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The Bioactive Compounds obtained from the Tomatoes (*Solanum lycopersicum* L.) act as Potential Anticancer agents against the Human Cervicle Adenocarcinoma

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Abstract

Tomato (Solanum lycopersicum L.) is one of the most important vegetables worldwide because of its high consumption, year round availability and large content of health related bioactive compounds. Tomatoes contain a variety of bioactive compounds such as lycopene, vitamin-C, quercetin, naringenin, chalcone and chlorogenic acid and others health protectives like amino acids, phytosterols, amines and carbohydrates etc. The extract was prepared by reflux condensation method. The objectives of the present work are to search anticancer activity. Based on this,a new series of constituents have been planned to extract by Methanol (E1), Ethanol (E2), Acetone (E3), and chloroform (E4) a from ripen tomatoes. The *in-vitro* anticancer studies were performed against human cancer cell line (HeLa) and MTT assay was used to analyze the cell growth inhibition and doxorubicin and dactinomycin were used as a standard antineoplastic agents. The results showed that the various extracts of tomato possessed a very good to moderate anticancer activity and the **IC50 values of E1, E2, E3, and E4 extracts were found to be 63.3, 68.2, 57.5 and 62.1.**

Keywords: *Solanum lycopersicum L.*, Bioactive compounds, Lycopene, Anticancer, HeLa, MTT assay, IC50 etc.

Contents

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1. Introduction

Tomato (Solanum lycopersicum L.) is one of the most important vegetables worldwide because of its high consumption, year round availability and large content of health related components [1]. Mounting evidence over the past decade suggests that the consumption of fresh and processed tomato products is associated with reduced risk of prostate cancer. The emerging hypothesis is that lycopene, the primary red carotenoid in tomatoes, may be the principle phytochemical responsible for the reduction in risk [2]. In addition tomato consumption leads to decreased serum lipids levels and low density lipoprotein oxidation [3].Tomatoes contain a variety of phytochemicals

including carotenoids like lycopene (the highest concentration:85%), phytoene, phytofluene and provitamin A, β-carotenoid, polyphenols including quercetin, kaempferol, naringenin, neutrients like vit-C, vit-E, vit-K, vit-B, P, S, K^+, Ca^{++} (significant quantities), sugars, like aldoses, ketoses, disaccharides, polysaccharides mainly starch proteins and amino acids, enzyme-polyphenoloxidase, phytosterols like cholesterol, sitosterol and small quantities of fats. All of these are known to contribute significantly to the antioxidant activity of tomato fruit [4,5].

The literature survey revealed that the tomatoes extracts were possessed a wide range of pharmacological activities: Anticancer, Antidiabetic Antiobesity ,Antidiarrhoeal ,Antioxident Antibacterial Antihypertensive, Antiasthamatic, Antitubercular, Immunomodulatory, Antihypercholesterolemic Wounds healing ,Renal calculus inhibitors ,Antisylorotic, Antiatherogenic, Antiviral. The number of infections which are caused by multi drug resistant gram positive and gram negative pathogens and viruses are life threatening for human being. Infections caused by these organisms pose a serious challenge to the scientific community and need for a effective therapy has lead for novel antimicrobial and anticancer agents. The objectives of the present work are to search anticancer activity against human cervical adenocarcinoma. Based on this, a new series of constituents have been planned to extract by methanol (E1), ethanol (E2), acetone (E3) and chloroform (E4) from tomato fruits.

2. Materials and Method

General laboratory techniques recommended by Purvis *et al* (1966) was followed for the preparation of media, inoculation and maintenance of cultures.

Chemicals and drugs:

The all chemicals used for the extraction and phytochemical screening were of LR and AR grade. Cell culture: The human cervical adenocarcinoma cell line (HeLa) was provided by National Centre for Cell Science (NCCS), Pune and was grown in Eagles Minimum Essential Medium (EMEM) which contained 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 100% relative humidity, 5% CO2, 95% air and the culture medium was changed twice a week. The standard drugs doxorubicin and dactinomycin were purchased from Local. Whole sale Pharmacy shop and other chemicals and solvents were used from Institutional store and were of AR grade.

