Original Article

Spectrophotometric Characterization of lycopene Phytosomes from Fruit Peels of *Lycopersicum esculentum*

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ABSTRACT

Among all colored fruits tomatoes are one of the rich source of lycopene, which is essentially known for its anti-oxidant activity and effective in treating major diseases like cancer, heart diseases. It is insoluble in water and sensitive to light and temperature but, it is soluble with various proportions in aprotic solvents like petroleum ether, diethyl ether, and acetone. Best combination of these solvents was made used to extract lycopene and characterized for its standardization by means of phytochemical testing, melting point, λ max, FTIR and DSC studies showing the effective way of extraction with good yield up to 8%, phytochemical investigation showed presence of flavonoids, turpinoids and steroids. UV Spectrophotometric analysis shows λ max at 471nm. FTIR studies show the presence of conjugated carbon double bonds essential to get red color to the product and open ring structure on both the ends. DSC endotherms, the melting peak obtained for the phytosomal complex was less than the melting point obtained for the lycopene and is due to the less cohesive force energy of crystal lattice of the complex.

Key words: Lycopene, FTIR, UV Spectrophotometer, DSC, Endotherms.

Recent intriguing possibilities for the treatment of chronic diseases stand out as phytopharmaceuticals. New therapy options for a variety of diseases are made possible by phytochemicals that have fewer side effects and cost less. Natural pigments called carotenoids are produced by microbes and plants to protect cells from photosensitization and act as light-blocking substances during photosynthesis [1]. About 60 of the more than 700 carotenoids that have been classified can be present in the human diet [2]. Carotenes and xanthophylls, including lutein and beta-cryptoxanthin, are important dietary groups [3]. Up to 95% of the total plasma carotenoid concentration is made up of six different compounds: -carotene, -carotene, -cryptoxanthin, lutein, zeaxanthin, and lycopene. Food is one factor that affects plasma concentrations of carotenoids [4]. In human plasma and tissue, lycopene is a very significant carotenoid and accounts for up to 50% of the body's total carotenoids.

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Lycopene is a carotenoid pigment that is primarily present in foods that are red in hue. Due to its conjugated double bonds, lycopene is a potent antioxidant. Lycopene prevents oxidation of vascular cells and lipoproteins through its antioxidant action. In vitro tests on human lymphoid cells, low-density lipoprotein, and plasma have shown that lycopene is an efficient singlet oxygen quencher [5]. Lycopene-rich meals have been demonstrated to reduce the risk of cancer and cardiovascular disease.

The primary red colour in fruits, lycopene is a linear, unsaturated hydrocarbon carotenoid. Lycopene, which is present in tomatoes, belongs to a class of carotenoids with a broad polyene chain that contains 35–40 carbon atoms. Some of these polyene chains are ended by two 6-carbon rings.Antioxidant capabilities of carotenoids have the potential to slow down the ageing process and many degenerative diseasbes. Lycopene is a necessary nutrient that must be consumed daily. Lycopene's potential health

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consequences have also recently been researched. Lycopene is believed to have antioxidant characteristics that are primarily responsible for these positive effects [6].

MATERIALS & METHODS

Collection of Tomato fruits: Tomatoes were collected from local market at Mangalore of Karnataka in the month of January 2018. The fruit was authenticated by Botanical department Regional Science Center Pilikula Mangalore.

Morphological study of Tomato fruit

Tomato fruit: The tomato is the edible, often red, fruit/berry of the plant *Solanumlycopersicum*, *Syn: Lycopersicumesculentum*, commonly known as a tomato plant. The plant belongs to the nightshade family, *Solanaceae*. Tomato is consumed in diverse ways, including raw, as an ingredient in many dishes, sauces, salads, and drinks. While tomatoes are botanically berry-type fruits, they are considered culinary vegetables as an ingredient or side dish for savory meals. Numerous varieties of tomato are widely grown in temperate climates across the world, with greenhouses allowing its production throughout the year. The plants typically grow to 1–3 meters (3–10 ft.) in height and have a weak stem that sprawls. It is a perennial in its native habitat, and cultivated as an annual. Fruit size varies according to cultivator, with a width range of 0.5–4 inches (1.3–10.2 cm)

Preliminary preparation for the peel of tomato: The ripe tomatoes stored at 4ºC were used within 48 h for isolation of lycopene. The peels of tomato were separated using blanching method. The removed peels were dried at room temperature (24-25°C) for 2-3 h and then packed in zip-lock polyethylene bags.

