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Identification of Lycopene extracted from Papaya Using Thin Layer Chromatography and FT-IR Studies

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A B S T R A C T

Living tissues have a control mechanism to keep Reactive Oxygen spp, (**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. Researches show that lycopene can be a natural aid in this. It is absorbed more efficiently by the body after it has been processed into juice, sauce, paste, or ketchup. In fresh fruit, lycopene is enclosed in the fruit tissue. Therefore, only a portion of the lycopene that is present in fresh fruit is absorbed. Processing fruit makes the lycopene more bio-available by increasing the surface area available for digestion. More significantly, the chemical form of lycopene is altered by the temperature changes involved in processing to make it more easily absorbed by the body. Also, because lycopene is fat-soluble (as are vitamins, A, D, E, and beta-carotene), absorption into tissues is improved when oil is added to the diet. Although lycopene is available in supplement form, it is likely there is a synergistic effect when it is obtained from the whole fruit instead, where other components of the fruit enhance lycopene's effectiveness. The present study is aimed at extracting and identifying Lycopene from Papaya using TLC and FTIR method.

Keywords: Lycopene, Papaya, Extraction, TLC and FT-IR

A R T I C L E I N F O

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1. Introduction

Lycopene belongs to the family of carotenoids. It has a structure that consists of a long chain of conjugated double bonds, with two open end rings. The structure lycopene is the longest of all carotenoids (**Simran Lilwani and Vrinda Nair 2015).** Lycopene ([C40H56], molecular weight 536.85) is an unsaturated hydrocarbon carotenoid containing 13 carbon-carbon double bonds, 11 of which are conjugated and arranged in a linear array. These conjugated double bonds are responsible for the vibrant red color of lycopene. Lycopene is a lipophilic compound that is insoluble in water, but soluble in organic solvents **(Sanjay Metkar *et al.*, 2014)** (**Figure 1).**

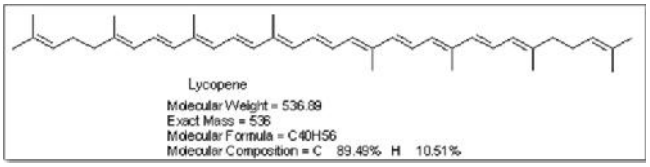


Figure 1: *Structure of Lycopene*

Lycopene is phytochemically synthesized by plants and microorganisms but not by animals. It is an acyclic isomer of beta-carotene (**Suraj Ashok Bhagat *et. al.*, 2012).** This highly unsaturated hydrocarbon contains 11 conjugated and 2 unconjugated double bonds, making it longer than any other carotenoid. As a polyene, it undergoes cis-trans isomerization induced by light, thermal energy, and chemical reactions (**Kalaivani, G. 2015**). Lycopene obtained from plants tends to exist in an all-trans configuration, the most thermodynamically stable form. Humans cannot produce lycopene and must ingest fruits, absorb the lycopene, and process it for use in the body. In human plasma, lycopene is present as an isomeric mixture, with 50% as cis isomers (**Amany M. Basuny *et. al.*, 2009)**.

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 defence system (**Neelu Malviya, 2014)**. 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 **(Adsule, P. G. and Dan, A. 1979).**

Researches show that lycopene can be absorbed more efficiently by the body after it has been processed into juice, sauce, paste, or ketchup. In fresh fruit, lycopene is enclosed in the fruit tissue. Therefore, only a portion of the lycopene that is present in fresh fruit is absorbed. Processing fruit makes the lycopene more bio-available by increasing the surface area available for digestion (**Di Mascio, P, *et. al.*, 1989)**. More significantly, the chemical form of lycopene is altered by the temperature changes involved in processing to make it more easily absorbed by the body. Also, because lycopene is fat-soluble (as are vitamins, A, D, E, and beta-carotene), absorption into tissues is improved when oil is added to the diet (**Lticia G, *et. al.*, 2003)**. Although lycopene is available in supplement form, it is likely there is a synergistic effect when it is obtained from the whole fruit instead, where other components of the fruit enhance lycopene's effectiveness **(V. Kalai Selvan *et. al.*, 2011).** The present study is aimed at extracting and identifying lycopene from Papaya using TLC and FTIR Method.

