NEW SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF β-CAROTENOID CONTENT IN THE FRUIT EXTRACTIONS OF CAPSICUM FRUTISCENS

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Abstract: **The experiment was carried out on capsicum fruits which include spectrophotometric determination of β-carotene by using cyclohexane as solvent. The extraction of capsicum was done with cyclo hexane and the absorbance was determined by UV Spectrophotometer. β-carotene obtained from Red pulp from Red capsicum shows maximum absorbance like pure drug. Remaining extracts have the order of maximum β-carotene are Red peel, Green pulp, Green peel respectively. This study reveals that, Red capsicum contains more amount of β-carotene when compared with Green capsicum. The Beer's Law Limits, sensitivity data (Correlation coefficient, Regression equation, Precision data, Accuracy data) are in near correlation with the pure drug. It is also proved that the extracted β-carotene has nearly 80o% anti oxidant activity.**

1. INTRODUCTION

Beta-carotene was first isolated by Wackenroder in 1831, and many other carotenoids were discovered and named during the 1800s, although their structures were still unknown. Not until 1907 was the empirical formula of beta-carotene, $C_{40}H_{56}$, established by Willstatter and Mieg. The structure was elucidated by Karrer in 1930-31. This was the first time that the structure of any vitamin or provitamin had been established, and he received a Nobel Prize for his work.

Steenbock suggested in 1919 that there could be a relationship between beta-carotene and vitamin A. The concept of provitamins (molecules which are converted into vitamins by the body) was entirely new, and proved to have great significance scientifically and commercially.

The first total syntheses of beta-carotene were achieved in 1950, and Roche started producing it commercially in 1954. Various studies were carried out throughout the 1970s-80s to determine its suitability for use in food, and its activity in the body. In the early '80s it was suggested that beta-carotene might be useful in preventing cancer, and it was found to be an antioxidant. More recently beta-carotene has been claimed to prevent a number of diseases, including cystic fibrosis and arthritis, and there is a flourishing trade in vitamin supplements containing beta-carotene.

Green Capsicum

Red Capsicum

Capsicums are beautifully colored fruits available in green, red, and yellow colours. They are mild-flavoured and used as vegetables. They are known to be sweet peppers and are named differently according to where it comes from. It is a vegetable that is adored all over the world not only for its delicious taste but also for its benefits to human body.

2. UV SPECTROPHOTOMETRIC DETERMINATION OF β-CAROTENE

EXPERIMENTAL:

Hot Ethanol Extraction of Capsicum Fruits:

Materials and Sample Pre-Treatment

A systematic selection of fresh capsicum fruits collected from a local market. The different types of capsicum like Red and Green were used in our study. The capsicum fruits were thawed, grinded and homogenized using a blender. About 2 g of the grinded samples were used for the extraction. When dispersing agents were used, they were mixed together with the weighed-in samples before transferring the whole mixture to the extraction cell. These extractions are admixed with the solvent Ethanol. After that they are transferred to the extraction cell. The extraction process is carried up to 20 minutes. Then after the extract was collected and filtered using wattsman filter paper. The filtrate was collected and it is subjected to evaporation. Then after it was dried by natural drying. Finally a powdered form of β-carotene was obtained. This form of beta carotene was used for the analysis by UV Spectrophotometer.

PREPARATION OF REAGENTS:

PREPARATION OF SOLUTION A: 50mg of beta carotene is weighed accurately and it was added with 10 ml of chloroform, make it to dissolve completely and volume made up to 100ml in volumetric flask, with cyclohexane.

PREPARATION OF SOLUTION B: Pippet out 5ml of solution A and make up to 50 ml in volumetric flask with cyclohexane. From solution A, take 0.2, 0.4, 0.6, 0.8 and 1.0 ml respectively and make up the volume to 10ml with cyclohexane.

INSTRUMENT:

UV SPECTROPHOTOMETER.

The absorbance was noted as follows.

The absorbance of solution B is determined at 455nm and at 483 nm using cyclohexane as blank. The absorbance of solution B at 455nm and that of A at 340 nm are determined.

