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RESEARCH ARTICLE

# Spectral Identification and *In –Vitro* Antioxidant Evaluation of All Trans-Isomers of Lycopene from *Solanum lycopersicum* Seedless Paste

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## ABSTRACT

Current study performed on seedless paste of *Solanum lycospercium* for achieving isolation of Trans-lycopene and searching its antioxidant effects by in-vitro method. Trans-lycopene compound went through Spectral evaluation like UV, IR, GC-MC and PMR where molecular validity of Lycopene checked on functional group and on its structural traits. TLC performed in Dichloromethane-Pet Ether. Column Chromatography conducted to isolate high yield of Trans-lycopene compounds. It was 5.2mg/10g of Trans-Lycopene from Seedless Paste. In-Vitro study carried on this Trans-Lycopene using DPPH Radical, Nitric Oxide assay. It shows good dose dependent antioxidant effect. $IC_{50}$ value was found to be 20.60 µg/ml & 18.21µg/ml for DPPH & Nitric Acid Assay. It indicates that seedless paste gives not only good yield of pure Trans- lycopene but also proves equivalent antioxidant potency to that of Standard Ascorbic Acid.

## KEYWORDS

Trans-lycopene, UV, IR, GC-MS, Nitric Oxide Scavenging

## INTRODUCTION

India is biodiversity rich country. From Ayurveda to Herbal Medicine, Plants plays vital role in Human Health of Indians. From ancient time till todays date it had been utilized by many peoples as treatments for their diseases and disorders. Last two decades vigorously research was carried on these systems of medicine for its authenticity. It proved good health beneficial impact in-vitro & in vivo too. *Solanum lycospercium* commonly known as Tomatoes. It's readily available all across globe. Its red pulp besides pleasant sensory characteristic like flavor, color, sour- sweetness and succulence has showed as an alternate source of Lycopene.

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Lycopene ($C_{40}H_{56}$) is carotenoids which found in many fruits, vegetables, herbal medicine. Lycopene is without provitamine-A activity. This compound had showed good response against Cancer, Cardiovascular diseases. Cis & Trans isomers of lycopene commonly exist. Various researches had been done on these forms with respect to its structural characteristic. Still all trans-lycopene study has been remains pertaining to its antioxidant activity from seedless paste of fruits. Study showed that cis-trans isomerization took place by light, thermal energy or chemical reactions.[1] In my work, I had develop method were all trans and 5-cis,9-cis,13-cis form get separated. One of most prominent identification of compound from its crude form is nothing but spectral analysis. UV, IR, GC-MS, PMR i.e.(NMR) are practices use in Pharmaceutical assays and Industry too. It will try to categorically figure out Molecule identification of compound. UV is technique where

electromagnetic radiation and substance get interacted. Beer Lambert law should get follow in this method. IR is method which is very fast & easy technique. Its sensitivity beyond micrograms levels. GC (Gas chromatography) – MS (Mass spectroscopy) recent advance method where retention time makes critical role in analysis of compound. NMR i.e. Nucleus Magnetic Resonance also known as Proton Magnetic resonance where molecules identified in solvent where proton is absent.2,3 Antioxidant study reveals whether compound possess free radical scavenging activity or not. This free radical theory in various kinds of disease and disorder are well known. It had been proved that many Cancer, Cardiovascular diseases, Ageing, Diabetes complications like Retinopathy, Nephropathy are occur due to imbalance of chemical reaction in body or generation of excess amount of free radicals. These free radical also inhibited by in build antioxidants that are present in our system. So, drugs that show free radical scavenging activity is nothing but having antioxidant activity in it. To determine particular antioxidant activity we have to check initially it's in vitro activity. Many assays are mentioned for antioxidant activity determination but DPPH, Nitric Oxide assay plays significant role in evaluating antioxidant potential of compounds.[4, 5] In this research we carried both assay as per standard procedure mention in literature.

