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COMPARATIVE ANALYSIS OF LYCOPENE CONTENT IN NATIVE AND HYBRID SOLANUM LYCOPERSICUM

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Abstract

Solanum lycopersicum is one of the most important vegetable plants in the world. It includes bioactive compounds which have a wide range of physiological properties. It contains many health-sympathetic or health-advanced compounds such as phenol compound arytenoids and vitamins which enact powerful roles related to the fine fettle. It has economic and nutritional significance Thin-layer chromatography has become the most potent tool for the qualitative analysis of compounds because of its simplicity and reliability. It shows a high level of lycopene in the Desi variety when compared with native Solanum Lycoperisum L.Lycopene acts as a cancer-fighting agent.

KEY WORDS: Solanum lycopersicum, phenol

Introduction

Solanum lycopersicum originated in western South America, and domestication is thought to have occurred in Central America. Solanum lycopersicum is a popular vegetable/fruit also because it is an important source of vitamins and minerals in diets. One medium-sizedSolanum lycopersicum provides 57% of the recommended daily allowance (RDA) of vitamins 25% RDA of vitamin A And 8% RDA of iron, yet with only 35 calories. Solanum lycopersicum is an important food; it has been bred to improve productivity, fruit quality, and resistance to biotic and Biotic stresses. Solanum lycopersicum has been widely used not only as food but also as research material. The red pigment contained in Solanum lycopersicume is called lycopene.Solanum lycopersicume is the most important non-starchy vegetable in the American diet. Research undergoes the relationship between consuming Solanum lycopersicume and reduced risk of cancer, heart disease, and other conditions,", the others conclude.[2]

Scientific classification

- Kingdom : planate
- (Unranked) : Angiogenesis
- (Unranked) :Endicott
- (Unranked) :Asteroids
- Order : Sultanates
- Family : Solanaceae
- Genus : Selenium
- Species : S.Lycopersicum

Fig : Native Solanum lycopersicum

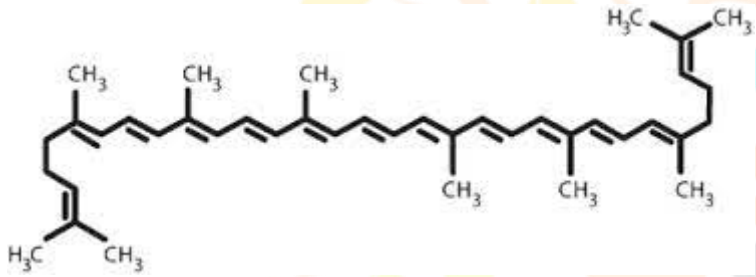
Health benefits:

Solanum lycopersicume, which are a fruit and not a vegetable, are loaded with all kinds of health benefits for the body. One of the most well-known Solanum lycopersicum eating benefits is its lycopene content. Lycopene is a vital anti-oxidant that helps in the fight against cancerous cell formation as well as other kinds of

health complications and diseases. Lycopene is not a naturally produced element within the body and the human body requires sources of Lycopene to make use of this powerful antioxidant. These studies have proven not only the benefits in preventing cancer; heart disease as well as high cholesterol are also in the Solanum lycopersicume sights. Cancers such as prostate cancer, cervical cancer, colon cancer, rectal cancer and cancers of the stomach, mouth, pharynx and oesophagus have all been proven to be staved off by high levels of Lycopene.[1]

Lycopene:

Lycopene is a bright red carotenoid pigment and phytochemical found in Solanum lycopersicume and other red fruits. Fruits and vegetables that are high in lycopene include gas, Solanum lycopersicume, watermelon, pink grapefruit, pink guava, papaya, red bell pepper, sea buckthorn, wolfberry (Gobi, a berry relative of Solanum lycopersicum), and rosehip. Solanum lycopersicum family is one of the most popular vegetable crops. In plants, algae, and other photosynthetic organisms, lycopene is an important intermediate in the biosynthesis of many arytenoids, including beta carotene, responsible for yellow, orange or red pigmentation, photosynthesis, and photoprotection.