Apparatus and chemicals required:

Round bottom flask, water condenser, heating mantle, motor and pestle, methanol , dichloromethane, sodium chloride solution, magnesium sulfate, ethanol , acetone , chloroform.

Extraction:

Weigh 20 g of red tomato paste (ripe tomatoes can be mashed to prepare apaste) into a250 ml round-bottomed flask. Add 50 ml of methanol and 60 ml of dichloromethane. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separating funnel. Wash this mixture containing lycopene with three portions of 150 ml each with sodium chloride solution. Dry the organic larger over anhydrous magnesium sulfate. Filter and evoprate most of the solvent in vacuume without heating [6]. Same procedure have been followed for the preparation of extracts E2, E3 and E4.

Preliminary Phytochemical screening [7, 8, 9, 10, 11, 12, 13, 14] :

Preliminary Phytochemical tests of various extracts of Tomato have shown the presence of following bioactive compounds: E1(+), E2(+), E3(+), E4(-)-reducing sugar, E1(-), E2(-), E3(-), E4(-)-pentoses, E1(+), E2(+), E3(+), E4(-)- ketohexoses, E1(+), E2(+), E3(+), E4(-)- disaccharides, E1(+), E2(+), E3(+), E4(-)- aromatic aminoacids, E1(+), E2(+), E3(-), E4(-)-tyrosin, E1(+), E2(+), E3(+), E4(-)-tryptophan, E1(+), E2(+), E3(+), E4(-)-Arginine, E1(+), E2(+), E3(-), E4(-)-Alpha amino acids and dipeptides, E1(+), E2(+), E3(+), E4(+)- polyphenols, E1 (-), E2(-), E3+), E4(+)- phytosterols, E1 (+), E2(+), E3(+), E4(-)-carotenoids, E1(+), E2(+), E3(+), E4(-)- flavanoids and E1 (+), E2(+), E3(+), E4(-)-long chain fatty acids - palmitic, stearic, arachidic, hexadecenoic, oleic, linoleic acid etc [**(+) = Presence, (-) = Absence].**

In-Vitro Evaluation of Anticancer Activity by MTT Assay [15,16,17]:

Cell culture:

The human cervical adenocarcinoma cell line (HeLa) was provided by National Centre for Cell Science (NCCS), Pune and was grown in Eagles Minimum Essential Medium (EMEM) which contained 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 100% relative humidity, 5% CO2, 95% air and the culture medium was changed twice a week.

Cell Treatment: The monolayer cells were detached and single cell suspensions were made using trypsin-ethylenediaminetetraacetic acid (EDTA). A hemocytometer was used to count the viable cells and the cell suspension was diluted with a medium containing 5% FBS in order to obtain final density of $1x10^5$ cells/ml. 96-well plates at plating density of 10,000 cells/well were seeded with one hundred microlitres per well of cell suspension and incubated for cell attachment at 37° C, 5% CO2, 95% air and 100% relative humidity. The cells were treated with serial concentrations of the test samples after 24 hr. Serial dilution method was used for preparing test samples of different concentrations. Cells were initially dissolved in dimethylsulfoxide (DMSO) and further diluted with

serum free medium to obtain twice the desired final maximum test concentration. The required final drug concentrations of 1.25, 2.5, 5 and 10 µg/ml were obtained by adding aliquots of 100 µl of the different drug dilutions to the appropriate wells already containing 100 µl of medium. After addition of the drug the plates were incubated for an additional 48 hr at 37° C, 5% CO2, 95% air and 100% relative humidity. The medium without samples served as control and triplicate was maintained for all concentrations.

MTT assay:

After 48h of incubation, to each well 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added and incubated at 37° C for 4h. The medium with MTT was flicked off and the formed formazan crystals were solubilized in 100µl of DMSO. Using micro plate reader the absorbance was measured at 570 nm. The % cell inhibition was determined using the following formula.