## MATERIAL AND METHOD

Instruments: 1) UV- Shimadzu 2501PC spectrophotometer 2) Infrared Spectra- Shimadzu FTIR-8400S spectrophotometer 3) Proton resonance magnetic Spectra- Broot Spectrophotometer (300MHz). 4) GC-MS Spectra- Walters auto system. Chemicals: 1, 1-Diphenyl-2-picrylhydrzyl (DPPH) Procures from Hi Media Lab, Mumbai. All other chemicals used were of Ranbaxy Lab. Ltd. Delhi. All solvents including chemicals were of analytical grade.

### *Plant Material:*

*Solanum lycopersicum* Fresh fruits collected from Local Vegetable Market, Nashik, Maharashtra. It is authenticated by Dr. D.R.Mahajan, Botanist, Head of Botany Department, KTHM College, Nashik, Pune University. Voucher specimen number (No.12) had been deposited for future reference.

### *Drug Processing:*

Fresh material after collection washed using tap water. Once again washed with distill water. It then shade dried & whole fruits with peel cut into slices. Then all seed were removed from inner portion of fruits. These then immediately mix into grinder. Semi solid paste utilized for Extraction process.

### *Extraction of Trans-Lycopene*

10 gm of tomato paste (tomatoes from which seeds were eliminated) was taken in a 50 ml beaker. 25 ml of 95% ethanol was added to it and stirred vigorously or macerated the mixture with a glass rod for 2 min or until thickness of the paste disappears. After completing this dehydration of the paste the funnel with a loose plug of glass wool was fitted. The mixture is filtered and pressed the pulp to take off all the filtrate. The filtrate is kept in conical flask and the crude was taken in round bottom flask, 50 ml dichloromethane was added and vigorously macerated for half an hour. The red colored solution was filtered using the funnel with a loose plug of glass wool. This was repeated for three times and kept in dark bottle away from sunlight. This extract was concentrated on rotary evaporator and the solvent was recovered.

### *Phytochemical Screening:*

Preliminary investigation carried out on extract to find out exact natures of compounds that are present in it. [6, 7]

### *Column Chromatography:*

Lycopene extracted from the tomato paste using dichloromethane used for the column

chromatography. If the sample is dried, about 5 ml of hexane added and shaken it and the liquid portion are removed with the help of pipette. The liquid is filtered through a short-stem pipette, plugged at the bottom with cotton, into a collection flask. The dried organic phase is decanted into a clean beaker and most of the solvent is evaporated. While the solvent is evaporating, chromatography column made ready. A short-stem pipette was used, plugged at the bottom with cotton. Slurry of Alumina in hexane (1-1.5 g Alumina in ~5 ml hexane) was added. Alumina slurry transferred to the column-using pipette, collecting the run-off hexane in a beaker or test tube. The column is not allowed to go dry. When all of the slurry has been added to the column and the hexane is just above the top surface of the Alumina, the column is allowed to saturate. By now most of the solvent has evaporated from the sample, and the column is ready to be loaded. If necessary, a little hexane (1 ml or so) added to sample so that we can transfer it carefully to the top of the column using a pipette. Let the sample load onto the column, then eluted with hexane (The column is not allowed to go dry). The eluent collected in a series of test tubes. First a thin yellow band (carotene), then a reddish-orange band (lycopene) observed. When the carotene has completely eluted from the column, we can switch to a 20% solution of acetone in hexane to elute the lycopene. Meanwhile the TLC is taken for each eluted samples. The solvent from the lycopene is evaporated by vacuum. The IR, UV, GC-MS, and NMR spectra have been taken for that lycopene sample.

### *Spectral Identification*

To determine UV-VIS spectra of Lycopene from seedless paste of *Solanum lycospercium* were undergone scan from 200-800nm. Its characteristic peaks were noted on their mentioned wavelengths. Infrared spectra of Trans-lycopene were recorded using Chloroform on Shimadzu FTIR-8400S spectrophotometer. It will elucidate peaks & functional groups for unique identification of compounds. GC-MS spectra were recorded on Walters's auto system in NHRDF, Chitegaon, Nashik. Trans-lycopene also went for identification in PMR study. Proton resonance magnetic spectra (1H NMR) were recorded on Broot Spectrophotometer (300MHz) using $CDCl_3$ as a solvent.

