Carotenoids and Carotene Profile of Selected Vegetables with Reference to Some Unconventional Food

Masters of Philosophy (M. Phil) Thesis
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I dedicate this thesis to

my beloved family and my co-researchers for their

endless support, constant encouragement and unconditional love.

CERTIFICATE

This is to certify that the thesis entitled ‘Carotenoids and Carotene Profile of Selected Vegetables with Reference to Some Unconventional Food’, has been completed sincerely and satisfactorily by Samia Sams, Examination Roll.o2, Registrationo.183,Session: 2011-12,enrolled in Institute of Nutrition and Food Science ,University of Dhaka, Dhaka – 1000, She has carried out this research work under my supervision and guidance of this thesis and can be submitted to the examination committee for evaluation.

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Preface

I thereby humbly declare that this thesis entitled ‘Carotenoids and Carotene Profile of Selected Vegetables with Reference to Some Unconventional Food’, based on works carried by me. No part of it has been presented for any degree.

This dissertation is submitted for the degree of Master of Philosophy in accordance with the rules and regulations of The University of Dhaka. The research described herein was conducted under the supervision of Professor. Dr.Sheikh Nazrul Islam, Institute of Nutrition and Food Science, University of Dhaka and Professor Dr. Saiful Huque, Director, Institute of Energy, University of Dhaka; Previously Professor, Institute of Nutrition and Food Science, University of Dhaka.

Samia Sams

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Author

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Abstract

Aim of the study was to analysis of carotenoids and carotene profile in selected unconventional vegetables grown Bangladesh.

Twenty seven unconventional vegetables comprising 23 leafy and 4 non-leafy were collected from Gazipur, Mymensing and Chittagong Hill Tracts for analysis of carotenoids and carotene profile comprising *α*-carotene, *β*-carotene, lutein, lycopene, *β*-cryptoxanthin. Total carotenoids were estimated by 'acetone-petroleum-ether' extraction followed by passing through a chromatographic open column to remove chlorophyll (except OFSP root). Yellow color eluent thus obtained, was injected into reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate), flowing at 1.7 ml/min, detector set at 450 nm. Or 470 nm.

It was observed that among non-leafy vegetables, total carotenoid content was in the range of 65.18 (Rakhal shosha) to 1202.38 (mulachi) µg per 100g edible. In the leafy vegetables, carotenoids contained was noted highest (9696.82 µg per 100g edible) in the leaves of orange flesh sweet potato, next was highest in the tetul pata (5313.83 µg per 100g edible) and the lowest amount was in telakucha leaves (72.05 µg per 100g edible) .Of the leafy vegetables, shetoddhron contained highest amount of *β*-carotene (1422.55 µg per 100g edible) followed by chimti shak (1115.9 µg per 100g edible), orhor leave (994.81 µg per 100g edible); lutein content was highest (2303.01µg per 100g edible) in tetul pata, next in kachuripana (566.33 µg per 100g edible); α-carotene, lycopene, *β*-cryptoxanthin contents were in the range of 8.0 (shornalata) to 72.41 (orhor leave), 1.05 (venna pata) to 10.60 (shetodhron) and 2.13 (shetodhron) to 36.70 (tetul pata) µg per 100g edible respectively. mulachi contained all of the carotenes tested, of which leutin content was highest (165.51 µg per 100g edible); orhor seed contained 106.26µg *β*-carotene per 100g edible. It was seen that most of vegetables did not have α- carotene, lycopene, *β*–cryptoxanthin or it were in so small amount that are not detectable with HPLC.

It can be suggested that regular intake of these unconventional vegetables may provide provitamin A. In order to address the micronutrient deficiency and to maintain the biodiversity, analysis of nutrient composition of these unconventional rare foods is to be initiated and make it available to mass population.

Contents

LIST OF ABBREBATION	
AOAC	Association of Official Analytical Chemists
AI	Adequate Intake
AIDS	Acquire Immunes Deficiency Syndrome
AMD	Age Related Macular Degeneration
BHT	Butyrate Hydroxyl Toluene
CHT's	Chittagong Hill Tracts
VAD	Vitamin A Deficiency
DNA	Deoxyribonucleic acid
DKPM	Deshio Khaddodrobber Pushtiman
DRI	Daily Reference Intake
EP	Edible Portion
FAO	Food and Agriculture Organization of the United Nations
HDL	High Density Lipoprotein
HPLC	High Pressure Liquid Chromatography
OFSP	Orange Fleshed Sweet Potato
INFS	Institute of Nutrition and Food Science
IS	Internal Standard
icddr,b	International Centre for Diarrhoeal Disease Research, Bangladesh
IU	International Unit
LDL	Low Density Lipoprotein
IOM	Institute of Medicine
PE	Petroleum Ether
RAE	Retinol Activity Equivalent
RE	Retinol Equivalent
ND	Not Detectable
WHO	World Health Organization
RDA	Recommended Dietary Intake
RE	Retinol Equivalents
UV	Ultra Violet
WHO	Worlds Health Organization

Chapter 1 Introduction

1. Overview

Food is essential component of human survival. Maintenance of good health needs balance diet comprising adequate macro and micro-nutrients. In order to have a balance diet, it is important to identify the food sources of various nutrients for the maintenance of good health and make it well known and available to the mass population. There is a significant relationship between diet and health and diseases. Vitamins and minerals are involved in numerous biochemical processes and an adequate intake of micronutrient can prevent nutritional deficiency diseases.[1] Micronutrient deficiency, hidden hunger, is one of the major public health problems in the world, particularly in developing countries.[2, 3] It is because of poor or insufficient dietary intake of micronutrient rich foods. Fruits and vegetables are rich source of micronutrients. Many epidemiological studies have shown that regular intake of fruits and vegetables are often associated with a lower incidence of several chronic pathologies, including infections, cardiovascular, neurological diseases and cancer.[4]

1.1 Background

Diets are largely imbalanced with staple food cereals contributing around 73% of total energy intake and a substantial proportion of dietary protein. In addition to imbalance in macronutrient intakes, diets are highly deficient in micronutrients. Partly as a consequence of the paucity of diets, there is a high prevalence of malnutrition notably among young children's and mother along with emerging problems of diet related chronic diseases in the population. For the people of this country especially rural people, rice is the staple food, but there are hundreds of plants and plant products those are used as food. Fruits and vegetables are the excellent source of micronutrient. The high intake of cereal based food and low intake of micronutrient rich foods results in an imbalance diet and causes different health disorders. Diets rich in micronutrients that have specific antioxidant functions and many of which reduce the risk of health disorders including cardiovascular complications, diabetes related damage, cancers(Connealy,2008;2003;kaur and kapoor;2001)[5,6] also HIV infection(Oguntibeju,2009;Baeten et al,2001)[7]. Micronutrients are essential for good health and nutrition, advancing physical and intellectual development. Vitamin and Minerals are considered as micronutrient. Although vitamins are usually considered to be well absorbed and readily compared with mineral nutrients, there are situation where bioavailability is limited. Vitamin A is an essential micronutrient for human health. Vitamin A deficiency (VAD) undermines growth and immunity, while it increases morbidity and mortality, moreover it may increase spreading of HIV/AIDS virus infection. Vitamin A deficiency undermines growth and immunity , while it increases morbidity and mortality, moreover it may increases the risk of VAD disorders .Poor or insufficient dietary intake of vitamin A during nutrient demanding period in life increases the risk of VAD disorders.[8] Scientific interest in the quantities of carotenoids and their distribution pattern in different fruits and vegetables, has revived since it has discovered that pro-vitamin A carotenoids are the precursors of vitamin A(retinol),they also contribute to the prevention of cancer and cardiovascular disease(Michlin LJ,1995)[9,10].In developing countries like Bangladesh, young children and women of childbearing age are considered to be at greatest risk of VAD, more than 80% people are suffering from malnutrition(BBS,2006)[11] ,in spite of being endowed with plenty of green around almost everywhere in our country. Preformed vitamin A (retinol) occurs in animal foods, particularly in liver, egg, milk, which are expensive for poor communities in developing countries, thus plant foods such as green or green-yellow vegetables containing carotenoids are the primary sources of provitamin A for poor people.[12,]Micronutrient, particularly carotene is a strong antioxidant which reduces the oxidative stress, induced by free radicals oxidative stress is one of the primary risk for incidence of chronic disease(Nutri Clin Care,role of carotenoids)[12,13]. In addition to the most commonly available green yellow vegetables, there are some relatively rare/uncommon varieties that can be defined as unconventional vegetables, which are nutrient rich and consumed by specific groups of people living in particular local area. With the discovery of modern machine and their high level of accuracy, in an effort to update existing food value and to introduce some uncommon groups of vegetables, this present report investigates with the carotenoids and carotene profile content of some selected lesser known or unconventional leafy and non-leafy vegetables grown in some selected area of Bangladesh and hence to explore its importance as a diet for local people.

Micro-nutrient malnutrition

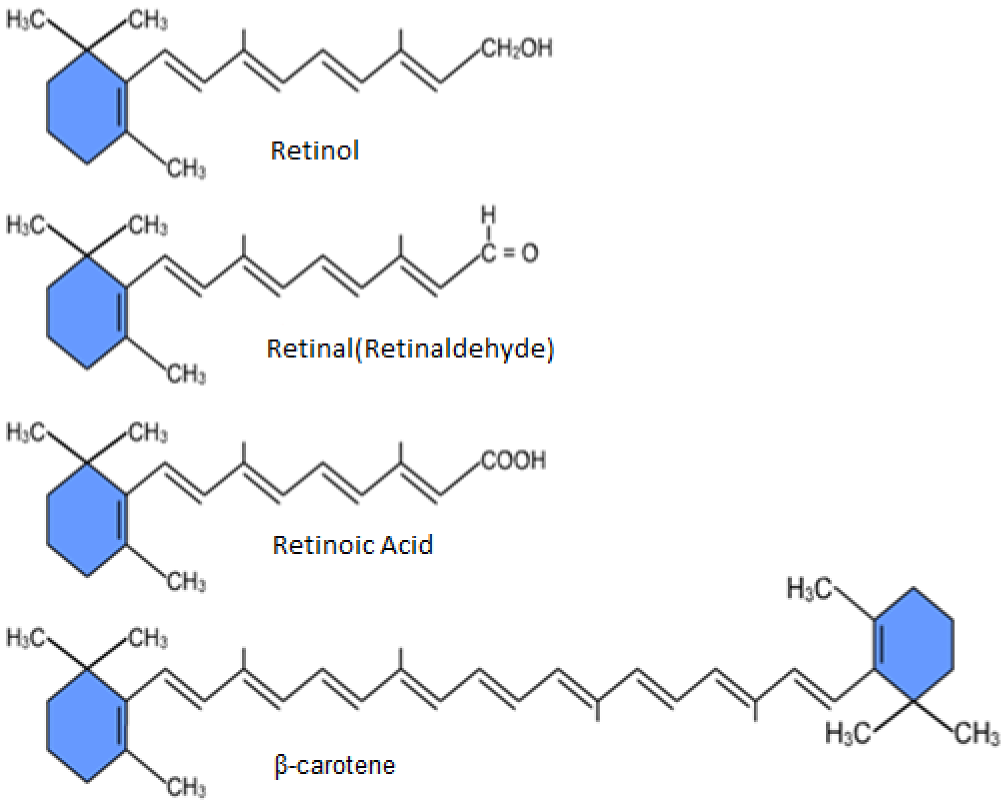
Micronutrients are different from macronutrients (like carbohydrates, protein and fat) because they are necessary only in very tiny amounts. Nevertheless, micronutrients are essential for good health, and micronutrient deficiencies can cause serious health problems. Micronutrients are what those commonly referred to as "vitamins and minerals." Micronutrients include such minerals as fluoride, selenium, sodium, iodine, copper and zinc. They also include vitamins such as vitamin C, A, D, E and K, as well as the B-complex vitamins. For human they include dietary trace minerals in amounts generally less than 100 mg per day. Micronutrients are different from the macronutrients protein, carbohydrate and fat, and micronutrients are called “micro”-nutrients because your body needs only very small quantities of them for survival. However, if our body doesn’t get small quantities of micronutrients that it needs, serious health problems can result. The World Health Organization feels that micronutrient deficiency presents a huge threat to the health of the world's population.[12]Some common micronutrient deficiencies include iodine deficiency, vitamin A deficiency and iron deficiency, often the signs of this form of malnutrition are “hidden”, as individuals may look alright but suffer extremely negative impacts on health and well being, that’s why known as “hidden hunger”.1 out of 3 people in developing countries suffers from hidden hunger, which increases their vulnerability to infection, birth defects and impaired development. But vitamin A deficiency is a leading cause of blindness in children; in pregnant women, it can cause night blindness and increases maternal mortality rates, which is one of the major public health problems all around the world, affecting more than 100 countries. Despite considerable improvement in the national rural health status, the nutritional well being of rural people continues to be neglected(World bank,2006) .Vitamin and Mineral deficiencies are thought to affect some 40% of the world population(MI &UNICEF,2004)[12].Insufficient energy and protein are derived from the diet could lead to lowered levels of retinol binding-protein in the blood, thereby impairing the transport of vitamin A in the body(Sommer,20004).[13]

Vitamin A deficiency (VAD) is widely prevalent in the young children of developing countries. Globally, 140 million children aged <5 yr are affected with subclinical VAD; nearly half (45-50) % of them live in south and Southeast Asia (American Journal of Clinical Nutrition)[15].In Bangladesh, the prevalence of subclinical vitamin A deficiency in the preschool aged children is 19.4 percent; in the slum it is significantly higher reaching at 38.1 percent(National Micronutrient Status Survey 2011-12,January 2013)[14].The VAD is the most common cause of childhood blindness worldwide, much of which is attributed to vitamin A deficient diets, about 10 million children become completely/partially blind every year in Bangladesh and it has been estimated that 30.8% and .62% of pre-school-aged children have VAD and xerophthalmia, respectively.[15] Micronutrient deficiencies are most prevalent where there is poverty and of social, economical ,environmental deprivation. Our current study focuses on one of the most important micronutrient, Vitamin A[15, 16].

Vitamin A

Vitamin A is a family of fat-soluble compounds ,which is a group of unsaturated nutritional organic compounds, that includes, retinal, retinoic acid, and several provitamin A carotenoids, and beta-carotene. There are two types of vitamin A available in foods; preformed retinol (vitamin A itself) typically found in animal food such as eggs, liver, milk, etc. Retinol is also called preformed vitamin A. It can be converted to retinal and retinoic acid, other active forms of the vitamin A family. And provitamin A carotenoids found in plant foods such as dark green leafy vegetables, yellow and orange vegetables and fruits. In the United States, approximately 26% and 34% of vitamin A consumed by men and women, respectively, is provided by provitamin A carotenoids (National Health and Nutrition Examination Survey (NHANES, 2007-2008)[17] Common carotenoids found in foods are beta-carotene, alpha-carotene, lutein, lycopene, and beta-cryptoxanthin. Of the 563 identified carotenoids, fewer than 10% are precursors for vitamin A.[10] Among these; beta-carotene is most efficiently converted to retinol. Alpha-carotene and beta-cryptoxanthin are also converted to vitamin A, but only half as efficiently as beta-carotene. Lycopene, lutein, are carotenoids that do not have vitamin A activity but have other health promoting properties. The Institute of Medicine (IOM) encourages consumption of carotenoid-rich fruits and vegetables for their health-promoting benefits.[18] Some carotenoids, in addition to serving as sources of vitamin A, have been shown to function as antioxidants in laboratory tests. However, this role has not been consistently demonstrated in humans. Antioxidants protect cells from free radicals, which are potentially damaging by-products of oxygen metabolism that may contribute to the development of some chronic diseases. Vitamin A helps regulate the immune system, which helps prevent or fight off infections by making white blood cells that destroy harmful bacteria and viruses. Vitamin A also may help lymphocytes, a type of white blood cell, fight infections more effectively .[19, 20]

Vitamin A promotes healthy surface linings of the eyes and the respiratory, urinary, and intestinal tracts. When those linings break down, it becomes easier for bacteria to enter the body and cause infection. Vitamin A also helps maintain the integrity of skin and mucous membranes, which also function as a barrier to bacteria and viruses.[20, 21, 22]



IUPAC Name:

Retinol: **(2E, 4E, 6E, 8E) -3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl) -2,4,6,8-nonatetraen-1-ol**

Retinal: **(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenal**

Retinoic Acid: **(2E, 4E, 6E, 8E)-3, 7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid**

Figure: 1.1 Chemical Nature of Vitamin A

Sources of Vitamin A

Retinol, the active form of vitamin A, is rarely found in foods. Instead, precursors to retinol, fatty acid retinyl esters, are found in the human diet. The esters are commonly found in foods of animal origin, such as egg yolks, liver, fish oil, whole milk and butter [17]. Plants can synthesize the carotenoids, but cannot convert them to retinoid; this process occurs in the human body.[23] The carotenoids are red, yellow, and orange in color and substantial in number (over 400 types). It is estimated that only 10% of the pigments have "vitamin A activity", with beta-carotene having the greatest activity, followed by the alpha and gamma forms [17]. Fruits and vegetables that appear bright orange or yellow in color are usually high in carotenoids. All green vegetables also contain substantial amounts of carotenoids, but the orange or yellow color is masked by chlorophyll .[17] The wide variety of vitamin A precursors allow for adequate amounts of the vitamin in all diet types.[23]

Metabolism of vitamin A

Absorption and bioavailability

Seventy to ninety percent of vitamin A from the diet is absorbed in the intestine. The efficiency of absorption for vitamin A continues to be high (60-80%) as intake continues to increase. Greater than 90% of the retinol store within the body enters as retinyl esters that are subsequently found within the lipid portion of the chylomicron .Absorption of vitamin A is very rapid, with maximum absorption occurring two to six hours after digestion. Within the intestinal lumen, the vitamin is incorporated into a micelle and absorbed across the brush border into the enterocytes. Within the enterocyte, precursors of vitamin A (carotenoids) are converted to active forms of the vitamin. The newly formed products and additional precursors are then packaged into chylomicrons and readied for transport throughout the body .[17, 18, 23]

Transportation

After leaving the enterocytes, chylomicrons which carry retinyl esters, carotenoids, and unesterfired retinol along with triglycerides, are circulated first through the lymphatic system and then through the general circulation. Upon arriving at extra-hepatic cells, chylomicrons release triglycerides; however, vitamin A remains within the chylomicron. The vitamin A is then incorporated into a chylomicron remnant .[17]The chylomicron remnant then travels back to the liver, where it is taken up and further metabolized or stored. When needed, retinol is mobilized from the liver and requires the use of a carrier for transport through the blood. Retinol-binding protein (RBP) is the specific carrier used to transport all-trans retinol in the plasma. The all-trans isoform accounts for more than 90% of all plasma vitamin A.[23]This specific carrier is manufactured and secreted by the parenchyma cells of the liver .Each mole of retinol released binds equivocally with RBP to form holo-RBP. This compound then binds with a molecule of transthyretin (TTR), formerly known as prealbumin. This newly formed retinol-RBP-TTR complex is not filtered by the glomerulus, but instead, freely circulates throughout the plasma. Tissues are then able to take the retinol up as needed via cellular retinoid-binding protein [22,23] .Retinoic acid is believed to be manufactured by the cells as needed via cellular retinoid-binding protein .Retinoic acid is believed to be manufactured by the cells as needed. Therefore, transport of retinoic acid is likely not substantial. Instead, the cell possesses intra-cellular proteins that regulate the amount of retinoic acid produced. The proteins also help to determine the intracellular usage of retinoic acid [17, 18, 23.]

