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# Extraction and Antioxidant Activity of Lycopene from Papaya

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**A B S T R A C T**

A modern life style keeps people away from healthy diet. For healthy dietary habits one should increase the consumption of food products which are helpful in the prevention of illness. Fruits and vegetables are main source of natural antioxidant components. Antioxidants give protection against harmful free radicals and reduce rate of cancer and heart disease. The most efficient carotenoid antioxidant is Lycopene. Lycopene a natural pigment in papaya protects the body by neutralizing the negative effects of oxidants. 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 whereas in processed fruits it is more bio-available and increases the surface area for digestion. The present study is aimed at extracting Lycopene from Papaya and conducting Antioxidant studies using DPPH method.

**Keywords:** Lycopene, Fruits and Vegetables, Papaya, DPPH and Natural Antioxidant Property.

**A R T I C L E I N F O**

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## 1. Introduction

Lycopene is soluble in fat and synthesized by plants and microorganisms. Regular intake of lycopene containing food reduces the risk of body tumour especially prostate cancer. Studies have shown that the antioxidants Vitamin E, selenium, and lycopene reduces risk of prostate cancer. Therefore, lycopene is considered very important for cancer

prevention besides reducing LDL cholesterol and cardiovascular diseases (**Di Mascio, P, *et. al.*, 1989)**. Lycopene in processed foods is mainly in the form of the isomers. Its molecular formula is C40 H56 and molecular weight 536.88. Lycopene is a highly unsaturated hydrocarbon with 13 double bonds and 11 unsaturated bonds which are conjugated. Conjugated bonds of lycopene molecule gives ability to act as an antioxidant and make it more efficient for the use of human health. Natural food sources of lycopene are tomatoes, watermelon, pink guava, pink grapes, papaya and apricots (**Gerster, H., 1997)**. Lycopene can be extracted from several different fruits and berries. It was first isolated from *Tamus communis* by Harsten in 1873. Consumption of lycopene from different products up to 150 mg daily shows no side effects. Recent studies have shown that ingested lycopene is metabolized in the body. Several metabolites have now been identified and characterized (**Joseph Levy and Yoav Sharoni, 2004).** Various researches show that lycopene can be used for the treatment of prostrate cancer. Patients of cancer when they increase the intake of tomato, watermelon and papaya in diet helps them more to fight against this disease.

**Papaya**

Papaya (***Carica papaya* L.)** is a fruit crop widely grown in tropical and sub-tropical environments. There has recently been increased interest in the study of the genome of papaya due to its small genome size of 372 Mb and its short life cycle compared with many other tropical fruit tree crops (**Neelu Malviya, 2014).** The two major papaya fruit flesh colours, red and yellow, are controlled by a single genetic locus with yellow being dominant over red (**Simran Lilwani and Vrinda Nair, 2015**). The fruit flesh colour of papaya is determined largely by the carotenoid content. Red-fleshed papaya fruit contain high levels of lycopene, whereas yellow-fleshed fruit contain minimal level (**Fig. 1**).

**Figure 1:** Papaya

**Lycopene**

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. 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 2).**

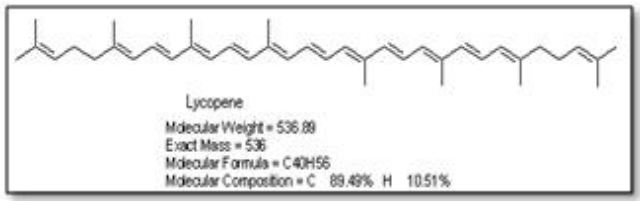


**Figure 2:** Structure of Lycopene

**Lycopene as an Antitoxidant Agent**

Lycopene a potent antioxidant found in watermelon, tomato, and red grapefruit may also exert positive effects on human health (**Wayne W. Fish *et al.*, 2002).** If patients of cancer increase the intake of tomato, watermelon and papaya in diet, it is proven that it helps them fight against this disease (**Adsule, P. G. and Dan, A, 1979).** 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 defence against oxidants, exogenous antioxidants may help restore the balance.

## 2. Materials and Methods

**Sample Collection:** Red – fleshed papaya was collected to extract lycopene (**Figure 3).**

**Figure 3:** 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

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 used for further processing.

**DPPH Radical Scavenging Assay**

**Materials Required**

- 2ml methanolic DPPH solution.
- 10mg/ml of crude extract.