PROCEDURE:

Evaluation of antioxidant activity based on coupled oxidation of ß-carotene and linoleic acid.ß-carotene (6mg) was dissolved in 20 ml of chloroform. A 3 ml of the solution was added to a conical flask with 40µl of linoleic acid and 400µl of Tween 20. Chloroform was removed with a evaporator under vacuum at 35ºC. Oxygenated distilled water (100 ml) was added to the ß-carotene emulsion and mixed well.3 ml aliquot of the ß-carotene emulsion and 0.2 ml of the diluted extract were placed in a test tube and mixed well. The tubes were immediately placed in a water bath and incubated at 50ºC. Oxidation of ß-carotene emulsion was monitored by measuring absorbance at 470 nm. Sample absorbance was measured at 60 min after incubation. A control consisted of 0.2 ml distilled water, instead of the extract using distilled water as blank. The degradation rate of the extracts was calculated by first order kinetics:

3. RESULTS AND DISSCUSION:

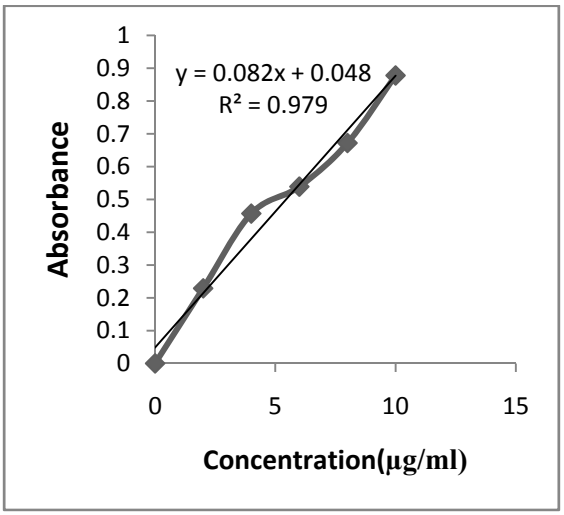

Fig.1: Normal plot of standard drug

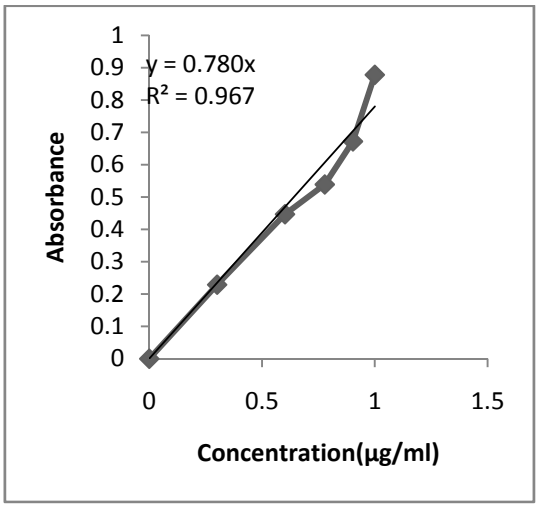

Fig.2: Ringbom plot of standard drug

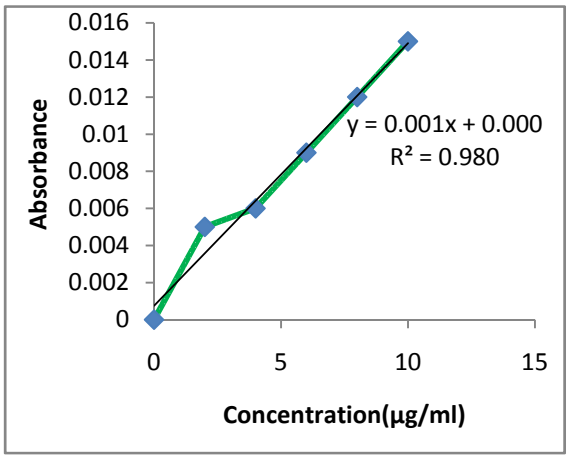

Fig .3: Normal plot of green peel

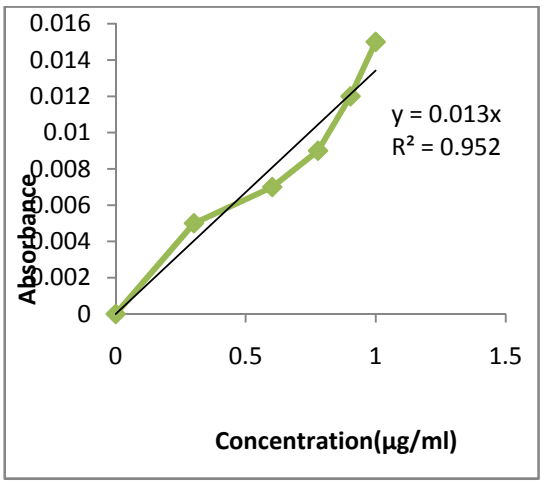

Fig .4: Ringbom plot plot of green peel:

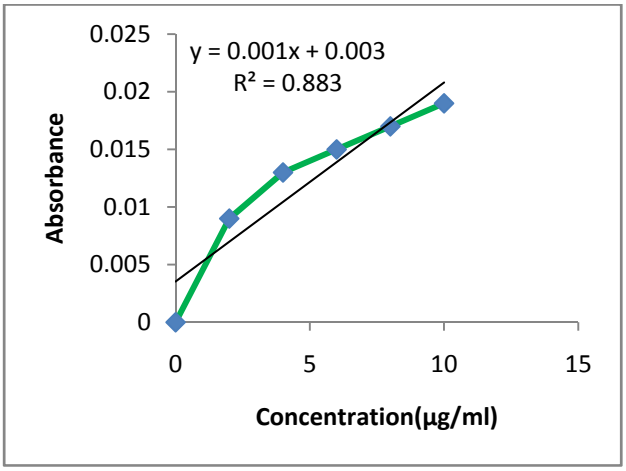

Fig. 5: Normal plot of green pulp

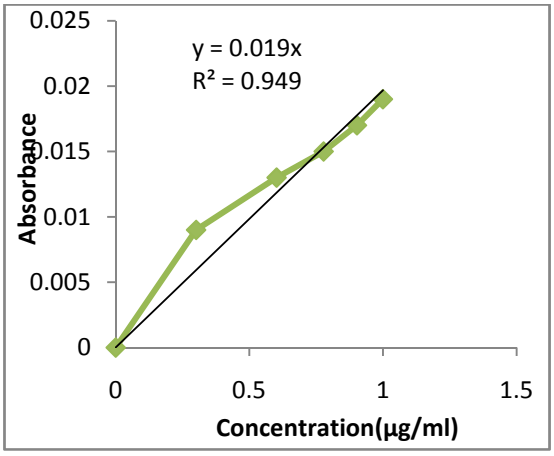

Fig .6: Ringbom plot of green pulp

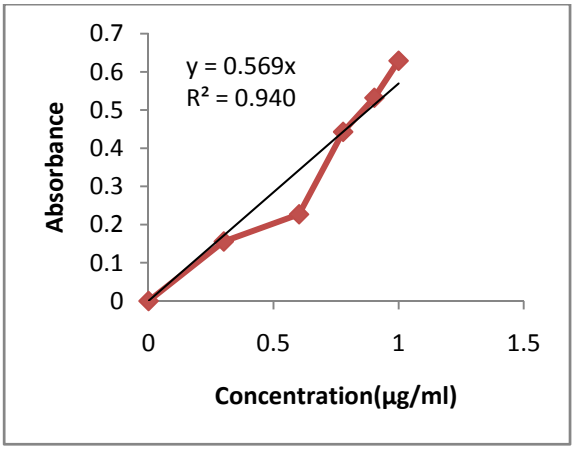

Fig.7: Normal plot of red pulp

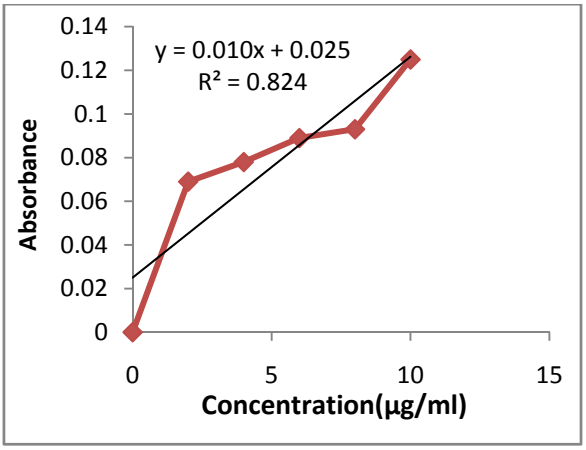

Fig .8: Ringbom plot of red pulp

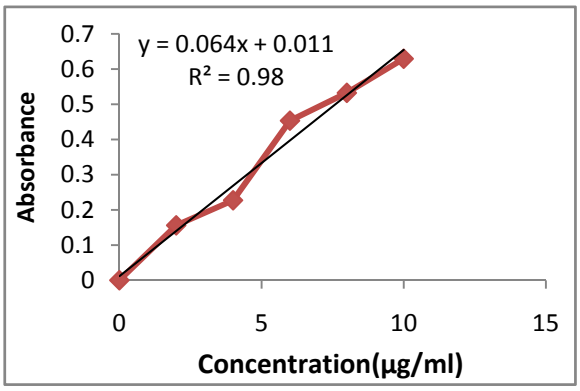

Fig .9: Normal plot of red peel

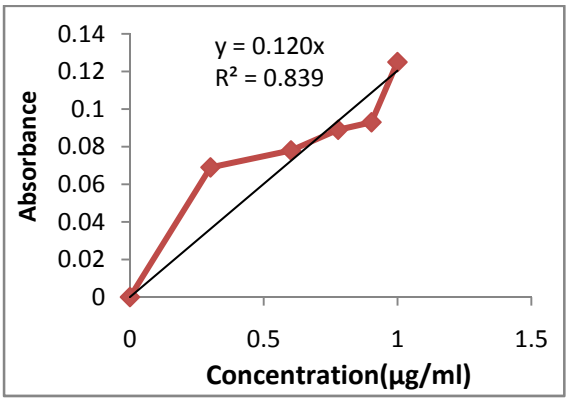

Fig .10: Ringbom plot of red peel

Table 1: Results of sensitivity of various extracts

Parameter	Beer's Limit (µg/ml)(c)	Correlation Coefficient	Regressionequation (I + aC): Slope; Intercept
Standard	2-4	0.979	0.082; 0.048
Green peel	4-10	0.980	0.000; 0.001
Green pulp	4-10	0.883	0.00; 0.003
Red peel	6-10	0.98	0.064; 0.011
Red pulp	2-8	0.824	0.010; 0.025

Table 2: Results of absorbance values of various extracts

SOLUTION			
Parameter	**Wave length**	**A**	**B**
Absorbance of pure drug at different wave lengths	455nm	0.965	0.599
	483nm	--	0.598
	340nm	0.588	--
Absorbance of green pulp at different wave lengths	455nm	0.043	--
	483nm	0.028	0.029
	340nm	0.079	--
Absorbance of red pulp at different wave lengths	455nm	0.956	0.583
	483nm	--	0.509
	340nm	1.37	0.463
Absorbance of green peel at different wave lengths	455nm	0.043	0.028
	483nm	--	0.029
	340nm	0.079	--
Absorbance of red peel at different wave lengths	455nm	0.738	0.483
	483nm	--	0.476
	340nm	0.729	--

EVALUATION OF ANTIOXIDANT ACTIVITY:

Sample degradation rate = ln (a/b) ×1/t,

where: ln = natural log;

a = initial absorbance at time 0;

b = absorbance at 60 min;

t =time (min).

Antioxidant activity (AA) was expressed as % inhibition relative to the control using the equation:

Determination of antioxidant activity:

$$\%Degradation = \log(a/b) \times 1/t$$

$$\%\ Antioxidant\ activity = Degradation\ of\ control - Degradation \frac{of sample}{of\ control}$$