Body storage

Approximately, 50 to 85% of the total body retinol are stored in the liver when vitamin A status is adequate [25]. Retinol returning to the liver is re-esterfied before storage. Because of this, over 90% of the retinol is stored in the form of retinyl esters. The retinol is stored in hepatic stellate (star-shaped) cells along with droplets of lipid .[17; 25]Thus constitutes the fat-soluble property of vitamin A. The size of stellate cells increases linearly with increasing retinol levels. Once hepatic stellate cells are saturated with all the retinol they can hold, hype-rvitaminosis can result. The precursor to vitamin A, beta-carotene, can be stored in adipose cells of fat depots throughout the body.[2]To date, the only side effect of excess beta-carotene supplementation appears to be yellowing of the skin. Serum levels of beta-carotene are an indicator of recent intake and not body stores [22, 25, 17.]

Elimination

The kidneys are the main paths of RBP and retinol excretion from the body. This is achieved mainly via renal catabolism and glomerular filtration.[17]Those persons suffering from renal disease often experience elevated serum levels of RBP and retinol and therefore, must be more aware of vitamin A toxicity.

Conversion of carotene to vitamin A

Retinol is referred to as *pre-formed* vitamin A. This means that it can be used directly by the body .Provitamin A Carotenoids are Vitamin A *precursors*. This means that they are converted to Vitamin A by the body. However, conversion of the carotenoids is less efficient than that of retinol. The Provitamin A Carotenoids are beta-carotene, alpha-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin (lutein and zeaxanthin are combined). The carotenoids are responsible for the red and yellow pigments of plants.

A unit called Retinol Activity Equivalents (RAE) is used to compare the Vitamin A activity of the different forms of Vitamin A. 1 µg (microgram) of retinol is equivalent to 1 µg RAE. However it takes 12 µg beta-carotene to equal 1 µg RAE, and 24 µg of the other carotenoids to equal 1 µg RAE.

Previously, a unit called International Units (IU) was used to describe Vitamin A activity. However, at the time International Units for Vitamin A were defined (1989),[11] it was thought that beta-carotene was half as concentrated as retinol (beta-carotene is now considered to be only 1/12 as concentrated as retinol).Because of this, Vitamin A measurements expressed as IU tend to over-state the contribution of the provitamin A carotenoids. [22, 26]

In 2001, the National Academy of Sciences Institute of Medicine (NAS IOM) determined that Vitamin A activity from carotenoids is only half of what was previously believed.[20] To account for this difference, and to avoid confusion, the new unit Retinol Activity Equivalents (RAE) was defined. Retinol Equivalents (RE) are no longer used. Every 12 micrograms of beta-carotene in diet from vegetables and fruits can be converted in our body to an average of 1 microgram of vitamin A, or retinol activity equivalent, RAE. However, exact quantities vary from person to person. In cells of our small intestine and liver, beta-carotene is first split in half to form retinal. Retinal can then be converted into retinol or retinoic acid, all of which are active forms of vitamin A. [25, 26, 27]

The following table summarizes the relationship between the different measures:

Table 1.1: Technical Details of Vitamin A

Nutrient	µg	µg RE	µg RAE	IU
Retinol	1	1	1	3.33
Beta-carotene	12	2	1	20
Alpha-carotene	24	2	1	20
Beta-cryptozanthin	24	2	1	20
Lycopene	24	2	1	20
Lutien+zeaxanthin	24	2	1	20

Traditionally vitamin A activity of beta carotene has been expressed in International Units (IU;1 IU=0.60 µg of all-trans beta-carotene).However, this conversion factor does not take into account the poor bioavailability of carotenoids in humans. Thus the FAO/WHO Expert Committee proposed that vitamin A activity be expressed as retinol equivalent (RE). 6µg beta-carotene provides 1 µg retinol. For leveling official national directives should be followed.[27]

1 RE = 1 µg retinol
= 6µg β-Carotene
= 3.33 IU vitamin A activity from retinol

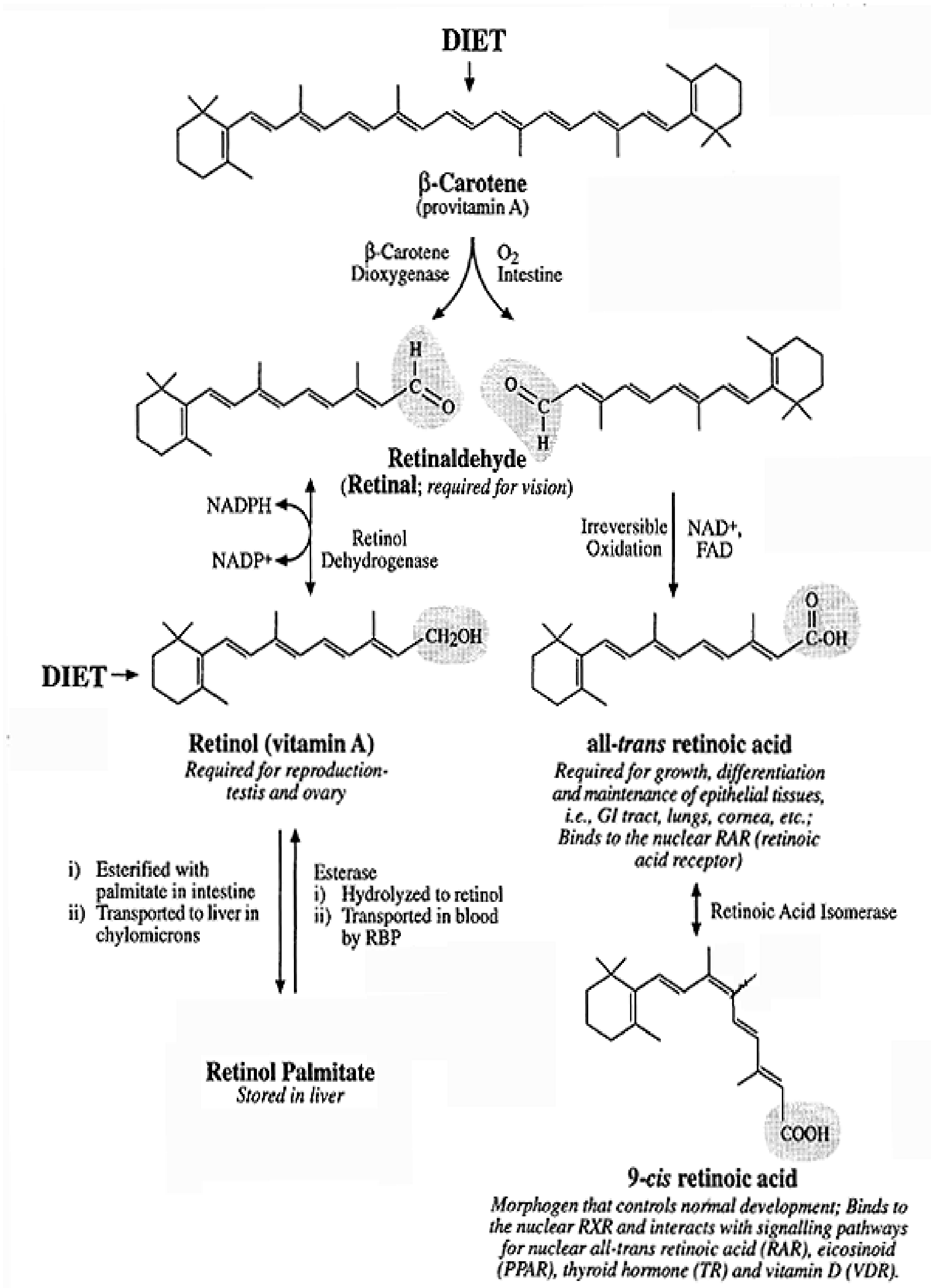


Figure 1.2 : Conversion of carotenoids to vitamin A

Table 1.2: Recommended Dietary Allowance Vitamin A (µg RAE)

Age	Male	Female	Pregnancy	Lactation
0–6 months	400	400		
7-12 months	500	500		
1-3 years	300	300		
4-8 years	400	400		
9-13 years	600	600		
14-18 years	900	700	750	1,200
19-50 years	900	700	770	1,300
51+ years	900	700		

(DRI-2013)

* Adequate Intake (AI), equivalent to the mean intake of vitamin A in healthy, breastfed infants[31]

Vitamin A Deficiency (VAD)

Vitamin A is an essential nutrient which cannot be synthesized by the body and therefore must be provided through diet and needed in small amounts for the normal activities in human body (WHO,2009). Vitamin A deficiency (VAD) is a lack of vitamin A in human. It is common in poorer countries but rarely seen in more developed countries. It is rare in the developed world to have a serious deficiency of vitamin A. Symptoms include. However, vitamin A deficiency is common in many developing countries, often because residents have limited access to foods containing preformed vitamin A from animal-based food sources and they do not commonly consume available foods containing beta-carotene due to poverty .[31]

According to the World Health Organization, 190 million preschool-aged children and 19.1 million pregnant women around the world have a serum retinol concentration below 0.70 micromoles/L. In these countries, low vitamin A intake is most strongly associated with health consequences during periods of high nutritional demand, such as during infancy, childhood, pregnancy, and lactation [22].VAD can occur in individuals of any age. However, it is a disabling and potentially fatal public health problem for children under 6 years of age.VAD related blindness is most prevalent in children under 3 years of age.[32]VAD also common in pregnant and lactated ,as well as women reproductive age thought to consider as vulnerable group.VAD affect them a lot ,also for children ,because their demand of VAD is higher. There are some major symptoms of VAD described below,

- Nyctalopia (night blindness) is one of the first signs of VAD, affects vision by inhibiting the production of rhodopsin, the eye pigment responsible for sensing low light situations. Rhodopsin is found in the retina and is composed of retinal (an active form of vitamin A) and opsin (a protein). Because the body cannot create retinal in sufficient amounts, a diet low in vitamin A will lead to a decreased amount of rhodopsin in the eye, as there is inadequate retinal to bind with opsine results Night blindness.[14,15]Xerophthalmia, keratomalacia, and complete blindness can also occur since Vitamin A has a major role in photo transduction. [32,34]
- The common cause of blindness in developing countries is VAD. The World Health Organization (WHO) estimates 13.8 million children to have some degree of visual loss related to VAD.1 Night blindness and its worsened condition, xerophthalmia, [35, 26] are markers of VAD, as it can also lead to impaired immune function, cancer, and birth defects. Collections of keratin in the conjunctiva, known as Bitot's spots, are also seen.
- Vitamin A deficiency is the leading cause of preventable childhood blindness and is critical to achieving Millennium Development Goal 4:2 to reduce child mortality.[37]
- Approximately 250,000 to 500,000 malnourished children in the developing world go blind each year from a deficiency of vitamin A, approximately half of whom die within a year of becoming blind. The United Nations Special Session on Children in 2002 set a goal of the elimination of VAD by 2010.[35,26]
- The prevalence of night blindness due to VAD is also high among pregnant women in many developing countries.[34,35]
- VAD also contributes to maternal mortality and other poor outcomes in pregnancy and lactation.[36]

- Getting enough vitamin A in diet is essential for good vision. Research shows that people who eat more foods with vitamin A are less likely to develop age-related macular degeneration (AMD)[30]
- VAD is one of the strongest reasons of heart disease like heart attack, blood pressure, heart failure, etc.
- Cancer cause for VAD ,now a days there are different types of cancer such as, lung, prostate ,colon and other types of cancer affecting human health, because of insufficient intake of Vitamin A as well as carotene rich food in daily .[31,32]
- VAD also diminishes the ability to fight infections. In countries where children are not immunized, infectious diseases like measles have higher fatality rates, its work against immune system of our body.[32]
- It may increase children's risk of developing respiratory and diarrheal infections, reproduction, inflammatory bowel disease(IBD).decrease growth rate, slow bone development, skin problem and decrease livelihood of survival from serious illness.[35]

What are Some Current Issues and Controversies about Vitamin A?

Vitamin A, Beta Carotene and Cancer: surveys suggest an association between diets rich in beta- carotene and vitamin A and a lower risk of many types of cancer. A higher intake of green and yellow vegetables or other food sources of beta carotene and/or vitamin A may decrease the risk of lung cancer.[23]

Vitamin A and Health

Vitamin A is required for several vital functions in the body. Some of the most *important* functions of *Vitamin A* are described below.

There are several forms of Vitamin A that are needed by the body. These include:

1. Retinal - a metabolite of vitamin A required for vision.
2. Retinol - the form of vitamin A that can be stored by the body and converted to retinal when needed .
3. Retinoic acid - a growth factor needed primarily to regulate genes.

- **Visual Process:** Located at the back of the eye, the retina contains two main types of light-sensitive receptor cells − known as rod and cone photoreceptor cells. Photons (particles of light) that pass through the lens are sensed by the photoreceptor cells of the retina and converted to nerve impulses (electric signals) for interpretation by the brain. All-TRANS-retinol is transported to the retina via the circulation and accumulates in retinal pigment epithelial (RPE) cells (Figure 1.3). Here, all-TRANS-retinol is esterifies to form a retinyl ester, which can be stored. When needed, retinyl esters are broken apart (hydrolyzed) and isomerizes to form 11-CIS-retinol, which can be oxidized to form 11-CIS-retinal. 11-CIS-retinal can be shuttled across the interphotoreceptor space to the rod photoreceptor cell that is specialized for vision in low-light conditions and for detection of motion. In rod cells, 11-CIS-retinal binds to a protein called opsin to form the visual pigment rhodopsin (also known as visual purple). Absorption of a photon of light catalyzes the isomerization of 11-CIS-retinal to all-TRANS-retinal that is released from the opsin molecule. This photoisomerization triggers a cascade of events, leading to the generation of a nerve impulse conveyed by the optic nerve to the brain's visual cortex. All-TRANS-retinal is converted to all-TRANS-retinol and transported across the interstitial space to the RPE cells, thereby completing the visual cycle. [35,36]

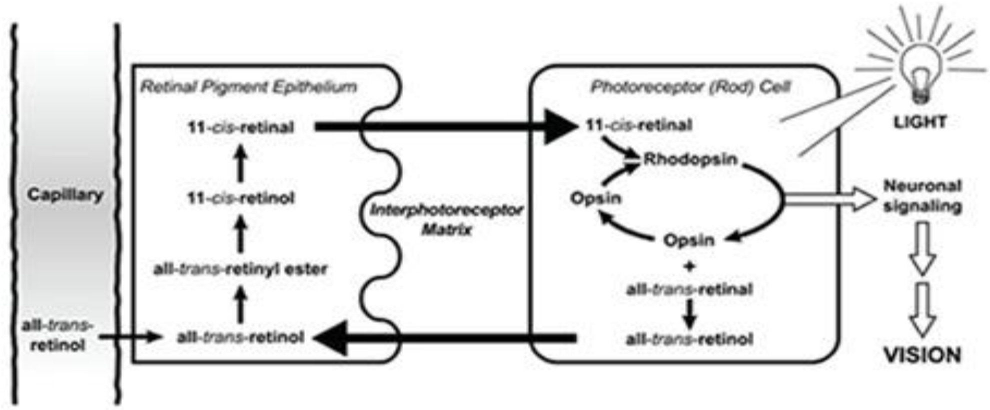


Figure 1.3: The Visual Cycle

Retinol is transported to the retina via the circulation, where it moves into retinal pigment epithelial cells. There, retinol is esterified to form a retinyl ester that can be stored when needed,retinyle esters are broken apart(hydrolyzed) and isomerized to form 11-cis-retinol,which cn be oxidized to form 11-cis-retinal. 11-cis-retinal can be shuttled to the rod cell, where it binds a protein called opsin to form the visual pigment, rhodopsin(also known as visual purple).Absorption of a photon of light catalyzes the isomeriztion of 11-cis-retinalto all-trans-retinal and results in its release. This isomerization trigger's a cascade of events, leading to the generation of an electrical signal to the optic nerve. The nerve impulse generated by the optic nerve is conveyed to the brain where it can be interpreted as vision. One released all-trans retinol is converted to all-trans-retinal which can be transported across the interphotorecepter matrix to the retinal epithelial cell complete the visual cycle.