Method 1: Extraction of Lycopene from Tomato Peel: The dried peels were stored at 4°C before grinding with a small amount of solvent system (n-hexane: acetone: ethanol in 2:1:1 v/v) in the presence of carbon dioxide. It was then sonicated using ultrasonic crusher followed by magnetic stirring for 4 h in an inert environment (carbon dioxide) under dark conditions. The resulting extract was collected in an amber color glass container with the presence of carbon dioxide environment and stored at 4ºC for further processing. The extract was then dried using rotary flash evaporator by heating at 60°C at 50 rpm in dark condition [1-2].

Method 2: Raw tomato slices were blended with water (1:1) and boiled for 10 Minutes. The aqueous suspension was vacuum filtered to reduce water to 15% and treated with Acetone (1:1) under high speedy blender and filtered, filtrate containing β- carotenes and other carotenoids was separated. Residue was treated with diethyl ether (1:1) under high speedy blender and filtered. Filtrate containing lycopene was separated and lyophilized.

Pre-Formulation Investigation of Lycopene Extract

1. Shinoda test: a little quantity of extract was dissolved in alcohol + few fragments of Mg turnings + conc. HCl drop wise.
2. Lead acetate test: lead acetate solution was added to small amount of extract.
3. Alkaline reagent test: Increasing amount of sodium hydroxide was added to the sample of extract.
4. Ferric chloride test: Extract + Ferric chloride solution

Detection of Steroids

1. Lieberman Burchard's test: 2mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of conc. H_2SO_4 added.
2. Salkowski reaction: 2mg of dry extract was shaken with $CHCl_3$. To the $CHCl_3$ layer, H_2SO_4 was added slowly along the sides of test tube [4].

UV-VIS Spectrophotometric Determination of Lycopene in Tomato Peel extract.

Determination of λ max: Solution of Lycopene in Diethyl ether was prepared and scanned using UV–VIS spectrophotometer to read λ max in the wavelength of 200–600 nm.

Determination of Standard Graph: 100 mg of tomato extract (Lycopene) was dissolved in diethyl ether and the obtained solution is serially diluted to obtain descending concentrations and the final volume was made up to 10 ml using diethyl ether. Finally the absorbance values were plotted on a graph with variable concentration on x-axis and absorbance at 471 nm on Y-axis. The regression coefficient value and the equation for the graph were also derived so as to ease the calculations of concentration of lycopene in tomato samples [8].

FTIR spectrum of lycopene was taken by FTIR Spectrophotometer: Lycopene was scanned between wave number ranges of 4000 cm^{-1} to 650 cm^{-1}. Major peaks of the spectra were interpreted to determine the respective functional groups present. To authenticate the spectrum, FTIR spectrum of the extracted lycopene was compared with spectrum of imported lycopene, spectrum given by HisarPhytoextracts [9].

Differential Scanning Calorimetric (DSC) studies of Lycopene Phytosomes: Lycopene Phytosomeswere

subjected to Differential Scanning Calorimetric using SDT Q600 V20.9 Build 20 calorimeter. The instrument comprised of calorimeter, flow controller, thermal analyser and operating software. The instrument was designed to supply heat to the sample, so that its temperature was raised precisely. The sample was heated in sealed aluminium pans under nitrogen flow (30 ml/min) at a scanning range of 30 to 800°C. Empty aluminium pan was used as a reference. The heat flow as afunction of temperature was measured for the Lycopene Phytosomes. The DSC of Lycopene Phytosomeswas obtained from which melting point was recorded [10].

RESULTS

Phytoconstiuents investigation found to possess flavonoids and Terpenoids in tomato peel extract (Table 1 – 3).

Qualitative determination of λ_{max} and FTIR studies. UV-VIS Spectrophotometric Detection of Lycopene in Tomato Peel Extract.

