

Cytotoxic Activities of Ethanolic Extract of *Allium Sativum* against Colon Cancer Cell Lines

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ABSTRACT: *Allium Sativum* is a perennial plant with narrow flat leaves and several small bulbs, known as clove, enclosed in a white skin. *Allium sativum* belongs to the family *Amaryllidaceae*. The objective of the study was to determine the anticancer properties of ethanolic extract of *allium sativum* against human cancer cell lines. The cytotoxic activities of the ethanolic extract of bulb of *Allium sativum* determined by SRB assay. A comparative study was done with two other Cell Line namely Central Nervous System Cell Line and Colon Cancer Cell Line. The cytotoxic activity shown by the ethanolic extract of *Allium sativum* against Human Cancer Cell Lines SF-295, Colon 502713, and Colo-205, were 0 %, 54 %, 6 %, respectively. The maximum cytotoxic activity shown against Human Cancer Cell Lines Colon 502713 was 54 %.

KEYWORDS: *Allium Sativum*, Colon Cancer Cell Lines, SRB assay

I. INTRODUCTION

Colon or colorectal cancer refers to malignant neoplasia of the large intestine. It comes second in the group of tumor related deaths [1] and ranks third in frequency in men and second in woman. In UK and U.S.A its incidence and mortality rates are second only to those of lung cancer and its peak onset is at the age of 65. Less than one third of these cancers occur in developing countries [2].

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of these indigenous medicinal plants are used as spices and food plants.

Plants constitute one of the major raw materials for drugs for treating various ailments of human being, although, there has been significant development in the field of synthetic drug chemistry and antibiotics, but during the last two decades considerable changes have taken place in the medicinal system all over the world. Because of the general awareness of the wide spread toxicity and harmful after effects associated with the long use of synthetic drugs and antibiotics, the Western Society prefers the drugs from natural sources. Taxol is the latest addition of anticancer drugs which was first isolated in 1971 from a crude extract of *Taxus brevifolia*. Taxol as a single chemotherapeutic agent has been proven effective against a variety of tumors including breast, bladder, esophageal, head and neck, ovarian and lung carcinomas. Important drugs introduced in this way include digoxin, digoxin, colchicines, atropine, hyoscine, reserpine, tubocurarine, codeine, podophyllotoxin, artemisinin, plumbagin and many more.

Allium sativum belongs to *Amaryllidaceae* family, commonly known as Garlic, Stinking Rose. It is claimed that garlic is effective against a broad spectrum of diseases. During the past 20 years, studies on garlic have been conducted primarily in the field of cardiovascular and cancer research. It has been suggested that garlic decreases cholesterol and triglyceride levels and may reduce the atherosclerosis process. Garlic is rich with many active compounds which have various distinctive functions such as antibacterial, antibiotic, anticoagulant, antihistamine, antiparasitic, antifungal,

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antiprotozoan and antiviral properties, expectorant, diaphoretic, alterative, Antidiabetic, diuretic, stimulant and antispasmodic. Present study was to determine anticancer study of ethanolic extract *Allium sativum* by SRB assay.

II. MATERIALS AND METHODOLOGY

2.1 *Allium sativum* Ethanolic Extract: The *Allium sativum* was ground (500 g) and was placed in a glass percolator of appropriate size. Sufficient quantity of solvent was added to submerge the plant material. After standing for about 16 hours percolate was collected and filtered if required. The process was repeated four times for exhaustive extraction of the plant material. The ethanolic extract was evaporated to dryness under reduce pressure at 50°C using rotavapor and round bottom flask. The final drying was done in vacuum desiccators. The extract was transferred to glass container of appropriate size. This form the stock extract.

2.2 Source of Human cancer cell line: Human cancer cell line were obtained from National Centre for cell science, Pune- 411007 (India) and National Cancer Institute, DTCD, Fredrick Cancer Research and Development Centre, Fairview centre, Suite 205, 1003, West -7th Street Frederick MD – 21701- 8527 (USA).

2.3 Selection of Human cancer cell line: The cell line were selected in such a way that almost all the cell line grow on a single growth medium (RPMI-1640) in tissue culture flask (TCP) and the mass doubling time was such that enough cell were obtained for screening. Cell which were used were free from bacteria, yeast, mould, mycoplasma and in special cases from viruses at all the stages. If contamination appeared at any stage, the stock in which it occurred was discarded immediately. Cancer of central nervous system CNS, Lung cancer cell line A-549, Colon cancer cell line, Colo- 205, Colon 502713, Liver cancer cell line, Hep-2, Ovarian cancer cell line, OVCAR-5, Prostrate cancer cell line PC-5.

