IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

EXTRACTION AND SEPARATION OF LYCOPENE FROM NATURAL SOURCES USING CHROMATOGRAPHIC TECHNIQUE AND ITS APPLICATION

Authers: Abhishek daga*, Trupti Tope[1], Vikas Shukre[2], Ganesh Kore[3], Komal Kadam[4]

MGM College of Agricultural Biotechnology, Gandheli, Aurangabad- 431003

Abstract

Lycopene is a functional component of great dietary importance obtained from many plant sources. In this article, we address the extraction of lycopene using different extraction methods,acetone petroleum ether extraction method, methanol Extraction method acetone and petroleum ether extraction method and hexane Extraction method and screening was carried out by TLC method using standardlycopene .

We also describe the different applications of lycopene hydrogen paraoxide scavenging effect, RNA Damage estimation and lycopene as food colorant

Keywords -.lycopene, Tomato sample, Extraction method, TLC , Applications

INTRODUCTION:

Fruits and Vegetables are main source of natural antioxidant. Antioxidant give protection against harmful free radicals and reduce rate of cancer and heart disease. The most efficient carotenoid antioxidant is Lycopene. Lycopene is one of the carotenoids that naturally occurred in many fruits and vegetables and found to be measured in blood serum. It is mainly found in Tomatoes and other red fruits and vegetables. Lycopene is a pigment principally responsible for the characteristic deep-red color of ripe fruits and vegetables. In the synthesis of Vitamin-A lycopene plays an important role as an intermediate and carotenoid like β-carotene and β- cryptoxent in, influences its development. Lycopene is soluble in fat and synthesized by plant and microorganisms. Lycopene has antioxidant and anti-tumor properties. Regular intake of lycopene containing food reduces the risk of body tumor especially prostate cancer. It also reduces LDL cholesterol and cardiovascular disease. Like essential amino acid, they are not made in the human body and therefore, can only be obtained through diet or supplementation.

Biosynthesis of Lycopene in Tomato: Lycopene biosynthesis in plant cell have been explained by metabolic pathway of carotenoid synthesis in a model plant, Arabidopsis thaliana (1980). The first step specific to the pathway of carotenoid biosynthesis is the production of the symmetrical 40-carbon phytoene from 20-carbon geranyl geranyl pyrophosphate. Phytoene then undergoes a series of four desaturation steps to form a first phytofluene and then, in turn zeta- carotene, neurosporene, and lycopene. Cyclization reaction at each end of lycopene molecule results in the formation of β- carotene, which may they serve as a substrate for production of the xanthophylls (oxygenated carotenoids). Thus, lycopene is a precursor of β- carotene and lutein. Due to common pathway of synthesis of carotenoids, other carotenoids may also be available in small quantities in tomatoes.

Lycopene is synthesized in chromoplast of fruit cells. Most of the cell in the pericarp near the epidermis synthesize higher lycopene levels than the inner tissues of tomatoes (Sharma and Le Maguer, 1999). In tomatoes, full ripening takes place 40-60 days after planting, during which chloroplast change to chromoplast upon synthesis of lycopene.

Biochemistry of Lycopene: Lycopene a carotenoid in the same family as beta-carotene, is what gives tomatoes, pink grapefruit, apricots, red oranges, watermelon, and guava their red color. Lycopene is not merely a pigment, it is a powerful antioxidant that has been shown to neutralize free radicals, especially those derived from oxygen, thereby conferring protection against prostate cancer, breast cancer, atherosclerosis, and associated coronary artery disease. Lycopene's configuration enables it to inactivate free radicals. Lycopene participates in a host of chemical reactions hypothesized to prevent carcinogenesis and atherogenesis by protecting critical cellular biomolecules, including lipids, proteins, and DNA. Lycopene is the most predominant carotenoid in human plasma, present naturally in greater amounts than beta-carotene and other dietary carotenoids. This perhaps indicates its greater biological significance in the human defense system. Its level is affected by several biological and lifestyle factors. Because of its lipophilic nature, lycopene concentrates in low-density and very-low-density lipoprotein fractions of the serum. Lycopene is also found to concentrate in the adrenal, liver, testes, and prostate. However, unlike other carotenoids, lycopene levels in serum or tissues do not correlate well with overall intake of fruits and vegetables.

