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Original Research Article

Identification and isolation of the pigments from M*usa acuminata* by TLC and HPLC method

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ABSTRACT

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The flower peel of the *Musa acuminata* have been possess the high antioxidant content and it is a good source for the Beta-carotene and it can be used for the health and its utilization. The flower peel was grinded with acetone to extract the pigment and dried. The extracted pigments were scrutinized using TLC applying different solvent system to identify the type of natural pigments and flower peel extract is compared with the standard Beta-carotene. The study was progressed to extract and find out the natural pigment from flower peel. The flower peel extract was determined for its total antioxidant, total carotenoids and Beta-carotene was measured spectrophotmetrically. Beta carotene content was evaluated for its quality and quantity by HPLC.

Keywords: *Musa acuminate,* pigment, TLC, Carotenoids

1. INTRODUCTION

Pigments are chemical compounds that absorb light in the wavelength range of the visible region. Produced color is due to a molecule-specific structure (chromospheres) this structure captures the energy and the excitation of an electron from an external orbital to a higher orbital is produced; the non-absorbed energy is reflected and/or refracted to be captured by the eye, and generated neural impulses are transmitted to the brain where they could be interpreted as a color. (Hari *et al*., 1994).

Pigments are functionally important molecules in photosynthetic organisms. They not only harvest the light energy necessary for carbon reduction but some serve to protect the organism from excess light. The balance of photosynthetic pigments is dynamic and contributes to the maintenance of photo stasis within the cell (Huner *et al*., 1998). In chloroplasts, chlorophyll and some carotenoids are lipophilic and occur as pigment–protein complexes within the thylakoid membrane. Therefore, the membrane has to be disrupted and the pigments must be separated from their associated proteins during their extraction. Carotenoids also occur within of the chloroplast envelope, particularly violaxanthin (Young, 1993).

Banana is referred as "Kalpatharu", a plant of all virtues, with each and every part of the plant being used for various purposes. The importance of banana as a food- fruit crop can hardly be exaggerated adding to its multifaceted uses as food, fibre, fuel and therapeutic values. Leaf is the most popular hygienic bio-plate for dining, male flower is much preferred as vegetable and for making pickles, and stem is also a vegetable in demand with lot of therapeutic uses. Banana is also a good fiber yielding plant and its corm is mostly exploited as animal feed, as composite mixture with others (Uma *et al.,* 2005). Bananas and plantains are therefore the important components of food security in the tropical world and they provide income to the farming community through local and international trade. The two species *M. acuminata* and *M. balbisiana* are widely distributed *M. acuminata* though originated either in Malaysia (Simmonds, 1962), they are predominantly spread in Pacific Islands, Indonesia, Malaysia, Philippines, Myanmar and India. The aim of present study is to extract, and identify the pigments by TLC & HPLC from *Musa acuminata* flower peel.

2. MATERIALS AND METHODS

2.1. PLANT COLLECTION AND EXTRACTION

The flower (*Musa acuminate)* was collected from local vegetable market in Puducherry. Freshly collected flower were grinded with acetone using an electric blender and the filterate was used for the identification of pigments.

2.2. IDENTIFICATION OF PIGMENTS BY TLC

The Acetone extract was monitored by thin layer chromatography. Readymade TLC plates (TLC silica gel 60 F254) were used for the analysis. Petroleum ether (40-60^{0}C): Acetone (7:3, v/v) was used as TLC Mobile phase. The sample spot was applied above 0.5cm of TLC and eluted on the mobile phase. Once it reached the solvent front, the TLC was removed from the Chamber and immediately observed the results due to evaporation and degradation of pigments. The different colors and R_f values was noted. The R_f values of Acetone extract was compared with the standard Spinach R_f values.As the pigments have its own colour and visualized by the eye in a fluorescent lamp light (Around 150-300lux) and Retention value was calculated by using the below mentioned formula.

$$R_f = \frac{\text{Migration distance of solute}}{\text{Migration distance of solvent}}$$

2.3. DETERMINATION OF ANTIOXIDANT PROPERTY BY DPPH METHOD

The free radical scavenging activity was performed by using DPPH as the diluents. The molecule of 1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 520nm.When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, and then this gives rise to the reduced form with the loss of this violet color.

