A

PROJECT-REPORT ON

"Study of estimation of vitamin C and extraction of betacyanin pigment from dragon fruit as a tracking dye for gel electrophoresis"

SUBMITTED BY,

Mr. Aniket Balasaheb Kadam

SUBMITTED TO,

VIVEKANAND COLLEGE, KOLHAPUR

DEPARTMENT OF BIOTECHNOLOGY

FOR PARTIAL FULFILMENT OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY

THE YEAR - 2022-2023

UNDER THE GUIDANCE OF,

Mr. S. G. Kulkarni (H.O.D- Biotech. Entire) and

Mr. A. L. Upadhye

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"Education for Knowledge, Science and Culture"

- Dr Bapuji Salunkhe

Vivekanand College (Autonomous), Kolhapur

Department of Biotechnology

Certificate

This is certified that Mr. /~~Ms.~~ Aniket Balasaheb Kadam

Roll No 9315 has satisfactorily completed his/her Project work on the topic Estimation of vitamin C and Betacyanin as a tracking dye., as a part of syllabus prescribed by Board of Studies, Department of Biotechnology, Vivekanand College (Autonomous), Kolhapur for B.Sc. III (Entire) Biotechnology and this report represents his/her bonafied work in year 2022 - 2023.

Date: 28-04-2023

Place: Kolhapur

VIVEKANAND COLLEGE ESTD. JUNE 1964 KOLHAPUR-416 003

Project Guide

Examiner 28/4/2023

Head 28/4/23.

Department of Biotechnology (Ent
Vivekanand College, Kolhapur (Auton

DECLARATION

I hereby declare that the project work entitled "**Estimation of Vitamin C from Dragon Fruit and use of natural pigment Betacyanin as a tracking dye, extracted from dragon fruit for gel electrophoresis"** submitted to Vivekanand College, Kolhapur for the award of the degree of "Bachelor of Science in Biotechnology" is the result of bonafied work carried out by me under the guidance of **Asst. Professor and H.O.D-Biotech entire Mr. S. G. Kulkarni and Asst. Professor. Mr. A. L. Upadhye.**

I further declare that the results presented here have not been the basis for the reward of any other degree.

Place: Kolhapur

Date: 28-04-23.

Akadam.

Mr. Aniket Balasaheb Kadam.

Department of Biotechnology (Entire), Vivekanand College, Kolhapur.

ACKNOWLEDGEMENT

This project work is a successful outcome of the contribution and guidance of other person which I express my deep gratitude.

I also express our thanks to **Prof. Mr. S. G. Kulkarni** Head of Department of Biotechnology (Entire), Vivekanand College, Kolhapur for availing me with the laboratory facilities to the biotechnology Department to carry experiment work.

I also express our gratitude towards Pro. Mr. A. L. Upadhye and Pro. Mr. S. G. Kulkarni, my project guide for their guidance and who gave me encouragement and support throughout the course of study so that could complete my project work.

I also wish to express my gratitude to the laboratory staff for completing the project work. Lastly, I express my gratitude to my parents, all our friends and classmates for their support and co-operation. I am also grateful to all those who have directly or indirectly supported me in completion of this work.

Akadam.

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AIM AND OBJECTIVES

In this study there are two types of experiment have been done, they are as follows:

- **Estimation of Vitamin C from white fleshed dragon fruit juice (Hylocereus undatus)**

AIM 1:

To estimate vitamin C from white fleshed dragon fruit juice (Hylocereus undatus) by using titrimetric methods:

a) by 2,6-Dichlorophenol Indophenol Method,

b) by Iodometric method.

OBJECTIVE 1:

Describe the various methods of estimation of ascorbic acid and compare the ascorbic acid content by different methods.

- **Natural pigment Betacyanin as a tracking dye for gel electrophoresis, isolated from red fleshed dragon fruit (Hylocereus polyrhizus)**

AIM 2:

To use natural pigment betacyanin as a tracking dye for gel electrophoresis isolated from red fleshed dragon fruit (Hylocereus polyrhizus).

