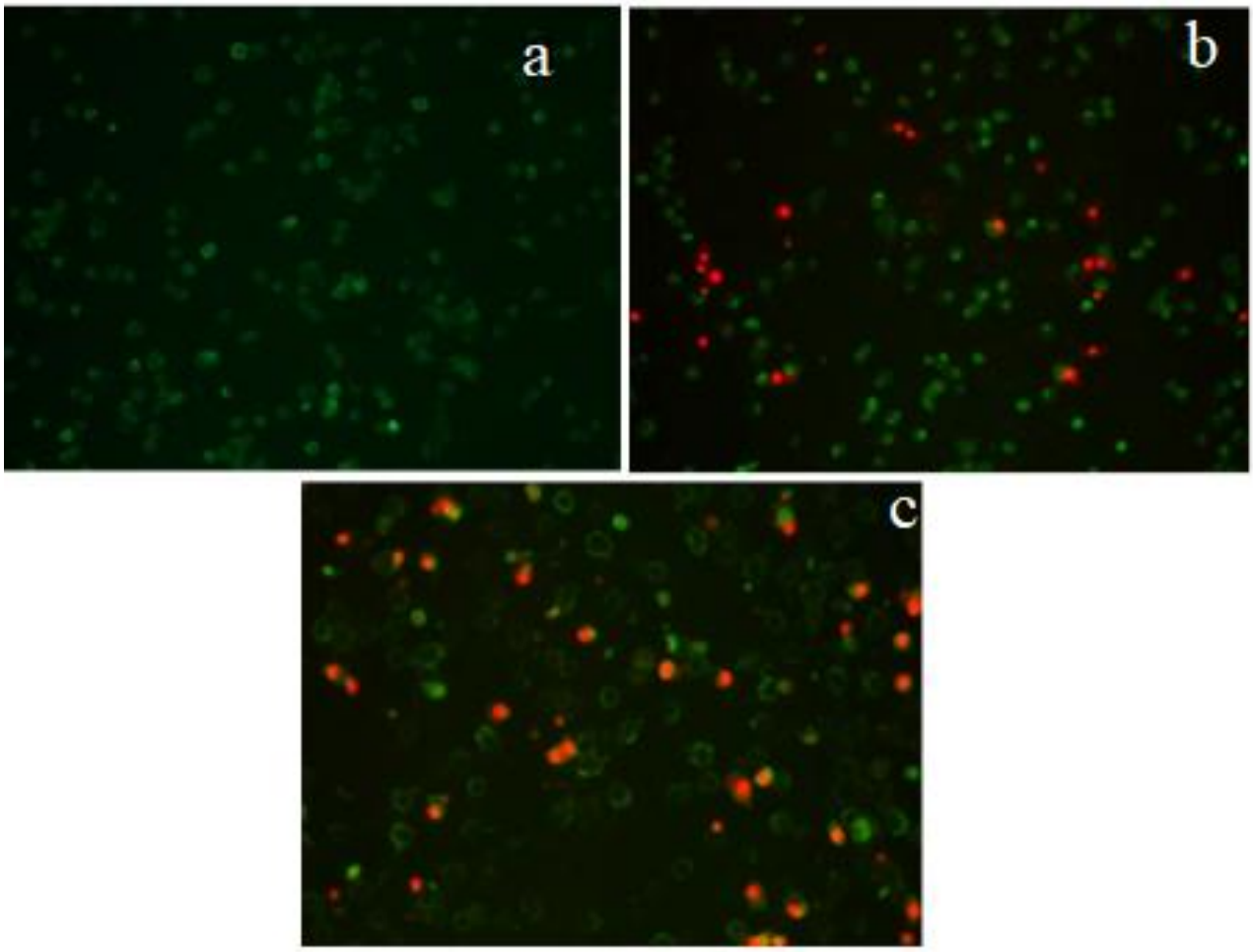

Plate 4. Acridine orange and ethidium bromide staining assay of *F. filamentosus* in MCF-7 cells (a. **Control, b - 6.25 µg/ml, and c - 12.5 µg/ml).**

Apoptotic cell nuclear morphological changes in MCF-7 cells by the *F. filamentosus* methanolic extract

Apoptosis can be differentiated from necrosis by their characteristic nuclear changes. DAPI is a nuclear stain which is observed as blue fluorescence when excited under fluorescence microscope. In the present study, DAPI staining revealed the changes associated with apoptosis in MCF-7 cells treated with the methanolic extract of *F. filamentosus*. The methanolic extract of *F. filamentosus* treated
MCF-7 cells appeared as bright blue with apoptotic nuclear morphological changes. The morphological changes include chromatin condensation nuclear fragmentation and marginalization, DNA condensation and fragmentation and formation of apoptotic bodies in MCF-7 treated cells. In untreated cells, there were no morphological changes, nuclei fluoresced as faint blue which was homogenous (Plate. 5) and (Fig .5).