% Cell Inhibition = [100- Abs (sample)/Abs (control)] x100

Table 1: For Percentage (%) of Cell Growth Inhibition of Methanolic Extract (E1) of Tomato *(Solanum lycopersicum L.)* on Hela Cells by MTT Assay

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	10 µg/ml	1.520	70.9
2	5 µg/ml	1.511	80.3
3	2.5 µg/ml	1.502	89.9
4	1.25 µg/ml	1.506	87.5
5	Control	1.88	0

Table 2: For Percentage (%) of Cell Growth Inhibition of Ethanolic Extract (E2) of Tomato *(Solanum lycopersicum L.)* on Hela Cells by MTT Assay

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	10 µg/ml	1.411	64.61
2	5 µg/ml	1.466	61.56
3	2.5 µg/ml	1.499	59.94
4	1.25 µg/ml	1.501	58.04
5	Control	1.88	0

Table 3: For Percentage (%) of Cell Growth Inhibition of Acetone Extract (E3) of Tomato *(Solanum lycopersicum L.)* on Hela Cells by MTT Assay

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	10 µg/ml	1.602	55.62
2	5 µg/ml	1.745	51.56
3	2.5 µg/ml	1.77	49.94
4	1.25 µg/ml	1.811	45.04
5	Control	1.88	0

Table 4: For Percentage (%) of Cell Growth Inhibition of Cloroform Extract (E4) of Tomato *(Solanum lycopersicum L.)* on Hela Cells by MTT Assay

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	10 µg/ml	1.488	60.1
2	5 µg/ml	1.591	56.56
3	2.5 µg/ml	1.745	51.94
4	1.25 µg/ml	1.751	49.04
5	Control	1.88	0

Table 5: For Percentage (%) of Cell Growth Inhibition of Standard Drugs on Hela Cells by MTT Assay

Serial no.	Concentration of Doxorubicin.	Absorbance of Doxorubicin	Inhibition of cell growth (%)
1	10 µg/ml	1.201	94.5
2	5 µg/ml	1.302	88.8
3	2.5 µg/ml	1.399	84.9
4	1.25 µg/ml	1.401	79.9
5	Control	1.91	0

Table 6: For Percentage (%) of Cell Growth Inhibition of Standard Drugs on Hela Cells by MTT Assay

Serial no.	Concentration of Dactinomycin.	Absorbance of Dactinomycin.	Inhibition of cell growth (%)
1	10 µg/ml	1.101	97.2
2	5 µg/ml	1.21	90.4
3	2.5 µg/ml	1.301	89.1
4	1.25 µg/ml	1.309	85.5
5	Control	1.91	0

Table 7: For IC_{50} Values of Various Extracts of Tomato and Standard Drugs

Name of the Extracts and standard drugs	**IC_{50} values**
E1	63.3
E2	68.2
E3	57.5
E4	62.1
Doxorubicin, Dactinomycin	96.2, 99.1