OBJECTIVE 2:

Betacyanin isolated from red fleshed dragon fruit as alternative to conventional dye like bromophenol blue used in the loading dye preparation for agarose gel electrophoresis.

Fig. 3) Hylocereus undatus

Fig. 4) Hylocereus polyrhizu

ESTIMATION OF VITAMIN C

❖ ESTIMATION OF VITAMIN C (ASCORBIC ACID) BY 2,6-DICHLOROPHENOL INDOPHENOL METHOD

A. MATERIALS:

1) APPARATUS

Burette- 50 ml

Pipettes- 5 ml, 10 ml

Conical flask- 100 ml, 250 ml

Measuring cylinders

Beakers

Glass marker

Glass wool

Funnel

Whatman No.1 filter paper

Mortar and pestle

100 ml volumetric flask

2) REAGENTS

2,6 dichlorophenol indophenol solution

3% metaphosphoric acid

6% metaphosphoric acid

Dragon fruit juice sample (Hylocereus undatus)

Ascorbic acid standard solution (20 mg/100 ml)

D/W.

B) METHODS:

REAGENT PREPARATION:

Standard ascorbic acid (20mg/100ml):

- 20 mg of ascorbic acid prepared in 100 ml 3% metaphosphoric acid.
- 3% metaphosphoric acid:
- 3 gm of metaphosphoric acid in 50 ml water. This solution is mixed properly after boiling and diluted to 100 ml.
- 2,6 Dichlorophenol Indophenol solution:
- 40mg of $NaHCO_3$ dissolved in 90 ml of hot water. 50 mg of 2,6 Dichlorophenol Indophenol is added, this solution is heated, filtered and after filtration diluted to 100ml.

STANDARD TITRATION:

- Pipette 10 ml of standard ascorbic acid solution into a 100 ml conical flask.
- Fill the burette with the dye solution.
- Titrate the solution against the dye solution till a light pink color appears which persists for 30 seconds.
- Standardize the dye solution.

SAMPLE PREPARATION FOR TITRATION:

- Sample and standard preparation and quantification of vitamin C in titration method was performed using the method described by Tee et al. (1996).
- In brief, fruit samples (200 g-300 g) were blended with 6% metaphosphoric acid (HPO_3) with an equal volume to homogenous slurry and made it up until 500 ml of volume.
- The homogenous mixture was measured around 10 to 30 ml and diluted into 100 ml volumetric flask with 3% of metaphosphoric acid (HPO_3).
- The diluted sample was then filtered to remove away suspension using vacuum pump before 10 ml aliquote of the filtrate was pipetted into a small Erlenmayer flask.
- The filtrate was immediately titrated with a dye solution 2, 6-dichlorophenolindophenol to a faint pink end point. Triplicate titration was conducted for all samples.

PRECAUTIONS

- Rinse all glassware with 3% metaphosphoric acid before you begin your practical and subsequently each time you wash your flasks, cylinders, etc.
- Make up all volumes with 3% metaphosphoric acid.
- Use a stainless-steel knife for cutting the lemon.
- After cutting the dragon fruit, immediately pour the juice into 6% metaphosphoric acid.

RESULTS

❖ **ESTIMATION OF VITAMIN C (ASCORBIC ACID) BY 2,6-DICHLOROPHENOL INDOPHENOL METHOD**

STANDARD TITRATION:

- Solution in burette: 2,6 Dichlorophenol Indophenol.
- Solution in conical flask: standard ascorbic acid solution
- Endpoint: colourless to pink.

SR.NO.	BURETTE READING (ml)		
	INITIAL	FINAL	DIFFERENCE
1	0.0	10.2	
2	0.0	10.2	10.2
2	0.0	10.2	

Titration reading = A ml = 10.2 ml

SAMPLE TITRATION:

- Solution in burette: 2,6 Dichlorophenol Indophenol.
- Solution in conical flask: dragon fruit juice sample
- Endpoint: colourless to pink.