Solution type	**Absorbance at 455 nm**	
	Before Incubation	**After Incubation**
Sample	1.588	1.405
Blank	0.287	0.154

CALCULATION:

Standard:

$$\%Degradation = \log\left(\frac{1.588}{1.405}\right) \times \frac{1}{60}$$

= log 1.13 × 1/60

= 0.053/60

= 0.0008

Test:

$$\%Degradation = \log\left(\frac{0.287}{0.154}\right) \times \frac{1}{60}$$

= 0.269 × 1/60= 0.004

$$\% \, Antioxidant \, activity = 0.004 - \frac{0.0008}{0.004} \times 100$$

$$= 0.8 \times 100$$

$$= 80\%$$

4. RESULTS AND CONCLUSION

The experiment carried out on capsicum fruits which include spectrophotometric determination of β-carotene by using cyclohexane as solvent.It opens with the introduction about β-carotene includes its history, chemical & physical properties, sources, health benefits deficiency symptoms capsicum health benefits etc.., The extraction of capsicum was done with cyclo hexane and the absorbance was seen in UV Spectrophotometer. The results obtained shows that the pure drug which is used to compare with extracted crude drug has maximum specified amount of β-carotene when analysed in UV shows maximum absorbance. In our extractions β-carotene obtained from Red pulp from Red capsicum shows maximum absorbance like pure drug. Remaining extracts have the order of maximum β-carotene are Red peel, Green pulp, Green peel respectively. This study reveals that, Red capsicum contains more amount of β-carotene when compared with Green capsicum. In each case like, the Beer's Law Limits, sensitivity data (Correlation coefficient, Regression equation, precision data, Accuracy data)are in near correlation with the pure drug. It is also proved that the extracted β-carotene has nearly maximum anti oxidant activity.

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