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# Evaluation of Antioxidant and Anticancer activity of *Aspergillus terreus* against ovarian cancer (PA1)

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**Abstract**

**Aim:** to study the antioxidant and anticancer activity of *Aspergillus terreus* against ovarian cancer (PA1).

**Material and Methods:** Herbal extraction was done by Soxhlet extraction method with increasing polarity of solvents viz., Ethyl acetate, Chloroform, Methanol and Water. Antioxidant activity was evaluated by *in-vitro* Phosphomolybdenum (PM) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Whereas anticancer activity was evaluated using *in-vitro* MTT assay against ovarian cancer cell lines (PA1)

**Results:** *In-vitro* antioxidant activity revealed that among all the tested extracts aqueous extract exhibited potent antioxidant activity in both Phosphomolybdenum (PM) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay with good absorbance and percentage of inhibition. Anticancer studies showed that in case of ovarian cancer PA1 cell lines tested all extracts showed noticeable activity with increasing in concentration among all treated samples aqueous extract shows significant activity with good $IC_{50}$ value.

**Conclusions:** The present study revealed that different solvent extracts of *Aspergillus terreus* exhibited high antioxidant activity and anticancer activity. Among all extracts aqueous extract shown significant activity in both antioxidant and anticancer activity. Further study requires purification, Characterization and structural elucidation of compounds in aqueous extract that may help in the development of new phytopharmaceuticals.

**Key words:** *Aspergillus terreus*, MTT assay, Ovary cancer, *In-vitro* Antioxidant and Anticancer.

## INTRODUCTION

Marine environment is considered as a prominent source of fungi that possesses pharmacologically important and novel active compounds [1, 2]. In marine ecosystem, approximately 444 species of fungi have been characterized. In addition basidiomycetes, includes 7 genera and 10 species and 360 ascomycetes species.chytrids and mitosporic or asexual fungi are the other fungi found in marine environment. Most of the marine species of fungi are recognized only from spores and many more species have yet to be explored. Among these some of the fungi are unculturable, but they can be detected by observing sea water samples and conducting rDNA analysis. Most of the bioactive molecules like peptides, terpenoids and polyketides have been obtained from marine isolated fungi. A few renowned fungal polyketides exhibit anticancer property are associated with the statin group. The statins are known for inhibiting biosynthesis of cholesterol and are used to cure cardiovascular diseases and hypercholesterolemia [3-6]. In addition, *A. terreus* produces other group of statins that contain one more methyl group joined to the dicyclohexene ring [7].

Cancer has been developed as one of the considerable cause for human suffering with remarkable morbidity and mortality. Every year the American Cancer Society measures and consolidates the present cancer incidents, death rates and survival rates. According to the recent data, in 2019, 6, 06,880 cancer mortality and 1,762,450 new cancer cases are estimated to appear in the United States. In the past few decade of evidence, in female the cancer incidence data (2006-2015) was constant and in male it has decreased nearly 2%, at the same time mortality rate (2007-2016) was declined yearly by 1.4% in female and 1.8% in male respectively by cancer has emerged as expanding public health challenge [8].

The disadvantage of regular chemotherapy technique used for treating cancer is multi drug resistance resulted by exceeded activity of integral membrane transporters like P-gp that leads to outflow of anticancer drugs that can cause excess drug storage. These (MDR) cells are resistant to toxic property of different chemotherapeutic substances. Various workers have studied the production of novel drugs that are prominent to resolve the multi drug resistance cells [9-11].

Oxidation process is essential for living organisms to generate energy for their various biological activities [12, 13].Free radicals, for example ROS (reactive oxygen species) or RNS(reactive nitrogen species) are produced during normal metabolic activities of all living cells cause cell injury. The more significant class of free radicals produced in living systems are obtained from molecular oxygen [14]. Deprivation of natural antioxidant activity or excess supply of free radicals (ROS) leads to oxidative stress. This can cause to the oxidation of protein or DNA, lipids, enzymes, this is a crucial factor which is responsible for aging, [15 &13]. Degenerative disorders like cancer, cardiovascular disease, renal failure and chronic inflammation [16 & 17].