SR. NO.	BURETTE READING (ml)		
	INITIAL	FINAL	DIFFERENCE
1	0.0	1.5	
2	0.0	1.5	1.5
3	0.0	1.5	

Titration reading = E ml = 1.5 ml

CALCULATION:

i)Strength of standard ascorbic acid solution = **20 mg/100 ml**

ii) Volume of standard ascorbic acid solution = **10 ml**

iii) Volume of dye solution required

iv) **(A) 10.2 ml** of dye solution is reduced by 10 ml of standard ascorbic acid solution but 20 ml of standard ascorbic acid solution contains 4 mg of ascorbic acid hence, (A) 10.2 ml of dye solution is reduced by 4 mg of ascorbic acid.

v) Volume of 6% HPO3 = **X ml = 10 ml**

vi) Volume of 6% HPO3 + dragon fruit juice = **Y ml = 20 ml**

vii) Volume of dragon fruit juice = **y – x = Z = 10 ml**

(Z) 10 ml of dragon fruit juice diluted to 100 ml Volume of dilute dragon fruit juice = 10 ml

viii) Volume of dye solution required,

ix) (A) 10.2 ml of dye solution is reduced by 4 mg of ascorbic acid

hence, I 1.5 ml of dye solution is reduced by:

$$\frac{4 \times E}{A} = \frac{4 \times 1.5}{10.2} = \mathbf{F = 0.5882\ mg}\ \text{ascorbic acid}$$

x) But I 1.5 ml of dye solution is reduced by 10 ml of dilute dragon fruit juice

hence, 10 ml of dilute dragon fruit juice contains (F) 0.5882 mg ascorbic acid.

100 ml of dilute dragon fruit juice contains:

$$\frac{F \times 100}{10} = \frac{0.5882 \times 100}{10} = \mathbf{G = 5.882\ mg}\ \text{ascorbic acid}$$

xi) But 100 ml of dilute dragon fruit juice contains **(Z) 10 ml** of dragon fruit juice

(Z) 10 ml of dragon fruit juice contains (G) 5.882 mg ascorbic acid

Hence, 100 ml of lemon juice contains:

$$\frac{G \times 100}{Z} = \frac{5.882 \times 100}{10} = H = 58.82 \text{ mg ascorbic acid.}$$

RESULT:

A 100 ml of dragon fruit juice contains 58.82 mg of ascorbic acid.

