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Research Article

Thin Layer Chromatographic Characterization of Carotenoid Isolates in Sugar Date Palm (*Phoenix sylvestris*) Fruit Epicarp and Inflorescence Axis

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ABSTRACT
Plant carotenoids are one of the most important classes of plant pigments and play a crucial role in defining the quality parameter of fruits and vegetables. They possess a large numbers of important biological activities such as antioxidant properties, anti – carcinogenic, precursor of vitamin A and preventive in a large number of chronic diseases. The objective of the present study was to characterize the carotenoids present in *Phoenix sylvestris* fruits and their inflorescence axis which are orange red in colour. So, in this study we isolated carotenoids from both the epicarp and the extensively large inflorescence axis by preparative TLC and also characterized some of the major components like β-carotene and lutein using HPTLC analytical techniques. The present findings suggest that the consumption of β-carotene and lutein from this fruit crop can be helpful in providing potential targets for combating vitamin A deficiency in countries where vitamin A deficiency is a public health problem.

Keywords: *Phoenix sylvestris*; Epicarp; Inflorescence axis; HPTLC; β-carotene; Lutein.

INTRODUCTION

In today's nutritionally cognizant society, the metabolic potentiality of human diet containing carotenoids has become an imperative issue. In addition to their role as provitamin A, carotenoids may play a key role as free-radical scavenger and antioxidants in body tissues[1,2]. Despite their significance, there are still many unreciprocated questions concerning carotenoid stability and their bioavailability in foods we ingest.

Health professionals, bioscientific researchers and regulatory authorities have specified substantial interest to carotenoids, in view of their antioxidant potentialities. Carotenoids are the only unit of pigmented compounds that are synthesized by plants and microorganisms but not by any animals. In plants, the fundamental roles of carotenoids are to protect the photosynthetic machinery against photo-damage and are also essential structural components of photosynthetic reaction centre complexes. Fruits and vegetables constitute the major sources of carotenoid in human diet[3-5]. Carotenoids are present as micro-nutrients in fruits and vegetables and are responsible for their bright orange, red and yellow colors. Carotenoids are thought to be responsible for the beneficial properties of fruits and vegetables in preventing human diseases including cardiovascular diseases, cancer and other chronic diseases[6,7]. They are important dietary sources of vitamin A. In recent years the bioactive properties of carotenoids has been one of the major highlights of research[6].

All carotenoids possess a polyisoprenoid structure, a long conjugated chain of double bond and a near bilateral symmetry around the central double bond, as common chemical features[8]. Different carotenoids are derived essentially by modifications in the base structure by cyclization of the end groups and by introduction of oxygen functions giving them their characteristic colors and antioxidant properties[8].

Based on epidemiological studies a positive link is suggested between higher dietary intake and tissue concentrations of carotenoids and lower risk of chronic diseases[4,5,9]. β-Carotene and lycopene have been shown to be inversely related to the risk of cardiovascular diseases and certain cancers whereas lutein and zeaxanthin to the disorders related to the eye[4,10]. The antioxidative potentialities of carotenoids have been recommended as being the chief machinery by which they afford their protective effects. Recent studies are also showing that carotenoids may mediate their effects via other mechanisms such as gap junction communication, cell growth regulation, modulating gene expression, immune response and as modulators of Phase I and II drug metabolizing enzymes [6,7,11,12].

