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Research Article

TOXICOLOGICAL EFFECT OF PLANT FLAVONOIDS ON MCF-7, A-549 AND PA-1 CANCER CELL LINES

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ABSTRACT

The research aimed in the direction of five different medicinal plants (hydro alcoholics extract) to evaluate anticancer activity and cytotoxcity. The most important purpose of this study is to evaluate cytotoxicity of these medicinal plants with facilitate of MTT assay. Concentrations are prepared of each plant extracts which are 100 µg/ml, 10 µg/ml, 0.1 µg/ml, 0.01 µg/ml and 5-10Ч10^3 cells/ml are taken into each well which are exposed to different Concentrations of plant extracts crude and elute of column chromatography [CC] for 96 hrs and then treated with MTT and take absorbance at 570 nm. Standard drug was used for IC50 of Doxorubicin MCF-7 500nm, A549- 550nm, PA-1- 580nm.

KEY WORDS: Toxicology, Medicinal Plants

INTRODUCTION

Globally, cancer represents a substantial burden of disease in the community and appears to be a prime cause of concern. According to estimates published in the Lancet, in India, around 5.5 lakhs people died of cancer in 2010. Tobacco-related cancers represented around 42% of male and 18% of female cancer deaths. Furthermore, cervical, stomach and breast cancers accounted for 41% of cancer deaths in women in rural and urban areas. Most of the chemotherapeutic drugs available today exert various side effects and are also immunosuppressive. To overcome the undesired effect, plant-based immunomodulators are often employed as supportive or adjuvant therapy to restore normal health. The aim of present study was to investigate the cytotoxic activity of elute (E) and crude (C) constituents isolated from five Indian medicinal plants Table 1 and identify their active principles. The ethanolic extracts of plants were evaluated using MTT assay on three human cancer cell lines, i.e. A549 (lung carcinoma), PA1 (ovarian cancer) and MCF7 (breast cancer) cell lines.

MATERIALS AND METHODS

Reagents

Alcohol 70%, 100% Alcohol, MEM media (Minimal Essential Media) [1], Trypsin [2], MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5- diphenyl tetrazolium bromide, a tetrazole) 3, Distilled Water, Dimethyl sulphoxide (DMSO) [3][4].

Preparation of plant extracts

Different parts of plant material ware collected from Sanjivani Ayurvedic Nursery, Bhopal during october month (2012), collected plants materials were dried under the shed for three week and then powdered. All five plants were extracted in Soxhlet Apparatus using Hydroalcoholic solvent (1:1). Phytochemical Analysis of the hydroalcolic extracts all five plants was tested for the presence of various phytoconstituents such as Carbohydrate, Starch, Protein, Amino acids, Steroids, Flavanoids, Alkaloids, Tannins, Phenolic compounds, Oxalic acid and inorganic

compounds [5].

Separations analysis

TLC

TLC analysis was performed on aluminum plates pre-coated with silica gel 60 (0.063-0.200 mm mesh) several solvents used were assessed for their ability to fractionate the chemical constituents in the starting material. The mobile phase consisted of solvents (To facilitate mobile phase migration, blotting paper (Whatman 3 mm) were allowed to soak and equilibrate for 20 to 30 minutes previous to TLC runs. Detection was carried out by UV light at 254 and 365 nm, and with iodine crystals as developer.

Column chromatography

After phytochemical analysis bioactive compounds present in extract was separated out by column chromatography in a proper solvent system. Column chromatography was performed on a classic 20 cm long Ч 2 cm diameter glass column packed with 50 g Silica gel of 60-120 mesh size as stationary phase and crude drug were further subjected to column chromatography [CC] and eluted with specific solvent to obtain pure compounds. Silica gel for column chromatography was used as stationary phase. The flow rate used was 5 ml/min. Three and four elutes for each solvent were taken.