A similar cycle occurs in cone cells that contain red, green, or blue opsin proteins required for the absorption of photons from the visible light spectrum [2.] Vitamin A is also essential for mammalian eye development [17.] Thus, because vitamin A is required for the normal functioning of the retina, dim-light vision, and color vision, inadequate retinol and retinal available to the retina result in impaired dark adaptation. In the severest cases of vitamin A deficiency, thinning and ulceration of the cornea leads to blindness (sommer, 1994)[13]

- **Eye Health**: *Vitamin A* is commonly known as the *vitamin* needed for good eyesight. Along with promoting vision. Vitamin A is the key to keep eye good. There are two types of vitamin A, one is come from animal source another which is carotene (provitamin A) comes from vegetables sources. For a healthy vision carotene is acts strongly as it's a antioxidants, which can protect eye (cornea) from any kind of damage (night blindness, cataract, xerophthalmia, age related macular etc).[35,36]
- **Immune System and growth:** We need vitamin A for a strong immune system to protect our body from any kind of damage. for healthy skin, for a healthy growth of human body. The retinol form of vitamin A is responsible for maintaining the function of the cells that make up these barriers.[37]
- **Cancer:** Vitamin A regulates hundreds of genes. It is a key modulator of the immune system. It helps aim cells along the tricky trajectory from primitive to adult forms, a job that may play some role in preventing the development of cancer. Whether vitamin A can reduce the risk of cancer is not clear. People who eat a healthy diet with enough beta-carotene and other carotenoids from fruits and vegetables seem to have a lower risk of certain cancers, such as:
  - Breast cancer,
  - colon Cancer
  - Esophageal cancer
  - Cervical cancer
  - Melanoma
  - Lung cancer
  - Prostate cancer

  Some laboratory studies suggest that vitamin A and carotenoids may help fight certain types of cancer in test tubes. One preliminary study suggests that a topical form of vitamin A may reduce abnormal growth of cells on the cervix, called cervical neoplasia.[37, 39, and 40]

  Researchers are also investigating retinoid, a synthetic form of vitamin A, for skin cancer. People with certain types of skin cancer tend to have lower levels of vitamin A and beta-carotene in the blood. However, studies that have looked at whether taking higher amounts of vitamin A or beta-carotene would prevent or treat skin cancer have had mixed results.

- **Heart disease:** The American Heart Association recommends obtaining antioxidants, including beta-carotene, by eating a well- balanced diet high in fruits, vegetables. Getting antioxidants from foods is a great part of a heart-friendly diet. Vitamin A rich as well as carotene rich food, such as green or dark green or yellow or colored fruits and vegetables are really work as a great dietary prevention of cardiovascular disease(CVD) ,blood pressure, blood clot in artery, and other heart diseases. Carotenes are antioxidants which may cause lower risk of heart problem.[35,37]

- Vitamin A also works for **maternal health** and **reproduction.**
- It may helpful to **lung, kidney** any other body organ acts properly.
- Vitamin A is vital for **regulating genes**, **producing red blood cells** and **formation and activation of white blood cells.**[36,37]

Nutrient interaction of Vitamin A

Zinc (Zn)

Zinc deficiency is thought to interfere with vitamin A metabolism in several ways.zinc deficiency results in decreased synthesis of retinol-binding protein (RBP), which transports retinol through the circulation to peripheral tissues and protects the organism against potential toxicity of retinol; zinc deficiency results in decreased activity of the enzyme that releases retinol from its storage form, retinyl palmitate, in the liver; and zinc is required for the enzyme that converts retinol into retinal [40] The health consequences of zinc deficiency on vitamin A nutritional status in humans are yet to be defined [41.]

IRON (Fe)

Vitamin A deficiency often coexists with iron deficiency and may exacerbate iron deficiency anemia by altering iron metabolism[42]. Vitamin A supplementation has beneficial effects on iron deficiency anemia and improves iron nutritional status among children and pregnant women. The combination of supplemental vitamin A and iron seems to reduce anemia more effectively than either supplemental iron or vitamin A alone. Moreover, studies in rats have shown that iron deficiency alters plasma and liver levels of vitamin A [35, 41, 42]

Carotenoids

Carotenoids, the colorful plant pigments which are widely distributed group of naturally occurring, usually green, red, orange or yellow in color of fruits and vegetables, and many dark green vegetables(Delgado et al.,2000).The color of carotenoids in green plant tissues is covered by chlorophyll and becomes only evident after degradation of green pigment in the fall(Stahl,2002).The most abundant carotenoids in the nature are beta carotene, alpha carotene, lutein, lycopene, beta-cryptoxanthin, etc(Delgado et.al.,2000).These precursors to vitamin A are sometimes called pro-vitamin A. Approximately50 carotenoids ,over 600 known as provitamin,can convert them into retinol,an active form of vitamin A.As a results,foods containing carotenoids can helps to prevent vitamin Adeficiency.The most commonly consumed provitamin A carotenoids are beta-carotene, alpha carotene and beta cryptoxanthin(Delgado.,et al,2000); The institute of medicine(IOM) encourages of all carotene-rich fruits and vegetables for their health benefits. Current USDA dietary guideline recommended eating 5-9 serving of fruits and vegetables per day.[43, 45, and 46]

Properties of Carotenoids

Carotenoids are belongs to the family of tetrapinoids(40 carbon atoms, being built from four tarpene units).Structurally,craotenoids take the form of a polyene hydrocarbon chain which is sometimes terminated by rings, and may or may not have additional oxyzen atoms attached.

- Carotenoids split into two classes, carotenes (which are purely hydrocarbons, contain no oxyzen),such as alpha carotene, beta carotene,lycopene are known as carotenes, typically contain only carbon and hydrogen(hydrocarbons),and are in the sub-class of unsaturated hydrocarbons.

- The other is carotenoids with molecules containing oxygen, such as lutien, zezanthin.are known as xanthophylls.

Vitamin A water insoluble but in soluble in fat and organic solvent. Vitamin A alcohol occurs as light coloured viscous oil which is heat labile and subject to air oxidation.Carotenoids absorbs light energy for use of photosynthesis and they protect chlorophyll from photo damage. They are used extensively as safe, natural colorants for food some of which the body can turn into vitamin A are powerful antioxidants that can help prevent some forms of cancer and heart disease, and act to enhance immune response to infections. Dietary carotenoids are thought to provide health benefits in decreasing the risk of disease, particularly certain cancers and eye disease. Epidemiologic studies have shown strong associations between diets rich in carotenoids and a reduced incidence of many forms of cancer, and that finding led to the suggestion that the antioxidant properties of those compounds might help protect immune cells from oxidative damage, thus enhancing their ability to detect and eliminate tumor cells. In addition to their role in cancer prevention, the carotenes offer us protection from heart disease, too. Again, it's their antioxidant behavior that protects the lining of the arteries and the fats in the blood from free radicals' oxidative damage. And age-related macular degeneration of the eye, which leads to vision loss, may be counteracted by carotenes' antioxidant power. Humans and Animals are mostly incapable of synthesizing carotenoids, and must obtain them through their diet.[49, 52]

Sources of carotene

Carrot, pumpkin, sweet potatoes, spinach, grapefruits, broccoli, melons, tomatoes, colorful fruits, and dark green or green yellow leafy or non leafy vegetables are the good sources of carotene, but less in animal products. In meat, fish, oils, from animals and fish, 10% of the vitamin activity occurs as carotenoids, while in poultry, eggs and milk 30% occur as carotenoids (Wu Leung et al,1968)[45]

Pro vitamin A activity of carotenoids

Vitamin A that is found in colorful fruits and vegetables is called provitamin A carotenoids. Carotenoids are absorbed into the small intestine by passive difficulties by passive diffusion.It is estimated that carotenoids pigment have ‘vitamin A activity’, with beta carotene having the greatest activity, followed by the alpha and gamma forms(Fumich,1983) [63].Absorption efficiency is estimated to be between 9-22 %.The absorption and conversion of carotenoids may depend on the form that the beta carotene is in(cooked vs.raw vegetables or in a supplement) Intake of fats and oil at that time and current levels of vitamin A and beta carotene.In the United States approximately 26 % of vitamin A consumed by men and 34% of vitamin A consumed by women is in the form of pro vitamin A carotenoids. Among all these carotenoids, beta carotene is most efficiently made into retinol.Alpha carotene,beta cryptoxanthin ,and others are also converted to vitamin A but only half as efficiency as beta carotene. Researchers suggest the following factors that determine the pro vitamin A activity of carotenoids(Tanumihardjo,2002).[36] Species of carotenoid,molecular linkage, amount in the meal,effectors,nutrient status,genetics,host specificity, interaction between factors,etc.

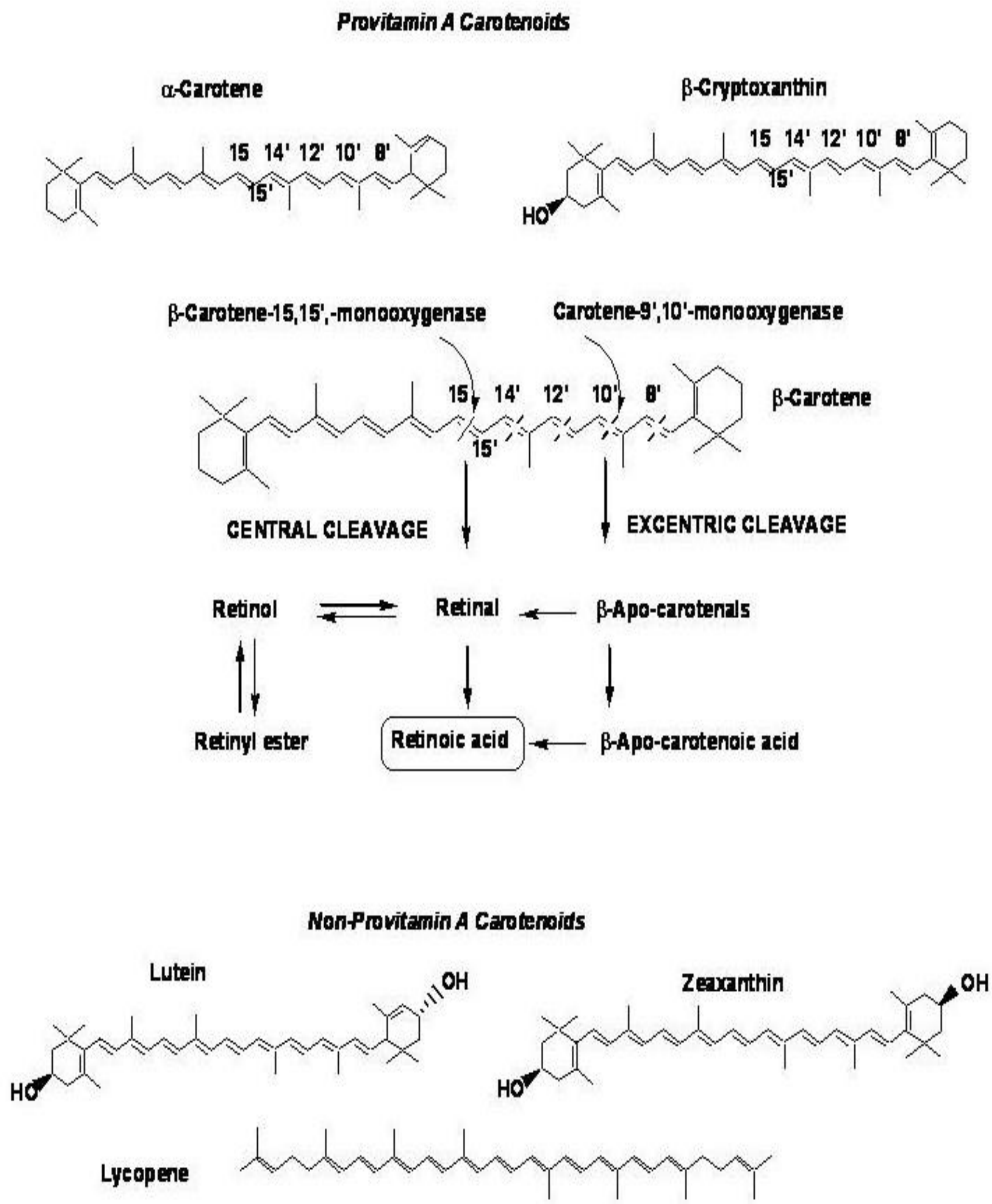


Figure 1.4: Structure of Carotenoids

Beta Carotene

The name β (beta)-carotene comes from the Greek *beta* and Latin *carota* (carrot). It is the yellow/orange pigment that gives vegetables and fruits their rich colors. It was discovered by Heinrich Wilhelm Ferdinand Wackenroder in 1831 in the roots of carrots and named the substance "carotene." Wackenroder was an analytical chemist at the Pharmaceutical Institute in Jena, Germany (www.life.illinois.edu/govindjee/CarFin 1.html). β-Carotene chemical formula-$C_{40}H_{56}$ was discovered in 1907. The structure of beta carotene was clucidated by Karrer in 1930-1931.This was the first time that the structure of any vitamin or provitamin had been established, and he received a noble prize for his work. Various studies has carried throughout the year1970s-1980s to determine the suitability for use in food and its activity in 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..Beta carotene has been claimed to prevent a number of diseases (Coultate,1996).[54,57]The human body converts β-Carotene into vitamin A(retinol) ,is a precursor of vitamin A.

Beta-carotene, like all carotenoids, is an antioxidant. An antioxidant is a substance that inhibits the oxidation of other molecules; it protects the body from free radicals. Free radicals damage cells through oxidation. Eventually, the damage caused by free radicals can cause several chronic illnesses. Several studies have shown that antioxidants through diet help people's immune systems, protect against free radicals, and lower the risk of developing cancer and heart disease. Some studies have suggested that those who consume at least four daily servings of beta-carotene rich fruits and/or vegetables have a lower risk of developing cancer or heart disease[.53]

Bile salt and fat are needed for the absorption of β-Carotene in the upper small intestine. Many dietary factors, e.g.fat and protein, affect absorption. Therefore, β-Carotene is a very safe source of vitamin A and high intakes will not lead to hypervitaminosis A.

Bioavailability refers to the proportion of β-Carotene that can be absorbed, transported and utilized by the body once it has been consumed, its better absorbed from dietary supplements than food.[55]

Beta-carotene is a carotenoid that is a precursor of vitamin A. It is administered to reduce the severity of photosensitivity reactions in patients with erythropoietin protoporphyria .[59]

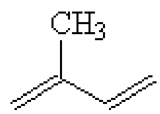
Beta-Carotene is a naturally-occurring retinol (vitamin A) precursor obtained from certain fruits and vegetables with potential antineoplastic and chemo preventive activities. As an anti-oxidant, beta carotene inhibits free-radical damage to DNA. This agent also induces cell differentiation and apoptosis of some tumor cell types, particularly in early stages of tumor genesis, and enhances immune system activity by stimulating the release of natural killer cells, lymphocytes, and monocytes. (NCI04)(Pharmacology from NCIT).Beta carotene absorbs most strongly between 400-500 nm which is the green or blue part of spectrum and so beta carotene appears as orange ,because the red /yellow colors reflected to us(Coultate,1999)[57,64]

“Antioxidants have been associated with cancer reducing effects—beta carotene, for example—but the mechanisms, the genetic evidence, has been lacking. Now we have genetic proof that mitochondrial oxidative stress is important for driving tumor growth,” says lead researcher Michael P. Lisanti, M.D., PhD [58]

Chemical Name: Beta-carotene; Beta Carotene: Beta, beta-Carotene; Solatene; Provitamin A Carotaben, etc. [59]
Molecular Formula: $C_{40}H_{56}$, **Molecular Weight**: 536.87264 g/mol

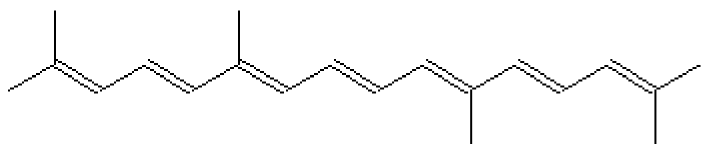
IUPAC Name :1,3,3-trimethyl-2-[(1E,3E,5E,7E,9E,11E,13E,15E,17E)-3,7,12,16-tetramethyl-18-(2,6,6-trimethylcyclohexen-1-yl)octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]cyclohexene.