Identification of Lycopene: -

i) Determination of λ_{max}

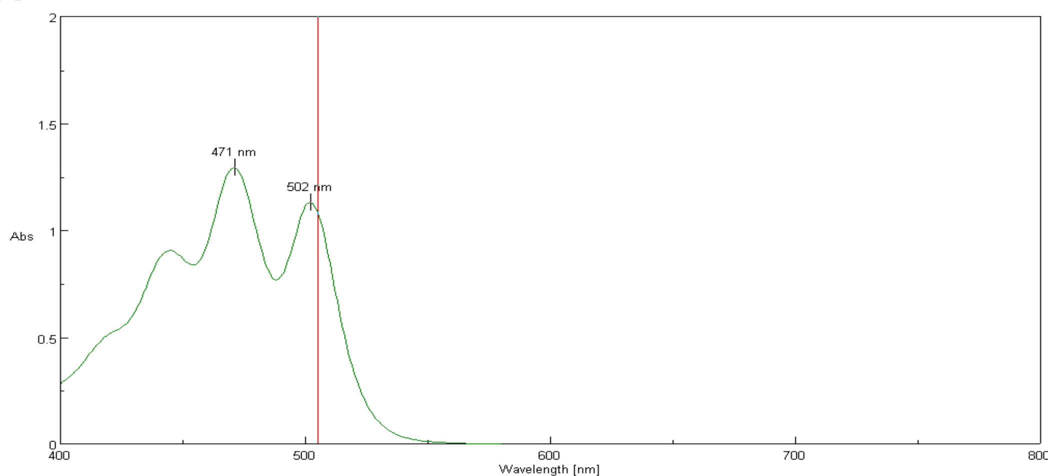

Figure 1: Determination of λ_{max} of Lycopene form Tomato Peel extract.

As reported λ max for solution of lycopene in diethyl ether is 472 nm and during investigation it was found to be 471 and 502 nm (Figure 1 & Figure 2).

Quantitative determination –The Percentage yield from marketed powder and Tomato peels was found to be 8.5% and 8.0 % respectively.

From fruit authentication and from the qualitative & quantitative determination it was concluded that selected component from tomato was Lycopene.

The phytochemical studies confirms the presence of the above said constituents and further the spectrophotometric determination was compared with that of the marketed tomato extract containing Lycopene, confirming the presence of lycopene which showed same λ Max as that of the standard Figure 2. FTIR spectral studies confirms about the presence of the same functional groups as that of the standard (Figure 3, 4, 5).

Drug Excipients Compatability Study

FTIR Studies of Tomato Extract

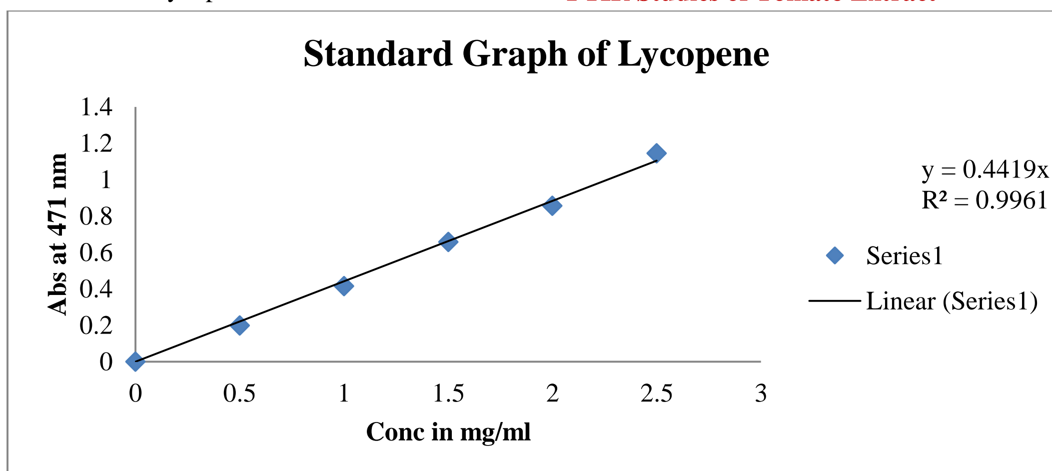

Figure 2: Standard Graph of Lycopene.