FTIR Analysis

FT-IR analysis of the extracts for the detection of functional groups associated was done. The FT-IR spectrum of the plants extracts recorded the number of peaks. IR analysis was done by IR spectrometer using Potassium Bromide (KBr) pellets. The methodology involved mixing a small quantity of the sample along with a specially purified KBr. This powder mixture was then crushed in a pellet press in order to form a pellet through which the beam of the spectrometer could pass. This pellet was crushed under high pressures in order to ensure that the pellet was rendered translucent.

Human cancer cells

All the three **Human cancer Cell lines** were perches from NCCS Pune.

Seeding of Cells

A sub confluent monolayer culture was prepared & collected in growth medium containing serum. The cells were suspended in growth medium & counted. Then the cells were diluted to 2.5 – 5.0 x 10^3 cells / ml, depending on the growth rate of the cell line, allowing 20ml of cell suspension per microtitration plate. The cell suspension were transferred to 9 cm petridish & 200 µl of the suspension were added into each well of the 96-well plate, placing 3 x 10^3 cells into each well, with a multichannel pipette. 200 µl of growth medium were added to the wells in column 1-12. Columns 1 & 2 were used as blank. The same plate was incubated at 37^0C for 1-3 days, such that the cells were in the exponential phase of growth at the time when the drug was added [6].

Assay performed

MTT assay method

Laminar air flow was prepared. Dilutions of concentration 100 µg/ml, 10 µg/ml, 1 µg/ml, 0.1 µg/ml, 0.01 µg/ml from stock solution (test drug +DMSO) having concentration 10mg/ml was done. Then normal count on haemocytometer before seeding the cells in plate was done. 10µl from each conc. in 4 wells i.e. 20 wells for one drug was added. Plate contained 5,000-10,000 cells/ml into each well of 96-well culture plate. The cells were incubated for 96 hrs in CO2 incubator. After it cells were incubated with basal medium containing 0.5 mg/ml MTT in CO2 incubator at 37ºC for appropriate duration of time. The medium is aspirated, and the formazan product is solubilized with dimethyl sulfoxide (DMSO). Absorbance at 570 nm is measured for each well using a microplate reader on colorimeter. Analyzed data of test with standard drug and plot graph.[6][7][8][9][10]

Drug Addition

Serial dilution of the cytotoxic compound, in growth, was prepared to give different concentration and incubated at 37^0C for overnight.

Estimation of Surviving Cell Numbers

The % viability was calculated on the basis of reading taken on plate reader with radiation at 590 nm.

Calculation of Cell Viability and IC_{50}

$$\text{Cell viability (\%)} = \frac{(\text{OD value of treated well} - \text{OD value of the blank}) * 100\%}{(\text{OD value of untreated well} - \text{OD value of the blank})}$$

RESULTS

TLC and FT-IR

After phytochemical analysis bioactive compounds present in extract was separate out by TLC Rf value were identify of all selected plant which is 0.43, 0.50, 0.56, 0.63 and 0.6. (*Withania somnifera, Terminalia arjuna, Aegle marmelcos, Azadirachta indica,* and *Ocimum sanctum* respectively). And the FT-IR functional groups analyses of the samples were done, the spectrum of the samples recorded a number of peaks lying between 3300.29 cm^{-1}, 510.12 cm^{-1}

Table 1: *Final Cell growth (MTT Assay) of column Elutes / Crude*

S.No	Plants Names	IC_{50} value					
		MCF-7 (E)	MCF-7 (C)	A-549 (E)	A-549 (C)	PA-1 (E)	PA-1 (C)
1	*Terminalia arjuna*	±6 µg/ml	±10	±5	±15	±10	±20
2	*Withania somnifera*	±10	±3	±11	±20	±12	±10
3	*Ocimum sanctum*	±10	±15	±8	±10	±7	±35
4	*Azadirachta indica*	±17	±20	±5	±15	±14	±30
5	*Aegle marmeleos*	±10	±25	±16	±25	±18	±20