The FAO/WHO Expert committee proposed that vitamin A activity be expressed as retinol equivalent (RE).6μg β-Carotene provide 1 μg retinol.
Beta-carotene is a member of a family of molecules known as the carotenoids. These have a basic structure made up of isoprene units:



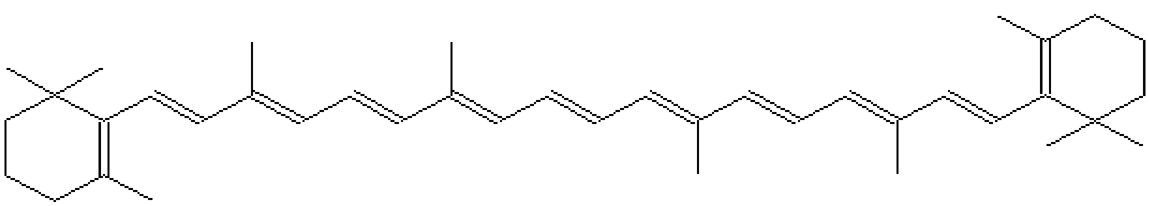
Isoprene

These are joined end-to-end to give a conjugated chain which is common to all carotenoids. Notice that the two centre isoprene units are joined differently to the others ("head-to-head" rather than "head-to-tail") so that the chain has a centre of symmetry.



Central part of carotenoids

Beta-carotene is made up of eight isoprene units, which are cyclised at each end. It looks like,



Beta-carotene

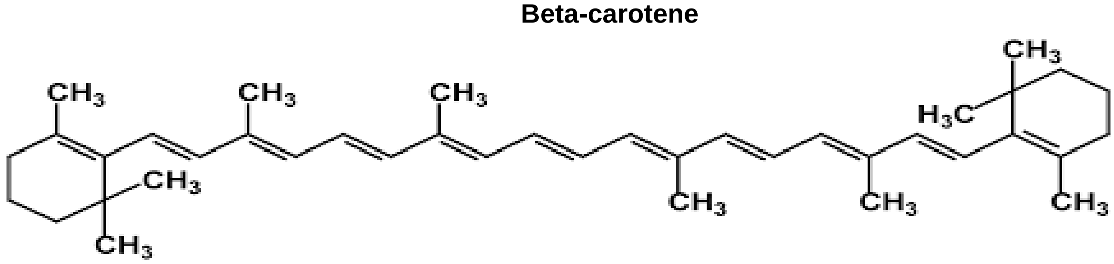


Figure 1.5: Chemical Nature of Beta Carotene

Factors influencing the conversion of beta carotene to vitamin A

1) To increase our understanding of the potential benefits of carotenoids,it is important to obtain more insight into their bioavailability.
2) Factors that may interfere with rate of each of the step will affect the overall bioavailability of the carotenoids ingested "SLAMENGHTI".
3) Effect of Dietary factors that is matrix and effectors, on the bioavailability of carotenoids.

Beta carotene (pro-vitamin A), which found in plants. It is cleaved in the intestine to produce 2 molecule of retinal. In human this conversion is inefficient. Beta carotene in human possesses only one sixth vitamin activity compared to retinal.[60, 62]

Absorption and body stores

Bile salts and fat are needed for the absorption of beta carotene in the upper small intestine. Many dietary factors, e.g. fat and protein, affect absorption. Within the intestinal wall (mucosa) beta carotene is partially converted into vitamin-A(retinol)by the enzyme deoxygenize. If the body has enough vitamin A, the conversion of beta-carotene decreases. Therefore beta-carotene is a very safe source of vitamin A and high intake will not lead to hypervitaminosis A.

Excess beta-carotene is stored in the fat tissues of the body and liver. The adult's fat stores are often yellow from accumulated carotene white carotene while the infant's fat stores are white.[62, 64]

Deficiency of beta carotene:

People with deficiency of Beta carotene may sometimes be suggested to take test for deficiency of vitamin A. Beta carotene is a fat soluble nutrient just like retinol. The fact that how much fat can be absorbed by the body can be found out but measuring the beta carotene levels in the blood.

- It may become the cause of a lot of medical complications. The person may experience a lack of appetite, intestines might not able of absorb entire food the mouth had chewed (celiac disease), roots of hair may start losing their grip, fat absorption maybe inadequate, inflammation or dryness of eyes, teeth of children might develop abnormalities, bones may remain as strong as before, rashes on the skin causing redness and certain infections.[54]
- The lack of beta carotene rich foods in daily diet is the main reason for beta carotene deficiency. A mother who does have enough vitamins A in her diet will produce breast milk which is deficient in this nutrient.
- There are innumerous causes for lack of beta carotene. This includes consuming unwanted amounts of alcohol and deficiency of iron in blood. That is because it would cause reactions leading to production toxic substances in their body.[55]
- If we keep on having insufficient amounts of beta carotene with diet every day then we have to face chronic diseases. They include various heart related diseases and cancer of different organs. The reason behind this is that as there are less amounts of antioxidants in your body those free radicals will more the pleased to destroy all the healthy cells organs are made of. The will result in improper functioning of the organs and a high probability of having cancer.[53,54]
- Low beta carotene diets make the immune system ineffective against carcinoma causing agents. White blood corpuscles will be just a joke to them.
- Type 2 diabetes patients from the elderly group have been known to have vitamin A deficiency.
- All these above mentioned ill effects must make us understand that we must have a balanced diet each and every day.[55,56]

Health Benefit of beta-Carotene

- **Eye health.** Plays a significant role in prevention problems, Beta-carotene is thought to possess many positive health benefits and in particular helps prevent night blindness, vision and other eye problems. Beta carotene is a rather cheap vitamin to produce, which means this type of blindness is easily preventable, also works against cataract.[57]
- **Skin disorders**. Enhances immunity, protects against toxins, colds, flu, and infections. It is an antioxidant and protector of the cells while slowing the aging process.[55]

- **Heart disease.** People whose diets are rich in beta carotene have lower risk of heart disease. Beta carotene works with vitamin E to reduce the oxidation of LDL-cholesterol, which lowers the risk of atherosclerosis, High blood pressure and coronary heart disease.
- **Maternal health.** Beta carotene also helps in used malnourished (underfed women to reduce the chance of death, as well as diarrhea and fever after giving birth.
- **Respiratory system problems.** High intakes of beta carotene and vitamin C were found to increase lung capacity and relieve respiratory problems, as well as protecting from breathing disorders such as asthma, bronchitis and emphysema.
- **Cancer**. Studies show that people who eat a diet rich in carotenoids, had a much lower risk of breast, colon cancer and lung cancer. One way that carotenoids fight cancer is through their antioxidant capacity. Another way that they help is to keep our cells in proper communication, an effective preventive measure against the growth of cancer cells. [64,65]
- **Immune system.** Beta carotene helps activate our thymus gland, one of our most important sources of immune protection. The thymus gland stimulates immune system to fight off infections and viruses, and destroy cancerous cells before they can spread.
- **Radiation.** Combining beta carotene with vitamins E & C offers significant protect against ultraviolet radiation from the sun, as well as from chemotherapy. It's interesting to note that vitamin C and vitamin E do not offer this protection on their own. This reinforces the fact that antioxidants work more effectively as a team. [64,65]
- **Diabetes**. Studies have shown that people with low levels in their bodies are much more likely to suffer from impaired glucose tolerance and diabetes.
- **Rheumatoid Arthritis**. Studies have shown that low levels of beta carotene and vitamin C have been found to be a risk factor for rheumatoid arthritis.[54,55,64,65]

Additional beta carotene benefits include.

- Effectives as treatment for Alzheimer's disease, epilepsy, depression, Parkinson's Disease, eczema [.55].
- prevents oxidative damage from strenuous exercise etc.

Recommended Dietary Allowance (RDA) for beta-Carotene

Until now, dietary intake of beta carotene has been expressed as part of the RDA for vitamin A. The daily vitamin A requirements for adult men and women are 900 µg and 700 µg of preformed vitamin A(retinol)respectively. Apart from its pro vitamin A function, data continue to accumulate supporting a role for beta-carotene as an important micronutrient in its own right(FNB,2001).[75,76] Consumption of food rich in beta carotene is recommended by scientific and government organizations' such as the UN National Cancer Institute(NCI) and the US Department of Agriculture(USDA).If this dietary guidelines are followed, dietary intake of beta-carotene(about 6 mg) would be several times the average amount presently consumed in the US(about 1.5 mg daily) [76]

Bioavailability of beta-carotene

Bioavailability of β-carotene depends of many factors' trace amount of fat is necessary for absorption and conversion of β-carotene to retinol. β-carotene to retention and bioaccessibility determine the bioavailability of the provitamin A. Food matrix and processing improve of the carotenoids. It has been noted that maceration and heat processing β-carotene in food and it is probably due to rupture of microstructure of plant tissue and subsequent release of nutrients from the complex food matrix[33, 77].

Alpha -Carotene

The word α(Alpha)-Carotene is a form of with a β-ionone ring at one end and an α-ionone ring at the opposite end. It is the second most common form of carotene. Yellow-orange-dark-green vegetables are good source of α-Carotene. Because the body converts alpha-carotene to vitamin A, alpha-carotene is called a precursor to vitamin A, or a provitamin A compound. As a precursor to vitamin A, alpha-carotene is only about half as effective as beta-carotene, another well-known carotenoid. Alpha-carotene is found in vegetables like green peas, broccoli, green beans, spinach, collards and turnip greens, as well as in pumpkins and other squash, carrots and sweet potatoes.[78]

Researchers were able to determine that blood levels of alpha-carotene were inversely associated with risk of death from cancer, cardiovascular disease and all other causes. For years, alpha-carotene played second fiddle to its more famous cousin, beta-carotene, but both are rich sources of antioxidants; numerous studies have shown that fruits and vegetables, which contain antioxidants, seem to help prevent or delay the development of cancer, cardiovascular disease and other conditions. Alpha-Carotene can be used to make vitamin A, though not very efficiently. Its main role is more likely to be as a cofactor for other substances. Alpha-carotene has been implicated in helping prevent cervical cancer. Nutrition “experts” often recommend supplementation with beta-carotene to prevent cancer. But sometimes it’s alpha-carotene that actually works. Alpha-carotene is a substance some plants make from lycopene that they can further transform into lutein. Plants use this intermediary chemical to manage their ability to deal with sun and shade. It enables the plant to use different wavelengths of light in shade to continue to make carbohydrates and oxygen through photosynthesis.[79, 80]

Because the role of alpha-carotene in plants is helping it deal with sun and shade, plants tend to concentrate this substance in their fruit, leaves, and stems. Alpha-carotene is usually bound to proteins that are themselves bound in fiber. It is not soluble in water and but it is soluble in fat.[79]

What does Alpha-carotene do in the human body?

For alpha-carotene to get into the human body from food, the food has to be thoroughly chewed. Proteins in the fibers that carry the tiny crystals of alpha-carotene have to be digested by stomach acid, and then there has to be the right mix of foods in the small intestine for alpha-carotene to be absorbed through the lining of the small intestine and into general circulation.[81]

Chemical Name: ALPHA-CAROTENE; Beta, epsilon-Carotene; All-trans-alpha-Carotene; Hi-Alpha; (+)-alpha-Carotene; (6'R)-beta, epsilon-Carotene.
Molecular Name: $C_{40}H_{56}$, **Molecular Weight**: 536.87264 g/mol
IUPAC NAME: 1,3,3-trimethyl-2-[(1E,3E,5E,7E,9E,11E,13E,15E,17E)-3,7,12,16-tetramethyl-18-(2,6,6-trimethylcyclohex-2-en-1-yl)octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]cyclohexene

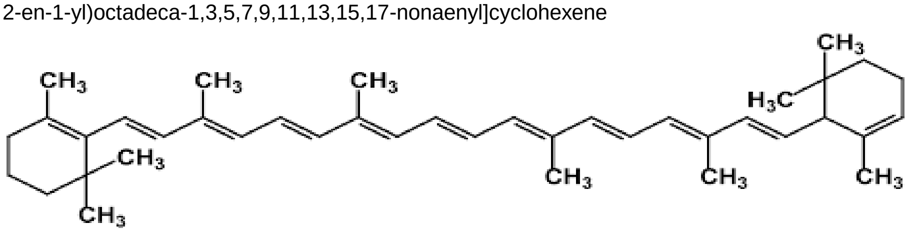


Figure: 1.6: Chemical Nature of Alpha-Carotene

Health benefit of alpha -Carotene

- Alpha-carotene is part of the carotenoid family, strong antioxidant and is one of the most abundant carotenoids in a healthy diet. Our body can convert alpha and beta-carotene into vitamin A for the maintenance of healthy skin and bones, good vision, and a robust immune system.[82]
- Though alpha-carotene is similar to beta-carotene, it “may be more effective at inhibiting the growth of cancer cells in the brain, liver, and skin,” according to Natural society study.[83]
- These orange and green vegetables are more “strongly associated with a decreased risk of lung cancer,” than all other vegetables. We all know vegetables can improve our health and even increase our life span. These findings merely substantiate those beliefs and give them scientific credence.[83]
- However, alpha-carotene may be even more effective than beta-carotene in its role as an antioxidant.

- Antioxidants are enzymes that stop free radicals from causing cells to break down, or oxidize. Powerful antioxidants like alpha-carotene remove destructive free radicals from the body before they cause the tissue damage that can lead to chronic diseases like heart disease and cancer.[84]
- In addition, alpha-carotene may help prevent cancer by stimulating cell-to-cell communication, a process which researchers now believe is necessary to ensure proper cell division.
- Alpha-carotene contains flavonoids, which are antioxidant substances that give color and flavor to many orange- and red-colored fruits and vegetables. Many flavonoids in fruits and vegetables are in the skin, can actually improve the body's ability to absorb them.[83]
- Dietary intake affects blood levels of α-carotene, which in one study was associated with significantly lower risk of death.

Lutien

Lutein from Latin *luteus* meaning ("yellow") is a xanthophylls and one of 600 known naturally occurring carotenoids. Lutein is synthesized only by plants and like other xanthophylls is found in high quantities in green leafy vegetables. In green plants, xanthophylls act to modulate light energy and serve as non-photochemical quenching agents to deal with triplet chlorophyll (an excited form of chlorophyll), which is overproduced at very high light levels, during photosynthesis. Lutein is a natural part of human diet when fruits and vegetables are consumed. Lutein is obtained by animals directly or indirectly, from plants.[85] Lutein is apparently employed by animals as an antioxidant and for blue light absorption. Lutein is found in egg yolks and animal fats. Lutein is called a carotenoid vitamin. It is related to beta-carotene and vitamin A. Foods rich in lutein include broccoli, spinach, kale, corn, orange pepper, kiwi fruit, grapes, orange juice, zucchini, and squash. Lutein is absorbed best when it is taken with a high-fat meal**.**[88, 89]

The carotenoid lutein is, like its sister compound zeaxanthin, a natural colorant or pigment, which appears yellow at low concentrations and orange-red at high concentrations. As the human body cannot produce lutein and zeaxanthin, they need to be obtained through food. Lutein is present in the eye, blood, skin, brain and breast. As antioxidants, potentially protecting the body against cell-damaging effects of free radicals, lutein and zeaxanthin have been linked to disease prevention, especially age-related eye diseases.[92]

Chemical Name: Lutein; XANTHOPHYLL; Trans-?Lutein; Bo-Xan; Luteine; Trans-Lutein;
Molecular Name: $C_{40}H_{56}O_2$, **Molecular Weight**: 568.87144 g/mol
IUPAC NAME: (1R)-4-[(1E,3E,5E,7E,9E,11E,13E,15E,17E)-18-[(1R,4R)-4-hydroxy-2,6,6-trimethylcyclohex-2-en-1-yl]-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaenyl]-3,5,5-trimethylcyclohex-3-en-1-ol

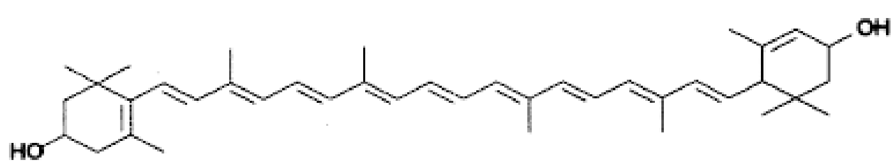


Figure 1.7: Chemical Nature of Lutien

Disease Risk Reduction

Lung cancer

In a population study, dietary intakes of lutein and zeaxanthin were associated with a decreased lung cancer risk. Other studies did not show such beneficial effects [87]

Age-related eye disorders

As lutein and zeaxanthin are present in high concentrations in the center of the eye's retina, the macular, where they are efficient absorbers of blue light, they may protect against light-induced damage, which is thought to play a role in the development of age-related macular degeneration (AMD). As light absorbers, lutein and zeaxanthin may also prevent clouding of the eye's lens, known as cataracts. Several studies found that men and women with the highest intakes of foods rich in lutein and zeaxanthin were less likely to develop cataracts. [88]

Blood vessel health

One of study suggested that high levels of lutein in blood may be linked to less thickening of blood vessel walls, a component of atherosclerosis. More research is needed to clarify this effect.[88]

Eye Health

It is noted that,several studies have suggested lutein and zeaxanthin may lower the risk for forming age-related macular degeneration (AMD) and cataracts. [87]

Visual performance under glare conditions

As lutein and zeaxanthin are thought to block out blue light and to shield from strong light, they may increase the eyes' tolerance for bright lights (e.g. glare from the sun).