The fruits of Sugar Date Palm or *Phoenix sylvestris* Roxb. (Arecaceae) are cultivated agriculturally as a major source of sugar in India, and the sap is sometimes fermented into a drink called “toddy,” which explains the names “sugar date palm” and “toddy palm”. This palm is native to India. In India, it occurs in areas where there is

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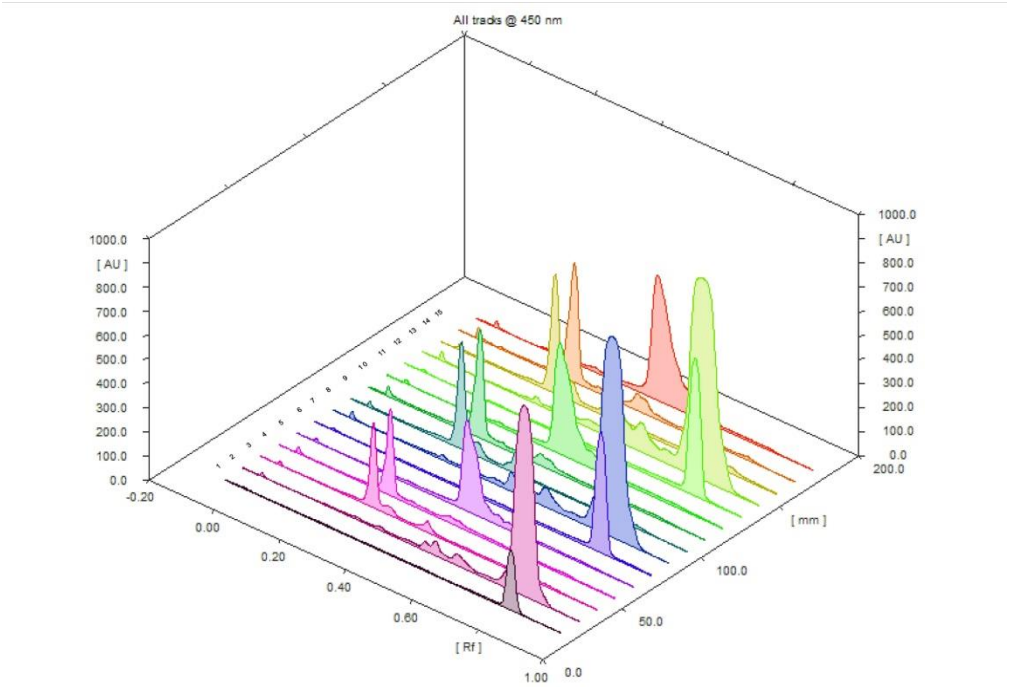
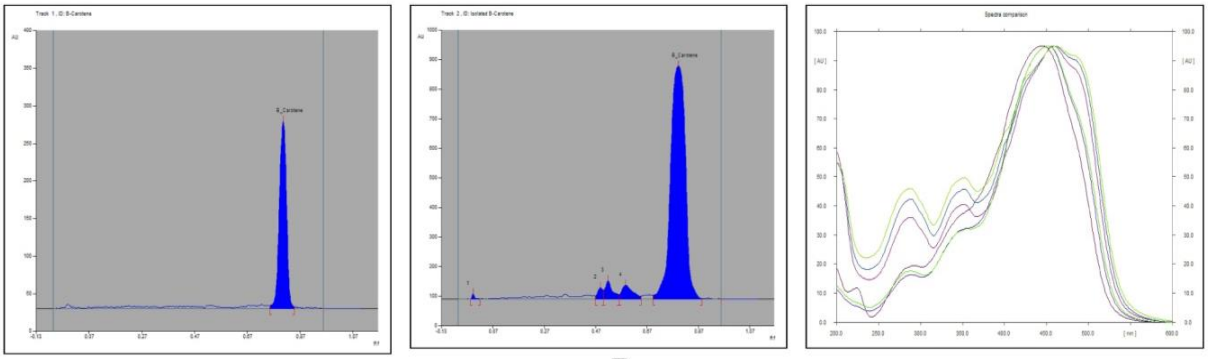
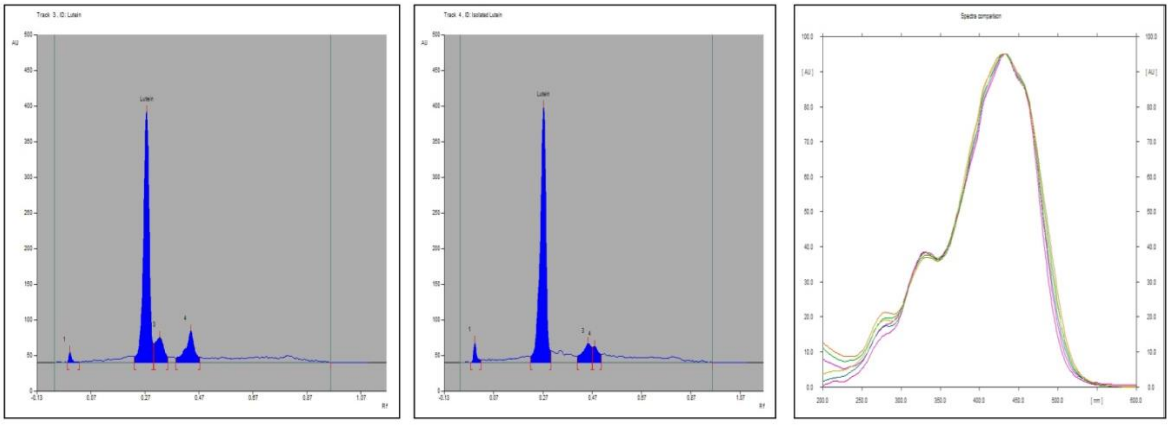