Lycopene as an Antioxidants Agents: Living tissues have a control mechanism to keep ROS in balance. When ROS are generated in vivo, many antioxidants come into play. Their relative importance depends upon which ROS are generated, how and where they are generated, and which target of damage is considered. Our body defends itself from these phenomena via endogenous antioxidants. However, when endogenous antioxidants become insufficient or imbalanced in defense against oxidants, exogenous antioxidants may help restore the balance. Tomatoes are widely known for their outstanding antioxidant content, including, of course, their often times-rich concentration of lycopene. Researchers have recently found an important connection between lycopene, and its antioxidant properties. Lycopene, a red carotenoid pigment, $C_{40}H_{56}$ found in blood, the reproductive organs, tomatoes and palm oils. It is a Carotenoid without provitamin A activity and present in many fruits and vegetables. It is a red fat-soluble pigment found in certain plants and microorganisms,

where it serves as an accessory light gathering pigment and protect the organisms against the toxic effect of oxygen and light. As an antioxidant its consumption can reduce the risk of some cancers. The FDA has approved Generally Recognized as Safe (GRAS) status to lycopene. Recently the FDA has also given a limited health claim declaration for lycopene, stating "very limited and preliminary scientific research suggests that eating one of the cup of tomatoes and/or tomato sauce a week may reduce the risk of prostate Cancer". Consuming cooked tomato sauces, tomato ketchup, tomato soup, stewed tomatoes and other cooked tomato dishes are excellent sources of lycopene.

MATERIALS AND METHODOLOGY

Isolation of Lycopene by Using Liquid-Liquid Extraction-

Plant Materials

Fresh fruit, ripe fruits All tomato fruits collected from market in Aurangabad. It was identified as a *Lycopersicum esculentum* (Solanaceae)

A. Acetone-Petroleum Ether Extraction Method:

Reagents-

1. Acetone 2. Petroleum ether 3. Anhydrous sodium sulphate 5. 5% sodium sulphate

Two-Three tomato fruit (samples) was taken and pulped well to a smooth consistency in a wearing blender. Weigh 10-15 grams of this pulp. The pulp was extracted repeatedly with acetone by using pestle and mortar or wearing blender until the sample was colorless. Acetone was pooled, extracted and transferred to a separating funnel containing about 20ml of petroleum ether and mixed gently. Added about 20ml of 5% of sodium sulphate solution and shook well in a separating funnel gently. Volume of petroleum ether might be reduced during these processes because of its evaporation. Then 20ml of petroleum ether was added to the separating funnel for clear separation of two layers. Most of the color noticed in the upper petroleum ether layer. Separated the two phases and re-extracted the lower aqueous phase with additional 20ml of petroleum ether until the aqueous phase was colorless. The petroleum ether extract was pooled and washed once with little distilled water. Poured the wash petroleum ether extract containing carotenoids into a brown bottle containing about 10g of anhydrous sodium sulphate. It was kept aside for 30 minutes or more. Decant the petroleum extract into a 100ml volumetric flask through a funnel containing cotton wool washed sodium sulphate slurry with petroleum ether until it was colourless and transferred the washing to the volumetric flask. It was measured at the absorbance in spectrophotometer at 503nm using petroleum ether as a blank.

Fig:3. Acetone Petroleum Ether Extraction Method

B. Methanol Extraction Method:

Reagents-

1.Methanol 2. Carbon tetrachloride 3. Benzene 4. Boiling methanol

Fifty grams of tomato paste was dehydrated by adding 65ml of methanol. This mixture was immediately shaken vigorously to prevent the formation of hard lumps. After two hours, the thick suspension was filtered. The dark red cake had shaken for another 15 min with 75ml mixture of equal volume of methanol and carbon tetrachloride and separated by filtration. The carbon tetrachloride phase had transferred to a separating funnel, added one volume of water and shook well. After phase separation, the carbon tetrachloride phase had evaporated and the residue was diluted with about 2ml of benzene. Using a dropper, 1 ml of boiling methanol was added in portion, the crystals of crude lycopene were appeared immediately and the crystallization was completely by keeping the liquid at room temperature and ice bath, respectively. The crystals were washed 10 times using benzene and boiling methanol. After that measured the absorbance in spectrophotometer at 503nm using methanol as a blank.