Carotenoids such as lutein and zeaxanthin are fat-soluble substances, and as such require the presence of dietary fat for proper absorption through the digestive tract. [87, 88,90]

Health Benefit of Lutein

- Lutein is one of two major carotenoids found as a color pigment in the human eye (macula and retina). It is thought to function as a light filter, protecting the eye tissues from sunlight damage [86]
- Many people think of lutein as "the eye vitamin." They use it to prevent eye diseases including age-related macular degeneration (AMD), cataracts, and retinitis pigmentosa.
- Some people also use it for preventing colon cancer, breast cancer, type 2 diabetes, and heart disease.
- Lutein was found to be concentrated in the macula, a small area of the retina responsible for central vision. The hypothesis for the natural concentration is that lutein helps keep the eyes safe from oxidative stress and the high-energy photons of blue light. Various research studies have shown that a direct relationship exists between lutein intake and pigmentation in the eye. [87,88]
- There is epidemiological evidence of a relationship between low plasma concentrations of lutein and zeaxanthin, and an increased risk of developing age-related macular degeneration (AMD). Some studies support the view that supplemental lutein and/or zeaxanthin help protect against AMD. [87]
- There is also epidemiological evidence that increasing lutein and zeaxanthin intake lowers the risk of cataract development.

Lycopene

Lycopene from the neo-Latin *lycopersicum*, the tomato species, is a bright red carotene and carotenoid pigment and photochemical found in tomatoes and other red fruits and vegetables. Although lycopene is chemically a carotene, it has no vitamin A activity. [93]

In plants, algae, and other photosynthetic organisms, lycopene is an important intermediate in the biosynthesis of many carotenoids, including beta carotene, which is responsible for yellow, orange, or red pigmentation, photosynthesis, and photo-protection. Lycopene is not an essential nutrient for humans, but is commonly found in the diet mainly from dishes prepared from tomatoes. [94] When absorbed from the intestine, lycopene is transported in the blood by various lipoproteins and accumulates primarily in the blood, adipose tissue, skin, liver, and adrenal glands, but can be found in most tissues. Lycopene is a naturally occurring chemical that gives fruits and vegetables a red color. It is one of a number of pigments called carotenoids. Lycopene is found in watermelons, pink grapefruits, apricots, and pink guavas. It is found in People take lycopene for preventing heart disease, "hardening of the arteries" (atherosclerosis); [95] and cancer of the prostate, breast, lung, bladder, ovaries, colon, and pancreas. Lycopene is also used for treating human papilloma virus (HPV) infection, which is a major cause of uterine cancer. Some people also use lycopene for cataracts and asthma. Particularly high amounts present in tomatoes and tomato products. Lycopene, similar to other carotenoids, is a natural fat-soluble pigment (red, in the case of lycopene) which is synthesized by some plants and micro-organisms but not by animals, where it serves as an accessory light-gathering pigment .Unlike other micronutrients, such as vitamin C, lycopene content does not decrease during processing. protect these organisms against the toxic effects of oxygen and light. Because lycopene is so insoluble in water and is so tightly bound to vegetable fiber, the bioavailability of lycopene is increased by processing. Lycopene is a substance that has many health benefits for us and our body. Most health agencies encourage individuals to increase their intake of food that has high levels of carotenoids, lycopene included.[95, 96, 97]

Lycopene is an acyclic isomer of ß -carotene. It is a 40 carbon atom, open chain polyisoprenoid with 11 conjugated double bonds.

Chemical Name: LYCOPENE; All-trans-Lycopene; Psi,psi-carotene; Trans-Lycopene; Lycopene 7;
Molecular Name : $C_{40}H_{56}$, **Molecular weigh**t :536.87264 g/mol
IUPAC Name : (6E,8E,10E,12E,14E,16E,18E,20E,22E,24E,26E)-2,6,10,14,19,23,27,31-octamethyldotriaconta-2,6,8,10,12,14,16,18,20,22,24,26,30-tridecaene.

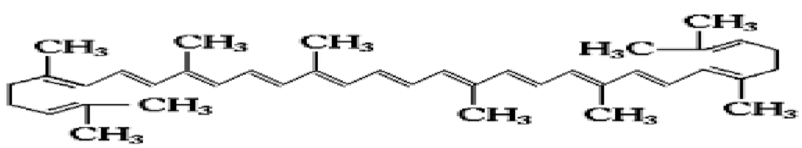


Figure 1.8: Chemical Nature of Lycopene

How Lycopene work for our body

Lycopene is allegedly a more effective antioxidant than other of its carotenoid cousins which include beta-carotene. Its powerful antioxidant actions are effective in maintaining the strength, thickness and fluidity of cell membranes. Cell membranes are the guardians of cells. They are responsible for screening what goes in and out of cells. They allow good nutrients in and remove cellular junk and prevent toxins from entering the cells. Strong healthy cell membranes are vital in the prevention of many diseases. [98]

Health benefits of Lycopene

Lycopene is well known specifically to help prevent many forms of cancer as well as the prevention and treatments of many illnesses and diseases such as:

- Lycopene is a powerful antioxidant that may help protect cells from damage. Lycopene is a strong antioxidant, works against free radical and can affect mechanisms of prostate cancer, liver disease and gall bladder. [98]
- Lycopene is a key intermediate in the biosynthesis of many important carotenoids, such as beta-carotene, and xanthophylls. [97]
- Heart diseases-Lycopene stops LDL cholesterol from being oxidized by free radicals and in turn cannot be deposited in the plaques which narrows and hardens the arteries.
- Infertility-Research suggests that lycopene may help in the treatment of infertility. Results from tests showed that lycopene can boost sperm concentration in men.

- Helps to prevent diabetes.
- Prevents age-related macular degeneration and cataracts.
- Prevents the aging of skin and keeps it younger looking.
- Acts as an internal sunscreen and protects skin from sunburn.
- Lycopene is also been known to help prevent osteoporosis. [98,99,100]

Beta -Cryptoxanthin

β (Beta)-Cryptoxanthin is a natural carotenoid pigment. It has been isolated from a variety of sources including the petals and flowers of plants. In terms of structure, cryptoxanthin is closely related to β-carotene, with only the addition of a hydroxyl group. It is a member of the class of carotenoids known as xanthophylls.In a pure form, cryptoxanthin is a red crystalline solid with a metallic luster. It is freely soluble in chloroform, benzene, pyridine, and carbon disulfide. In the human body, cryptoxanthin is converted to vitamin A (retinol) and is, therefore, considered a provitamin A. As with other carotenoids. Beta-cryptoxanthin belongs to the class of carotenoids, more specifically the xanthophylls. In the human body, beta-cryptoxanthin is converted to vitamin A (retinol) and is therefor considered as a pro-vitamin A. The phytochemical beta-cryptoxanthin can be found in many vegetables and fruits, mainly in papaya, mango, peaches, oranges, tangerines, bell peppers, corn and watermelon. Beta-cryptoxanthin is also found in some yellow coloured animal products such as egg yolk and butter. Beta-cryptoxanthin is a source of vitamin A, but about 2 times less strong than beta-carotene. Cooking of fruit and vegetables do not break down much the beta-cryptoxanthin. In order to absorb the beta-cryptoxanthin is essential that the diet contains fat, because beta-cryptoxanthin is fat-soluble substances.[101, 102]

Cryptoxanthin, also known as beta-cryptoxanthin, is a member of the carotenoid family, a group of flavonoids that provide color and flavor to fruits and vegetables. Carotenoids are proven antioxidants, and their role in protecting the body from free-radical damage has been well established. Although some carotenoids, such as alpha-carotene, beta-carotene, and lycopene, have been the subject of exhaustive research, scientists are just beginning to explore the possible benefits provided by cryptoxanthin. [100]

Like alpha- and beta-carotene, cryptoxanthin is a powerful antioxidant that can be converted to active retinol, or vitamin A, in the body. Vitamin A is crucial to the maintenance of healthy vision, reproduction, and body tissues. Recent studies have shown that cryptoxanthin also plays an important role in preventing many forms of cancer. [102,103]

A study published in the January 2004 issue of Cancer Epidemiology Biomarkers and Prevention reported that people with diets high in cryptoxanthin were 30 percent less likely to develop lung cancer. A lack of cryptoxanthin in the diet appears to leave the body vulnerable to other types of cancer as well. Preliminary studies also suggest that women with cervical cancer and patients with colon cancer have low levels of cryptoxanthin. Researchers theorize that cryptoxanthin protects against cancer both by neutralizing free radicals and by stimulating the Beta-Cryptoxanthin is a major source of vitamin A, often second only to beta-carotene, and is present oranges, tangerines, and papayas. Beta-Cryptoxanthin is a member of xanthophylls a class of carotenoids group. [100] Beta-cryptoxanthin is converted to vitamin A. Beta-cryptoxanthin is an antioxidant and may helps to prevent oxidative damage to DNA. Thus, beta-cryptoxanthin is believed also used to have health benefits on people at risk of certain chronic diseases, especially certain kinds of cancers. Anyway, beta-cryptoxanthin is also used to have health benefits for human health. [102,103,104]

Chemical Name: Beta-Cryptoxanthin; Cryptoxanthin; Cryptoxanthine; Beta-Caroten-3-ol; (3R)-beta,beta-caroten-3-ol;
Molecular Name: $C_{40}H_{56}$, **Molecular Weight**: 552.87204 g/mol

IUPAC NAME: (1R)-3,5,5-trimethyl-4-[(1E,3E,5E,7E,9E,11E,13E,15E,17E)-3,7,12,16- tetramethyl-18-(2,6,6-trimethylcyclohexen-1-yl)octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]cyclohex-3-en-1-ol

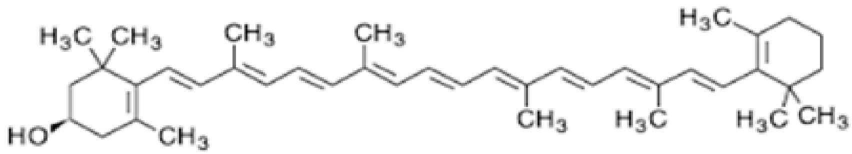


Figure 1.9: Chemical Nature of Beta-Cryptoxanthin

Health Benefit of beta-Cryptoxanthin

- β-Cryptoxanthin is an antioxidant and may help prevent free radical damage to cells and DNA, as well as stimulate the repair of oxidative damage to DNA.
- Recent findings of an inverse association between β-cryptoxanthin and lung cancer risk in several observational epidemiological studies suggest that β-cryptoxanthin could potentially act as a chemo preventive agent against lung cancer. [101]
- Beta-cryptoxanthin seems to reduce the risk of lung cancer and colon cancer. Studies have demonstrated that beta-cryptoxanthin can reduce the risk of lung cancer by more than 30 per cent. Researchers believe that the anti-cancer effect is linked to the antioxidant effect of beta-cryptoxanthin, but also to a specific expression of a gene that protects cells from becoming cancerous. [102]
- Beta-cryptoxanthin reduces risk for rheumatoid arthritis by 41 per cent. [102]
- Cryptoxanthin may protect against other diseases associated with aging, including heart disease, skin cancer, prostate cancer, and arthritis. Like other carotenoids, cryptoxanthin almost certainly plays a key role in keeping the eyes healthy and preventing against age-related macular degeneration (AMD) and cataracts. [105]
- Research also indicates that carotenoids may play a role in the prevention of many other serious health conditions, including Acquired Immunodeficiency Syndrome (AIDS), asthma, chronic yeast infection, and infertility.
- Cryptoxanthin is a fat-soluble substance, and needs to be taken with fat to be properly absorbed by the body. Consequently, certain conditions that reduce the body's ability to absorb fat, such as pancreatic enzyme deficiency, Crohn's disease, celiac sprue, cystic fibrosis, surgical removal of part or all of the stomach, gall bladder disease, and liver disease, may lead to a carotenoid deficiency. [105]
- Beta-cryptoxanthin may also benefit people at risk of diabetes.
- Oral administration of β-Cryptoxanthin repressed body weight, abdominal adipose tissue weight. [105,106]

Table 1.3: Sources, deficiencies and health benefits of carotenoids and carotene Profile

Compound	Deficiency Disease	Health Benefits	Good Sources
Carotenoids	free radicals oxidative damage, age-related macular degeneration of the eye, which leads to vision loss, heart ,disease, skin problem, cancer.	There is strong relation between diets rich in carotenoids and a reduced incidence of many forms of cancer, and that finding led to the suggestion that the antioxidant properties of those compounds might help protect immune cells from oxidative damage, thus enhancing their ability to detect and eliminate tumor cells. In addition to their role in cancer prevention, the carotenes offer us protection from heart disease and vision.	Carrot, pumpkin, sweet potatoes, spinach, grapefruits, broccoli, melons, tomatoes, colorful fruits, and dark green or green yellow leafy or non leafy vegetables are the good sources of carotene, but less in animal products
Beta carotene	Inflammation or dryness of eyes, night blindness teeth of children might develop abnormalities, , rashes on the skin causing redness and certain infections, free radicals will destroy all the healthy cells organs, cancer.	Plays a significant role in prevention problems, Beta-carotene is thought to possess many positive health benefits to eye health, skin disorder, maternal health and immune system.	Vegetables and fruits their rich colors.
Alpha Carotene	Eye problem, weakness of skin and bone, damage free radicals, destry healthy tissues.	Maintenance of healthy skin and bones, good vision, and a robust immune system.	Yellow-orange-dark-green vegetables like green peas, broccoli, green beans, spinach, collards and turnip greens, as well as in pumpkins and other squash, carrots and sweet potatoes.
Lutein	Age-related eye disorders, Blood vessel health, Lung cancer	Effect of protecting the eye tissues from sunlight damage, lowers the risk of cataract development and lung cancer.	Green leafy vegetables, broccoli, spinach, egg yolks and animal fats.
Lycopene	It s a strong antioxidant cancer, liver disease and gall bladder	Lycopene is a key intermediate in the biosynthesis of many important carotenoids, such as beta-carotene , reduce, cancer, diabetes.	In plants, algae, and other photosynthetic organisms and especially in tomatoes.
Beta-cryptoxanthin	Cataract, cancer, rheumatoid arthritis.	It has anti-cancer effect is linked to the antioxidant effect of beta-cryptoxanthin, but also to a specific expression of a gene that protects cells from becoming cancerous.	fruits, mainly in papaya, mango, peaches, oranges, tangerines, bell peppers and watermelon

Vegetables of Bangladesh

Bangladesh is an agriculture based country. Agriculture produces around 90% of its food including cereals and vegetable (WFP, 2010).Vegetables are important for nutritional, financial and food security in Bangladesh. Fruits and vegetables are highly valued in human diet mainly for vitamins and minerals. High prevalence of micronutrient deficiency i.e. hidden hunger is common in the society, and the situation could be improved significantly by increasing the consumption of high quality and micronutrient-rich foods like fruits and vegetables. Due to tropical and subtropical climates, a variety of fruits and vegetables are grown in Bangladesh. However, the present consumption of fruits and vegetables in Bangladesh is 126 g/day/capita (23g leafy vegetables, 89 g non-leafy vegetables and 14 g fruit), which is far below the minimum average requirement of 400 g/day/capita (FAO/WHO 2003).As our country is so fertile, so that varieties of vegetables grown in Bangladesh all over the year. [106,107,108]

There are 141 varieties of leafy vegetables (commonly known as sak) and 25 varieties of non leafy vegetables in Bangladesh (Maksuda, 2010).[111]Among the leafy vegetables,97 items are identified as ethnic varieties and the rest are consumed by both the general and ethnic people. Vegetable or any herbaceous plant whose fruit, seeds, roots, tubers, bulbs, leaves etc is used as food. Nearly 100 different types of vegetable comprising both local and exotic type are grown in Bangladesh (table). Vegetable is important for nutritional, financial, and food security in Bangladesh. However, the availability of vegetable is only about 1/5th of the recommended requirement of 200 g/person/day. Based on the growing season, vegetables are categorized as summer/rainy, winter and all season vegetables. The major winter vegetables are cabbage, cauliflower, tomato, brinjal, radish, hyacinth bean, bottle gourd, etc while major summer vegetables are pumpkin, bitter gourd, teasle gourd, Bitter gourd, ladies finger, ribbed gourd, ash gourd, okra, yard-long bean, and Indian spinach among others. Some vegetables like brinjal, pumpkin, okra, regular potatoes, and sweet potatoes and red amaranth are found to grow in both the seasons. Vegetable production on homestead Vegetables differing in morphology, growth habit, light, and nutrient requirement are grown in the homestead under complex multiple cropping system. [106,107,110]

Traditionally, farm families grow vegetables using local varieties and indigenous technologies mainly for family consumption and sell their surplus production. Women play the dominant role in this system. Some vegetables have localized areas of production because of favorable agro ecological condition, and better marketing infrastructure.[109]Pointed gourd in Bogra, onion in Faridpur, hyacinth bean in Chittagong, early cauliflower in Tangail, tomato in Jessore, varieties of potatoes in rangpur and Nawabganj are some of the examples of concentrated zones. There are lots of varieties of vegetables grown or exist in Bangladesh, which are good in quality , but not so much familiar to people. These local vegetables are consumed by our people, most of these vegetables have nutritive, but some of which are extremely good for human health . [106,109]

Unconventional Vegetables

Bangladesh having the congenial atmosphere and fertile land for farming vegetables, it still remains in the list of non-conventional items. The demand for Bangladeshi vegetable has been rapidly boosting up day by day because of being tasty, delicious and healthy quality. In addition to the most commonly available green-yellow vegetables, there are some relatively rare or uncommon varieties that can be defined as unconventional vegetables, which are nutrient rich and consumed by groups of people living in particular local area. [107]

Anything that's nonconformist or out of the ordinary can be described as **unconventional**. Every culture has its own conventions — or norms — and what may be unconventional in one region might be typical in another. The perception of what's unconventional is determined by context.

Exporters told BDNEWS that if initiatives would be taken to increase the production and export of these types of unconventional items including vegetables and crabs it can play an important role in the field of economic.