Figure 1: HPTLC densitogram at 450 nm in the order of standard β-carotene (track 1), isolated β-carotene (track 2), standard lutein (track 3), isolated lutein (track 4) and isolated unknown compound (track 5) shown in triplicates isolated from *P. sylvestries* fruit epicarp.



A B C

Figure 2: chromatogram showing standards β-carotene in track 1 (A) and isolated β-carotene in track 2 (B) and superimposed spectra β-carotene (C) in 200nm to 600 nm spectral range.



A B C

Figure 3: Chromatogram showing standards lutein in track 3 (A) and isolated standards lutein in track 4 (B) and superimposed spectra of lutein (C) in 200nm to 600 nm spectral range.

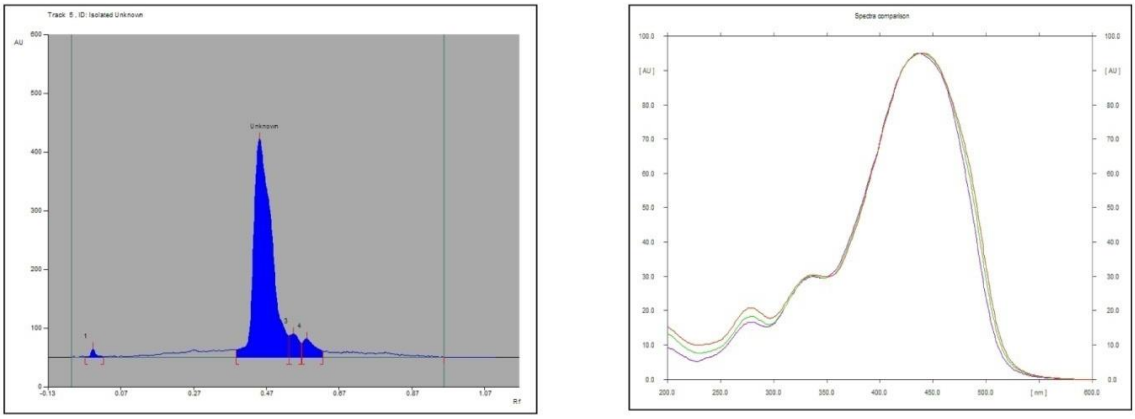


Figure 4: Chromatogram showing an unknown compound in track 5 (A) and superimposed spectra of that compound (B) in 200nm to 600 nm spectral range.