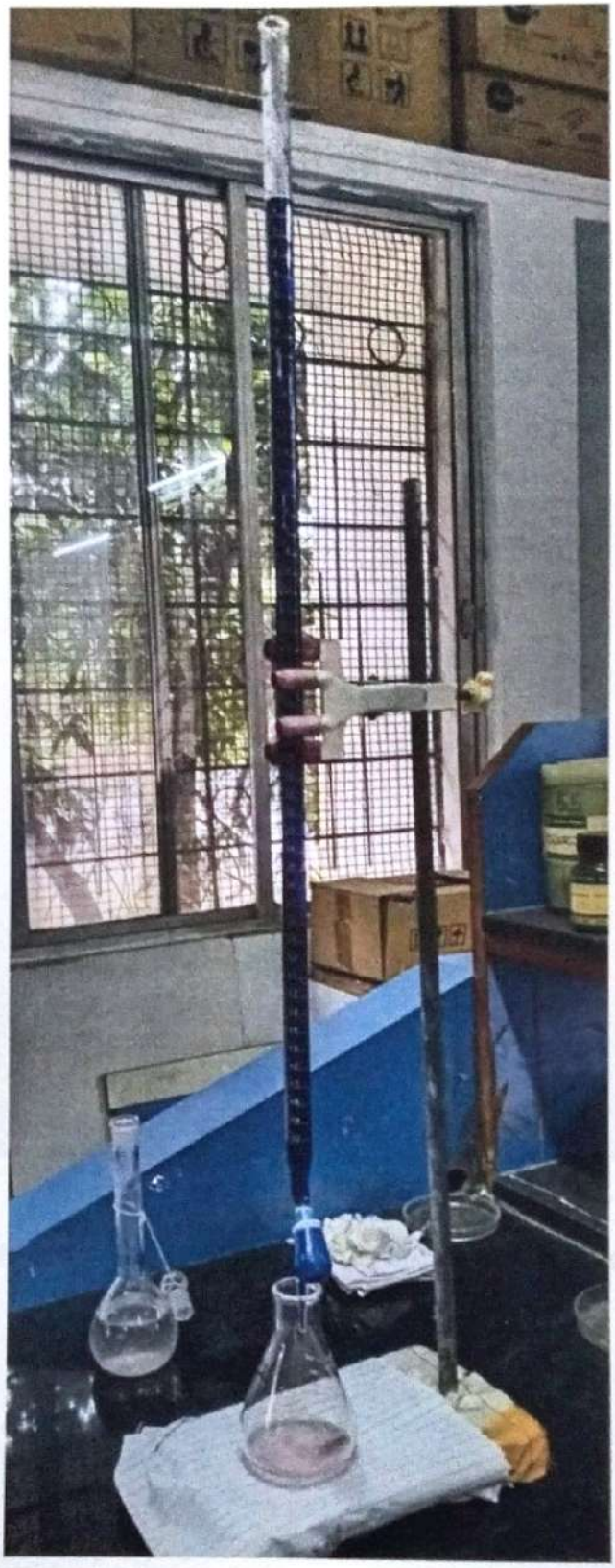

Fig. 5). Titration of dragon fruit juice using 2,6 dichlorophenol indophenol method.

Fig. 6). Result of titration by 2, 6 dichlorophenol indophenol method (colourless to light pink)