### *In- Vitro* Evaluation

### *DPPH Radical Scavenging Activity*

Diphenyl picryl hydrazyl (DPPH) nitrogen-centered free radical. Its reaction rates correlate directly with the antioxidant activity. Two mechanisms for antioxidants scavenge DPPH free radical have been proposed. The odd electron in DPPH free radical gives a strong absorption maximum at 517nm and is purple in colour. The colour turn from purple to yellow as the molar absorptivity of DPPH radical at 517 nm reduces when odd electron of DPPH radical becomes paired with hydrogen from free radical scavenging antioxidant to from the reduced DPPH-H.[8, 9] The free radical scavenging activity of the different fractions of lycopene was measured using DPPH, employing the method of Blois. One ml of each fraction of lycopene and the reference compound in various concentrations (25, 50, 75, 100, & 125 µg/ml) was added to one ml of 0.1 mM solution of DPPH in methanol. After 30 minutes, absorbance was measured at 517 nm, using a spectrophotometer. A 0.01mM solution of DPPH in methanol was used as control, whereas Ascorbic acid was used as a reference material. Percent inhibition was calculated using common equation.[10]

DPPH Radical Scavenging Activity%= [$Abs_{(control)}$-$Abs_{(standard)}$/$Abs_{(control)}$] x100

Where Abs control= Absorbance of DPPH Radical + methanol

Abs standard= Absorbance of DPPH Radical+ extract/std.drug

### *Nitric Oxide Assay*

Nitric oxide exhibited numerous physiological properties and it is also implicated in various pathological states. It has important role in the defense against pathogens as well as control of blood pressure. NO is produced in various cells including neurons, endothelial cells and neutrophils by three isoform of nitric oxide synthase enzyme, from nitrogen of guanidine group of L-arginine and from molecular oxygen. Nitric oxide was generated from sodium nitroprusside which at physiological pH liberates nitric acid. This nitric acid gets converted into nitrous acid and further forms nitrite ions on contact with air. The nitrite ion diazotized with sulphanilic acid and get coupled with naphthyl-ethylenediamine (griess reagent) producing pink color which can be measured at 546nm. Three ml of 10 mM sodium nitroprusside in phosphate buffer was added to two ml of each fraction of lycopene and the reference compound in different concentrations (25, 50, 75, 100, 125 µg/ml). The resulting solutions were then incubated at 25ºC for 60 minutes. A similar procedure was repeated with methanol as a blank, which served as control. To 5 ml of the incubated sample, 5 ml Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% $H_3PO_4$) was added. The absorbance of the chromophore formed was measured using a spectrophotometer at 546 nm. Percent inhibition of the nitric oxide generated was measured by comparing the absorbance values of control and test preparations (equation1). Ascorbic acid was used as a reference material. [11, 12, 13]

NO Scavenging %= [$Abs_{(control)}$-$Abs_{(standard)}$/$Abs_{(control)}$] x100

Where Abs control= Absorbance of control reaction

Abs standard= Absorbance of extract/standard

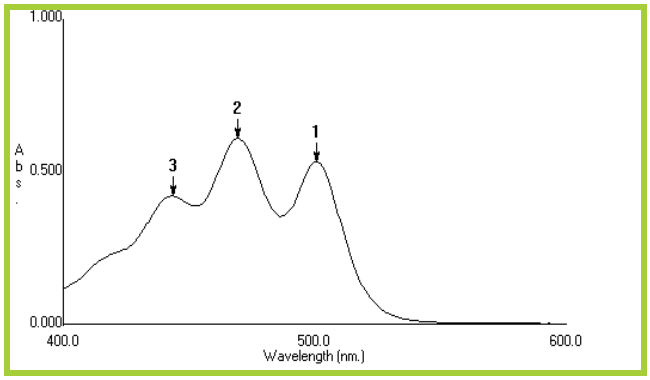
***Statistical Analysis***

Data reported are means of three assays. All measurements performed in triplicate. The data given as means ± standard error of mean. (S.E.M.) $P \leq 0.05$ considered as significant. Version 5.0 Graph Pad Software, CA, USA utilized.