ROLE OF MCF-7 CELL LINE:

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females, accounting for 23% of the total cancer cases and 14%of the cancer deaths; thus, research in this field is important to overcome both economic and psychological burdens. In recent years it has become clear that breast cancer does not represent a single disease but rather some molecularly-distinct tumours arising from the epithelial cells of the breast. Cell lines are a key element for the molecular diagnosis in breast cancer as they can be widely used in many aspects of laboratory research and, particularly, as in vitro models in cancer research. As for breast cancer, MCF-7 cells represent a significant candidate as they are used ubiquitously in research for estrogens receptor (ER)-positive breast cancer cell experiments and many sub-clones, which have been established, represent different classes of ER-positive tumours with varying nuclear receptor expression levels.[3]

Materials and methods

Detection of different compounds in hybrid, standard and native Solanum lycopersicum using TLC

Sample collection:

Solanum lycopersicum (Solanum Lycopersicon) was purchased from the local market. (Country and hybrid Solanum lycopersicum)

Reagent used:

Ethyl acetate

Acetone

TLC plate

Lycopene sample

UV trans – illuminator

Ethyl acetate: Acetone (1:19)

Silica plate

Preparation of sample extract

Solanum lycopersicumes are washed in distilled water.Peeling out the Skin of the Solanum lycopersicumes in both country and hybrid.Crushing, the Solanum lycopersicumes into crude juice with the use of mortal and pistil.5 µL of the crude juice Solanum lycopersicumwas taken and placed in a Petri plate it was incubated for 48 hours at 60˚C.The dry content of the Solanum lycopersicum pulp is placed in the beaker with the use of a spatula.

The powder form of the Solanum lycopersicum pulp is then extracted with 50 ml Ethyl acetate and acetone.The final product is obtained after solvent removal by evaporation under vacuum at 40-60˚CLycos red tablet 200 mg was taken and mixed with Ethyl acetate and Acetone 1:19 ratios.The three samples hybrid, country, and standard are taken and loaded with TLC.

Fig:Sample extraction

Fig:Lycored

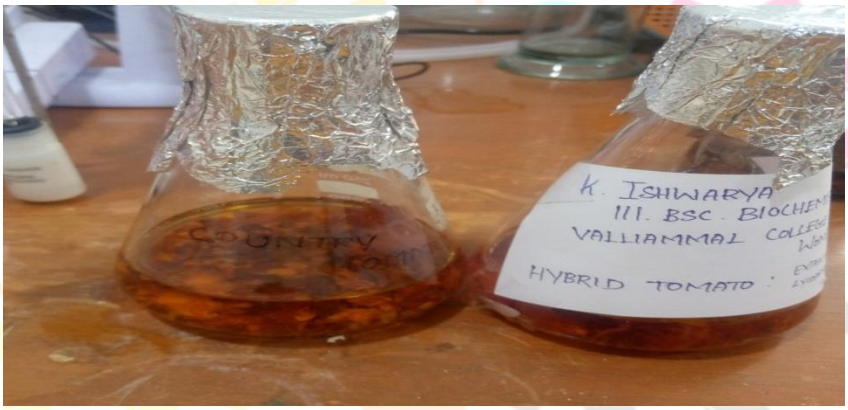


FIG:6

Thin layer chromatography

The solvent ethyl acetate: Acetone (1:19) was used as a mobile phase for the compound in the sample to be identified. Silica plate coated 5×2.5 precondacted. TLC plate was used as a stationary phase. A line was drawn at the bottom of the TLC plate and the sample (standard, hybrid, country) was placed using the capillary tube over the line marked. The TLC plate was placed in the beaker containing the mobile phase and was left undisturbed for the solvent to reach the top of the TLC plate. The TLC plate was removed and air-dried. The pigment was identified by observing under UV trans-illuminator. The Retention Factor (R_F) of the compound was calculated using the formula.

$$\mathrm{RF} = \frac{\text{Distance travelled by the compound}}{\text{Distance traveled by the solvent front}}$$

Fig:Standard count

2.Spectrophotometric determination of Lycopene from Solanum lycopersicumes by extraction with hexane/ ethanol/ acetone and absorbance measurement at 530 nm.

Collection of plant material:

Solanum lycopersicum was purchased from local market.

Preparation of extract:

Acetone and hexane are HPLC grade from Fisher. The ethanol used is 200-proof absolute ethanol, which can be obtained from spectrum chemicals (Gardena, CA). Mix in a ratio of two parts hexane to one part acetone and one part ethanol. Only mix as much solvent as you plan to use in the next few days and keep it in a well-stoppard bottle.

Reagent used:

- Hexane
- Ethanol
- Acetone

Procedure:

1. Starting with well-homogenized Solanum lycopersicum juice (prepared under vacuum to minimize the introduction of air bubbles), use a 100 Drummond micropipette to take a sample. After drawing the sample into the pipette, wipe any Solanum lycopersicum juice from the outside of the glass bore with a kewpie then inspect the pipette to be sure no large air bubbles have been included. Dispense the sample into a 20×125 screw cap tube. Also, prepare several blank samples with 100µL water instead of Solanum lycopersicum pulp.
2. Let samples stand for 10 minutes to allow phases to separate and all air bubbles to disappear.
3. Rinse the curette with the upper layer from one of the blank samples. Discard, then use a fresh blank to zero the spectrophotometer at 503 nm (see comment#5 below) Determine the A 530 of the upper layers of the lycopene samples.