*Aspergillus* species are the potential secondary metabolite producers [18]. According to previously reported data, Many researchers have reported that *Aspergillus candidus* produces various antioxidants for example candidusin B, 3-OH terphenyllin and 3,3-di-OH terphenyllin these exhibit radical scavenging activity on DPPH radicals [19 & 20]. *Aspergillus terreus* is distributed both in marine and terrestrial ecosystems [18 & 21] and it is a potential producer of economically important bioactive compounds for example sulochrin, terrain that exhibit antibiotic property [22] and anti-hypercholsterolemic compounds mevastatin, lovastatin etc. In this presentstudy investigation was carried to study the antioxidant and

anticancer activity of different solvent extracts of *Aspergillus terreus* using *in-vitro* assays.

## MATERIAL AND METHODS:

### Preparation of Fungal extract

About 25g of powdered biomass of *Aspergillus terreus* was extracted with 250 ml of different solvents i.e. Ethyl acetate, Chloroform, Methanol and Distilled water by using Soxhlet extraction method by marinating temperature at $50^0$ C for 4 hours. The resultant extract was filtered through whatman filter paper and dried through Rota evaporator and dessicator further dried extract was stored in air tight bottle at 4°C until use.

### Solvents and Reagents

All the solvents and chemicals used were analytical grade and were obtained from Hi-media, India.

### DPPH free radical-scavenging ability assay

Radical scavenging activities of different solvent extracts of *Aspergillus terreus* were tested through DPPH radical as a reagent, by following the methods of Rice-Evans *et al*., (1997) [23]. In this procedure 100 µL of a DPPH radical solution in ethanol (60 µM) was mixed with 100 µL of sample solution (different concentrations, w/v). The mixture was incubated for 30 min in the dark at room temperature and then absorbance was measured at 517 nm using a UV-VIS Spectrophotometer. Ascorbic acid was used as a reference standard. The DPPH scavenging activity of each sample was calculated using the following equation:

% inhibition= Ac-At/Ac x 100

Where, Ac is the absorbance of the control reaction (100 µL of ethanol with 100 µL of the DPPH solution), and it is the absorbance of the test sample. The experiment was done in triplicate. The $IC_{50}$ value was calculated for all the samples used. Lower absorbance of the reaction mixture indicated higher free radical activity.

### Phosphomolybdenum (PM) assay

Total antioxidant activity was estimated by PM assay using standard procedure of Prieto *et al*., (1999) [24]different solvent extracts of *Aspergillus terreus* are taken in different concentrations ranging from 100 µL to 500 µL were added to each test tube individually containing 3 ml of distilled water and 1 ml of molybdate reagent solution. These tubes were kept incubated at 95°C for 90 min. After incubation, these tubes were normalized to room temperature for 20–30 min and the absorbance of the reaction mixture was measured at 695 nm. Ascorbic acid was used as reference standard.

### Determination of anticancer activity by using cell viability by MTT Assay

The effect of different solvent extracts of *Aspergillus terreus* were tested on the viability of ovarian cancer (PA1) cells was determined using the standard colorimetric MTT assay using the 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyl tetrazolium bromide dye (Sigma, St. Louis, MO, USA), according to Carmichael *et al*. (1987) [25]. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% ($IC_{50}$) values is generated from the dose-response curves for each cell line. This assay is based on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product [26].

% Inhibition = OD of Test sample ÷ OD of control × 100

### Statistical analysis

All experiments were performed in triplicates (n=3) and the data are represented as the mean ±standard deviation and standard error. Differences between the means of the individual groups were analyzed using the one way ANOVA analysis SPSS software 20 Version (IBM).

## RESULTS AND DISCUSSION:

The naturally derived compounds cover largest space than the simulated compounds and they exhibit similar property to the drugs that are used in present. Nowadays about 50% of the drugs used in pharmaceutical industry are natural byproducts and their derivatives, about 63% of these compounds are anticancer drugs [27 & 28]. The recent studies reveled that natural bioactive compounds derived from organisms found in marine ecosystem possess anticancer drugs than terrestrial organisms [29-34].Alzheimer's, Parkinson's disease, diabetes mellitus [35 & 36].Living organisms require antioxidants for their normal health. These are the molecules that inhibit the progressive oxidizing chain reactions and thus prevent oxidative damage. However animals can overcome the damage caused by free radicals by establishing natural machinery, but the antioxidant compounds synthesized during normal process are not sufficient to combat free radicals. Hence intake of exogenous antioxidants obtained from natural resources would inhibit the pathological events caused by free radicals [37].