## RESULT

The Trans-lycopene yield of *Solanum lycopersicum* was found to be 0.06%w/v. Phytochemical screening showed presence of Flavonoids, Phenols, and Saponins. TLC using Pet.Ether and Dichloromethane gives characteristics $R_f$ Value of 0.20.

Figure 1 TLC of Trans Lycopene

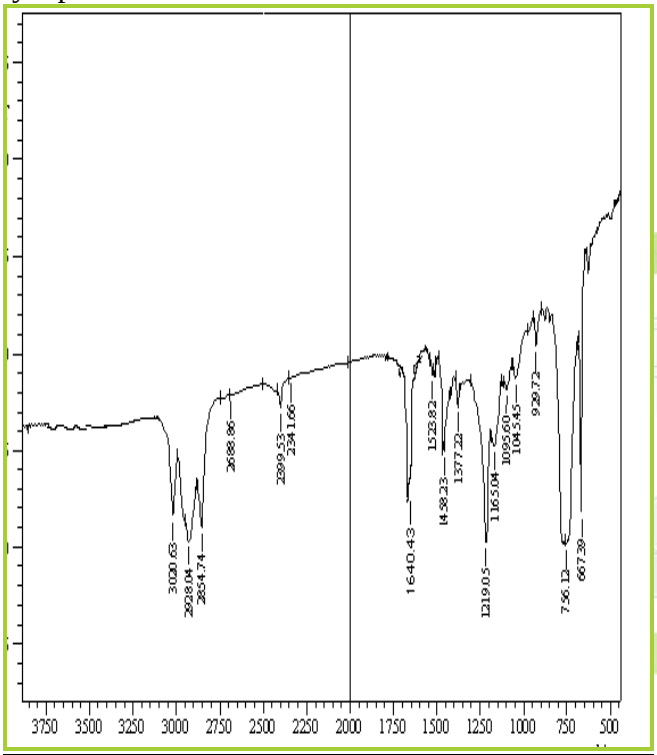



Graph 1: UV Spectral Analysis of Trans-lycopene

| Sr. No. | Λ max | solvent | % III/II or % all trans lycopene |
|---|---|---|---|
| 1. | 443 472 502 | Petroleum ether | 66 |

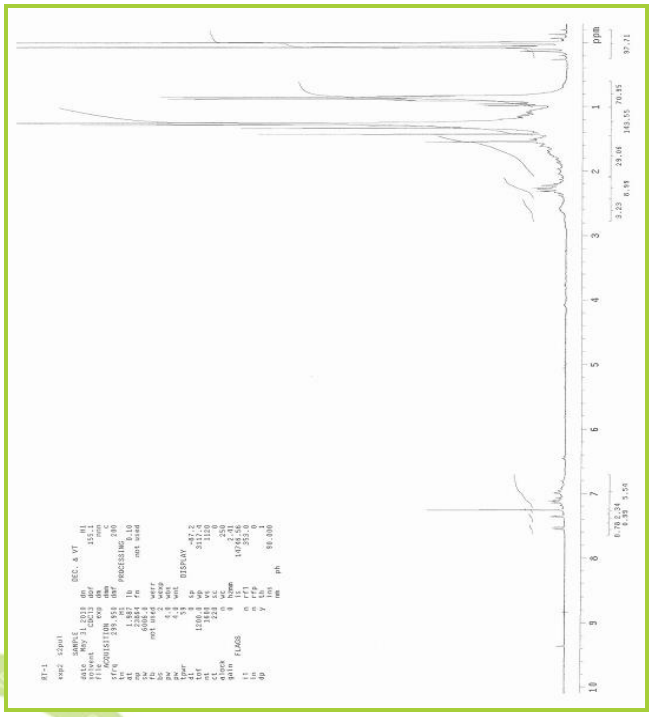
Table 1: UV Spectral Data of Trans-lycopene