Fig:lycopene juice

MTT ASSAY

Principal:

The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) tetrazolium reduction assay was the first homogeneous cell viability assay developed for a 96-well format that was suitable for high throughput screening (HTS). The MTT tetrazolium assay technology has been widely adopte and remains popular in academic labels as evidenced by thousands of published articles. The MTT substrate is prepared in a physiologically balanced solution, added to cells in culture, usually at a final concentration of 0.2-0.5mg/ml, and incubated for 1 to 4 hours. The quantity of Formosan (presumably directly proportional to the number of viable cells) is measured by recording changes in absorbance at 570 nm using a plate reading spectrophotometer. A reference wavelength of 630 nm is sometimes used, but not necessary for most assay conditions.

Viable cells with active metabolism convert MTT into a purple-coloured Formosan product with an absorbance maximum near 570 nm. When cells die, they lose the ability to convert MTT into Formosan, thus colour formation serves as a useful and convenient marker of only the viable cells. The exact cellular mechanism of MTT reduction into Formosan is not well understood but likely involves a reaction with NADH or similar reducing molecules that transfer electrons to MTT. Speculation in the early literature involving specific mitochondrial enzymes has led to the assumption mentioned in numerous publications that MTT is measuring mitochondrial activity.

The Formosan product of the MTT tetrazolium accumulates as an insoluble precipitate inside cells as well as being deposited near the cell surface and in the culture medium. The Formosan must be solubilised before recording absorbance readings. A variety of methods have been used to solubilise the Formosan product, stabilize the colour, avoid evaporation, and reduce the amount of signal generated is dependent on several parameters including the concentration of MTT. The length of the incubation period, the number of viable cells and their metabolic

The MCF-7 cell line was plated separately using 96 well plates with a concentration of 1×10^4 cells / well in DMEM media with IX Antibiotic Antimycotic solution and 10% fetal bovine serum (Hymenia, India) in a CO_2 incubator at 37% C with 5% CO_2. The cells were washed interference by phenol red and other culture

medium components. Various solubilisation methods include using: acidified isopropanol, DMSO, dimethylformamide, SDS, and combinations of detergent and organic solvent.

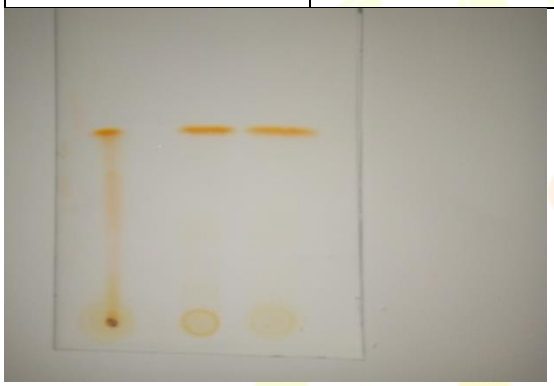
Cell culture and MTT assay:

The MCF-7 cell line was plated separately using 96 well plates with a concentration of 1×10^4cells / well in DMEM media with IX Antibiotic Antimycotic solution and 10% fatal Bovine serum (Himedia,India)in CO_2 incubator at 37%with 5%CO_2. The cells were washed with 200µL of IX PBS. Then the cells were treated with various test concentrations of a compound in serum-free media and incubated for 24 hours. The medium was aspirated from cells at the end of the treatment period. 0.5mg/ml MTT prepared in IX PBS was added and incubated at 37°C for 4 hours using a CO2 incubator. After the incubation period, the medium containing MTT was discarded from the cells and washed using 200 L of PBS. The formed crystals were dissolved with 100 L of DMSO and thoroughly mixed. The development of colour intensity was evaluated at 570 nm. The Formosan dye turns to a purple-blue colour. The absorbance was measured at 570 nm using a microplate reader.

Results

RESULT : Qualitative analysis of lycopene

Sample	Standard	Hybrid	Country
RF value	0.72	0.72	0.72



RESULT :

Calculation of Lycopene levels

Where 537 g / mole is the molecular weight of lycopene, 8 ml is the volume of mixed solvent. 0.55 is the volume ratio of the upper layer to the mixed solvents, 0.10 g is the weight of Solanum lycopersicum added, and 172 mM^{-1} is the extinction coefficient for lycopene n-hexane.