Prepared 0.1 M DPPH in methanol and allowed it to completely soluble and wrapped the vial using foil as it is light sensitive. Transferred 1 ml of 0.1M DPPH alone in a tube and 1 ml of respective sample's in a separate tube. A fresh tube was taken and added 1ml of respective sample and 1 ml of 0.1M DPPH. Mixed well. Incubated the mixture in dark for 30 minute at room temperature. Measured the absorbance at 517 nm using 0.1 M DPPH as standard and methanol as blank. Repeated the procedure for different dilution or sample.

2.4. ESTIMATION OF TOTAL CAROTENOIDS

The extraction was carried out as described by Kimura and Rodriguez-Amaya (2004), with slight modifications. The portion of the about 1.0g of sample was ground in a mortar and pestle of the homogenized sample was transferred to a mortar and 0.3 g of $MgCO_3$ was added. The mixture was ground with 25 ml of cold acetone (refrigerated for about 2 hours). The extract was filtered using a Whatmann filter paper (Kimura and Rodriguez-Amaya., 2004)

Twenty (20ml) of Petroleum ether were pipetted into a separating funnel with Teflon stopcock. Fifteen (15 ml) of the acetone extract were added and allowed to stand for 15 minutes. One hundred and fifty (150) ml of distilled water were added by flowing along the walls of the funnel. The mixture was allowed to separate into two phases, and the aqueous phase was discarded. The petroleum ether phase was washed 4 times with 100 ml of distilled water to remove residual acetone. The petroleum ether phase was collected in a 25 ml volumetric flask by passing the solution through a small funnel containing 7.5 g of anhydrous sodium sulfate to remove residual water. The separating funnel was then washed with petroleum ether and the washing was collected into the volumetric flask by passing it through the funnel with sodium sulfate. The volumetric flask was then made up to volume with petroleum ether and the total carotenoids content were determined from the molar absorptive Beta-carotene $E^{1\ cm}_{1\%}$ = 2590 at λ max 450nm, derived from the standard plots. The samples extracted with the petroleum ether solvent were scanned between 350 and 500nm using UV-Vis Scan Spectrophotometer and it shows individual peaks for respective carotenoids with their optical densities. The extracted carotenoid from each experimental sample was diluted to approximate

volume as to be obtaining the optical density value for that the solvent used for the carotenoid extraction were used. After proper dilution, the optical density was measured at 400-500nm. Total carotenoid in the sample was then estimated by using the formula given below.

$$\text{Total carotenoid content } (\mu g/g) = \frac{A \times \text{Volume (ml)} \times 10^4}{A^{1\%}\ 1\text{cm} \times \text{sample weight (g)}}$$

2.5. ESTIMATION OF BETA-CAROTENE PURITY BY HPLC

the acetone extract of sample was analyzed for the estimation of beta carotene by HPLC. An isocratic HPLC system (WATERS HPLC) consisting of WATERS 510 pump, UV visible detector (WATERS UV 486), a ODS C-18 Supelco C18 ODS (250× 4.6mm× 5µm) Rheondyne injection syringe and Windows-based Autochro 3000 software is used for analysis.

a. Preparation of Mobile phase: The mobile phase was prepared by mixture of 700 volumes of Acetonitrile, 200 Volumes of Dichloromethane and 100 volumes of Methanol, filtered and sonicated in a sonication bath.

b. Preparation of standard beta carotene solution: Standard of beta carotene (1g enclosed in vial) was obtained from Merck. Stock solution of beta carotene was prepared by taking 10mg in 100ml with mobile phase. The concentration of stock solution was equal to 100 ppm.

c. Sample Preparation: Sample was prepared by taking mg of betacarotene purified extract in 50 ml with mobile phase.

Chromatographic Condition:

Chromatograph the standard preparation and record the peak responses as directed under procedure. Five replicates of the injections to be injected and % Relative Standard Deviation is not more than 2.0 %. Before injecting the sample, the samples were filtered through micron filter paper (0.45 µm) and taken in a syringe and then injected on the HPLC.. Twenty microlitres of solution was injected into HPLC system to obtain chromatogram for standard solution (five replicates) and sample solution (two replicates).