Graph 2: IR Spectral Analysis of Trans – lycopene




| Sr.No. | Wave Number ($cm^{-1}$) | Assignment |
|---|---|---|
| 1. | 1523, 1640 | C=C str |
| 2. | 929 | C-H def |
| 3. | 3020 | Aromatic C-H |
| 4. | 1465 | Aromatic C=C |

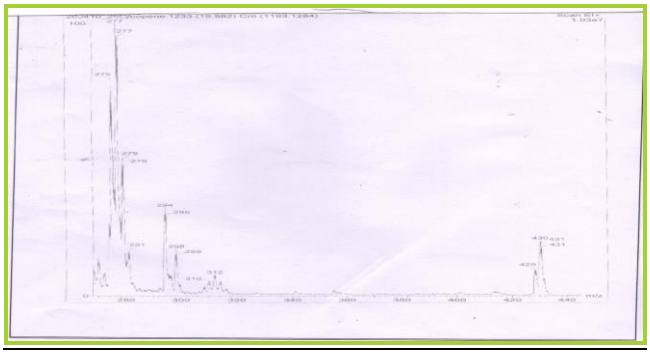
Table 2: IR Spectral Data of Trans-lycopene



Graph 3: PMR Analysis of Trans lycopene

| Sr. No. | δ ppm | Splitting | Assignment |
|---|---|---|---|
| 1 | 1.6 | Singlet | $CH_3$ |
| 2 | 2-2.4 | Quartet | $CH_2$ |
| 3 | 1.2-1.4 | Triplet | CH |

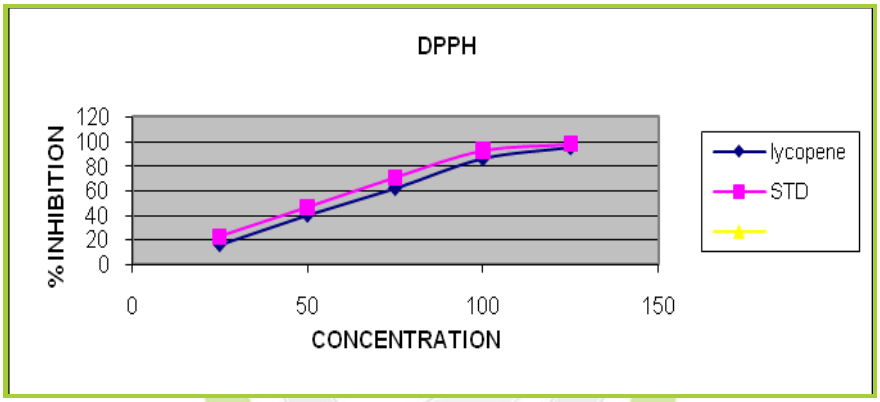
Table 3: PMR Data of Trans lycopene



Graph 4: GC-MS Analysis of Trans lycopene

| Drugs | % Scavenging | | | | | $IC_{50}$ µg/ml |
|---|---|---|---|---|---|---|
| | 25 µg/ml | 50 µg/ml | 75µg/ml | 100µg/ml | 125µg/ml | |
| Trans - Lycopene | 16.24± 0.82 | 40.38±1.02 | 62.12±0.01 | 86.4±0.32 | 95.2±1.16 | 20.61 |
| Std – Ascorbic Acid | 22.56±0.75 | 46.35±0.98 | 70.58±1.15 | 92.43±0.67 | 97.60±0.19 | 12.48 |