If 100µL of Solanum lycopersicum juice is analysed but the volume of mixed solvent is something other than 8ml then the lycopene concentration can be calculated by:

(Sample: country and hybrid Solanum lycopersicum)

Lycopene (mg/kg fresh wt.:

Country Solanum lycopersicum:

= A1.212×17.17×8

= 166.48 mg/kg

Hybrid Solanum lycopersicum:

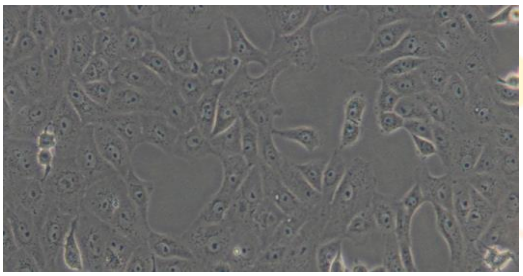
= $A_{1.802}$×17.17× 8

= 247.52 mg/kg

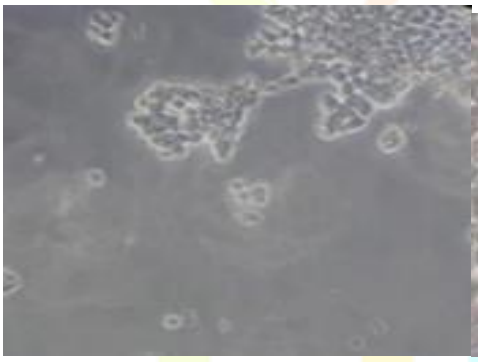
Where V is the volume of mixed solvent added, in ml.

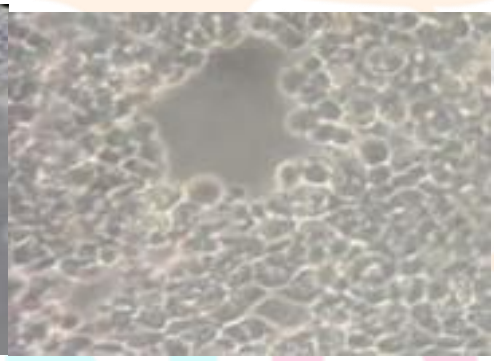
3. MTT ASSAY

CONTROL:500µg

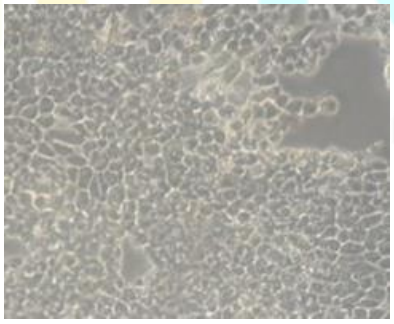


250µg **125µg**

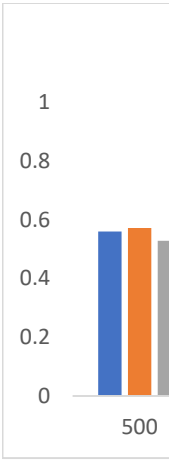




62.5µg



Tested concentration(µg/ml)	OD at 570nm(triplicate values)		
500	0.560	0.572	0.528
250	0.615	0.621	0.599
125	0.698	0.675	0.666
62.5	0.769	0.759	0.742
31.25	0.801	0.812	0.785
Control	**0.858**	**0.871**	**0.849**



Tested concentration(µg/ml)	% of cell viability (triplicate values)		
31.25	65.26807	65.67164	62.19081
62.5	71.67832	71.29736	70.55359
125	81.35198	77.49713	78.44523
250	89.62704	87.14122	87.39694
500	93.35664	93.22618	92.46172
	100	100	100

DISCUSSION

Solanum lycopersicum is an important vegetable. It is a popular fruit because it is an important source of vitamins, minerals, phytochemicals, antioxidants.Solanum lycopersicum contains powerful compounds such as comedic acid and chlorogenic acid. These compounds act as antioxidants and neutralize free radicals that can damage cells in the body. It shows that there is a high level of lycopene in the Desi variety when compared with native Solanum Lycoperisum L. performed by using Thin layer chromatography with mobile phase and stationary phase used in the ratio 0.72 and the compound was detected under spectrophotometric 530 nm.The MTT assay showed that Solanum Lycoperisum L. is not cytotoxic. At room temperature, It has a high cell survival rate compared with refrigerated.The current study helps in the determination of lycopene using thin layer chromatography and quantitative analysis using spectrophotometric revealed that the amount of lycopene is greater in the Desi Solanum Lycoperisum L. when compared with native solanum Lycoperisum L.

Conclusion

The present study compares native and Desi varieties of Solanum lycopersicum.Thin-layer chromatography has become the most potent tool for the qualitative analysis of compounds because of its

simplicity and reliability. From the above preliminary study, it is concluded that the Ethyl acetate and acetone extract of Solanum Lycoperisum L. Desi prove to be one of the lower risks of certain types of Lycopene as a cancer-fighting agent.

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