

Research Article



Anticancer Activity of *Allium sativum* (Bulb) Polyphenolic Compound

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ABSTRACT

The aim of the present research is to determine the use of *Allium Sativum* (Bulb) Polyphenolic Compound activity on MCF-7, A549 and PA-1 cancer cell line (breast, lung and ovary cancer respectively). Hydro alcoholic (1:1) sample of *Allium Sativum* (Bulb) were prepared and tested for their cytotoxic activities against cancer cell lines (MCF-7, A-549 and PA-1) with standard Doxorubicin. The most essential reason of this study is to estimate cytotoxicity of certain important Indian medicinal plants with facilitate of MTT assay. Concentrations are set of each plant extract which are 100 µg/ml, 10 µg/ml, 0.1 µg/ml, 0.01 µg/ml and 5-10×10³ cells/ml are taken into each well which are exposed to different Concentrations of *Allium Sativum* (Bulb) for 96 hr and then treated with MTT. For MTT absorbance in use at 570 nm. From IC₅₀ values of MTT assay of *Allium Sativum* (Bulb) for MCF7, A549 and PA1 cancer cell lines, from this it may conclude that *Allium Sativum* (Bulb) shows efficient cytotoxicity on MCF-7 (6 ± 1 µg) than PA-1(15 ± 1 µg) and A459 (28 ± 1 µg) cancer cell line.

Keywords: *Allium Sativum*, Anticancer activity, Soxhlet extraction.

INTRODUCTION

Allium sativum L. (GARLIC)

Scientific classification

Kingdom: Plantae; clade: Angiosperms; clade: Monocots; Order: Asparagales; Family: Amaryllidaceae; Subfamily: Alliioideae; Genus: *Allium*; Species: *A. sativum* L.

Description

Allium sativum is a bulb. It grows up to 0.5 m (2ft) in height. Its hardiness is USDA Zone. It produces hermaphrodite flowers. Pollination occurs by insects and bees.

Biological and Medicinal Property

The effects of garlic on health with its possible preventive effects on the development of cancer in humans have been mentioned in previous reviews. In addition *A. sativum* has free radical scavenging activity, immune system modulation and direct cytotoxic effect on cancer cells.¹ Now a day's numerous epidemiological, clinical and laboratory studies have demonstrated the role of garlic in cancer prevention²⁻³ especially in relation to digestive tract cancers, including esophageal and stomach cancers.⁴ There is also promising research evaluating the use of garlic in leukemic melanoma⁵ and neuroblastoma cell lines.⁶

MATERIALS AND METHODS

Requirements

Alcohol 70%, 100% Alcohol, MEM media (Minimal Essential Media) (Eagle H 1959), Trypsin⁷, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a

tetrazole)⁸, Distilled Water, Dimethyl sulphoxide (DMSO)⁹, etc.

Laminar air flow, Autoclave, N₂ liquid container, CO₂ incubator, Inverted microscope, Filtration assembly, Hemocytometer, Centrifuge machine, Micropipette, Soxhlet, Spectrophotometer.

Plant Material Collection

Allium Sativum (Bulb) of plants were collected from Bhopal during month of October. Then dried up under the shed dry for six week furthermore crush it.

Soxhlet Extraction: Hydroalcoholic (1:1)

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. *Allium Sativum* (Bulb) were extracted in Soxhlet Apparatus using Hydroalcoholic solvent (1:1).

Phytochemical Analysis

The hydroalcoholic extract of *Allium Sativum* (Bulb) was tested for the presence of various phytoconstituents such as Carbohydrate, Starch, Protein, Aminoacids, Steroids, Flavonoids, Alkaloids, Tannins, Phenolic Compounds, oxalic acid and inorganic compounds. All phytochemical tests were done as per the procedure given in the standard book (Practical Pharmacognosy by C.K. Kokate). The FT-IR analysis of the *Allium Sativum* (Bulb) extract was done and the functional groups associated were determined.

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 \times 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.