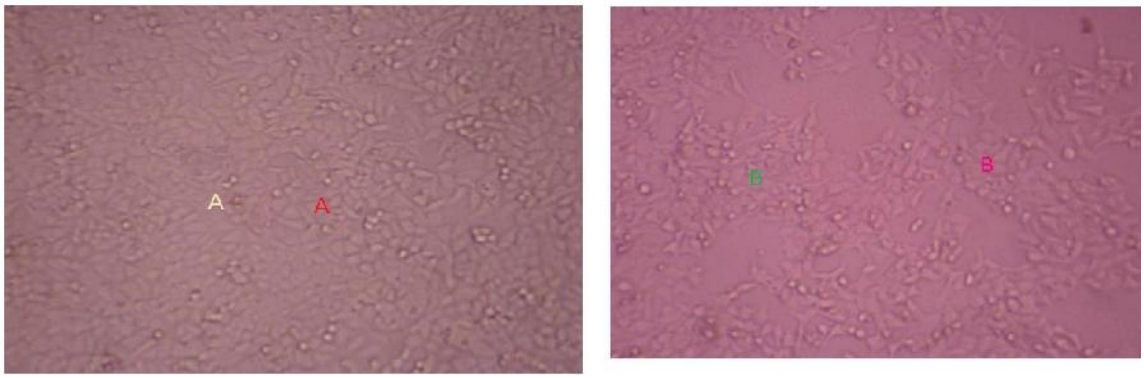

Figure 1: Anticancer activity of various extracts of Tomato *(Solanum lycopersicum L.)* against Hela cells; (A) control cells, (B) 10µg extract treated cells

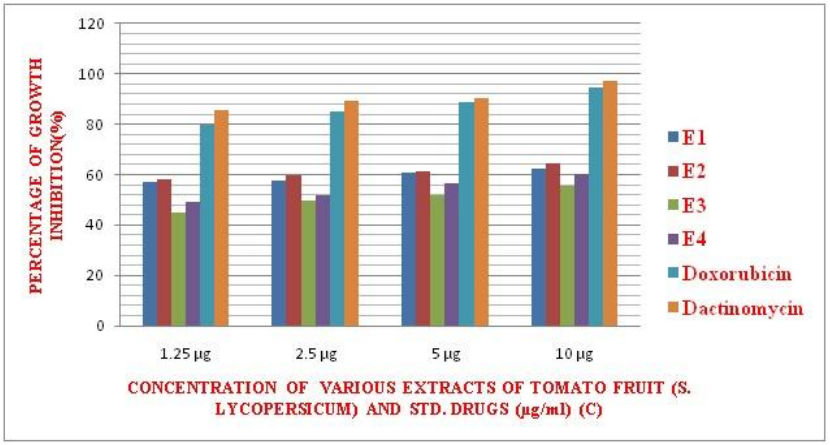

Figure 2: Anticancer activity of various extracts of Tomato *(Solanum lycopersicum L.)* against Hela cells; (C) Concentration Vs % growth inhibition.

IC50:

IC_{50} is the acronym for "half maximal inhibitory concentration". IC_{50} value indicates the concentration needed to inhibit a biological or biochemical function by half (e.g. inhibition of enzymes, affinity to cell receptors). In pharmaceutical research, it is a frequently used unit to specify the in vitro potency of a drug or a NCE. Amongst others, determination of IC_{50} is commonly calculated via linear interpolation: The activity of an enzyme is determined after exposure to a series of inhibitor concentrations.

3. Results and Discussion

The results for cell growth inhibition by various extracts of Tomato against Hela cell lines for various concentrations is shown in table 1, 2, 3 and 4. As the concentration increases there is an increase in the cell growth inhibition and it was found that the extract E2 with the highest 64.61 % , E1 with 62.61% , E4 with 60.1% and E3 with 55.62% growth inhibition at 10 µg. The IC50 values (68.2, 63.3, 62.1 and 57.62) were more than 100µg/ml and the regression values were difficult to analyze.

4. Conclusion

The results obtained from the *in-vitro* studies performed using the HeLa cell lines reveals that the various extracts of Tomato *(Solanum lycopersicum L.)* fruits have a very good to moderate anticancer activity, the IC50 values were more than 100 µg/ml for the cell line studies as shown by the MTT assay method. Hence the level of cytotoxicity of the extracts were effective. From the present studied it has been concluded that E2, E1, E4 and E3 extracts have good anticancer activity which is mainly due to the presence of lycopene, polyphenols, flavanoids, aminoacids etc. The intensity of spectrum of the cell growth inhibition of various extracts of Tomato *(Solanum lycopersicum L.)* fruits were given as below: E2 > E1 > E4 > E3. Now it is revealed that the tomato is not only used for the treatment of benign prostatic hyperplasia but also for the human cervical adenocarcinoma.

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