1.2 Rational of the study

Consuming a healthy diet throughout the life course helps prevent malnutrition in all its forms as well as a range of non communicable diseases and conditions. But the increased production of processed food, urbanization and changing lifestyles have led to a shift in dietary patterns. People are now consuming more foods high in energy, fats, free sugars or salt/sodium, and many do not eat enough fruit, vegetables, which are extremely good for human health.

Regular intake of vegetables and fruits can reduce the risk of vitamin A deficiency disorders. Fruits and vegetables are universally promoted as healthy; contain vitamins, minerals, dietary fiber and rich source of antioxidant. The Dietary Guidelines for Americans 2010 recommend make one-half of plate fruits and vegetables. Myplate.gov also supports that one-half the plate should be fruits and vegetables. Fruits and vegetables include a diverse group of plant foods that vary greatly in content of energy and nutrients. [113]

Green-yellow vegetables and fruits are rich source of provitamin A carotenes. Therefore, It is important to explore dietary rich source of vitamin A. A diet rich in vegetables and fruits can lower blood pressure, reduce risk of heart disease and stroke, prevent some types of cancer, lower risk of eye and digestive problems, and have a positive effect upon blood sugar which can help keep appetite in check.

In line to this view, a selected number of unconventional vegetables, consumed by specific group of very local communication, have been analyzed for their carotene profile. The findings not only suggest the importance of these vegetables in a healthy and balance diet, but also provide the motivation for consuming some uncommon varieties in our daily food menu.

It is also maintaining the biodiversity and to conserve these food sources from extinction, also from further destruction, in order to enrich plant food source.

1.3 Objective of the study

There are several known places in our country where varieties of foods can be found, which are not so well known but have better nutrition quality. In this research the focus was on unconventional plant sources belongs to some selected area and specially their uses as food and the nutritional contribution.

Aim of the study was to

- Estimate total Carotenoid content and
- analyses carotene profile, comprising Beta-Carotene, Alpha-Carotene, Lutien, Lycopene, Beta-Cryptoxanthin ; in selected unconventional vegetables.

Chapter 2

Materials and Method

Dietary carotenoids are thought to provide health benefits in decreasing the risk of disease. Food sources of these compounds include a variety of fruits and vegetables. Vegetables are one of the cheap sources of provitamin a carotenoids. Many people of Bangladesh could not afford to subsistence level of income for meeting their basic needs, as a result, they suffer from nutritional deficiencies as well as vitamin A deficiency from their very early days.

Besides local vegetables, people may keep some uncommon plant foods in their daily menu, which are act as nutritive food and also strong antioxidants.

This study has aim to prepare detail information of some unconventional vegetables, containing their vitamin A (carotene) presence level based on laboratory analysis.

2.1 Selection of vegetable items

The plant food approach is used to identify and select food items for analysis of carotene profile. The purposes of vegetable items are to choose important foods which have a positive effect on human health and nutrition with great advantage.

Over the past three decades a number of studies, which include national nutrition surveys, have been carried out to investigate the prevalence of vitamin A deficiency among different population groups in Bangladesh, and they have demonstrated a significant public health problem.(public health nutrition,2000) [110].To eradicate VAD in Bangladesh sources of vitamin A must be enrich more.

In this present study twenty seven vegetables comprising 23 leafy and 4 non-leafy, has been selected for analysis of total carotenoids and carotene profile. Vegetable items has been choosen from some of selected local area, due to selection of these plant foods there was some priority condition such as-

- food items consumed by local people of selected area, have a positive comment from them
- The group of vegetables which are not so much known to mass people, no carotene analyzed were done before.

2.2 Study Area

Sample were collected from some selected area of Gazipur, Mymensing and

CHT's (Chittagong hill tracts) in Bangladesh.

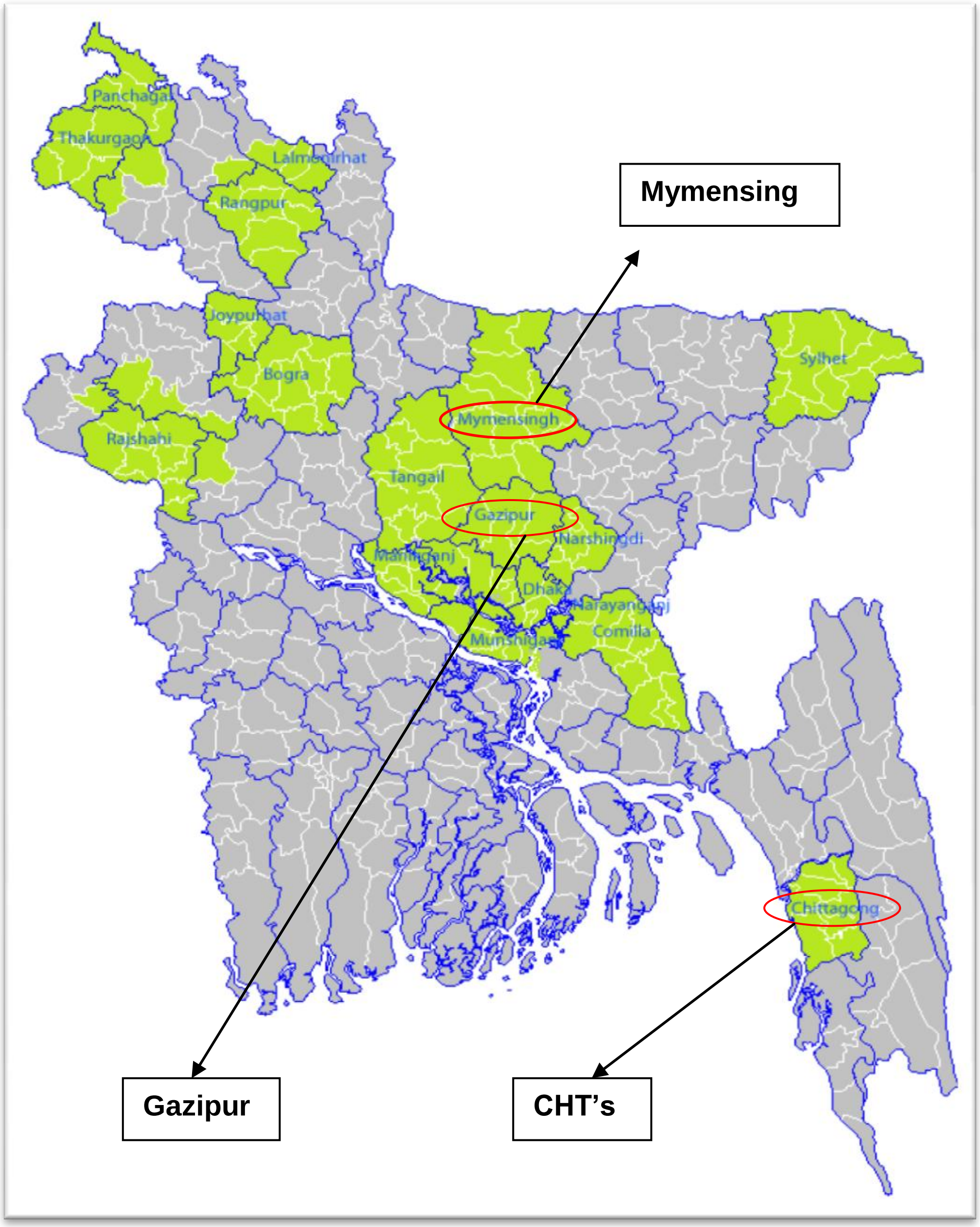


Figure: 2.1 Map of Study Area

Image 1.1 Collection & selection of vegetables

Image 1.1 Collection & selection of vegetables

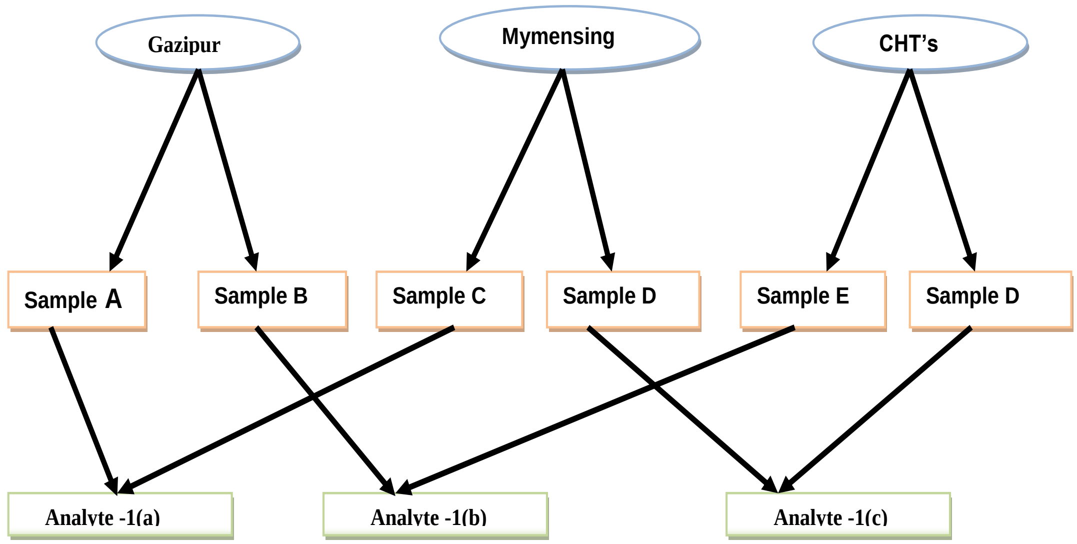


Figure: 2.2 Multi-region Sampling Designs for Unconventional Vegetables

2.3 Procedure of Food Sample Collection

Three replicate samples for each item were collected from each location in new clean plastic bags. Some water was sprayed on the vegetables samples during packing into the poly bag and thus kept it moistened during transportation from field to the lab, as for carotene analysis there needs fresh sample. These Replicate sample were mixed together to make a single sample for each collection point and thus made six samples for six collection sites. Two samples were then pooled to make a single test sample and thus made three test samples for each vegetables items. To avoid sampling error, a large portion (approximately 2.0kg) of replicates samples for every item were collected from each collection point. Since there is limited scope to study the seasonal variation, the vegetables were collected during their pick available period. [116]

2.3.1 Identification and Characterization of Food Sample

The food items (vegetable) were categorically identified and certified by personnel of the taxonomist of the Department of Botany, University of Dhaka. After taking food sample to the laboratory, taxonomist expert completed further identification of the plant items , for its common, local, English and scientific name with family and collection area.

Image 1.3 Identification and Characterization of vegetable Sample by Taxonomist

Table: 2.1 List of selected unconventional vegetables including Leafy and non-leafy

SL	Common Name	English Name	Local Name	Scientific Name	Family	Collection
	Leafy vegetable					
1	Shorno lota	Dodder	Torulota, Jigro	*Cuscuta reflexa Roxb.*	Cuscutaceae	Bandarban
2	Mandar	Indian coral tree	Madar	*Erythrina variegata L.*	Fabaceae	Bandarban, Gazipur
3	orhor leaf	Pigeon pea	Dumursumi, Crakchi	*Cajanus cajan Millsp.*	Fabaceae	Bandarban, Gazipur
4	Kantanotey	Spiny pigweeed	Katamaris, Khuriakanta	*Amaranthus spinosus L.*	Amaranthaceae	Bandarban, Gazipur,Dhaka
5	Shetodhron		Dhondokolos,Thorinlodi	*Leucas aspera (Roth) Spreng*	Lamiaceae	Bandarban, Gazipur,Modhupur
6	Shetokanchon	White Orchid Tree	Boro katamaris, Ghi kanchon	*Bauhinia acuminata*	Fabaceae	Bandarban
7	Parul	Parul	Parsia,Ghengha	*Stereoperum suaveolens*	Fabaceae	Bandarban
8	Helencha	Marsh herb	Helenche shak,Inlonchi	*Enhydra fluctuans*	compositeae	Bandarban
9	Amrul	Wood sorrel	Thingra	*Oxalis europaeajord*	Oxalidaceae	Bandarban
10	Sada koroi	Frywood tree/Labbec tree	Sada koroi	*Albizia procera*	Fabaceae	Bandarban
11	Telakucha	Ivy groud baby water melon	Telakucha	*Coccinia grandis*	Cuccer bitaceae	Bandarban
12	Holud pata	Turmaric	Holud Pata	*Curcuma domestica*	Zinger Beraceae	Bandarban
14	Kachuripana	Water hyacinth	Pana	*Eichhornia crassipes*	Pantederiaceae	Bandarban
15	Tatul Pata	Tamarind Tree	Tatul pata	*Tamarindus indica*	Fabaceae	Bandarban
SL	Common Name	English Name	Local Name	Scientific Name	Family	Collection
16	Muktajhuri	Indian acalypha	Muktajhuri	*Acalypha indica*	Euphorbiaceae	Modhupur
17	Roktodrone	Red verticulia	Roktodrone	*Leonurus sibiricus*	Labiatae	Modhupur
18	Jolpai pata	Indian olive leaves	Jolpai pata	*Elaeocarpus Roxb*	Elaeocarpaceae	Modhupur
19	Bondhonia	Wild coriander	Bondhonia	*Scroparia dulcis*	Scrophulariaceae	Modhupur
20	beth gach	korok bet	beth gach	*Calamus tenuis*	Arecaceae	MAU
21	Chimti shak		Chimti shak	*Polygonum plebeium*	Polygonaceae	MAU
22	Venna pata	venna leaves	Venna pata	*Ricinus communis L*	Euphorbiaceae	MAU
Non-Leafy vegetable						
23	Pahari kolar Thor	Dwarf cavendish	Kolattur,Feufu	*Musa acuminata Colla.*	Musaceae	Bandarban
24	Rakhal Sosha	Wood Cucumber	Rakhal Sosha	*Zineria seaber*	Fabaceae	Bandarban
25	OFSP	Sweet Potato	MISTI ALU	*Ipomoea batatas var.*		BARI, Gazipur
26	Orhor	Pigeon pea	Dumursumi, Crakchi	*Cajanus cajan Millsp.*	Fabaceae	Bandarban,Gazipur, Dhaka
27	Mulachi	Radish seed	Mulachi	*Raphannus sativus L.*	Brassicaceae	Bandarban

2.3.2 Introducing unconventional food sample

Leafy Vegetables

1. **SHETOKANCHAN**

Scientific Name: ***Bauhinia acuminata***

Family: Fabeceae

Medicinal Use: Leaves are used to treat ulcer, wound, stomach tumor, diarrhoea and also works against diabetes, cancer, has strong antioxidant properties [122-125].

Image: 2.1 Shetokanchon

2. AMRUL

Scientific Name: ***Oxalix europaejord***

Family: Oxalidaceae

Medicinal Use: A poultice of the plant has been used to treat swellings. [126-128]

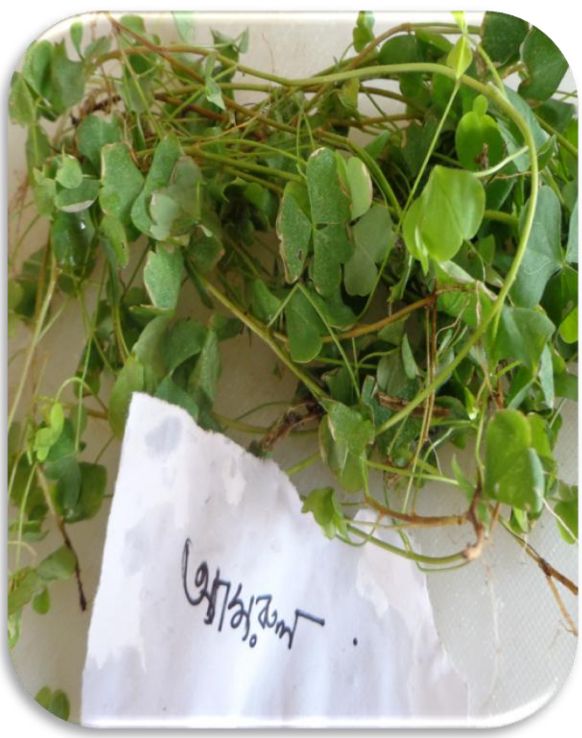


Image: 2.2 Amrul

3. MANDAR

Scientific Name: ***Erythrina variegata***

Family: Fabaceae

Medicinal Use: Leaves are used to treat eye ailments, relieve joint pain, work as antioxidant, reduce CVD effect, juice of this leaves stimulate lactation and menstruation period. [130,131]

Image: 2.3 Mandar

4. SAADA KOROI

Scientific Name: ***Albizia*** **procera**

Family: Fabaeace

Medicinal Use: This plant has antioxidant activity as well as works against cancer, diabetes, liver disorders, eye vision, atherosclerosis and inflammatory diseases**.** [133]

Image: 2.4 Saada Koroi

5. TELAKUCHA

Scientific Name: ***Coccinia grandis***

Family: Cuccer betaceae

Medicinal Use: In traditional medicine, its use to treat leprosy, eye health, fever, asthma, bronchitis, skin disease, jaundice and joint pain. (135-137)

Image:2.5 Telakucha

6. VENNA PATA

Scientific Name: ***Ricinus communis L***

Family: Euphorbiaceae.