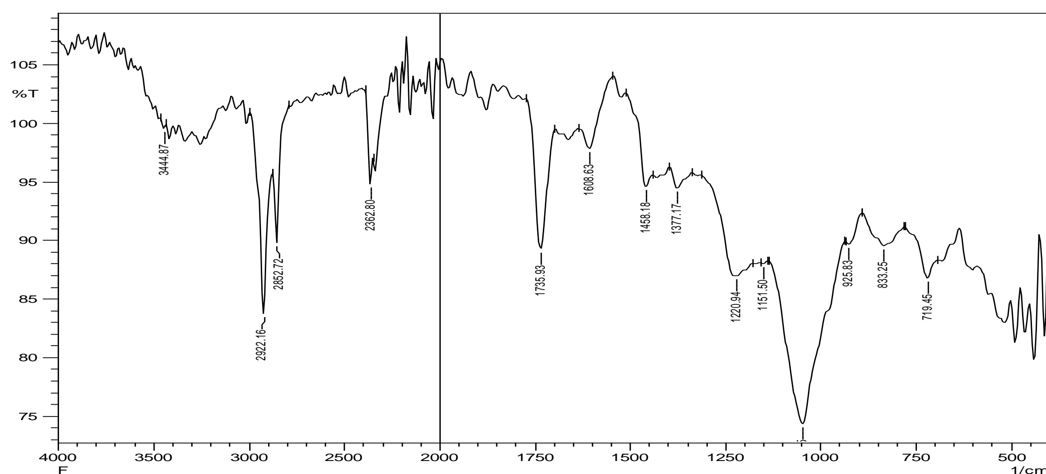

Figure 3: FT-IR spectrum of Lycopene

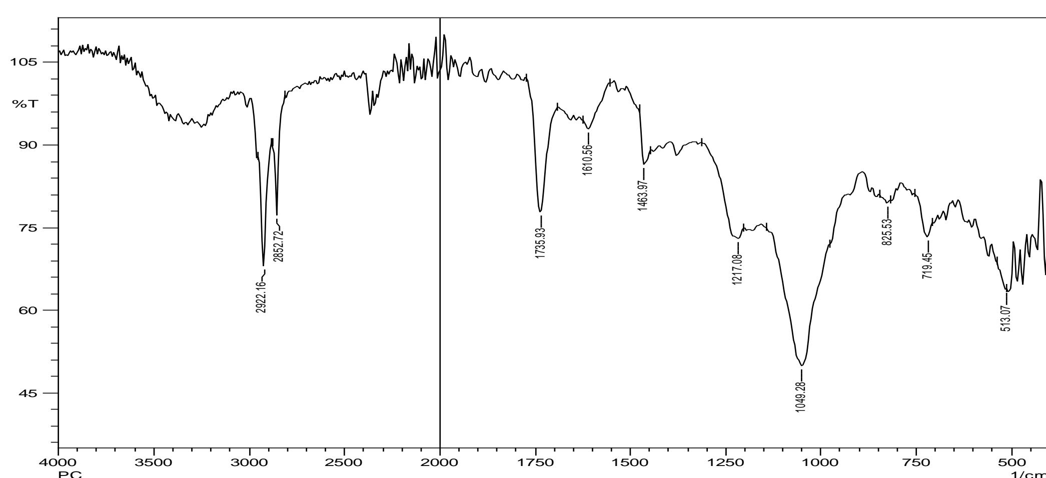

Figure 4: FT-IR spectrum of Phosphatidylcholine

Table 1: Peak picking of Lycopene

Frequency	Amide	Lipid	C-H bending C-C and C-C-H stretching	Water
Reported	1650 and 1540 cm^{-1}	1730-1765 cm^{-1} And 3000-2800 cm^{-1}	1100-1400 cm^{-1}	3700-3000&1600-1700
Observed	1610	1735	1049	2922 & 1610

Table 2: Peak picking of Phosphatidylcholine

Frequency	Amide	Lipid	C-H bending C-C and C-C-H stretching	Water
Reported	1650 and 1540 cm^{-1}	1730-1765 cm^{-1} And 3000-2800 cm^{-1}	1100-1400 cm^{-1}	3700-3000&1600-1700
Observed	1610	1735	1049	2922 & 1610

Table 3: Peak picking of Formulation C1

Origin	Frequency (Cm^{-1})	
	Observed	Given
C−C−H stretch,	3338.78	3072−3055, 3033−3007 m
methylene/methyl	2924.09	2981−2962, 2926−2894
H stretch, alkene	2852.72	2894 m to s
C=C stretch	1602.85	1636, 1558 m

C=O Stretch	1734.01	1735
C−H deformation, methyl	1373.32	1399−1375, 1364−1350 m
C−H out-of-plane, (E) disubstituted double bond	1004.91	976−946 s