Antioxidants are essential for keeping normal cell function and health. They are compounds that prevent the initiation or propagation of oxidizing chain reactions and thus inhibit or delay oxidative damage related to aging and disease. There are number of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA),utilization of these compounds leads to adverse effect on human health [13]. To develop customer's consciousness about preservatives prepared from synthetic antioxidants that has induced to enhance attentiveness in searching potential and secured preservatives from natural sources.

Fungi are a well-known producer of many bioactive products, such as antibiotics, immuno-suppressants, anti-cancers and antioxidants. Fungi produce diverse bioactive antioxidant secondary metabolites including phenolic compounds, polyketides and terpenes [38 & 39]. With this background in the present study the different solvent extracts of *Aspergillus terreus* were tested for *in-vitro* antioxidant activity by using DPPH and PM assay.

DPPH assay is most widely accepted method for evaluating antioxidant activity of many plant based drugs and crude extracts [40]. It is based on the reduction of

Methanolic solution of colored free radical DPPH by free radical scavengers. Concentration of free radical scavengers is proportional to scavenging DPPH with the absorbance at 517 nm. The reducing potential is measured by decreasing in absorbance by the action extracts. The antioxidant activity of different solvent extractsof *Aspergillus terreus* was compared with ascorbic acid as standard antioxidant. The results revealed that among the all tested extracts the aqueous extract exhibited highest antioxidant activity with 94.94 percentage of inhibition whereas the remaining tested extracts ethyl acetate, chloroform and methanol showed moderate activity with the percentage of inhibition 85.38, 86.35 and 83.81 respectively. As compared to all samples the standard ascorbic acid has the highest percentage of inhibition i.e. 98.50. The overall results are depicted in **Table.1 and Figure.1**.

PM assay is a quantitative method used to evaluate redox reaction by antioxidant, oxidants with the involvement of ligand molybdenum. It involves the longer incubation period at higher temperature which influences the auto-oxidation reaction in the mixture. It directly estimates the reducing potent of the extracts[41]. The reaction mixture forms green phosphomolybdenum colored complex at acidic pH which is measured at 695 nm. The reduction activity of extracts and standard drug was increased with increasing in concentrations. As the PM assay is related to the total antioxidant activity which is directly depends on the absorbance value. More absorbance implies highest antioxidant activity. In the present study known concentrations of different solvent extracts of *Aspergillus terreus* were subjected to PM assay along with ascorbic acid as standard. Overall results showed that aqueous extract possessed highest absorbance i.e. 1.9873 than remaining tested extracts. Whereas standard ascorbic acid showed absorbance of 2.1830. Overall results shown in **Table.2 and Figure.2**.

**Table.1. Percentage inhibition of DPPH free radical by different solvent extracts of *Aspergillus terreus***

| Sl.no | Concentration in µg | Std Ascorbic acid | Ethyl acetate extract | Chloroform extract | Methanol extract | Aqueous extract |
|---|---|---|---|---|---|---|
| 1 | 62.5 | 95.58±0.0021 | 26.05±0.0328 | 11.72±0.0033 | 5.976±0.0134 | 31.06±0.0014 |
| 2 | 125 | 95.92±0.0098 | 44.79±0.0026 | 14.25±0.0094 | 9.749±0.0118 | 52.75±0.0026 |
| 3 | 250 | 96.46±0.0084 | 79.63±0.0115 | 47.95±0.0006 | 32.26±0.0137 | 71.84±0.0055 |
| 4 | 500 | 97.89±0.0014 | 82.45±0.0130 | 75.71±0.0147 | 52.38±0.0004 | 80.27±0.0011 |
| 5 | 1000 | 98.50±0.0084 | 85.38±0.0004 | 86.35±0.0045 | 83.81±0.0042 | 94.94±0.0100 |

Results are expressed as Mean± SD (n=3)