Spectrophotometric Determination of Total Flavonoid Content (TFC)

Total flavonoid contents were measured by Aluminum chloride colorimetric assay. Hydroalcoholic extracts that has been adjusted to come under the linearity range and different dilution of standard solution of Quercetin (10-100 μ g/ml) were added to 3ml of water. To the above mixture, 0.1ml of 5% $C_4H_4O_6KNa_4 \cdot H_2O$ (Potassium Sodium L-(+) - Tartrate Tetrahydrate) was added. After 5 minutes, 0.1ml of 10% $AlCl_3$ was added and the total volume was made up to 3 ml with distilled water. It was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 430nm with a single beam spectrophotometer (Systronic).¹⁰⁻¹¹

Isolation of Human Cancer Cells

Human cancer cells are isolated from the patients and characterized at cellular and molecular levels. Isolated cells are cultivated in specialized mediums and specialized incubators to provide them physiological conditions required for the growth.¹²⁻¹³

Cell Line

The sub culturing of the primary culture gives rise to cell lines. The term continuous cell line implies the indefinite development of the cell in the successive sub culturing. On the other hand, finite cell lines symbolize the death of cell after several subcultures. The considered cell lines are MCF-7¹⁴⁻¹⁵ (breast cancer), A-549¹⁶ (lung cancer) and PA-1¹⁷ (ovary cancer).

Assay Performed

MTT Assay Method

Laminar air flow was prepared. Dilutions of concentration 100 μ g/ml, 10 μ g/ml, 1 μ g/ml, 0.1 μ g/ml, 0.01 μ g/ml from stock solution (test drug +DMSO) having concentration 10mg/ml is done. Then normal count on haemocytometer before seeding the cells in plate was done. 10 μ l from each conc. in 4wells i.e. 20 wells for one drug was added. Plate contained $5-10 \times 10^3$ cells/ml into each well of 96-well culture plate. The cells were incubated for 96 hr in CO_2 incubator. After it cells are incubated with basal medium containing 0.5 mg/ml MTT in CO_2 incubator at 37°C 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. Analyze data of test with standard drug and plot graph.^{14, 18}

RESULTS AND DISCUSSION

Phytochemical Evaluation: The results of preliminary phytochemical evaluation are summarized in Table 1.

Table 1: The results of preliminary phytochemical evaluation

Natural product	Test performed	Result
Carbohydrate	Molish test	+Ve
Starch	Iodine	-Ve
Protein	Millions	+Ve
Amino acid	Cysteine test	+Ve
Steroid	Salkowski test	+Ve
Flavonoids		+Ve
Alkaloid	Mayer's test	+Ve
Tannic and phenolic compound	%5 $FeCl_3$ test	+Ve
Oxalic acid		+Ve
Inorganic acid	Sulphate test	+Ve

Column chromatography

Column chromatography of *Allium Sativum* (Bulb) was performed on a classic 20 cm long \times 2 cm diameter glass column packed with 50 g Silica gel of 60-120 mesh size as stationary phase and *Allium Sativum* (Bulb) crude drug were further subjected to column chromatography [CC] and eluted with specific solvent chloroform methanol (1:2) to obtain pure compounds.

FT-IR Spectral Analysis

The FT-IR spectrum of the *Allium Sativum* (Bulb) leaves extract recorded the number of peaks lying between 3330.14 cm^{-1} , 2943.57 cm^{-1} , 2831.65 cm^{-1} , 17.64 cm^{-1} , 1453.30 cm^{-1} , 1453.30 cm^{-1} , 1239.30 cm^{-1} , 1111.50 cm^{-1} , 1021.76 cm^{-1} , 751.81 cm^{-1} , and 662.91 cm^{-1} respectively. This finding will help in further research in the investigation of medicinally active chemical compounds present in *Allium Sativum* (Bulb).

Quantification

Current study revealed the flavonoid contents of *Allium Sativum* (Bulb). (Quercetin standard plot: $y = 0.0966x$, $R^2 = 0.9878$).¹⁹ On the basis that calibration curve was plotted by preparing the Quercetin solutions at concentrations 12.5 mg/ml-1. Total flavonoid content of the extracts was expressed as percentage of flavonoid in plant extract 4.35 μ g./ml equivalents per dry weight of sample and take notice of $y = 0.002x + 0.0148$, $R^2 = 0.993$.