The effect of given samples on DPPH radical was estimated according to the procedure described by **Von Gadow *et. al.*, 1997**. 2 ml of methonolic solution of DPPH were added to 50 µl of a methonolic solution (10 mg ml-1) of the sample. Absorbance measurements commenced immediately. The decrease of absorbance at 515 nm was continuously recorded in a spectrophotometer for 16 minutes at room temperature. Methanolic solutions of pure compound [quercetin] were tested at 1 mg/ml concentration. The scavenging effect (decrease of absorbance at 515 nm) was plotted against the time and the percentage of DPPH radical scavenging ability of the sample was then calculated from the absorbance value at the end of 16 m in duration as follows: All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of **Yen and Duh, 1994**.

$$IP = [(AC(0)- AA(t)/ AC(0))] \times 100$$

Where AC(0) is the absorbance of the control at t = 0 min; and AA(t) is the absorbance of the
Anti-oxidants at t = 16 min.

## 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 4).**

**Figure 4:** 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. 5).**

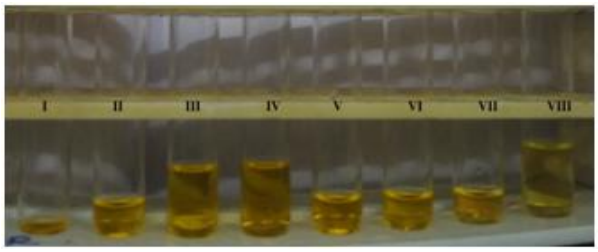


**Figure 5:** Fractions collected from Column Chromatography

**DPPH Radical Scavenging Assay**

DPPH reagent was used extensively for investigating the free radical scavenging activities of compounds. In the DPPH test, the dried extracts are potentially able to produce the yellow coloured diphenyl picryl hydrazine. The assay is based on the reduction of alcoholic DPPH solution in the presence of hydrogen – donating antioxidant due to the formation of the non-radical from DPPH-H by the reaction. The samples chosen for DPPH study were crude and purified samples **(Figure 6 & Table 1).**

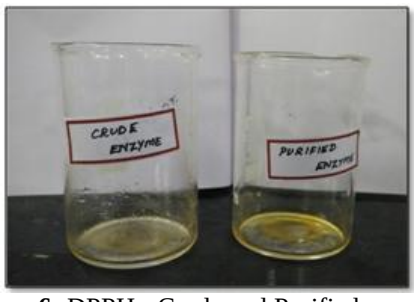


**Figure 6:** DPPH - Crude and Purified samples

**Table 1:** DPPH tabulation - results for crude and purified sample

| Name of the sample | Control OD | Sample OD | Antioxidant % |
|---|---|---|---|
| Crude sample | 0.618 | 0.287 | 53.55 |
| Purified sample | 0.618 | 0.364 | 41.10 |

## 4. Conclusion

Lycopene pigment was extracted from papaya by acetone – petroleum ether method. Antioxidant activity of lycopene was carried out by using DPPH. Although best known as an antioxidant, both oxidative and non-oxidative mechanisms are involved in lycopene's bio-protective activity. The nutraceutical activities of carotenoids such as beta-carotene are related to their ability to form vitamin A within the body. Since lycopene lacks a beta-ion one ring structure, it cannot form vitamin A and its biological effects in humans have been attributed to mechanisms other than vitamin A. Lycopene's configuration enables it to inactivate free radicals. Because free radicals are electrochemically imbalanced molecules, they are highly aggressive, ready to react with cell components and cause permanent damage. Oxygen-derived free radicals are the most reactive species. These toxic chemicals are formed naturally as by products during oxidative cellular metabolism. As an antioxidant, lycopene has a singlet-oxygen-quenching ability twice as high as that of beta-carotene (vitamin A relative) and ten times higher than that of alpha-tocopherol (vitamin E relative). One non-oxidative activity is regulation of gap-junction communication between cells. Lycopene participates in a host of chemical reactions hypothesized to prevent carcinogenesis and atherogenesis by protecting critical cellular biomolecules, including lipids, proteins, and DNA. As an antioxidant it can be used as a prominent medical tool in the treatment of cancer as well as Alzheimer's disease. Thus these promising results can be regarded as an initiatory step towards future modification and utilization of lycopene as a natural source for medical applications.

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