❖ NATURAL PIGMENT BETACYANIN AS TRACKING DYE FOR GEL ELECTROPHORESIS

Stability of betacyanin pigment at different pH

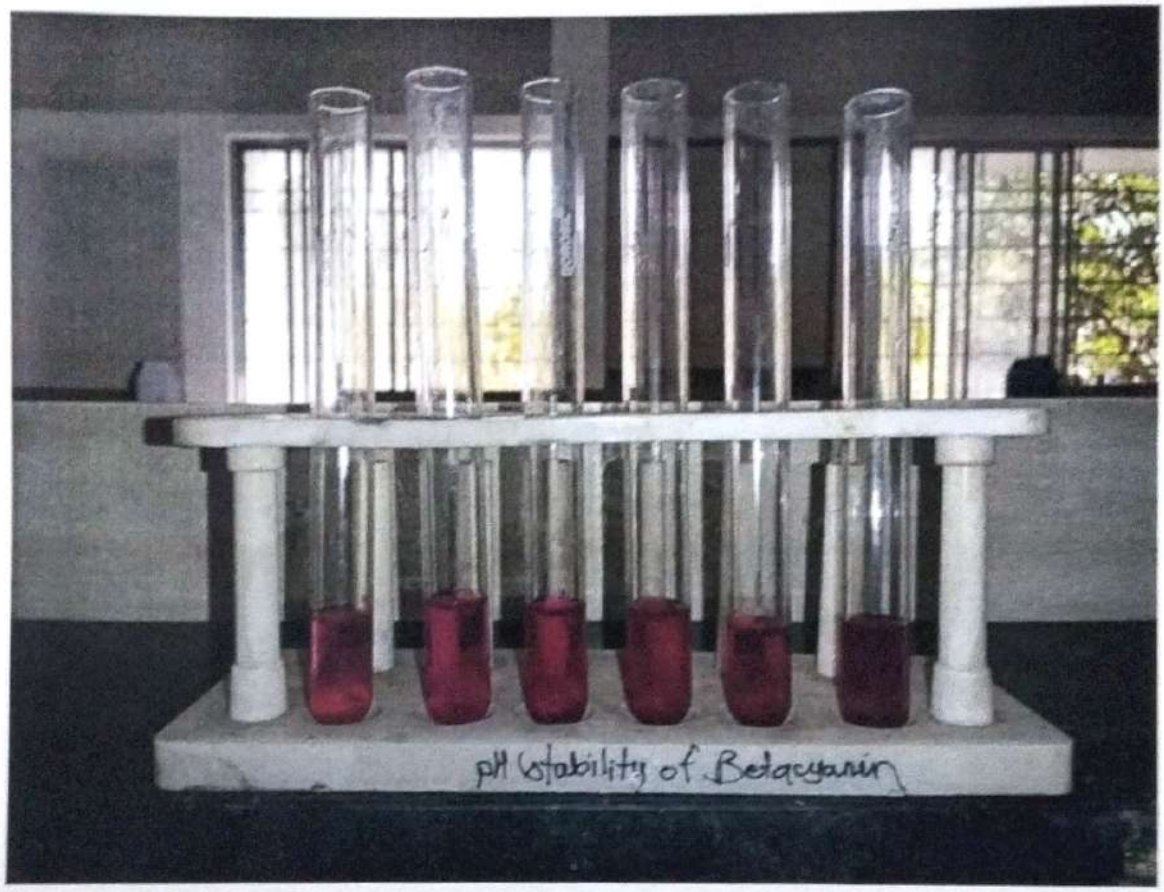

Fig. 9). Stability of betacyanin pigment at different pH. From left to right pH ranges from pH 9, 8,7, 6, 5, 4.

Betacyanin was used as an alternative dye to bromophenol blue in agarose gel electrophoresis. Migration of betacyanin was found to be parallel to that of bromophenol blue as shown in fig.10. The gel was examined after running the gel for 60-90 min. at 50 Volt.

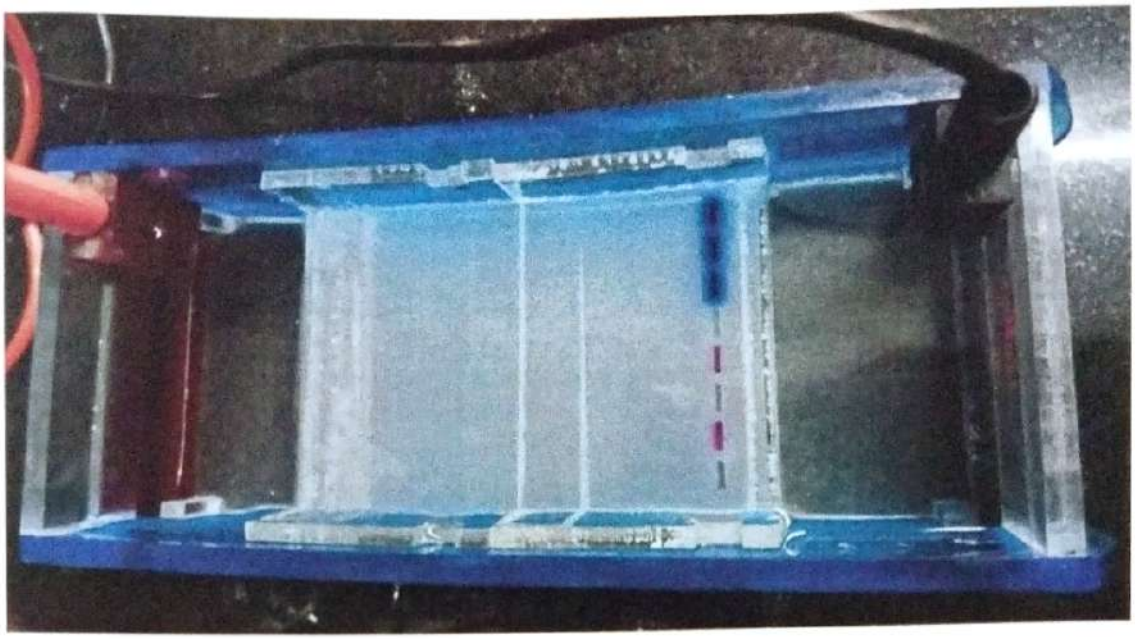

Fig. 10) before running the agarose gel electrophoresis, sample (different concentration of betacyanin as tracking dye) is loaded in 5 wells. (without DNA)