Cell Line	MCF-7	A-549	PA1
Sample Code	AS	AS	AS
IC ₅₀ (mg/ml)	6 \pm 1	20 \pm 1	11 \pm 1



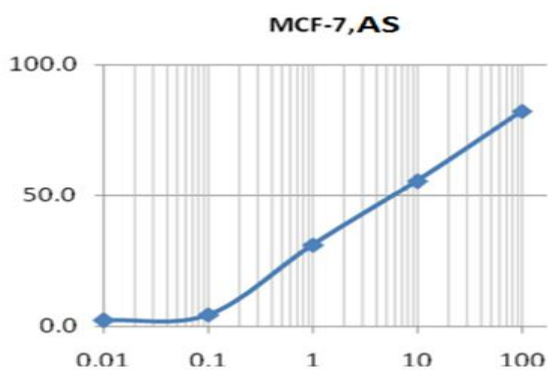
MTT Assay Result

Figure 1: MCF7 Cells were treated with hydro alcoholic extract of *Allium Sativum* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 conc. Cells were subjected to MTT within 1 hr-24 hr. Response of MCF7 Cell to *Allium Sativum* For % Inhibition on Y-axis and Concentration on X-axis.

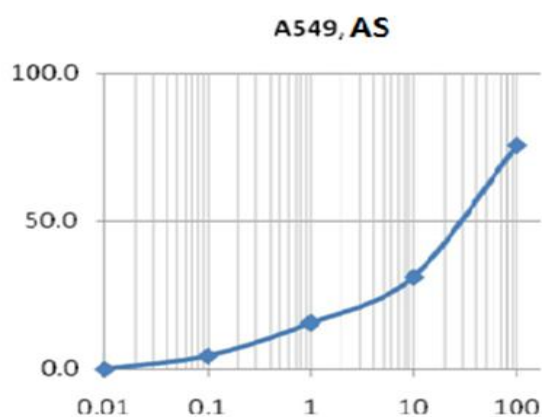


Figure 2: A549 Cells were treated with hydro alcoholic extract of *Allium Sativum* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 conc. Cells were subjected to MTT within 1 hr-24 hr. Response of A549 Cell *Allium Sativum* For % Inhibition on Y-axis and Concentration on X-axis.

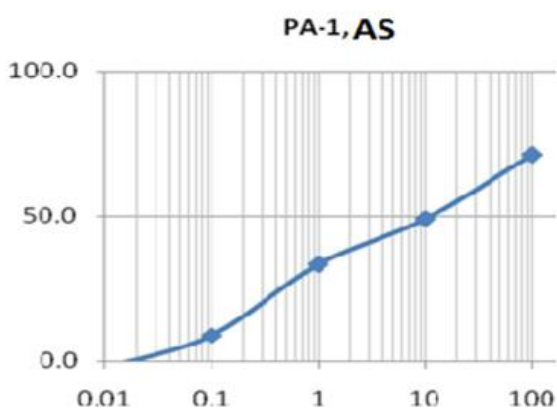


Figure 3: PA1 Cells were treated with hydro alcoholic extract of *Allium Sativum* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 conc. Cells were subjected to MTT within 1 hr-24 hr. Response of PA1 Cell to *Allium Sativum* For % Inhibition on Y-axis and Concentration on X-axis.

DISCUSSION

Allium Sativum (Bulb) extracts was investigated. The *invitro* cytotoxic potentiality was investigated as the ability of *Allium Sativum* (Bulb) extracts to inhibit tumour cell line growth. With this investigation we had also focused on angiogenesis. The studied cell lines are MCF7, A549 and PA1. After exposure of cells to *Allium Sativum* (Bulb) extract cell line were treated with MTT Dye which results into the live cells convert the MTT to purple colour formazan crystals, which are soluble in Dimethyl sulphoxide (DMSO). After solubilization of crystals then absorption is taken on spectrophotometer at 570 nm. With respect to readings the graphs were plotted for % inhibition on Y-axis and Conc. of drug on X-axis. The readings were directly converted into percentage. from this it may conclude that *Allium Sativum* (Bulb) shows efficient cytotoxicity on MCF-7 ($6 \pm 1 \mu\text{g}$) than PA-1 ($15 \pm 1 \mu\text{g}$) and A459 ($28 \pm 1 \mu\text{g}$) cancer cell line where Standard drug was used for IC50 of Doxorubicin MCF-7 500nm, A549- 550nm, PA-1- 580nm.

CONCLUSION

In the present time herbal products are considered to be symbols of protection in comparison to the synthetic product that are regarded as unsafe to human life and environment. Although herbs had been priced for their medicinal importance. But now everyday phytochemical and pharmacological studies are conducted on different parts of plants. More research can be done to investigate the unknown and unexplored potential of these plants. Further analysis of these plants (active compounds) can be carried out by way of making use of different analytical methods such as HPTLC, HPLC, NMR and UV spectrophotometer analysis.

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