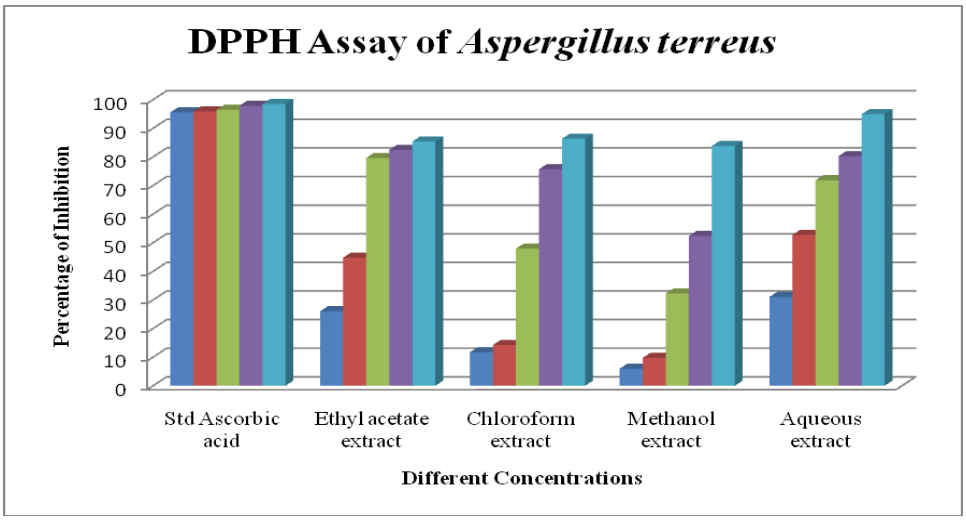


**Figure.1. Percentage inhibition of DPPH free radical by different solvent extracts of *Aspergillus terreus***

**Table.2. PM Assay of Different solvent extracts of *Aspergillus terreus***

| Sl.no | Concentration | Std Ascorbic acid | Ethyl acetate extract | Chloroform extract | Methanol extract | Aqueous extract |
|---|---|---|---|---|---|---|
| 1 | 100 | 0.638±0.0028 | 0.171±0.0043 | 0.155±0.0050 | 0.130±0.0007 | 0.523±0.0012 |
| 2 | 200 | 0.843±0.0063 | 0.370±0.0087 | 0.328±0.0127 | 0.342±0.0027 | 0.798±0.0027 |
| 3 | 300 | 1.385±0.0056 | 0.780±0.0002 | 0.647±0.0226 | 0.596±0.0103 | 1.163±0.0006 |
| 4 | 400 | 1.763±0.0042 | 0.930±0.0043 | 1.197±0.0179 | 0.964±0.0052 | 1.567±0.6056 |
| 5 | 500 | 2.183±0.0028 | 1.482±0.0670 | 1.697±0.0413 | 1.268±0.0593 | 1.987±0.0124 |

Results are expressed as Mean± SD (n=3)