Purity of beta carotene as %:

$$\%\ \text{Purity} = \frac{\text{Sample area} * \text{Standard wt} * \text{Std Dilution} * \text{Std purity} * 100}{\text{Standard area} * \text{Spl Dilution} * 100}$$

3. RESULT AND DISCUSSIONS

The study was progressed to find out the natural pigment from flower peel. The flower peel was grinded and was dried to extract the pigment aiding acetone to extract the pigments. The extracted pigments were scrutinized using TLC applying different mobile phase to identify the type of natural pigments, the experimental possess in it. Pigments are generally identified by using thin layer chromatography and in this case, flower peel is used as a standard to spot out the Beta-carotene after running the TLC and its R_f Values are calculated and listed in the table.1 where the R_f Values

compared with the beta carotene (STD) value. In the sample, there is an orange and yellow spot near to beta-carotene (STD) spot and the R_f values are near to 0.64 and 0.62, by the lecture review it was confirmed as a Beta-carotene.

Figure 1: Extract, TLC analysis & Carotenoid analysis of *Musa acuminate* flower peel

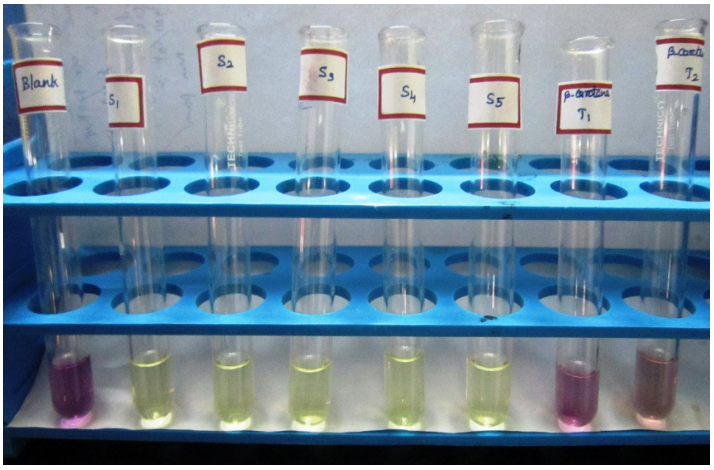


Figure 2: Total Antioxidant activity of *Musa acuminata* flower peel by DPPH method

Table: 1. R_f values of Betacarotene (Std) and Petroleum ether crude extract of *M.acuminata*

Compound Name	**R_F Value**	**Colour**
Beta Carotene (Std)	0.62	Orange
Crude	0.64	Orange

Table: 2. Estimation of Total Carotenoid, Total Antioxidant and Beta-carotene

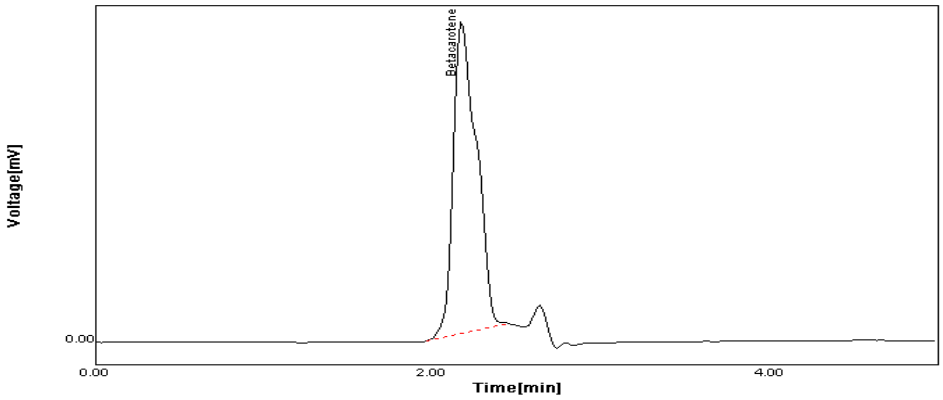
Name of the Sample	Beta carotene / in g	Total Carotenoid /ml	Total Antioxident (DPPH)
M.acuminata	0.44mg	0.56mg	83.6%