Medicinal Use: Leaves are helps to protect liver from damage, has antimicrobial properties, works against skin dryness, headache, swallowing etc. (138-140)

Image: 2.6 Mandar

7. ORHOR LEAVES

Scientific Name: ***Cajanus cajan (L.) Millsp***

Family: Fabaceae

Medicinal Use: Leaves are use to improve indigestion in traditional medicines as well as limit the excessive production of urine and also work against anemia, fever, ulcer and typhoid. [141,142]

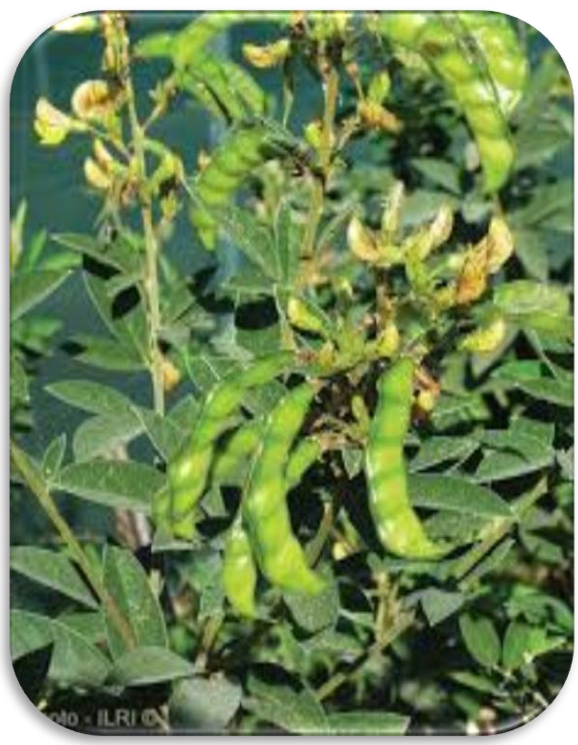


Image:2.7 Orhor Leaves

8. SHETODHRONE

Family: Lamiaceae

Scientific Name: ***Leucas aspera***

Medicinal Use: This plants are used to treat gout, liver ailment, constipation, kidney disorders. [144,146]

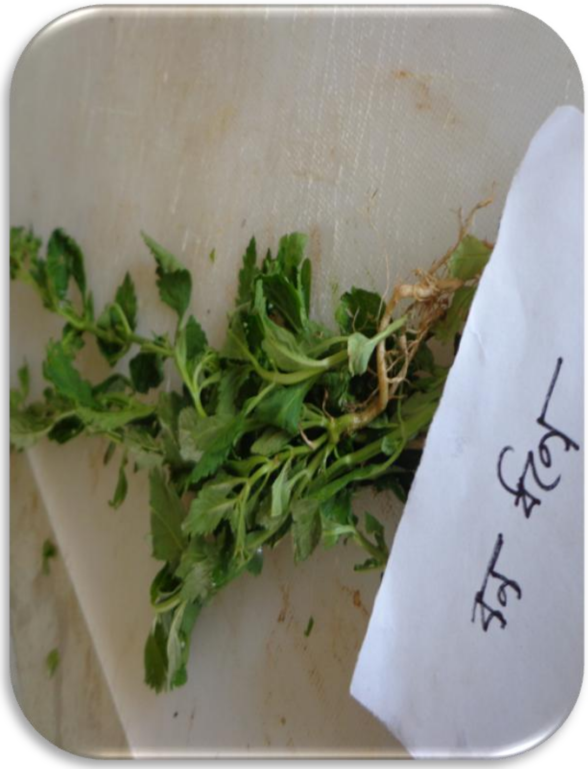
Image:2.8. Shetodrone

9. BON DHONE

Scientific Name: *Scroparia dulcis*

Family: Scrophulariaceae

Medicinal Use: Leaves are used to treat diabetes, hypertension, wounds, anemia, burns, headache, stomach ulcer, also has anti allergic activity. [147]



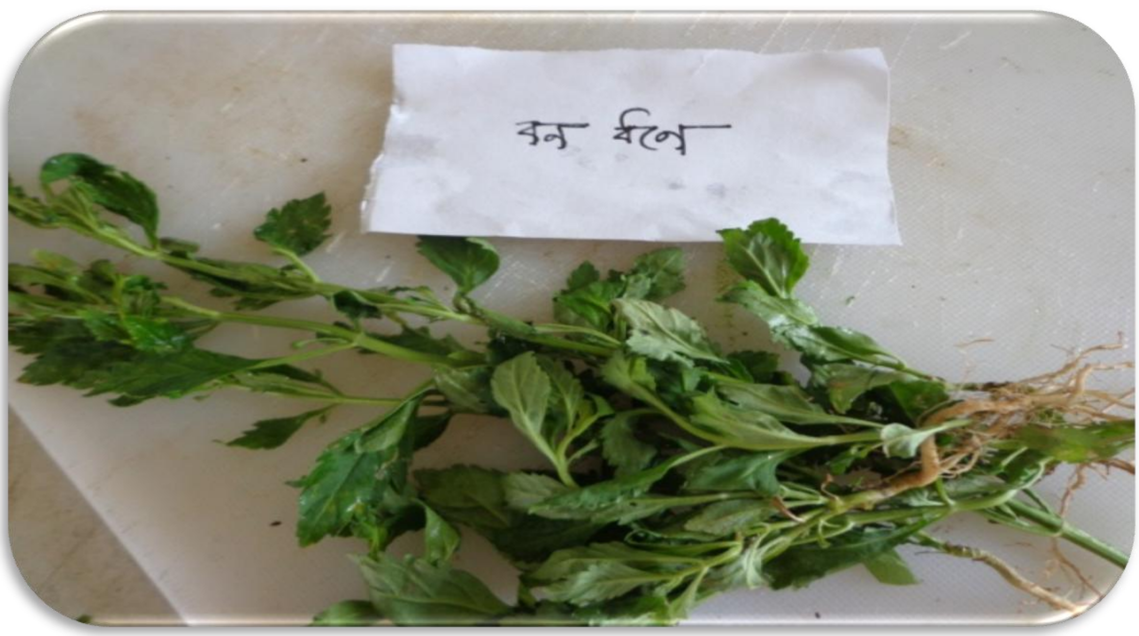


Image:2.9 Bon Dhone

10. ORANGE FLESHED SWEET POTATO LEAVES (OFSP leaves)/MISHTI ALOO PATA

Scientific Name: ***Ipomoea batatas L.var.***

Family: Convulvulaceae

Medicinal Use: This plant plays a role in health promotion by improving immune function ,has strong antioxidant properties as its reduce oxidative stress by damaging free radical, control blood pressure and suppress cancer cell growth**.** (185-187)

Image: 2.10 OFSP Leaves

11. PARUL

Scientific Name: ***Stereoperum suaveolens***

Family: Fabaceae

Medicinal Use: This plant helps to improve eye vision, improve eye health, and work as strong antioxidant. [148]

Image:2.11 Parul

12. SHORNOLOTA

Scientific Name: ***Cascuta reflexa roxb.***

Family: Cuscutaceae

Medicinal Use: Leaves are use as traditional medicine for osteoporosis, strengthen the liver and kidney. (49-151)

Image:2.12 Shornolota

13. JOLPAI PATA

Scientific Name: ***Eleocarpus roxb.***

Family: Elaceocurpaceae

Medicinal Use: Leaves are help to remove headache, cough, paralysis, heart disease, control blood pressure and improve maternal health. [152]

Image:2.13 jolpai pata

14. MUKTAJHURI

Scientific Name: ***Acalpha indica***

Family: Euphorbiaceae

Medicinal Use: Leaves are traditionally use to relieve from the pain of snakebite, skin disease, good stimulating application in rheumatism, work as antioxidant, has antifungal and antibacterial activity. (153-156)

Image:2.14 Muktajhuri

15. ROKTODRONE

Scientific Name: ***Leonurus sibricus***

Family: Labiatae

Medicinal Use: Plants are remedy for menstruation issue, has effect on heart health, liver, arthritis and female reproduction. [158,159]

Image:2.15 Roktodrone

16. BETH GACH

Scientific Name: ***Calamus tenuis***

Family: Aracacae

Medicinal Use: Reduce Blood pressure, has anti-diabetes, anti-cholesterol activity, work against gastritis, sweating, skin disease and stroke. [162]

Image:2.16 Beth Gach

17. TATUL PATA

Scientific Name: ***Tarmarindus indica***

Family: Fabaceae

Medicinal Use: Leaves are use as an antioxidant, has effect on fever, diabetes, control cholesterol, improve bone structure, protect eye health particularly cataract which can eventually lead blindness. [164]

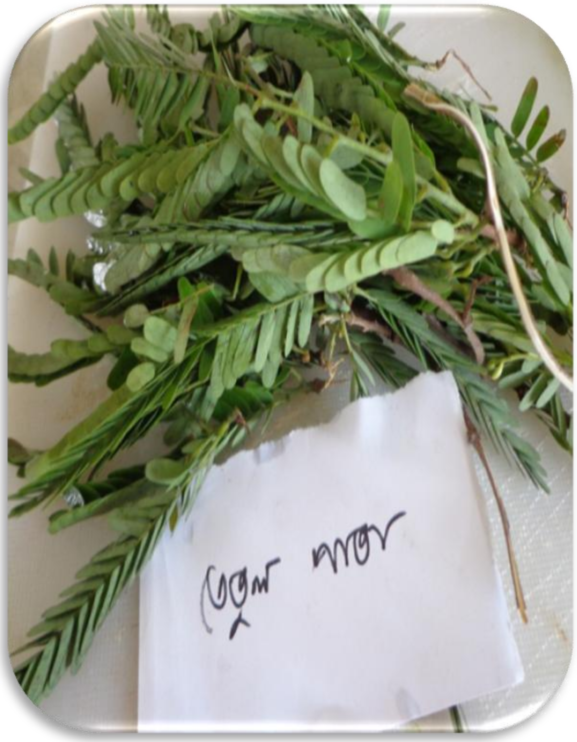


Image:2.17 Tetul Pata

18. CHIMTI SAK

Scientific Name*: Polygonum plebeium*

Family: Polygonaceae

Medicinal Use: The plant is effective in survey, liver diseases. People sap the leaf on the body during scorching heat of summers for relief in blisters and boils. [167,168]

Image:2.18 Chimti Sak

19. HELENCHA

Scientific Name: ***Enhydra fluctuans***

Family: Compositeae

Medicinal Use: Its can be use as a traditional medicine against constipation, asthma, neurological disorders, epilepsy, skin disease , has antioxidant activity, work as pain killer. [170]

Image:2.19 Helencha

20. KOCHURIPANA

Scientific Name: ***Eichhornia crassipes***

Family: Pantederiaceae

Medicinal Use: This free floating plant has health effect against CVD, neurologic disorders, diabetes and cancer. [172]

Image:2.20 Kochuripana

21. KATANOTEY

Scientific Name: ***Amaranthus spinosus***

Family: Amaranthaceae

Medicinal Use: Leaves are helps to improve appetite, dental health, constipation, gonorrhea, cardiovascular disease, also good for immune system. [173]

Image:2.21 katanotey

22. HOLUD PATA

Scientific Name: ***Curcuma domestica***

Family: Zinger Baraceae

Medicinal Use: This plant is a very good spice source. Its work as antioxidant treats and prevents neurodegenerative disease Alzheimer's, inhibit cancer cell growth in colon, prostate, lung and breast. [176]

Image:2.22 Holud Pata

23. RAKHAL SHOSHA

Scientific Name: ***Zineria seaber***

Family: Fabeceae

Medicinal Use: Can be use for dermatitis, skin care, constipation, diabetes, kidney health, also control blood pressure. [178]

Image:2.23 Rakhal Shosha

Introducing Unconventional Food Sample

Non-Leafy Vegetables

24. PAHARI KOLAR THOR

Scientific Name: ***Imusa acuminata Colla.***

Family: Musaceae

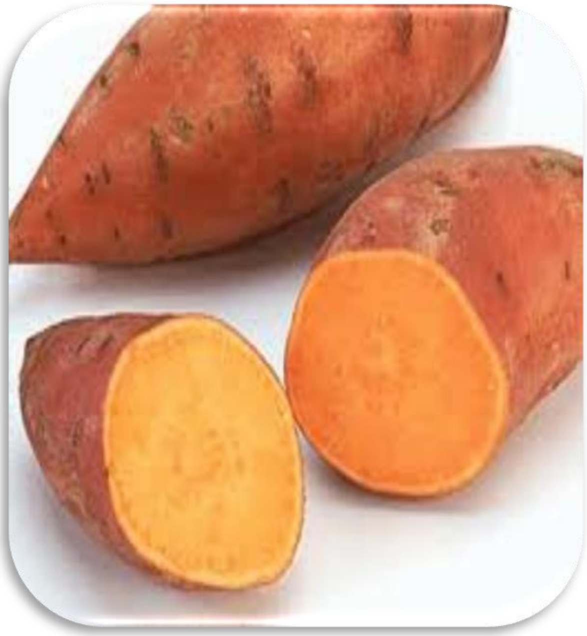
Medicinal Use: It's a high calorie food, work as antioxidant, control blood pressure and heart disease. [180,181]

Image:2.24 Pahari kolar thor

25. MISHTI ALOO/ ORANGE FLESHED SWEET POTATO (OFSP)

Scientific Name: ***Ipmoea batatus var.***

Medicinal Use: This food is high in beta-carotene, one of the versatile root vegetables, has strong antioxidant activity, fight against powerful diseases, has ant carcinogenic and ant diabetic properties, protect skin, hair, eye health etc. (185-187)



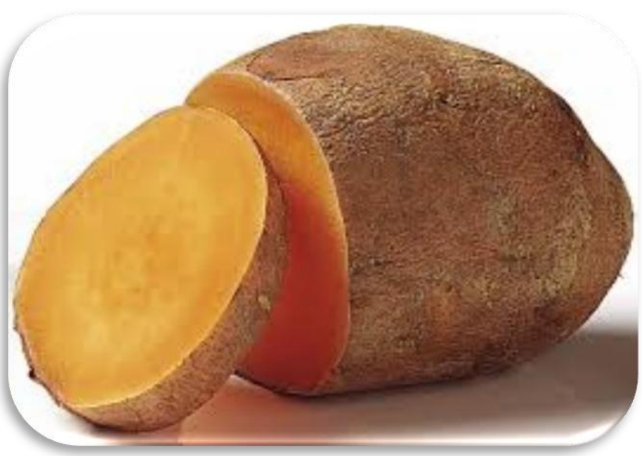


Image: 2.25 OFSP

26. MULACHI

Scientific Name: ***Raphanus sativus***

Family: Brassicaceae

Medicinal Use: Helps to prevent jaundice by remove bilirubin, keep it stable; control blood pressure, diabetes, CVD, fever, obesity, many types of cancer etc. [182]

Image:2.26 Mulachi

27. ORHOR SEEDS

Scientific Name: ***Cajanus cajan Millsp***

Family: Fabaceae

Medicinal Use: In combination with cereals make a well balanced diet. Helps to prevent ulcer, malaria, fever and aneamia. [187,188]

Image:2.27 Orhor Seeds

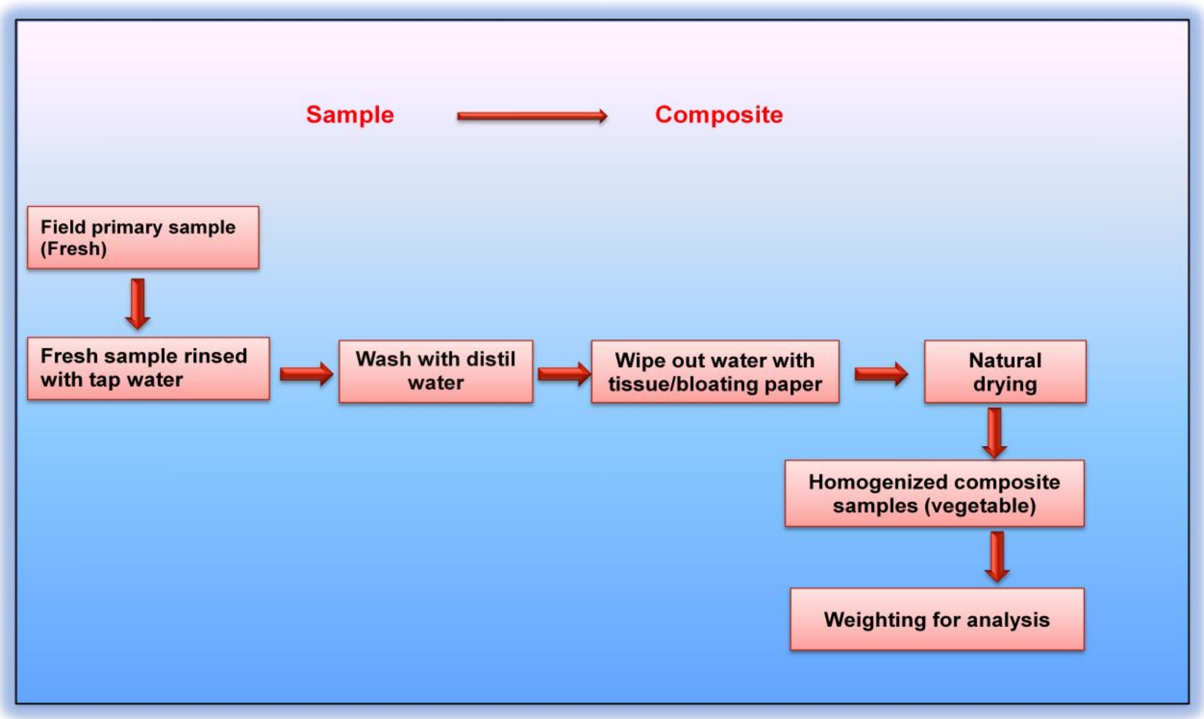


Figure: 2.3 Preparation of Food Sample

2.4 Procedure of Sample Preparation for Estimation of Total Carotenoids

Carotenoid content in the supplied sample was determined by acetone and petroleum-ether extraction followed by spectrophotometric measurement (Roriguez-Amaya and Kimura, 2004). It is notable that green colored (green edible portion) leafy vegetables and some non-leafy green vegetables are subjected to treat with "column chromatography" after extraction procedure. [116,117]

Reagents

- Acetone (Merck, Germany)
- Petroleum Ether (40/60, BDH, England)
- BHT (Butylated Hydroxy toluene)
- Alumina / Neutral Aluminum Oxide (E. Merck)
- Anhydrous Sodium Sulfate(Merck, Germany)

Preparation for analyses

- Distill petroleum ether through glass distillation plant.
- Acetones were kept in the refrigerator (4˚c) for 2 hours prior to the beginning of homogenization procedure.
- A solution of 1mg/1ml BHT in acetone (app 10 ml) were prepared for only few weeks and wraped and kept in refrigerator with date and label. For this, 10 mg (0.001g) BHT were pouring into 10 ml acetone.
- All apparatus including vacuum flask, funnel, sintered glass, conical flask, volumetric flask and even separating funnel were cover with black cloth/aluminum foil.
- There were dim light and cool environment (room temperature should <20˚c) of the working place.