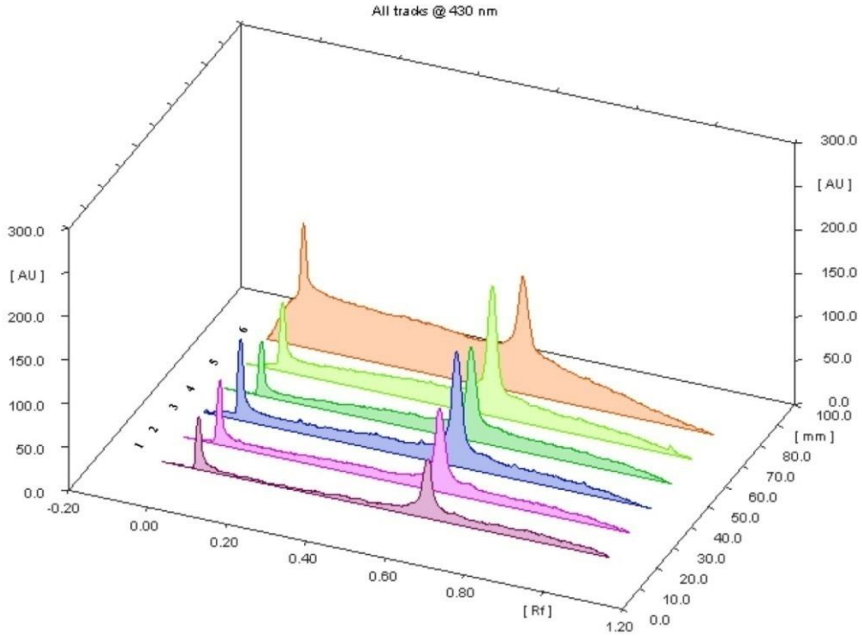


Figure 5: HPTLC densitogram at 430 nm in the order of standard lutein (track 1, 2 and 3), isolated isolated lutein (track 4, 5 and 6) shown in triplicates isolated from *P. sylvestris* inflorescence axis.

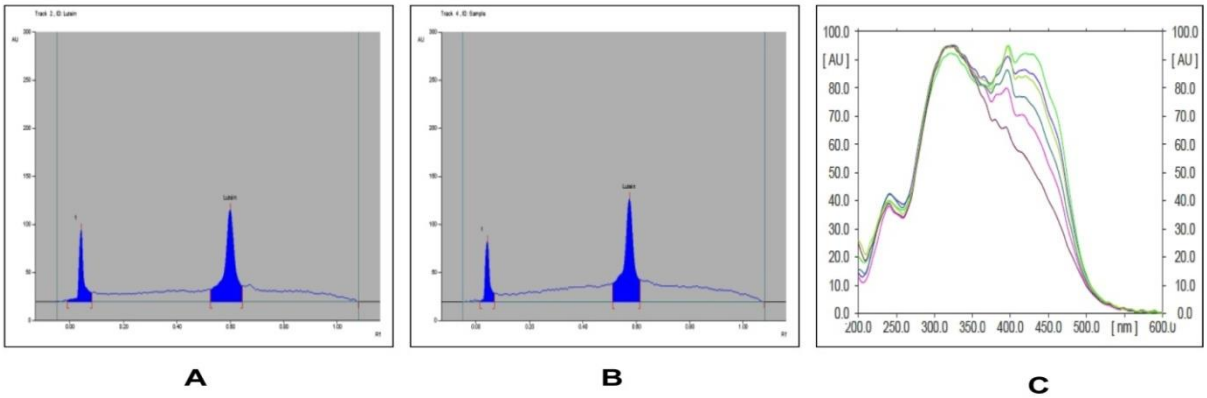


Figure 6: Chromatogram showing lutein in track 2 (A) and isolated lutein in track 4 (B) and superimposed spectra of lutein (C) in 200nm to 600 nm spectral range.

sparse vegetation mainly composed of scrub species and along flat lands where monsoons occur[13].