2. Materials and Methods

Red – fleshed papaya were collected to extract lycopene (**Figure 2).**

Figure 2: Sample collection – Papaya paste

Extraction of Lycopene by Acetone-Petroleum Ether Method

Materials Required

1. Red-fleshed papaya
2. Acetone
3. Petroleum Ether
4. Magnesium Sulphate
5. Whatman Filter Paper
6.

100 gms of papaya paste was weighed and 125ml of acetone was mixed and was allowed to stand for 3-4 mins to remove water. The mixture was filtered by using Whatman Filter paper. The filtrate was collected and squeezed by using a filter paper to dehydrate the paste. Then 125 ml of petroleum ether and magnesium sulphate was added to the filtrate and the content was stirred well for 3-4mins. It was then filtered by using Whatman Filter paper. Finally the filtered lycopene extract was collected and allowed to evaporate. Evaporated sample was used by adding petroleum ether: acetone in the ratio (9:1).

Column Chromatography

Materials Required

1. Silica (100-200) mesh size
2. Lycopene extract
3. Syringe – 12ml
4. Petroleum ether : acetone (9:1)
5. Cotton
6. Burette stand
7. Sterile beaker
8. Test tubes

12ml of syringe was taken and a piece of cotton was used as a filtrate. Silica (100-200 mesh) was used for packing the column by mixing it with Petroleum ether: Acetone (9:1). The silica was poured into the syringe for about 2cm and

Petroleum ether: Acetone (9:1) was used as a mobile phase. The sample was loaded in the syringe and allowed to settle on the silica packing. Petroleum ether: Acetone (9:1) was poured on top to avoid the breaking of silica. After few hours the fractions were collected in test tubes and were used for identification using TLC and FTIR Methods.

Thin Layer Chromatography

Materials Required

1. TLC plate
2. Petroleum ether : Acetone (9:1)
3. Lycopene sample
4. UV trans-illuminator

The solvent Petroleum ether: Acetone (9:1) was used as a mobile phase for the compound in the sample to be identified. Silica slurry coated TLC plate was used as a stationary phase. A line was drawn at the bottom of the TLC plate and the sample was placed using the capillary tube over the line marked. The TLC plate was placed in a 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 ($\mathbf{R_F}$) of the compound was calculated using the formula.

$$\mathbf{R_F} = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

Fourier Transform Infrared (FT-IR) Spectroscopy

The infrared spectroscopy exploits the fact that molecules absorb specific frequencies that are characteristic of their structure. These absorptions are resonant frequencies. Here the frequency of the absorbed radiation matches the transition energy of the bond or group that vibrates. FTIR is a technique used to identify and study the chemical structure of molecules. The analysis of the position, shape and intensity of peaks in the FTIR spectrum reveals details about the molecular structure of the sample. Characterization of sample by FTIR spectroscopy was done using Perkin Elmer Spectrum Version 10.4.00 within the range of 600 – 4000cm^{-1}.

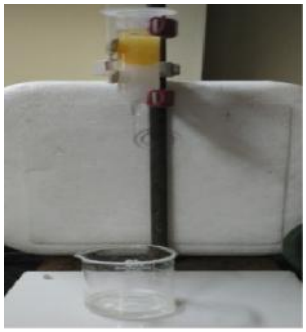
3. Results and discussions

Extraction of Lycopene by Acetone-Petroleum Ether Method: A simple liquid-liquid extraction method was employed to extract lycopene in minimum organic solvent. The yield of lycopene from papaya is extracted from acetone-petroleum ether method (**Figure 3).**

Figure 3: Extraction - Acetone-Petroleum ether method

Column Chromatography

Using a 12ml syringe and packing it with silica of 100-200 mesh size with the mobile phase as Petroleum ether: Acetone, the filtrate was added in the syringe layers and the fractions which were observed were collected using test tubes. Antioxidant activity was tested using the purified fractions obtained from Column Chromatography (Fig.4).