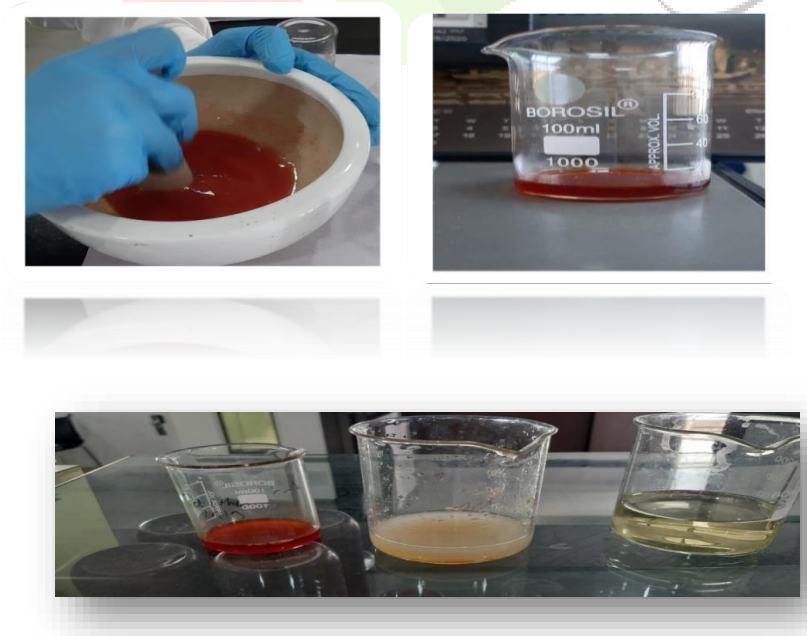


Fig:4. Methanol Extraction Method

C. Acetone-Petroleum Ether Extraction Method:

Reagents-

1.Acetone 2. Petroleum ether 3. Anhydrous sodium sulphate 4. 5% Sodium sulphate 5. Saturated NaCl

Weighted roughly 2 g of tomato paste was placed into a 15 mL centrifuged tube. 4 mL of 50/50 (% volume) mixture of petroleum ether and acetone was added. The centrifuge tube was caped and shaked until the solid becomes fluffy. Then opened the cap and crushed the solid with a spatula. Closed the tube and shook again. Repeated this crushing and shaking two more times. Centrifuge the tube to separate the extract and residues. Transfer the extract (liquid) to a clean centrifuged tube. In the original centrifuged tube, added a new 4 mL of solvent and repeated the entire extraction procedure. The resulting extract was added to the first extract (in the second centrifuged tube). Now washed the mixed extract with saturated NaCl solution (5mL), then with 10% aqueous potassium carbonate (5mL), then with saturated NaCl solution (5mL) again. The organic layer with anhydrous sodium sulphate was dried. Decant the organic layer into a small beaker and concentrated to roughly 0.2 mL by evaporation in the hood (did not applied heat).

D. Hexane Extraction Method-

Reagents-

1.Acetone 2. Hexane 3 Anhydrous Sodium Sulphate 4. 5% Sodium Sulphate 5. Saturated NaCl

Weighted roughly 2.0 g of tomato paste was placed into a 15 mL centrifuged tube. Added 4 mL of a 50/50 (% volume) mixture of Hexane and acetone. Caped the centrifuge tube and shook until the solid becomes fluffy. Opened the cap and crushed the solid with a spatula. Closed the tube and shook again. Repeated this crushing and shaking two more times. Centrifuge the tube to separate the extract and residues. Transfer the extract (liquid) to a clean centrifuged tube. In the original centrifuged tube, added a new 4 mL of solvent and repeated the entire extraction procedure. Added the resulting extract to the first extract (in the second centrifuged tube). Now washed the combined extract with saturated NaCl solution (5mL), then with 10% aqueous potassium carbonate (5mL), then with saturated NaCl solution (5mL) again. Dried the organic layer with anhydrous sodium sulphate. Decant the organic layer into a small beaker and concentrated to roughly 0.2 mL by evaporation in the hood hexane (0.2 mL).