In West Bengal, although the sweet sugary sap of *Phoenix sylvestis* is very popular, but the fruits of Sugar Date Palm is considered as a minor fruit. In our previous study we

have established the strong α- glucosidase, α- amylase[14] and ACE inhibitory activities[15] of the aqueous extract of this fruit pulp. But the rich occurance of carotenoids in this kind of less popular or underutilized fruit crop are not yet studied or reported. The purpose of the present study was to isolate, and identify the carotenoid composition of unripe but mature stage of the *Phoenix sylvestris* fruit epicarp and inflorescence axis.

MATERIALS AND METHODS

Plant Material

4.130 Kilograms of unripe fruits along with the entire inflorescence axis of *Phoenix sylvestris* were collected from a date palm farmer of Midnapur district, West Bengal, India during summer on the month of May, 2012 and stored at -20ºC prior to analysis. The oblong fruits were 1 inch long and occured in orange clusters, turning dark red when ripened. The inflorescence axis was orange in colour at the time of collection. The orange coloured (mature but unripe stages) fruits and the inflorescence axis were our plant part of interest to determine their carotenoid profile.

Extraction

Carotenoids were extracted by modifying the previously reported procedure[16]. First of all the epicarp and mesocarp tissues were separated from each fruit after protecting them from heat and light as far as possible. The epicarpic tissues (about 134 gms) were grinded well with sand in a mortar and pestle using liquid nitrogen. The whole inflorescence axis was cut into small pieces and crushed in the same manner. Then the carotenoids were extracted with ice-cold acetone. An intense orange-yellow coloured solution was obtained. The upper coloured layer was decanted after 10 – 15 minutes. This process was repeated for 4-5 times until the samples became colourless. The coloured solution obtained was then centrifuged at10, 000 rpm for 20 minutes. The crude extract (orange in colour) obtained was taken in a separating funnel. To each 25 ml of crude extract obtained, 50 ml of (25% w/v) aqueous NaCl solution & 50 ml of Petroleum ether (boiling range 60º – 80ºC) were added in the same separating funnel. Just after gentle shaking a sudden change in colour (intense yellow) was observed. After mixing well the upper layer was separated out. The extraction was repeated for several times until and unless all the coloured materials came out. The extract was evaporated to dryness in a rotary evaporator without applying heat and without exposing into light. The dried material was stored at -20º C. All the steps were carried out under dimmed light to prevent isomerization and photodegradation of carotenoids.

Apparatus and Reagents

Acetone, petroleum ether (boiling range 60º - 80º C) and sodium chloride of analytical grade were purchased from Merck. Commercial carotenoid standards (lutein and β-carotene) used for identification were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the other reagents were of analytical grade. A Camag (Camag, Switzerland) HPTLC instrumental set-up consisting of sample applicator Linomat 5, TLC scanner 3 and Digistore 2 Documentation system was used for the analysis under the control of the software platform winCats 14.4 Planer Chromatography Manager (Camag).

HPTLC Instrumentation and Conditions

Chromatographic analysis was done on HPTLC plates (10 x 20 cm^2) precoated with silica gel 60 F_{254} (0.25 mm thickness). The dried isolated pigment fractions both from fruit epicarp and from whole inflorescence axis and the standard samples were dissolved in acetone and the aliquots were applied with the standard samples viz., β-carotene and lutein as 7- mm wide bands, 20 mm from the side edges, 8 mm from the bottom and 12 mm apart. Samples (8-15 µl) and standard compounds (8-15 µl) were applied on plates by means of Linomat 5 applicator (Camag, Switzerland)) equipped with a 100 µl microsyringe with the nitrogen flow providing a dosage speed of 150 nL / sec from the syringe. The plates were run in the solvent system of Petroleum ether (60 - 80º C): Acetone (70: 30, *v/v*) as mobile phase following the modified method of Boudries et al., 2007 [17] in a twin trough chamber 20 cm x 10 cm (Camag) lined with filter paper and saturated for 30 minutes with 10 ml of developing solution Petroleum ether (60 - 80º C): Acetone (70: 30) in each trough. Developing time was 15 minutes. After development, the plates were dried under a steam of dry, cool air.