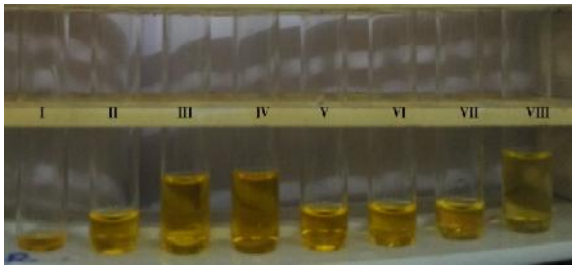


Figure 4: Fractions collected from Column Chromatography

Thin Layer Chromatography

TLC plate was done using Petroleum ether: Acetone (9:1) as a mobile phase for the identification of the compound. When the TLC plate was removed and air dried, the pigment was identified and observed as an orange colour band (**Figure 5).**

Figure 5: Pigment on TLC plate

The Retention Factor (Rf) of the compound was calculated using the formula

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

Sample ====> Rf = 3.2 / 5 = 0.64

Fourier Transform Infrared (FT-IR) Spectroscope

The FTIR spectrum was used to identify the functional groups of the active components based on the peak value in the region of infra red radiation. The crude and purified extract of Lycopene was passed into the FTIR and the functional groups of the components were separated based on its peak ratio (Figures 6–7).

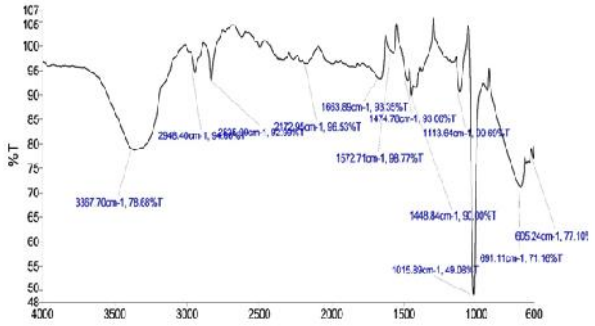


Figure 6: FT-IR results for the crude sample

Sample 1- The results of FTIR analysis showed different peaks at 3367.70, 2948.40, 2835.09, 2172.95, 1663.89, 1448.84, 1113.64, 1015.89, and 605.24 respectively **(Figure 6).**

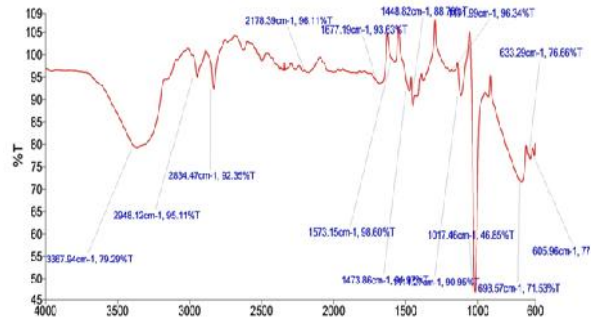


Figure 7: FT-IR results for the purified sample

Sample 2 - The results of FTIR analysis showed different peaks at 3367.70, 2948.40, 2835.09, 2172.95, 1663.89, 1448.84, 1113.64, 1015.89, and 605.24 respectively **(Figure 7).**

4. Conclusion

Lycopene sample was extracted by acetone – petroleum ether method. Results prove that the Crude sample exhibited more activity than the purified samples. Lycopene a carotenoid in the same family as beta-carotene 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. It reduces LDL (low-density lipoprotein) oxidation and helps reduce cholesterol levels in the blood. In addition, preliminary research suggests lycopene may reduce the risk of macular degenerative disease, serum lipid oxidation, and cancers of the lung, bladder, cervix, and skin. The chemical properties of lycopene responsible for these protective actions are well-documented. Thus the present study helped in the extraction and identification of Lycopene from Papaya using TLC and FT-IR Methods and paves way for future research owing to the multifaceted applications of Lycopene.

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