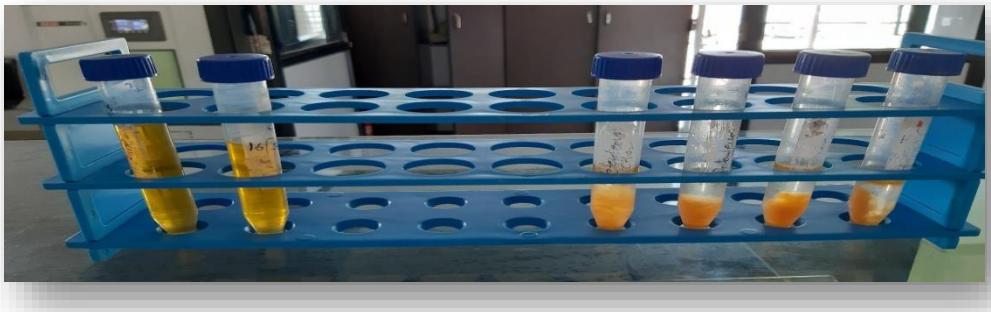
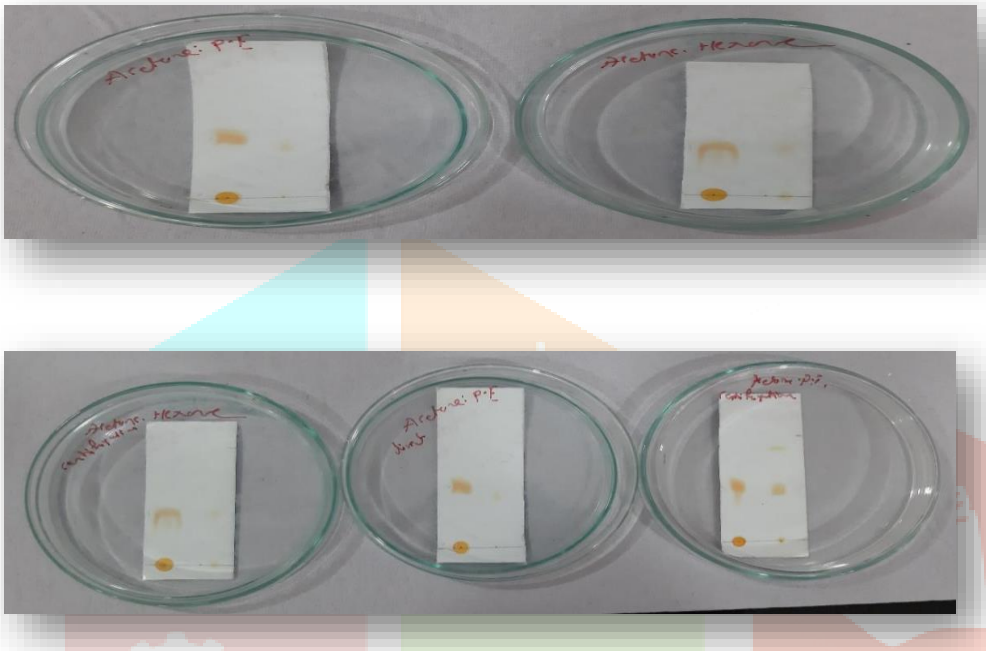


Fig:5. Acetone Petroleum Ether and Hexane Extraction

THIN LAYER CHROMATOPGRAPHY (TLC):

The purified lycopene was subjected to TLC screening method. The TLC was carried out using aluminum sheets (20 × 20cm) pre coated TLC silica gel 60F254 sheets. Three elution solvent systems were selected. (1) petroleum ether; dichloromethane 95:5 (2) 5% methanol in toluene, (3) toluene: hexane 1: 19. The solvent was used and eluted in covered TLC developing tank. Visualization was performed using UV lamp. The Rf solvent was measured;

Rf = **D**istance from origin to component spot (cm) / **D**istance from origin to solvent front (cm).



APPLICATION:

A.Hydrogen Peroxide Scavenging Effects:

The ability of the lycopene to scavenge hydrogen peroxide was assessed by the method of Ruch et al (1998).