Recently, the results of observational studies on supplemental β-carotene raised an active discussion regarding the role of carotenoids and other antioxidants in humans. Based on current scientific knowledge, the situation of antioxidant action of carotenoids *in vivo* is not clear. Although carotenoids have been proposed to prevent diseases through antioxidant action there is a need for further understanding in terms of the basic chemical and biochemical principles (Britton, 1995; Edge *et al*., 1997), rather than hypothesized from indirect associations from diet. In all, a number of areas of research need more investigation to have a more complete picture of the role of beta-carotene and other carotenoids in humans. It should be stressed that antioxidant mechanism is only one proposed mechanism for protective effects of carotenoids against cancer; others include regulation of gap functional communication, modulation of immune response and retinoid-like effects on cellular differentiation (Omaye *et al*., 1997)

Beta-carotene a pigment extracted and evaluated for its purity by HPLC (Figure-3 & 4) and the results are in the Table.2 respectively, where the antioxidant quantity of Beta-carotene of *Musa acuminata* flower peel were performed and the results are in the table2. Antioxidant assay determines the percentage of scavenging activity and the results of *Musa acuminata* flower peels possess low content of Beta-carotene among the total carotenoids.

Analysis:
Sample Name: STANDARD -1
Sample ID: BETACAROTENE
Date: 2014-05-29 PM 04:43:38

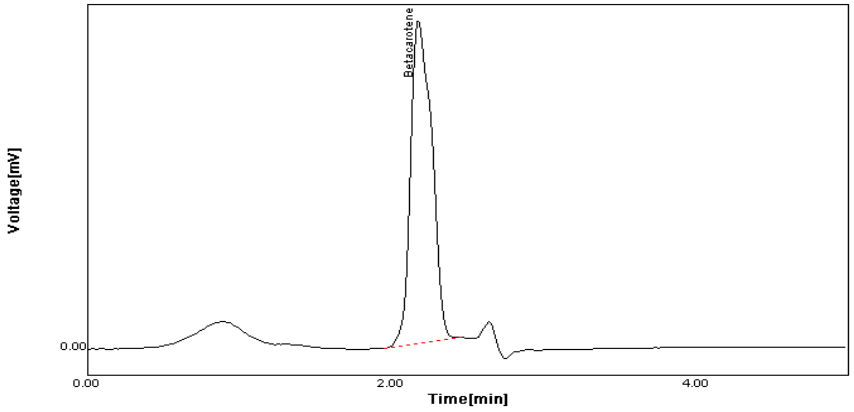
Chromatogram



No.	Name	RT[min]	Area[mV*s]	Height[mV]
1	Betacarotene	2.1667	459.2546	47.4573
Sum			459.2546	47.4573

Analysis
Sample Name: STANDARD 2
Sample ID: BETACAROTENE
Date: 2014-05-29 PM 04:50:55

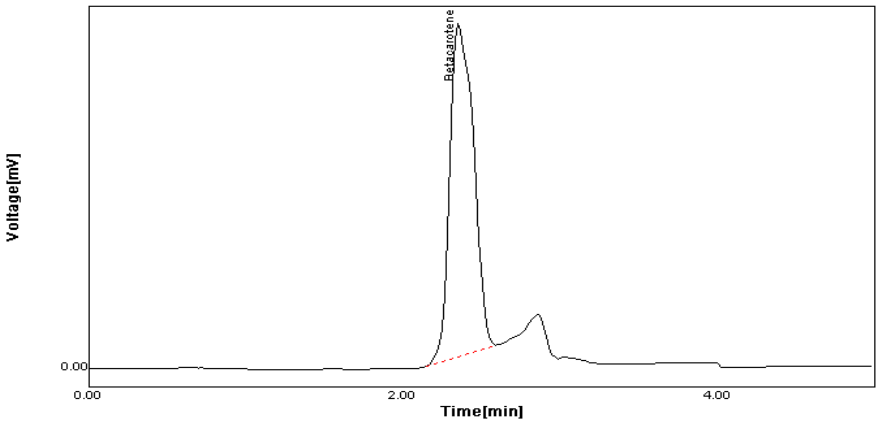
Chromatogram



No.	Name	RT[min]	Area[mV*s]	Height[mV]
1	Betacarotene	2.1667	465.1270	47.5402
Sum			465.1270	47.5402