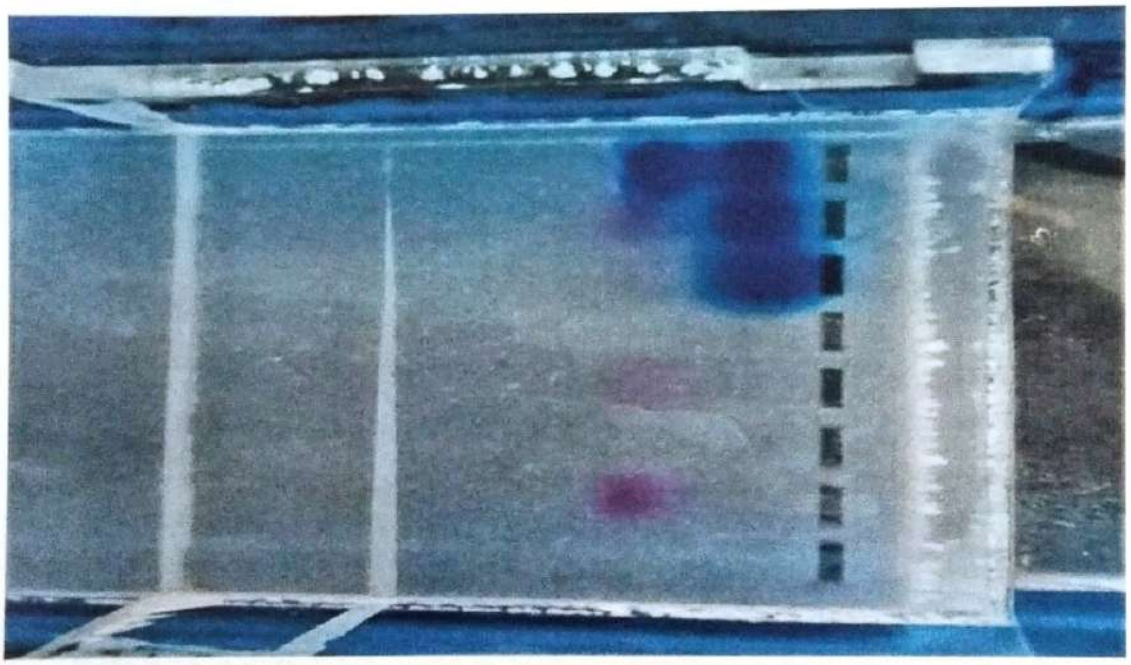

Fig.11). Agarose gel electrophoresis result. (without DNA)