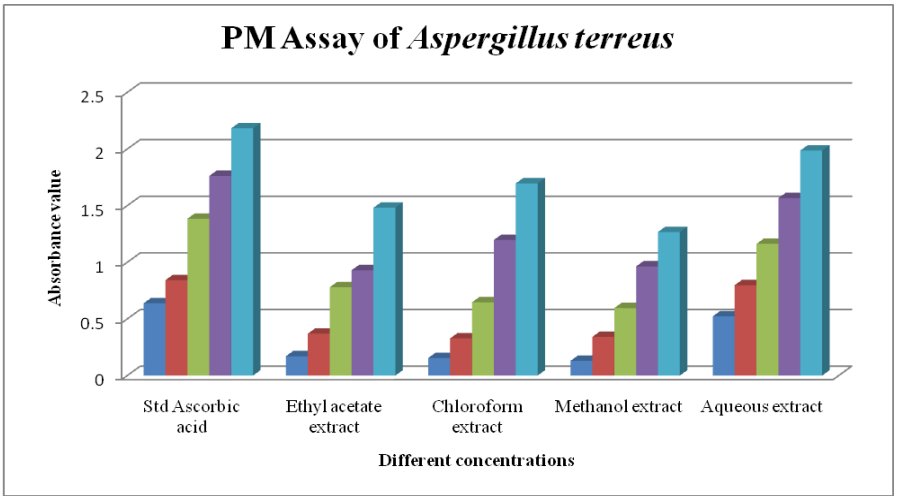


**Figure.2. PM Assay of Different solvent extracts of *Aspergillus terreus***

**Table.3. Effect of different solvent extracts of *Aspergillus terreus* on ovarian cancer (PA1) cell viability**

| Treatment | Cell Line | Concentration in µg | Cell Viability in Percentage (%) | $IC_{50}$ in µg |
|---|---|---|---|---|
| Ethyl acetate extract | PA1 | 50 | 80.73±0.0084 | **623.48** |
| | | 100 | 77.21±0.0254 | |
| | | 200 | 63.76±0.0077 | |
| | | 400 | 59.71±0.0063 | |
| | | 800 | 34.66±0.0130 | |
| Chloroform extract | PA1 | 50 | 83.32±0.0332 | **716.30** |
| | | 100 | 78.93±0.0106 | |
| | | 200 | 77.44±0.0028 | |
| | | 400 | 70.39±0.0003 | |
| | | 800 | 43.69±0.0205 | |
| Methanol extract | PA1 | 50 | 66.71±0.0176 | **585.30** |
| | | 100 | 64.60±0.0537 | |
| | | 200 | 59.98±0.0113 | |
| | | 400 | 51.43±0.0176 | |
| | | 800 | 41.42±0.0076 | |
| Aqueous extract | PA1 | 50 | 65.93±0.0148 | **194.89** |
| | | 100 | 58.49±0.0311 | |
| | | 200 | 51.31±0.0183 | |
| | | 400 | 46.30±0.0183 | |
| | | 800 | 27.48±0.0254 | |
| Standard drug Cisplatin | PA1 | 15 | 6.684±0.0091 | **<15** |

Results are expressed as Mean± SD (n=3)

Filamentous fungi such as *Aspergillus*, *Penicillium* and *Talaromyces* are some of the most exploited microbial sources for numerous bioactive compounds such as terpenes, statins, plant hormones, anti-fungal and anticancer agents. By keeping this point different solvent extracts of *Aspergillus terreus* were tested for anticancer activity against ovary cancer by using cell line PA1. In the present study untreated PA1 cell line taken as control group, whereas PA1 cell line treated with standard drug Cisplatin considered as positive control group. For the present study different concentration of standard drug and different solvent extracts of *Aspergillus terreus* were taken to study morphological changes as well as cell growth inhibition in PA1 ovarian cancer cell lines. It is already known that morphological changes occur in nucleus represents the apoptosis. Whereas normal cells appeared as regular and normal in shape, in case of treated group chromatin condensation, elongation of cells and decrease in cell count and density were observed which are the characteristic features of apoptosis. In the present study microscopic examination revealed that morphological changes and shrinkage of cells leading to cell apoptosis were observed in the treated cells by different solvent extracts of *Aspergillus terreus*, the results are expressed in **Figure.3 to Figure.4**.

In case of the cell viability tested extracts treated cells were compared to standard chemotherapeutic drug

Cisplatin. It was observed that in all treated samples, there was dose pendant manner of cell viability i.e. as the concentration increases viability of cells was decreased. Among the all tested extracts aqueous extract exhibited highest activity with low percentage of cell viability i.e. 27.48 at 800µg concentrations where as other solvent extracts ethyl acetate, chloroform and methanol shown percentage of cell viability 34.66, 43.69 and 41.42 respectively. In case of 50% of cell viability study (IC50) aqueous extract proven to be prominent with value 194.89µg for PA1 and for standard drug Cisplatin was observed to be less than 15 µg. $IC_{50}$ values for standard and extract along with cell viability were calculated and depicted in **Table.3.**Whereas the remaining tested extracts i.e. ethyl acetate, chloroform and methanol extract exhibited $IC_{50}$ value 623.48 µg, 716.30 µg and 585.30 µg respectively. Overall studies revealed that aqueous extract strong anticancer activity against ovarian cancer PA1 with $IC_{50}$ value 194.89 µg.

There are many plant species and mushrooms are renowned for production of various antioxidants whereas few information on lower fungal species [42]. Numbers of filamentous fungi are known for production of different kind of bioactive molecules those have a great impact on human health and are well - known for pharmacological and antibiotic activities like antioxidant and anticancer property [43].