Analysis
Sample Name: STANDARD 3
Sample ID: BETACAROTENE
Date: 2014-05-29 PM 04:56:32

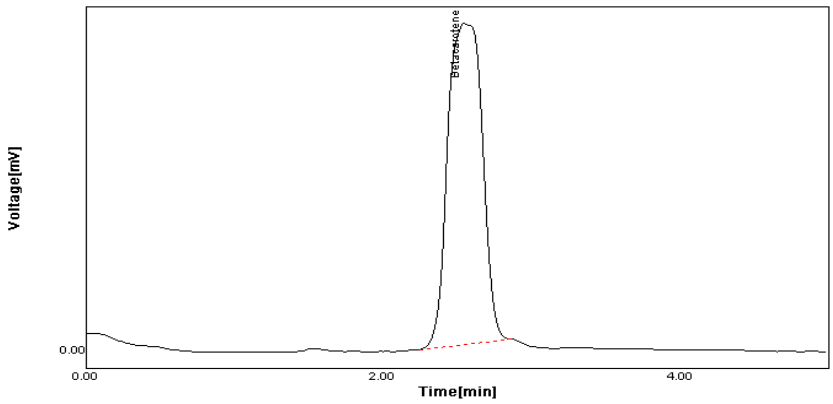
Chromatogram



No.	Name	RT[min]	Area[mV*s]	Height[mV]
1	Betacarotene	2.3500	484.6666	46.6966
Sum			484.6666	46.6966

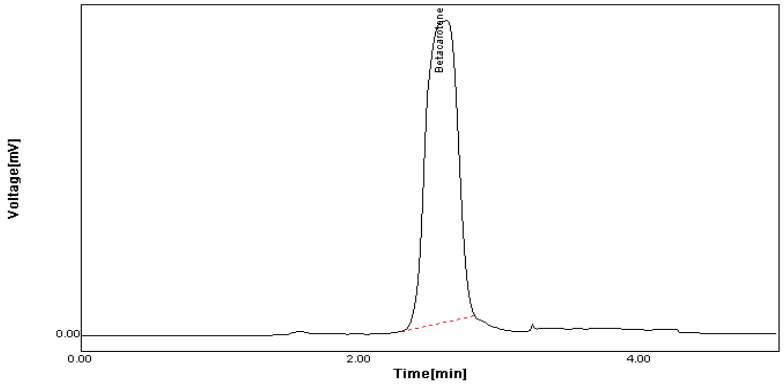
Figure 3: HPLC Analysis of Standard Beta carotene (Standard1, 2 & 3)

Analysis
Sample Name: SAMPLE
Sample ID: MUSAACCUMINATA
Date: 2014-05-29 PM 05:05:53
Chromatogram



No.	Name	RT[min]	Area[mV*s]	Height[mV]
1	Betacarotene	2.5333	1061.9277	67.5176
Sum			1061.9277	67.5176

Analysis
Sample Name: SAMPLE 2
Sample ID: MUSAACCUMINATA
Date: 2014-05-29 PM 05:13:11
Chromatogram



No.	Name	RT[min]	Area[mV*s]	Height[mV]
1	Betacarotene	2.6167	1020.0425	66.4085
Sum			1020.0425	66.4085

Fig: 4. HPLC Analysis of *Musa acuminate* flower extract

4. CONCLUSION

The flower peel of the *Musa acuminata* have been possess high antioxidant content and it is a good source for carotenoids and it can be used for the health and its utilization. When we are using the thin layer chromatography by using the mobile phase of (petroleum ether) in the ratio of 100% the R_f Value is around 0.64 and 0.62 by this we can confirm the presence of Beta-carotene. Beta carotene is present in the notable amount in the leaves of the plants which we through as the waste. The flower peels having high percentage of Antioxidant activity, proven by in-vitro method. To this, the flower peel transferred as the nutritional supplement because of Beta-carotene and a rich source of antioxidant. With this the banana peel acts as rich source of plant secondary metabolite.

5. REFERENCES

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests regarding the publication of this paper.