Apoptosis confirmation by DNA fragmentation assay

To gain further insights into the mode of cell death caused by methanolic extract of *F. filamentosus*, DNA fragmentation assay, a widely used technique for the detection of apoptosis was adopted. The treatment resulted in a dose dependent increase in the DNA fragmentation levels in MCF-7 cells. DNA fragmentation was clearly visible in the groups with the dose-dependent treatment of the methanolic extract (Lanes 3, 4) compared to the control group (Plate.5)

DNA laddering assay was performed on agarose gel electrophoresis, to determine methanolic extract of *F. filamentosus* cell death of MCF-7 breast cancer cell line via apoptotic. A clear fragmented DNA ladders were observed in MCF-7 breast cancer cell lines treated with methanolic extract of *F. filamentosus* and control didn't show any DNA fragmentation (Plate.6).

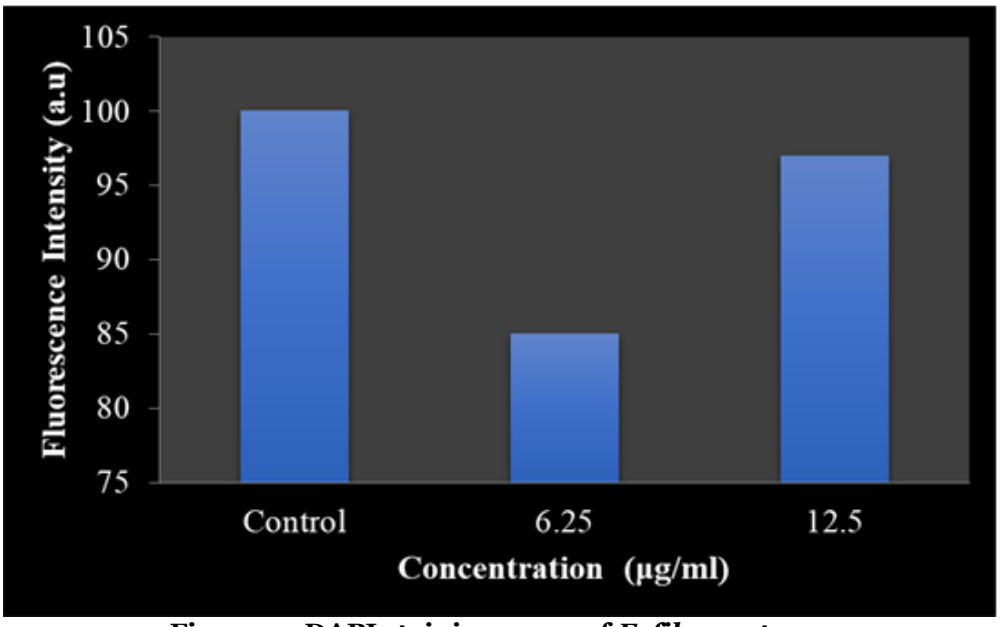

Figure 5. DAPI staining assay of *F. filamentosus*

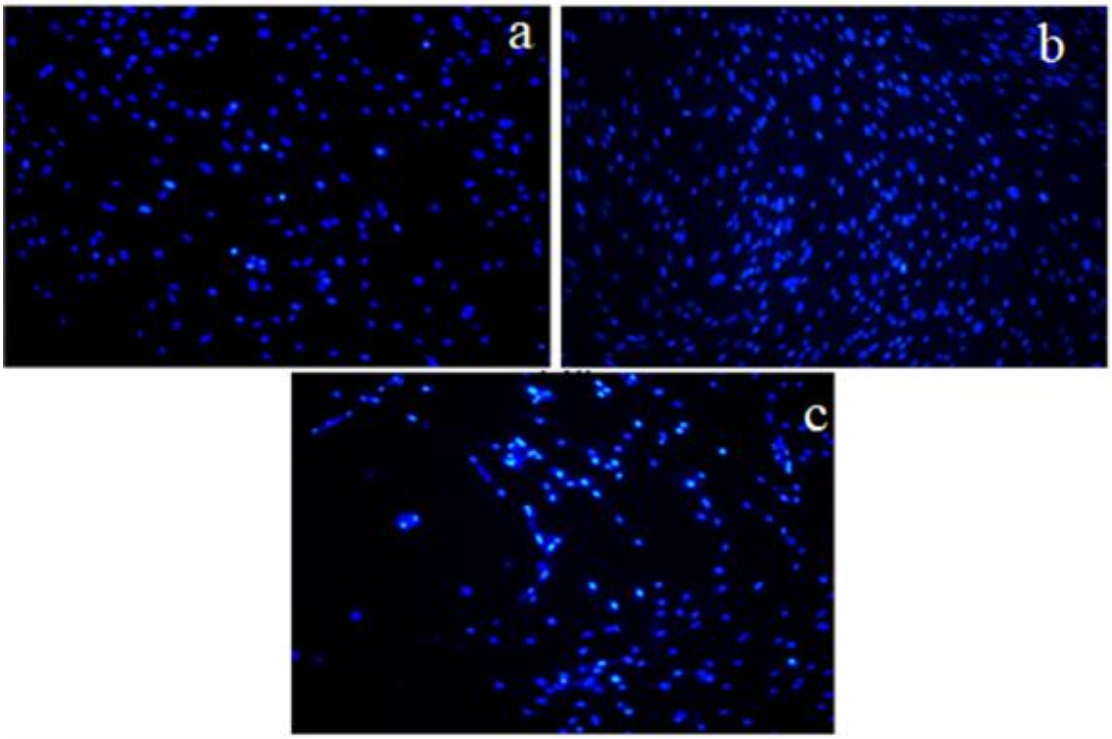

Plate 5 : DAPI Staining assay of *F. filamentosus* (a- Control, b - 6.25 µg/ml and c - 12.5 µg/ml).

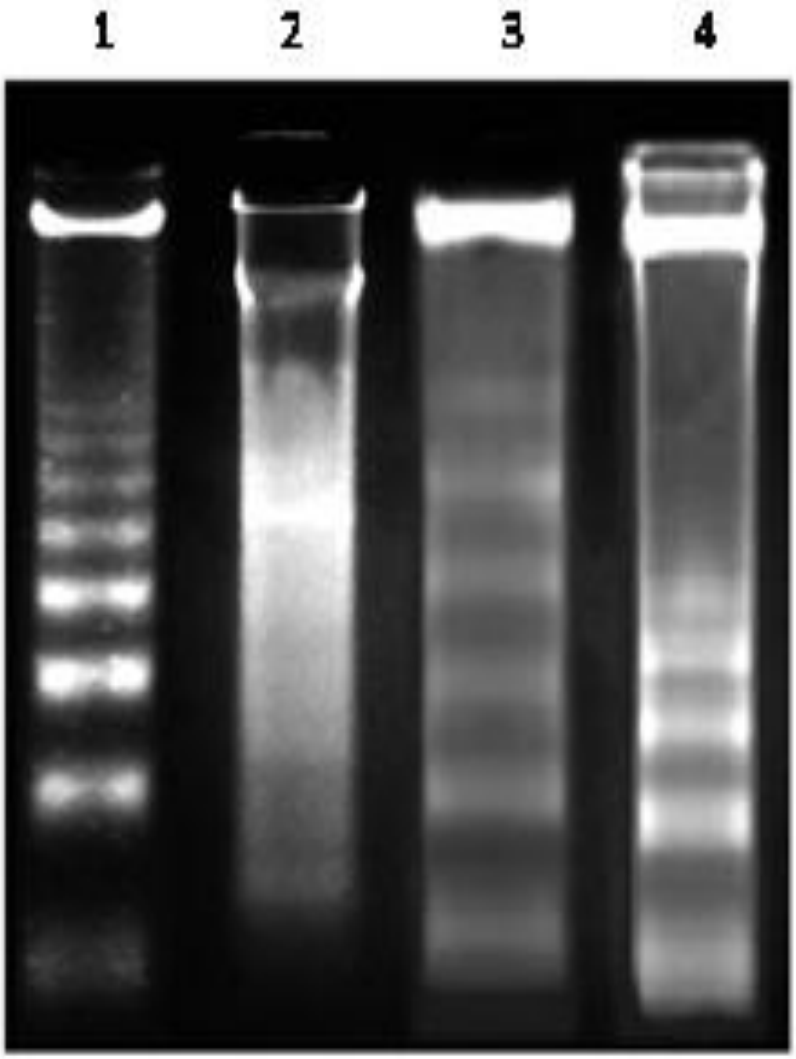

Plate 6. DNA fragmentation assay in *F. filamentosus* (Lane 1 – 1kb DNA Ladder, Lane 2 – Control, Lane 3 – 6.25 µg/ml, Lane 4 – 12.5 µg/m).

4. CONCLUSION AND SUGGESTION

The marine environment is a huge source for discovering many novel drugs. Apart from the food that is derived from the marine environment, wide varieties of drugs are being isolated and characterized with great promise for the treatment of human diseases. Studies on biochemical screening give new insights into the extraction of bioactive compounds from marine molluscs. The presence of various bioactive compounds justifies the use of *F. filamentosus* for various ailment. That it can be concluded from the above study that *F. filamentosus* extract act as therapeutic agent which is of great interest in pharmaceutical industry. This exploration creates the new standard where the extract can be a powerful weapon against cancer. This encouraging result provides useful information for designing a much better anticancer compound using *F. filamentosus* extract with minimal side effects. Hence *F. filamentosus* could proved to be a probable anticancer drug.

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