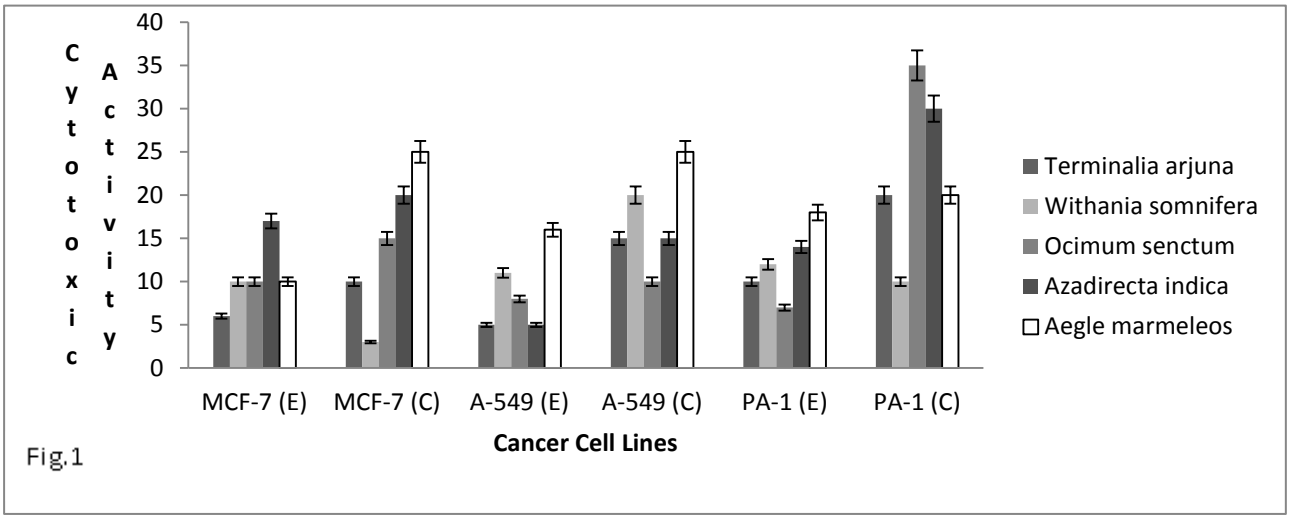

Fig.1

Figure 1: *Show comparative analysis of all the plants (crude and elute) on three cell line.fig.2 to fig. 6 explanted percentage values of crude and elute extracts.*