2.4. Procedure for *in vitro* cytotoxicity of extract: Cytotoxicity of test sample was performed against seven human cancer cell lines. 96 well flat bottom tissue culture plates were taken. There were four types of well in TCP, control blank (CB, without cells, complete growth medium only) and control growth (GC, with cell in absence of test material) to determine 100 % growth. The growth in the presence of test material was determined from the difference of test growth (GT, cell with test material) and test control (CT, test material without cells). The desired human cancer cell lines were grown in tissue culture flask at 37°C in an atmosphere of 5 % CO₂ and 90 % relative humidity in complete growth medium to obtain enough number of cells. The cells were harvested by the treatment of trypsin – EDTA and complete growth medium added. Viable cells were counted in haemocytometer by using trypan blue.

Viable cell density was adjusted 5000- 40,000 cells / 100 µl depending upon the cell line. Cell suspension 100 µl was added. Complete growth medium was added and incubated at 37°C for 24 hours in an atmosphere of 5 % CO₂ and 90 % relative humidity in a CO₂ incubator. After 24 hours test material was added. Plates were incubated at 37°C for 48 hours in an atmosphere of 5 % CO₂ and 90 % relative humidity in a CO₂ incubator. The growth was determined after 48 hours by SRB assay.

2.5. SRB assay: SRB assay was carried out as described by *Skehan et al* using SRB dye [3]. After 48 hours incubation of cells with test material, the plates were taken out and 50 µl of chilled 50 % TCA was gently layered on top of the medium in all the wells to produce a final concentration of 10 %. After that Tissue culture plate were incubated at 4°C in a refrigerator to fix the cells attached to the bottom of the wells. After one hour the plates were taken out from refrigerator and all the contents of all the wells were pipetted out and supernatant was discarded.

The plates were washed five times with distilled water to remove Trichloroacetic acid, growth medium, low molecular metabolites, serum protein etc. For washing, the wells of Tissue culture plates were filled with distilled water and the liquid in the wells was discarded by sharply flicking plate over sink. Plates were air dried and can be stored until use. SRB solution (100 µl) was added to each well of the plates and the plates were incubated for 30 minutes at room temperature. The unbound SRB was removed quickly (to avoid desorption of protein bound dye) by washing the wells

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of the plates five times with 1 % acetic acid. Plates were than air dried. After that Tris buffer (100 μ l /well) was added in the plates. The plates were gently stirred for 5 minutes on a mechanical shaker and optical. Density was recorded on ELISA reader at 540 nm. .

III. RESULTS AND DISCUSSION

In vitro cytotoxicity of *Allium sativum* (ethanolic extract) against human cancer cell lines