Reagents-

1. Phosphate buffer (0.1M, pH 7.4)

2. H_2O_2 (40Mm) in phosphate buffer

1. A solution of H2O2 (40mM) was prepared in phosphate buffer.
2. Lycopene of various concentrations from stock 5mg/ml were added to H2O2 solution (0.6ml) and the total volume was made up to 3 ml.
3. The absorbance of the reaction mixture was recorded at 230nm in a spectrophotometer.
4. The blank solution containing phosphate buffer, without H2O2 was prepared.
5. The extent of H_2O_2 scavenging of the plant extract was calculated as:

Where, A_0 = Absorbance of Control

A_1 = Absorbance in presence of Lycopene

A. Estimation of RNA Damage:

The method described by Chang et al. (2002) was used to assess the RNA damage.

Reagents-

1. **RNA`2. Tris buffer (30mM, Ph 7.4) 3. H_2O_2 (30%) 4. $FeCl_3$ (500M) 5. Agarose (1%) in 1X TAE buffer 6. EtBr (10mg/ml) 7. Gel loading dye (0.25% bromophenol blue, 0.25 % xylene cyanol, 50% glycerol) 8. 50X TAE buffer (Tris base 24.2g, EDTA 18.612g, glacial acetic acid5.7ml, in a total volume of 100ml, pH8.0)**

1. The reaction was carried out in tris buffer (pH 7.4) at 37 C. FeCl3 and H2O2 react with each other resulting in the generation of hydroxyl radicals.
2. Each reaction contained 5µl of tris buffer in RNA (2 µg) and 5µl of tris buffer in lycopene. FeCl3 (5µl) and 10 µl of H2O2 were added to test samples and incubated at 37 C for 15 minutes.
3. To the reaction mixture, 0.06 ml of gel loading was added and electrophoreses in 1% agarose gel containing 3 µl/ml EtBr, at 100V for 15 minutes.
4. Gels were viewed under trans-illuminating UV light and photographed.

B. Lycopene as Food Colorants Against Standard Colour:

Reagents-

1.Lycopene Sample 2. Standard Orange Red colour 3. Sugar Cubes

1. Sugar cube were made by using appropriate concentration of sugar and water.
2. Then by using distilled water the standard color and lycopene sample were diluted according to standard concentration that usually used in foods.
3. By using spreader both sample standard and lycopene was spread on the sugar cubes.
4. Then both sugar cubes were packaged by using aluminum foil and in polythene bags.
5. And then both stored at room temperature.
6. Observed the color efficiency and effect of light on lycopene colour sample against the standard colour.

RESULT:

Extraction Procedure:

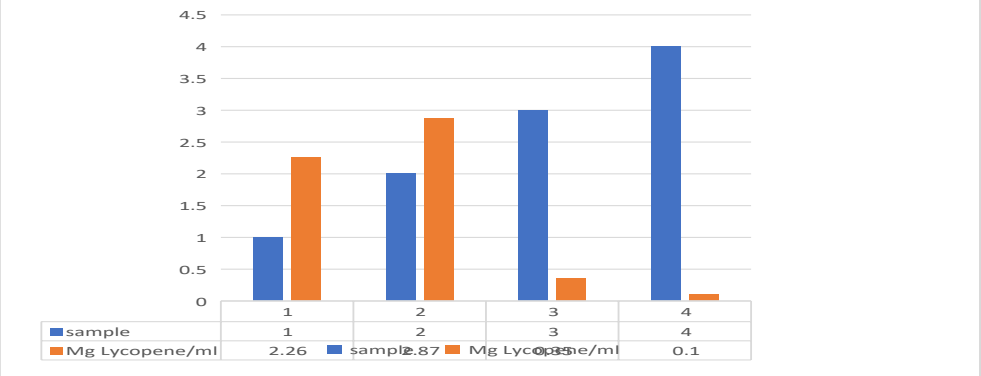
The highest yield of lycopene was found in procedure -2, that use liquid-liquid extraction by using methanol and carbon tetrachloride.

The yield was measured by using standard formulae:

Mg lycopene in 100g sample = 31.206 × Absorbance at 503nm / Weight of Sample.