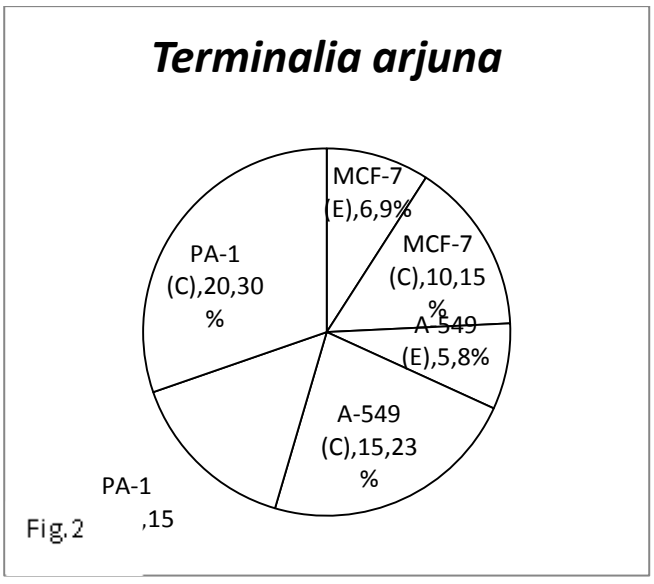

Fig.2

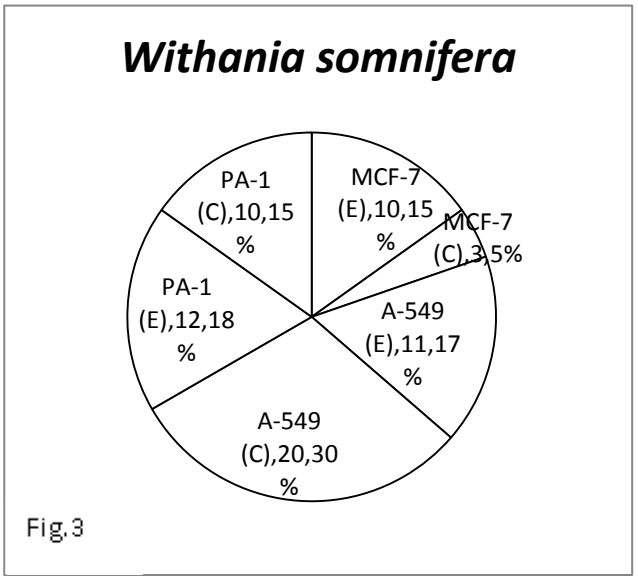

Fig.3

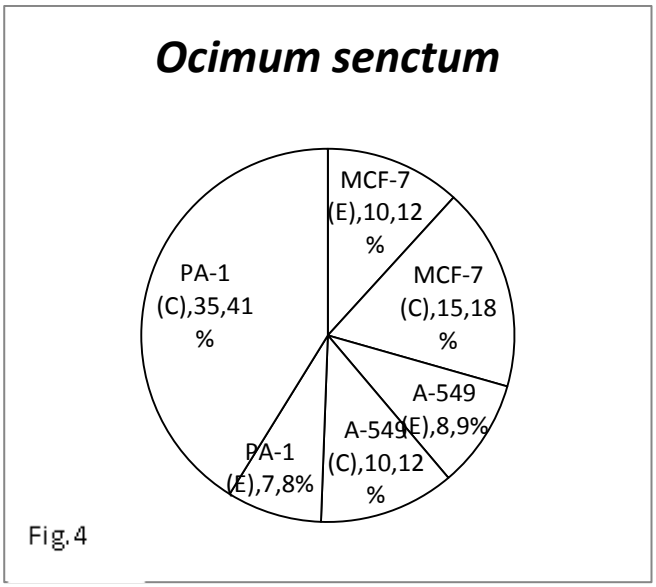

Fig.4

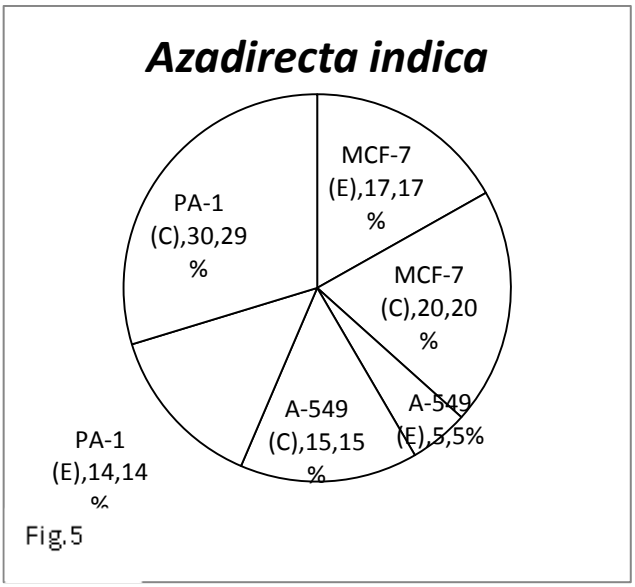

Fig.5

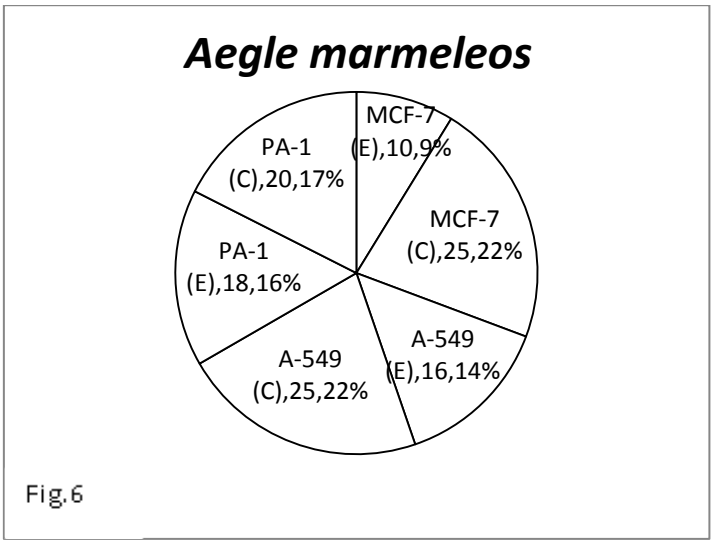

Fig.6

DISCUSSION

This research paper has brought out and highlighted some medicinal plants that have therapeutic potential, some of the plants selected for the present study. All the extracts were active against at least one or more of the cell line in a serial MTT assay for IC_{50} value calculation. The results showed that of all the 5 plants extracts tested, which included extracts are (*Withania somnifera,* IC_{50} 3 µg/ml; *Terminalia arjuna,* IC_{50} 10 µg/ml; *Ocimum sanctum,* IC_{50} 15 µg/ml; *Azadirachta indica,* IC_{50} 20 µg/ml; *Aegle marmeleos* IC_{50} 25 µg/ml), gave good IC50 value in MCF-7 cell line. Similarly five plants extracts showed cytotoxic activity in PA-1 cancer cell line with IC_{50} value of 10 µg/ml, 20 µg/ml, 20 µg/ml, 30 µg/ml, and 35 µg/ml for *Withania somnifera, Terminalia arjuna, Aegle marmeleos, Azadirachta indica,* and *Ocimum sanctum*, respectively. In the same way five plants extracts give good IC_{50} value when they were evaluated within A-549 cell line which are *Ocimum sanctum* (IC_{50} 10 µg /ml), *Terminalia arjuna* (IC_{50} 15 µg/ml), *Withania somnifera* (IC_{50} 20 µg/ml, *Aegle marmeleos* (IC_{50} 25 µg/ml), *Azadirachta indica* (IC_{50} 30 µg/ml). Percentages value were show that higher value of percentage have low potential than the value of percentage is low. The results showed that of all the 5 plants extracts tested, which included extracts are *Withania somnifera* on MCF-7 is 15% (E) and **5%** (C), *Withania somnifera* on A-549 is 17% (E) and 30% (C), *Withania somnifera* on PA-1 is 18% (E) and15% (C), *Azadirachta indica* on MCF-7 is 17% (E) and 20% (C), *Azadirachta indica* on A-549 is 5% (E) and 15% (C), *Azadirachta indica* on PA-1 is 14% (E) and 29% (C), *Aegle marmeleos* on MCF-7 is 9% (E) and 22% (C), *Aegle marmeleos* on A-549 is 14% (E) and 22% (C), *Aegle marmeleos* on PA-1 is 16% (E) and17% (C) ; *Terminalia arjuna* on MCF-7 is 9% (E) and 15% (C), ; *Terminalia arjuna* on A-549 is 8% (E) and 23% (C), ; *Terminalia arjuna* on PA-1 is 15% (E) and 30% (C) ; *Ocimum sanctum* on MCF-7 is 12% (E) and 18% (C), ; *Ocimum sanctum* on A-549 is 9% (E) and 12% (C), ; *Ocimum sanctum* on PA-1 is 8% (E) and 41% (C). The FT-

IR and UV- visible spectroscopy analysis of the five hydro-ethanolic extracts suggested the presence of Flavonoids.

CONCLUSION

The present research supports the potential of Indian medicinal plants. Although a large number of man-made drugs are being added to the world of modern pharmacopoeia, but still no system of medicine in the world has been able to solve all the health problems, which include diseases like Cancer. Therefore the search for new therapeutic constituents from plants is genuine and urgent. In India, there is an ocean of knowledge about medicinal plants and rich medicinal flora, but still only a few pearls have been searched as therapeutic agents. Now researches require to work in the field of herbal nano drug formulation or to formulate herbal nano chip expertise related for the same.

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