Afterwards the tracks were scanned by Camag TLC scanner 3 in absorption mode at λ = 450 nm and spectra were recorded from 400 nm to 600 nm with Deuterium (D_2) and Tungsten (W) lamp source. Slit dimensions were 6.00 x 0.45 mm, and scanning speed was 20 mm /s.

Pigment isolation and identification using Thin Layer Chromatographic analysis

The carotenoid fraction extracted was first dissolved in a very little amount (1-2 ml) of acetone. The pigments were then separated and isolated by preparative TLC on silicagel 60 GF_{254} plates (20 × 20 cm^2) and co-chromatographed with standard pigments (β-carotene and lutein) in a solvent system of Petroleum ether (60 - 80º C): Acetone (70 : 30). The samples were applied as bands on 6 such plates. The plates were run after protecting them from light by wrapping the developing chamber under black cloth. The three bands developed after the solvent run were scraped and eluted separately in acetone. After centrifugation at 10,000 rpm for 20 minutes the supernatant was evaporated to dryness and stored in -20º C. At all the stages precautions were taken to protect the elutes from light and heat. Out of three band zones developed, two pigment bands were very prominent, the bands with lowest and the uppermost Rf coinciding with that of the standard lutein and β-carotene respectively. Compounds present in the very faint intermediate band of unknown pigments could not be identified.

RESULTS AND DISCUSSION

Crude carotenoids were extracted from the fruit epicarp and inflorescence axis of *P. sylvestris*. Carotenoids were isolated from the crude extracts by preparative TLC. The identity of the carotenoids was confirmed by HPTLC by

comparing the R_f values and UV-VIS absorbances of the isolated compounds with that of the authentic carotenoids. The major carotenoid of the fruit epicarp was β-carotene.

The fruit epicarp extract showed three bands on TLC plates during preparative TLC. The carotenoid obtained from the upper band after elution and further HPTLC was identified to be β-carotene. The lower band was identified to be that of lutein. The middle band could not be identified. The chromatographic characters (R_f and UV-VIS spectra) of the identified carotenoids co-chromatographed with the authentic carotenoids are presented in Fig. 1-3. The spectral characters of the unidentified middle band are shown in Fig. 4.

The carotenoid fraction obtained from the inflorescence axis showed a single band. After isolation, the carotenoid was confirmed as lutein. The chromatographic characters of the carotenoid are presented in Fig. 5 and Fig. 6.

This seasonal and underutilized fruit of West Bengal is mainly eaten by the rural people. Due to thin flesh, this fruit has not received commercial status like that of many other species of *Phoenix* particularly *P. dactylifera*. The findings of the present study suggest that *Phoenix sylvestris* fruits could be a dietary source of β-carotene and lutein and many other carotenoids not reported yet. The extensively large inflorescence axes, which are usually wasted after fruit senescence, may also be considered and utilized as a good source of the important carotenoid, lutein.

CONCLUSION

Our results show that HPTLC can be a very powerful technique in qualitative analysis of carotenoid profile. Furthermore, carotenoids are the most widespread and important pigments in living organisms. β-carotene, the orange coloured principal carotenoid, as well as the yellow pigment lutein were found in *Phoenix sylvestris* fruit epicarp. Only lutein was identified from the inflorescence axis by HPTLC analytical method. The role of carotenoid composition in human health is still an important area of research. Based on our current scientific report, *Phoenix sylvestris* fruit epicarp and inflorescence axis could be an important source of such pigments and consumption of this fruit offer great hope for prevention of vitamin A deficiency and other stress related chronic human diseases.

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