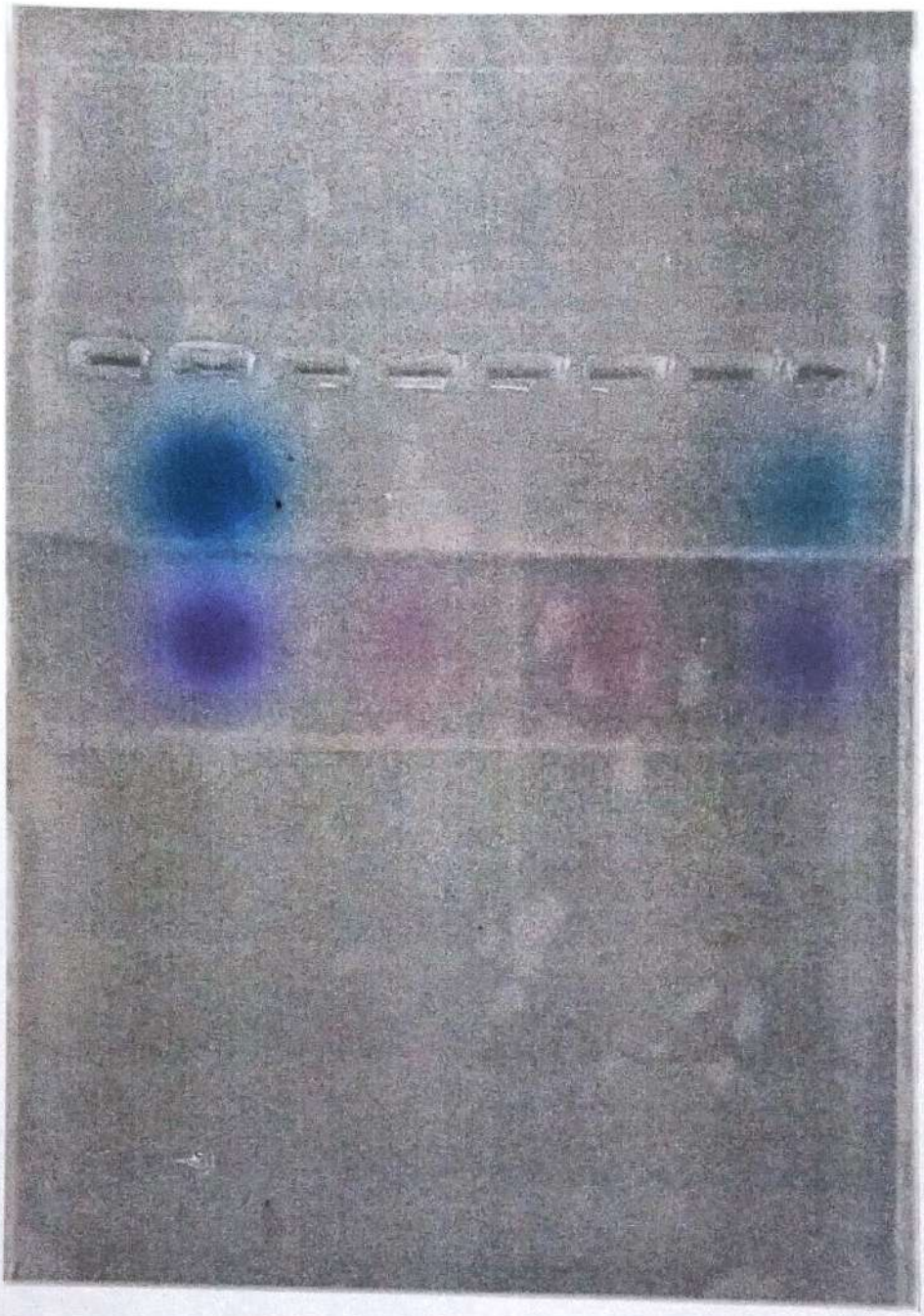

Fig.12). Agarose gel electrophoresis with DNA sample

(Lane 2- Standard gel tracking dye with DNA; Lane 4- 60% concentrated Betacyanin with DNA; Lane 6- 60% concentrated Betacyanin; Lane 8- Standard tracking dye with 60% Betacyanin)