Table-1: Comparative Yield of Lycopene (Without Any Dilution) Using Four Different Methods

Sr. No.	Sample	Procedure	Mg Lycopene/ml
1	FreshFruits	Acetone: Petroleum Ether	2.26
2	Fresh Fruits	Methanol: Carbon Tetrachloride	2.87
3	Fresh Fruits	Acetone: Petroleum Ether	0.35
4	Fresh Fruits	Hexane: Acetone	0.10



Comparative yield of lycopene using different methods

Thin Layer Chromatography:

Rf = **D**istance from origin to component spot (cm) / **D**istance from origin to solvent run.

Table-2: Comparison of standard sample w.r.t Test (Lycopene)

Sr. No.	Procedure	Rf Value (Standard)	Rf Value (Test)
1	Acetone: Petroleum Ether	0.69	0.59
2	Acetone: Hexane (Centrifugation)	0.51	0.48
3	Acetone: Petroleum Ether (Centrifugation)	0.57	0.51

Table-3: Carotenoid Content in Sample

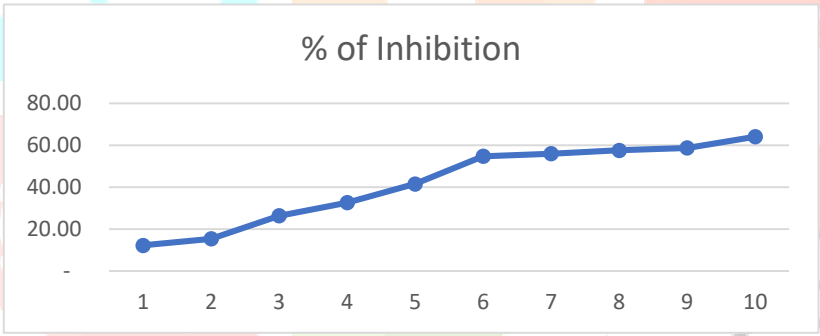
Sr. No.	Procedure	Rf Value
1	Acetone: Petroleum Ether	0.87
2	Acetone: Hexane	0.97
3	Acetone: Petroleum Ether	0.89

Hydrogen Peroxide Scavenging Effects:

The percent of inhibition was increase exponentially by increasing lycopene concentration. The highest percent of inhibition was found to be at 1.0mg/ml of lycopene concentration.

Table-4: Percent of Inhibition (H_2O_2) Against Lycopene Concentration

Sr. No.	Lycopene mg/ml	Phosphate buffer	O.D at 203 nm	% of inhibition
1	0.1	2.9	1.018	12.24
2	0.2	2.8	0.982	15.34
3	0.3	2.7	0.854	26.37
4	0.4	2.6	0.781	32.67
5	0.5	2.5	0.687	41.55
6	0.6	2.4	0.524	54.82
7	0.7	2.3	0.510	56.03
8	0.8	2.2	0.491	57.61
9	0.9	2.1	0.478	58.79
10	1.0	2.0	0.416	64.13



Percent of Inhibition(H_2O_2) Against Lycopene

ESTIMATION OF RNA DAMAGE:

The lycopene's antioxidant property was found to be effective against free radical of H_2O_2 that may be damage RNA. In this First and Third band contain lycopene, hydrogen peroxide and Std. RNA Sample. Second band contain damaged RNA due to Hydrogen Peroxide which has very low frequency compare to control sample.

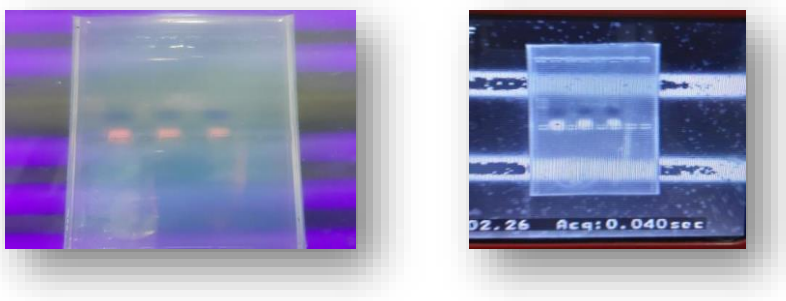


Figure No. 7: Estimation of RNA Damage

Component	Well-1	Well-2	Well-3
Standard RNA	✓	✓	✓
Hydrogen peroxide(H_2O_2)	X	✓	✓
Lycopene	X	X	✓