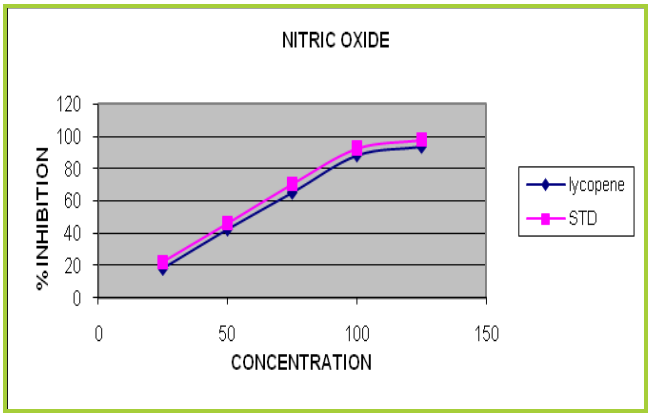
Table 4: In-Vitro Antioxidant Study by DPPH Method




Graph 5: % of Inhibition against Increase Concentration of Lycopene in DPPH Assay

| Drug | % Scavenging | | | | | $IC_{50}$ µg/ml |
|---|---|---|---|---|---|---|
| | 25 µg/ml | 50 µg/ml | 75µg/ml | 100µg/ml | 125µg/ml | |
| Trans - Lycopene | 18.38±1.08 | 42.68±0.44 | 65.39±0.05 | 88.45±0.87 | 93.80±1.06 | 18.21 |
| Std –Ascorbic Acid | 23.11±1.01 | 47.65±0.35 | 69.17±0.48 | 91.04±0.69 | 98.30±0.98 | 13.67 |

Table 5: In-Vitro Antioxidant Study by Nitric Acid Method




Graph 6: Graph showing nitric oxide antioxidant activity

## DISCUSSION

Fresh fruits always were utilized for research work. Unripe and ripe fruits possess varied concentration of lycopene and other phytochemicals. Ethanol extract made by using LeiBig condenser method where recycling of solvents took place. It makes method more cost effective. Column chromatography performed were Trans-Cis isomers get isolated.[14] Lycopene is highly unstable in nature as it is sensitive to air, light, heat and undergoes isomerization of trans to cis isomer. So the process was modified for the preparation of lycopene (cis as well as trans). The heating was avoided for Trans lycopene preparation. Extracts & Trans Lycopene Isomer get studied for TLC. In system of Dichloromethane & Pet Ether it showed characteristics Rf value 0.20 Spectral Study prima face carried on UV. Wavelengths had been checked from 200-600nm. In UV spectra, the λ max at 472nm indicates presence of all-trans lycopene. IR used for interpretation of functional groups. In IR spectra of each compound, different peaks were observed for each functional group. The characteristic peaks for Aromatic C—H and Aromatic C = C were present at 3020cm$^{-1}$ and 1465cm$^{-1}$ resp. For C=C stretching the peaks are present at 1523 and 1640 cm$^{-1}$. The C-H deformation shows peak at 929 cm$^{-1}$. [15, 16] The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in Graph. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios.

These mass spectra are fingerprint of that compound which can be identified from the data library.[17] PMR Studies where proper chemical shift in graph took place. It indicates we get approximate result of it. Apart from all fraction study of this material for antioxidant study, seedless paste study had been remains, which we carried in appropriate way.[18] Antioxidant activity evaluation, carried using DPPH Method.

As Trans-lycopene showed reducing power against DPPH so it acts as Antioxidant. $IC_{50}$ value to comparatively to Ascorbic acid $IC_{50}$ values proves its potential of antioxidant. Nitric oxide assay leads to reduced production of nitrite ions in presence of Trans- lycopene.[19] Nevertheless it proves that even seedless paste of *Solanum lycopersicum* has potential antioxidant activity.

## CONCLUSION

Current study in UV & IR interprets Trans-Lycopene functional groups exist in extract derived from *Solanum lycopersicum*. It becomes confirmatory after structural elucidation by LC-MS & NMR that it's Trans-lycopene. In vitro antioxidant potential of this molecule reveals molecule has to be study for Pharmacological actions & Therapeutic use. Further research on this molecule needed in area of its Stability, Preclinical & Clinical study.

## ACKNOWDLEGEMENT

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