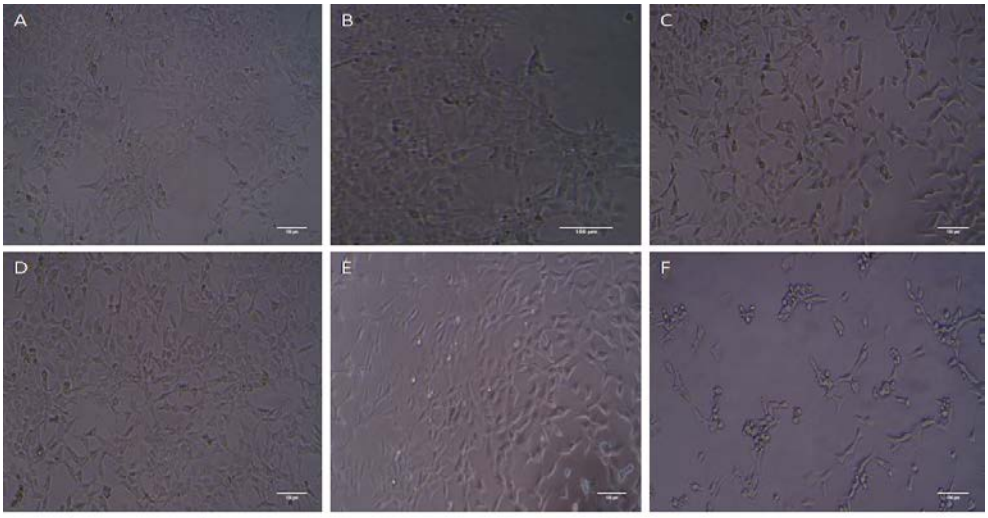


**Figure.3. Effect of ethyl acetate extract of *Aspergillus terreus* on ovarian cancer (PA1) cell morphology and viability**

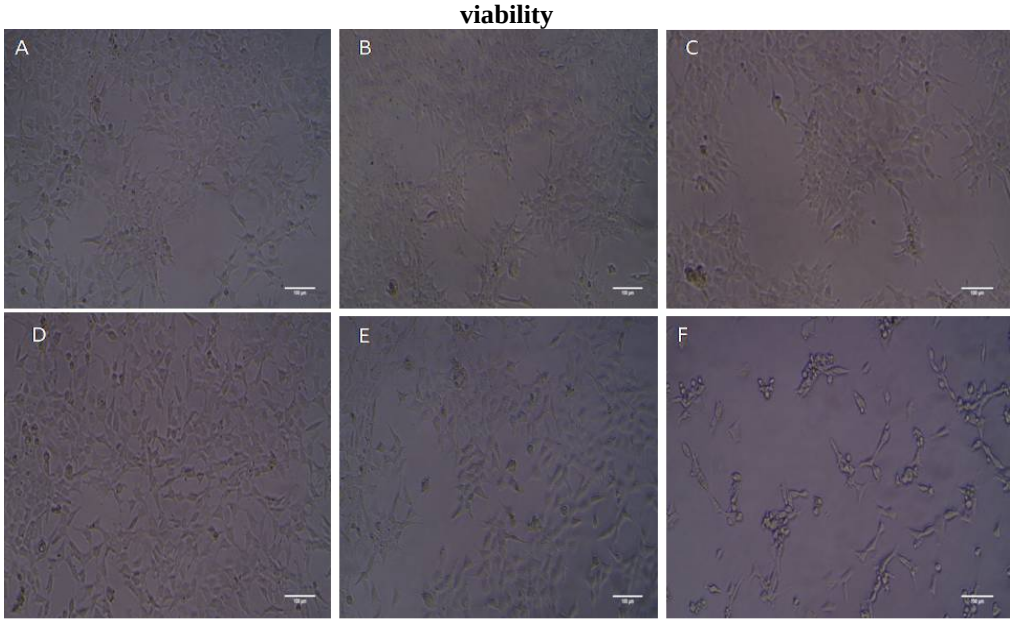


**Figure.4. Effect of Chloroform extract of *Aspergillus terreus* on ovarian cancer (PA1) cell morphology and viability**