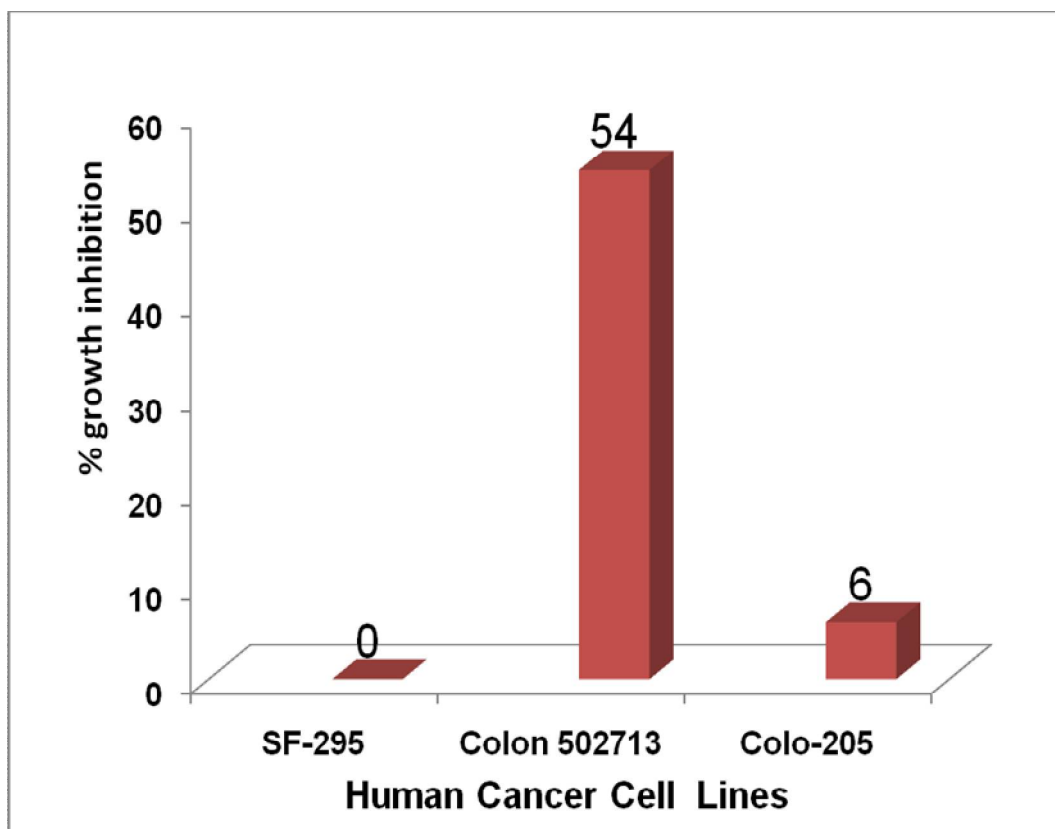


Fig 1: The Cytotoxic activity shown by the ethanolic extract of *Allium sativum* against Human cancer cell lines SF-295, Colon 502713, Colo-205 were 0 %, 54 % and 6 %. The maximum activity was shown by colon cancer cell line Colon 502713 as compared to Central Nervous System cancer cell line and Colon cancer line Colo-205.

3.1Result:

The main objective of this study was to evaluate the anti- cancer activity of some medicinal plants that has been used as spices and traditional medicines for management of cancer, by carrying out *in vitro* studies. *Allium sativum* were screened by using three human cancer cell lines by established in vitro cytotoxicity assays. *In vitro* cytotoxicity against human cancer cell lines was determined by SRB assay. The cells were allowed to grow for 24 hours 37°C in an atmosphere of 5 % CO₂ and 90 % relative humidity in a carbon di oxide incubator on 96- well flat bottom. Cells were further allowed to grow in the presence of test material for 48 h. Cell growth was terminated by addition of 50 % (w/v)

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TCA. Cells were stained with SRB dye, which binds to basic to basic amino acid residues in TCA fixed cells. Excess dye was removed by washing with 1 % (w/v) acetic acid and bound dye was dissolved in Tris buffer and read on ELISA reader. The Cytotoxic activity shown by the ethanolic extract of *Allium sativum* against Human cancer cell lines SF-295, Colon 502713, Colo-205 were 0 %, 54 % and 6 %. The maximum activity was shown by colon cancer cell line Colon 502713 as compared to Central Nervous System cancer cell line and Colon cancer line Colo-205.

3.2 Discussion:

The Cytotoxic activity shown by the ethanolic extract of *Allium sativum* against Human cancer cell lines SF-295, Colon 502713, Colo-205 were 0 %, 54 % and 6 %. The maximum activity was shown by colon cancer cell line Colon 502713 as compared to Central Nervous System cancer cell line and Colon cancer line Colo-205. It is claimed that garlic is effective against a broad spectrum of diseases. During the past 20 years, studies on garlic have been conducted primarily in the field of cardiovascular and cancer research. Aged garlic extracts have an immunomodulatory effect [4] and upon administration intravesically, it was found effective against murine transitional cell carcinoma, similar to that of BCG. Both preparations induced lymphocyte and macrophage infiltration and cytokine release. Much of the early work on garlic and cancer focused on its ability to suppress chemically induced tumors [5][6][7]. These studies revealed that consumption of garlic or associated compounds by experimental animals reduced chemically induced esophageal, skin, pulmonary, fore stomach, colon, mammary and lung tumors [8][9]. This widespread depression in chemically induced cancers suggests that compounds in garlic likely modify common metabolic events that are not tissue specific.

Cancer is a disease of complex etiology that is classically defined as uncontrolled cellular division. Dietary habits have been reported to significantly modify each of these phases. One of the dietary components reported to modify the cancer process is garlic (*Allium sativum*). Evidence for its anticancer protection comes from both epidemiologic and laboratory investigations [5][10]. Collectively, these studies provide evidence that increased consumption of garlic is accompanied by a marked reduction in cancer risk and tumor behaviour. Garlic constituents were shown to exhibit antitumor and cytotoxic action, in laboratory animals [11][12].

IV. CONCLUSION

The Cytotoxic activity shown by the ethanolic extract of *Allium sativum* against Colon cancer cell line Colon 502713 was 54% at low concentration of 100 µg /ml suggest that this compound can be considered as anticancer agent. more work is required to make natural anticancer drug which has less side effect. This can be consider as preventive agent for cancer. Dietary inclusion of *Allium sativum* can reduce the risk of cancer. More work is required to make natural anticancer drug from this plant extract which has no side effects.

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