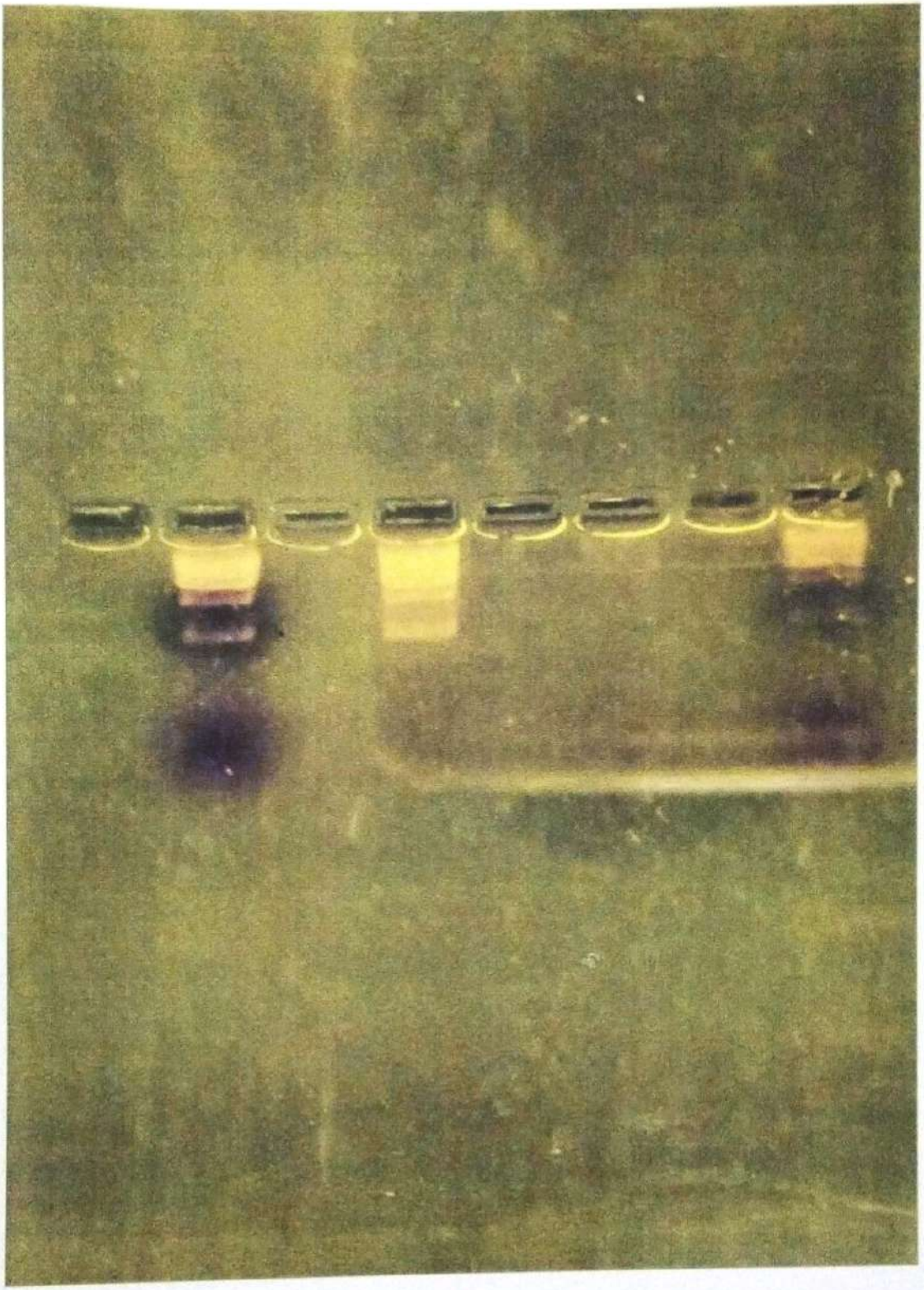

Fig.13). visualisation of results for the migration of DNA

CONCLUSION

From this study we concluded that,

The estimation of vitamin C by 2,6 dichlorophenol indophenol titration method as well as Iodometric titration method was performed. According to this study we concluded that Dragon fruit is a very good source of vitamin C.

To the best of our knowledge, our study is first of its kind to investigate betacyanin as an alternative to chemical dye in molecular biological experiment. The study revealed that the betacyanin pigment was stable showing pink colour between the pH range of 3 to 8.

Thus, we also concluded that the betacyanin pigment extracted from red fleshed dragon fruit can be used as novel tracking dye instead of bromophenol blue due to its stability at a broad pH range.