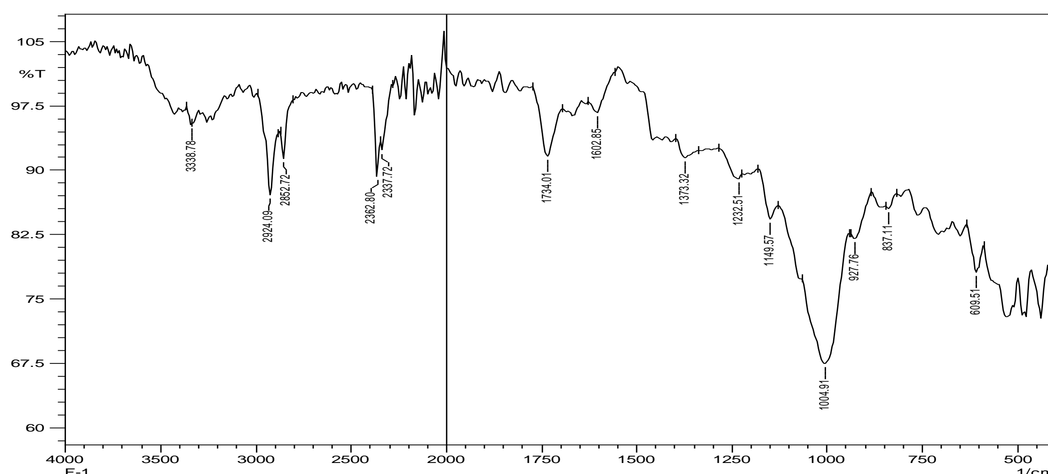

Figure 5: FT-IR spectrum of Formulation C1

Differential scanning calorimetric studies

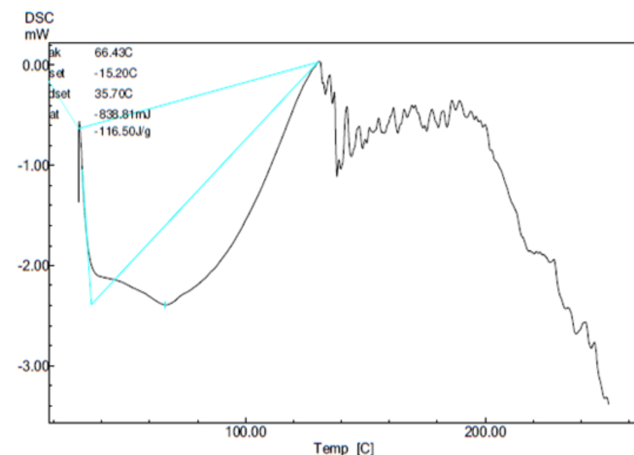

Figure 6: DCS of Lycopene phytosomes.

DISCUSSION

The maxima are decidedly red-shifted from the maxima at 505, 475, and 445 that are observed for lycopene in organic solvent such as hexane as observed by Davis AR et al. Sanchit S and Oishee C observed the absorbance maxima of lycopene at 502 nm using hexane. The FTIR spectra showed typical bands same as by Aghel N et al., observed arising from amide (1650 and 1540 [cm^{-1}]) and lipid (1730-1765 [cm^{-1}] and 3000-2800 [cm^{-1}]) groups. Other bands occur at 1477-1400 [cm^{-1}] (C-H bending), 1100-1400 [cm^{-1}] (C-C and C-C-H stretching), and 1170-1115 [cm^{-1}] (C-O stretching). Strong and broad absorption bands of water are shown in the 3700-3000 [cm^{-1}] and 1600-1700 [cm^{-1}] range. The frequency region between 1200 and 900 [cm^{-1}] shows intense bands attributed to (C-O-C) 1352 vibrational modes of various carbohydrates and acids, which are abundant groups in tomatoes. Frequencies of all types of deformations are found below 1000 [cm^{-1}]. The spectral signal obtained at a frequency of 957 [cm^{-1}] can be attributed to the presence of Trans CH out-of-plane deformation vibration of lycopene.

The DSC thermogram obtained for the phytosome is shown in Figures 6. The thermogram of the lycopene phytosomes showed endothermic peaks at 66.43ºC. These melting peaks obtained were less than the melting point obtained for the Lycopene (173.28°C). The decrease in the melting point of the lycopene might be due to the less cohesive force energy of crystal lattice of the complex formed as showed by Haixiang W et al one peak around 44 ◦C, probably because of loss of water, another peak at about 162.5 ◦C, likely due to its melting point. However, the DSC curve of the inclusion complexes showed different features of free molecules and the physical mixtures, indicating that there was probable interaction between the lycopene and β-CD. These results evidenced that the lycopene was embedded into the cavity of the β-CD. Phytochemical screening showed the presence of flavonoids, Terpenoids & Steroids as seen by Sravanthi J et al. Better yield was seen as compared with marketed spray dried tomato powder of 8%.

CONCLUSION

Hence, Tomato can be a good natural source of lycopene. Methods have been developed for extraction and analytical process for quantification and qualification. An absorption

maximum was found to be at 471 nm. FTIR, a better tool for understand drug excipient interactions and identifying the functional groups which complies with screening tests. In the DSC endotherms, the melting peak obtained for the phytosomal complex was less than the melting point obtained for the lycopene and is due to the less cohesive force energy of crystal lattice of the complex.

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