LYCOPENE AS COLOURING AGENT:

Fig:8. Lycopene as Coloring Agent

Summary:

Lycopene was extracted from tomato paste by simple liquid – liquid extraction using as minimum organic solvent as possible. The main problem was purification of the extract. Four different methods used for the extraction. After that the separation was carried out with the help of Thin Layer Chromatography. Then three types of application carried out. Hydrogen peroxide causes greater oxidation in cellular RNA than in DNA. In these free radicals that are generated in H_2O_2 causes damage the RNA. Lycopene protect the RNA from oxidative insult because lycopene has a great ability scavenging.lycopene has deep red colour and its antioxidants property makes it to use as food colorants. Its shows the good colour efficiency as compare to standard colour that normally use as a food colour.

References:

1. .Adhiraj Dasgupta et al (2012) Induced Mutations for Improved Lycopene, Total Antioxidant Properties and Other Quality Factors in Wild Tomato (Solanum pimpinellifolium). International Journal of Pharm Tech Research CODEN (USA): IJPRIF ISSN :0974-4304 Vol.5, No.4, pp 1655-1663.
2. Aghel N et al (2011). Isolation and Quantification of lycopene from tomato cultivated in dezofoul, IRAN. Jundishapur Journal of Natural Pharmaceutical Products 6(1): 9-15.
3. Amany M. Basuny et al (2009) Tomato lycopene is a natural antioxidant and can alleviate hypercholesterolemia. African Journal of Biotechnology Vol. 8 (23), pp. 6627-6633.
4. Amany M. Basuny et al, (2009) Tomato lycopene is a natural antioxidant and can alleviate hypercholesterolemia. African Journal of Biotechnology Vol. 8 (23), pp. 6627-6633.
5. Ambreen Naz et al (2013) Antioxidant Indices of Watermelon Juice and Lycopene Extract. Pakistan Journal of Nutrition 12 (3):255-260.
6. Ayed S. Amr and Deema S. Hussein (2013) Tomato Pomace Pigment: Extraction and Use as Food Colorant. Jordan Journal of Agricultural Sciences, Volume 9, No.1.

7. Bansuny M.A. et al (2008) Tomato lycopene is a natural antioxidant and can alleviate hyper cholesterolmia. Agriculture research center, Giza, Egypt.

8. D.K. Kang et al (2001) Use of Lycopene, an Antioxidant Carotenoid, in Laying Hens for Egg Yolk Pigmentation. (Asian-Aust. J. Anim. Sci. Vol 16, No. 12: 1799-1803)
9. Das S.et al. (2005) Lycopene, tomatoes, and coronary heart disease. Free Radical Research.39:449-455.
10. Demmig Adams B. and Adams, W.W. (2002) Antioxidants in photosynthesis and human nutrition. Science,298, 2149-2153(DOI:10.1126/science.1078002.
11. Elisa V.A.et al (2011) An Overview of Colorimetric Assay Method used to Assess Survival or Proliferation of Mammalian Cells. Proceeding Western Pharmacology Society's. 54: 10-14.
12. Emmanuel K.et al. (2012) Induced Mutations for Improved Lycopene, Total Antioxidant Properties and Other Quality Factors in Wild Tomato (Solanum pimpinellifoliumL) Advance Journal of Food Science and Technology 4(4): 182-188.
13. Jasmina Metal (2013) Antioxidant Capacity and Contents of Phenols, Ascorbic Acid, β-Carotene and Lycopene in Lettuce. Faculty of Agronomy, University of Kragujevac, Serbia.
14. Kin-Weng Konget al (2012) Revealing the Power of the Natural Red Pigment Lycopene. Advance Journal of Food Science and Technology 4(4): 182-188.
15. Mareike Kelkel et al. (2011) Antioxidant and anti-proliferative properties of lycopene. Free Radical Research,45(8): 925–940.