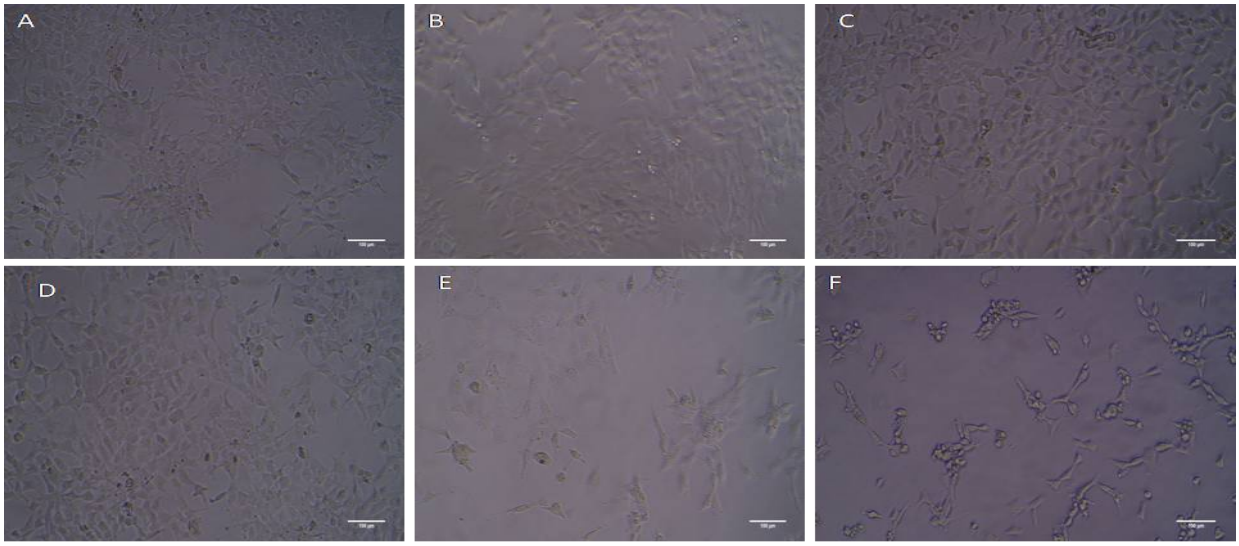


**Figure.5. Effect of methanol extract of *Aspergillus terreus* on ovarian cancer (PA1) cell morphology and viability**

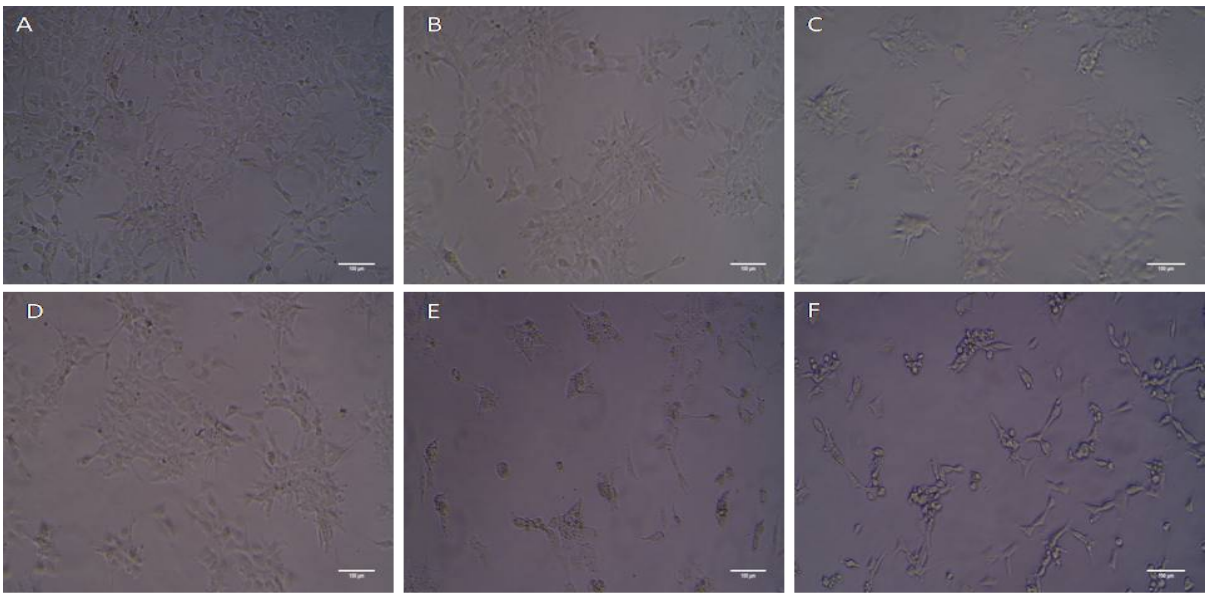


**Figure.6. Effect of aqueous extract of *Aspergillus terreus* on ovarian cancer (PA1) cell morphology and viability**

## CONCLUSION

In the present study biomass of *Aspergillus terreus* has taken and treated with different polar solvents to get different extracts. The isolated solvent extracts were subjected to *in-vitro* antioxidant and anticancer activity. *In-vitro* antioxidant studies concluded that all the tested extracts exhibited strong antioxidant activity at higher concentration. Whereas anticancer study revealed that all tested extracts shown noticeable activity against ovarian cancer PA1. Overall study concluded that aqueous extract of Aspergillus terreus shown significant activity in both antioxidant and anticancer activity. Further studies needed on the isolation, purification and structural elucidation of compounds required for particular activity with detailed mechanism.

## Conflict Of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication.

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