Extraction Procedure for total carotenoids

Extraction of carotenoid was performed by *grinding* of the supplied sample in mortar and pestle, filtration through sintered glass filter under vacuum and *separation* from acetone to petroleum ether. These 3 steps were followed consequently-

1. Grinding
2. Filtration
3. Separation

1. Grinding

- 2gm of sample (colored fruit/vegetables) were taken in a cleaned, dried mortar containing acid-washed sand (1g). It would mash and well mix with pestle under black cloth.
- Addition of 10-15 ml of cold acetone and 100 µL BHT solution (1mg/ml BHT in acetone) to the mortar contents and carefully homogenized with pestle for about 3 minutes.
- If required keep the mortar contents covered with black cloth in dark places for few minutes (15-20 minutes).

2. Filtration

- The homogenate (extract in acetone) were carefully transferred to a sintered glass filter (top of the Buchner funnel) underneath which vacuum flask wrapped with aluminum foil/black cloth. Thus, the deep colored (yellow/ orange/red/green) 'acetone extract' can be collect into the vacuum flask wrapping with black cloth.
- The mortar and pestle as well as very small particles/solid residue left on the sintered glass would wash with 5-10 drops of cold acetone (with the help of a Pasteur pipette) and added to the Buchner funnel to filter. This step, if required could be repeated 2-3 times until small particles/solid residue of the sample becomes colorless.

3. Separation of total carotenoids

- 20 ml of distilled petroleum ether (PE) will take in a 250ml capacity separating funnel with Teflon stop-cork which should also wrap with black cloth. The colored, filtered 'acetone extract' gently pour into the petroleum ether through a funnel allowing it pouring through the separating funnel's wall. The vacuum flask then rinses with drops of acetone (2-3 times, total acetone=1-5ml) with the help of a Pasteur pipette and pour through funnel.

- Now 150 ml of distilled water is added very slowly and carefully to the separating funnel allowing water to flow down the funnel wall gently. It was then left undisturbed for 5-10 minutes to allow separating into 2 layers.
- Then the lower aqueous layer was discarded carefully without any agitation. This step has to repeat for 3-4 times with 100ml of distilled water until the water completely removes the residual acetone. Finally, the lower aqueous phase is discarded as completely as possible, without discarding any part of the upper phase.
- The colored 'petroleum ether extract' in the separating funnel were collected through a funnel containing small amount of anhydrous sodium sulfate (15g) on a cotton plug placed into top of a 25ml volumetric flask wrapped with foil to prevent light. The separating funnel would rinse round its surface containing 'colored extract' with drops of petroleum ether (~2ml) by using a Pasteur pipette and thus the washings is also collected through funnel with the presence of anhydrous sodium sulfate .
- This colored 'eluent' were brought to its volume 25ml by distilled petroleum ether. The flask is capped and gently mixed and also wrapped up with aluminum foil.
- If the color of the 'eluent' is orange or yellow like, it would be ready for the colorimetric measurement for the concentration of "total carotenoids" and absorbance of the 'eluent' would read at 450nm with the help of a spectrophotometer (UV-1601, UV-Visible, Shimadzu, Tokyo, Japan). All preparative and extractive procedures should perform in dim lighting. Only Orange Fleshed Sweet Potato (OFSP) of all the unconventional vegetables, were orange in color, so it didn't need any column chromatography.
- Furthermore, if the color of the 'eluent' (separated from aqueous phase) is dark greenish it should further pass through a chromatographic column (2.5×40cm) to remove plant color (chlorophyll) for quantitative analysis of total carotenoids.

Chromatographic Separation of Photosynthetic Pigments

The yellow pigments present in leaves or other parts of the plants, are usually masked by the green colored pigments; chlorophylls. To resolve carotenoids from the PE (Petroleum Ether) extracts which are green or light green in color, the chromatographic separation should be carried out, prior to the spectrophotometer quantification. But for the samples(like Orange Fleshed Sweet Potato) for which the PE (Petroleum Ether) extracts are colorless or yellow or red in color this chromatographic separation does not need to perform,

Preparation of the Chromatographic Column

Open Column Chromatography or OCC is a descending, gravity-flow column which can be followed visually by a colored band formation inside column. In fact, it is the classical method for separating total carotenoids for quantitative analysis. The column that is usually used is a 'Pyrex chromatographic column' (2.5 × 40cm) with a sintered glass at the bottom. Here the stationary phase *or adsorbent* of the column chromatography is solid alumina and sodium anhydrous sulfate dry powder (1:1) and the mobile phase *or eluent* is the solvent Petroleum Ether (PE) which is flashed through the column. The column should pack for each analysis (each individual sample). The height of the column should ~15cm.

- To prepare the column, equal amount (1:1) of alumina and anhydrous sodium sulfate by weight is taken in a mortar and gently homogenize for 15-30 minutes using pestle and taken in a glass plate and should keep in the drier (45˚c-60˚c) over night by covering it with aluminum foil paper.

- To pack the column this dried salt is again taken in the mortar and sufficient amount of petroleum ether (PE) should add to it and mixed gently using pestle.

- This mixture is then transferred into the column through a big funnel placed on the top of the Pyrex column. While transferring the mixture, the parallel sides of the column should carefully, gently tap/slap several times by using a small rubber tube (~20cm) which is very important for every column pack because of the consistency, rigidity of the solids and overall better accommodation.

- Then about one bed column (full) of PE (Petroleum Ether) should pass through the column and the solvent flow should be adjusted to about 2-3 drops per second. The top of the column should cover with PE (Petroleum Ether) solvent at all time until chromatography is completed.

Chromatographic Separation procedure of "total carotenoids" from green Pigment (chlorophyll)

In this chromatographic technique, the components of the pigment mixture are partitioned between the mobile phase (solvent) and the stationary phase (alumina) due to their different adsorption and solubility strength. Since a high non-polar solvent (petroleum ether),were employed in this experiment, the pigments that were least polar (carotenoids) would be best solved in the non-polar solvent (*"similia similibus solvuntus"*) and therefore washed through the column much faster .

- At the start of the process the bottom tap of the column were open to allow the solvent (PE) already in the column to drain so that it could level equally with the top of the packing material.
- Then 10 ml of green colored PE (Petroleum Ether) extract of the plant sample add carefully to the top of the column using a 10 ml pipette. To do this, pipette should insert into the column's neck and allow 'green extract' to pour down drop by drop round the column's wall without disturbing the column's bed (top surface level). After that, the pipette should rinse with small amount of PE (Petroleum Ether) and the rinsing content should add to the column.
- And again tap of the column were open.
- The sample layer is then allowed to go down almost to the surface of the adsorbent layer and 10-20 drops (by Pasteur pipette) of PE should add to the top as frequently as possible before it get dried and at the same time, column should fill with PE fully as soon as green extract go down.
- A yellow band which is formed soon after the extract goes down and adsorbed containing carotenoids, would travel down the column, leaving the green chlorophyll pigments adsorbed onto the bed.
- The yellow band were collect in a 25ml volumetric flask with a final volume of 25ml.The flask should be foiled, capped and gently mix and is ready for spectro-photometric quantification.

Precautions

- The column should always cover with black cloths to prevent oxidation of carotenoids .
- Fresh solvent i.e. PE should continuously add as gently as possible without disturbing the packing materials. As the solvent is traveled down the column, it should collect in a beaker/conical flask and should keep adding (reuse) to the top so that the column never dries out.
- The samples should be protected from exposure to light, oxygen and heat. As exposure to light, especially direct sunlight or UV light induces *trans-cis* photo isomerization and photo destruction of carotenoids.
- The analysis should complete in one session as quickly as possible to prevent losses of carotenoids from exposure to air, light and heat.
- All procedures should be carried out in subdued light and cooled environment. Open column and vessels containing carotenoid solutions should wrap with aluminum foil and/or pieces of black colored cloths, as added protections.
  Distilled water should add gently in the separating funnel, along with the walls of the funnel to prevent formation of emulsion and the two layers (i.e. aqueous and petroleum ether phase) would allow to separate without agitation.

Calculation (without chromatography)

Carotenoids in solution obey the Beer–Lambert law, that is, their absorbance is directly proportional to the concentration. In OCC (Open Column Chromatography) methods, the quantification step was fairly straightforward. The absorption coefficient $A^{1\%}_{1cm}$ of a carotenoid (absorbance at a given wavelength of a 1% solution in1 cm light-path spectrophotometer cuvette), used in the calculation of the concentration. For example, beta carotene has an absorption maximum (λ max) of 450nm in PE (Petroleum Ether).

In this experiment the absorbance of the eluent was estimated at 450nm and 470nm .Spectrophotometer was initially set to zero absorbance with the solvent PE (Petroleum Ether), to ensure that the absorbance reading would reflect only the absorbance of the molecules to be quantified i.e. carotenoids in plant samples.

The total carotenoid content was calculated using the following formula:

Total carotenoid content (µg/g) = $\frac{A \times volume\,(mL) \times 10^4}{A_{1cm}^{1\%} \times sample\ weight\,(g)}$

Where;

A= absorbance;

Volume = total volume of extract (25 ml);

$A_{1cm}^{1\%}$ =Absorption coefficient of β carotene in Petroleum Ether (2592).

Multiplying by 100 gives the carotenoid content in µg/100 g.

Calculation (with chromatography)

In case of green sample,

As only 10 ml extract is taken for chromatography, so the weight of the sample -

25 ml PE extract has 2 gm sample

So, 10 ml has (2*10)/25=0.8 gm

Total carotenoid content (µg/g) = $\frac{A \times volume\,(mL) \times 10^4}{A_{1cm}^{1\%} \times sample\ weight\,(g)}$

$$= \frac{absorbance*25*10^4*100}{2592*0.8}$$

Expression of Data

The nutrient content in the leafy and non-leafy vegetables wrere expressed as the amount per 100g of edible portion.

Edible portion is the part of the food that is customarily eaten by the people depending on their cultures or food habits. Edible portion is expressed as percent. The edible portion is the proportion of edible matter in the food as collected, expressed on the basis of weight. It is obtained using following formula:

'Edible' Weight = 'As Purchased (A.P) –'Refuse' Weight (R)

% Edible Portion (E.P) = $\frac{\text{'Edible' Weight}}{\text{As Collected' (A.C) Weight}} \times 100$

In the study, the symbol '-' indicates only that these are not analyzed as well as authentic data are not available and it does not mean total absence of the nutrient in the foodstuff.

flow chart

2.4.2 Procedure of Analysis of Carotene Profile by HPLC

After spectrophotometric quantification, Carotene profile was analyzed in HPLC (HIGH Performance Liquid Chromatography).[117,118]

Procedure

- About 2-3ml of carotenoid eluent was taken in 2 glass vials or drum vials(a small glass tube)
- Dried under nitrogen then reconstitute with 100µl mobile phase (if needed dilute with mobile phase) preserve at -20^{0}C temperature (covered by aluminum foil) for analysis of carotene profile.
- Nitrogen dry were performed in dim light and cool room to avoid damage by light and temperature.

Preparation of Mobile Phase

Reagents

- (acetonitrile: methanol; 85:15,)
- 10 ml of 5% Ammonium Acetate in methanol was prepared
- ml of ammonium acetate solution to 1 lit cylinder
- Methanol was added to 150 ml
- 850 ml acetonitrile added to make volume 1000ml
- Mobile phase B (Final mobile phase)
- 750 ml of mobile phase A in 1 lit cylinder
- 250 ml 2-propanol was added to bring volume 1000 m
- This solution was filtered using 0.22µm filter

HPLC system include for carotene profile analysis

Column	**VYDAC reverse C18 column with 5 µm particle size**
Integrator	SHIMADZU Chromatopac C-R8A
Detector	SHIMADZU UV-VIS Detector SPD-10A vp
Pump	SHIMADZU Solvent Delivery Module LC-10ATvp
Injector	Rheodyne 7202 50 µl syringe
Flow rate	1.7ml /min.
Injection volume	25 µl

UV-Visible spectrophotometer (UV-1601, Shimadzu, and Tokyo, Japan) were used to determine the concentrations of standard solution by using reference extinction co-efficient. and then injected into the HPLC. A calibration curve were prepared for each carotene, the retention time and result were calculated based on the respective calibration curve.

Detection of carotene profile in vegetables

The most commom carotenoids in the plants are beta carotene, alpha carotene, lutein, lycopene, beta-cryptoxanthin(Delgado et.al.,2000)[109].First of all single standard of each of those carotenes were run by HPLC to detect the exact time of their peak. Then a mix standard containing all those carotenoids was passess through to confirm the specific peak time. After the confirmation of carotene standard peak timing, it's the time to pass vegetables sample eluent by the same condition. There were different of peaks in chromatogram when sample passes through, but the time which match or most nearer to carotene standard peak time, detect the present of any carotenoids(beta carotene, alpha carotene, lutein, lycopene, beta-cryptoxanthin). The time taken for a particular compound to travel through the column to the detector is known as its *retention* *time*. Different compounds have different retention times. For a particular compound, the retention time will vary depending on:

- the pressure used (because that affects the flow rate of the solvent)
- the nature of the stationary phase (not only what material it is made of, but also particle size)
- the exact composition of the solvent
- the temperature of the column

This time is measured from the time at which the sample is injected to the point at which the display shows a maximum peak height for that compound. There are several ways of detecting when a substance has passed through the column. A common method which is easy to explain uses ultra-violet absorption. We used UV absorbtion of 450 nm for beta carotene, alpha carotene, lutein beta-cryptoxanthin and 470nm for lycopene, The output were recorded as a series of peaks - each one representing a carotene compound in the mixture passing through the detector and absorbing UV light. As long as we were careful to control the conditions on the column and use the retention times to help to identify the compounds present. The area under the peak is proportional to the amount of X which has passed the detector, and this area can be calculated automatically by the computer linked to the display. The area it would measure is shown in the (very simplified) diagram.[195]

Image: 3.1 Procedure to Estimation of Carotenoids

Image: 3.1 Procedures to Estimation of Carotenoids

Image 3.1 Estimation of Carotenoids by column chromatography

Image: 3.2 Analysis of carotene profile by HPLC

Chapter 3

Results

Vegetables and fruits are rich source of carotene – the provitamin A. Vegetables are most commonly consumed in our country. In order to improve the intake of and introduce to carotene rich food to mass people, this study has investigated 27 selected unconventional vegetables including leafy and non-leafy, carotene were estimate and analyzed by using high accuracy of technologies.

3.1 Estimation of total carotenoids of selected unconventional vegetables

It was observed that among the non-leafy unconventional vegetables, total carotenoid content was obtained highest in Mulachi (1202.38µg per 100g edible), second high amount was in Orange fleshed sweet potato/OFSP (776.56 µg per 100g edible), nearer amount was Pahari kolar thor(615.15 µg per 100g edible); while Orhor seed contained the lower amount of carotenoid, it was 200.82 µg per 100g edible. (Table 3.1)

Among the leafy unconventional vegetables, total carotenoids contained noted highest (9696.82 µg per 100g edible) in leaves of orange flesh sweet potato(OFSP) ,next highest was in the Tetul pata (5313.83 µg per 100g edible), it was also observed that total carotenoids contained respectively high amount in Orhor leaves (1581.74 µg per 100g edible), Shetodhron (1568.73 µg per 100g edible),Chimti shak (1519.43 µg per 100g edible) and Kochuripana(1531.24 µg per 100g edible), comparatively of all other leafy plants .The lowest amount of total carotenoids was in Telakucha leaves (72.05 µg per 100g edible) or in Jolpai pata (87.26 µg per 100g).(Table 3.1)

3.2 Analysis of the carotene profile

Carotenoids, the colorful plant pigments some of which the body can turn into vitamin A, are powerful antioxidants that can help prevent some forms of cancer and heart disease, and act to enhance immune response to infections and many other chronic diseases. Carotenoids contain several numbers of carotenes, some of which are potentially bioactive having health benefit such as alpha(α)- carotene, beta(β)-carotene, lutein, lycopene, β-cryptoxanthin etc. In this present study carotene profile has been estimated.

Among selected unconventional leafy vegetables, Shetoddhron contained high amount of Beta carotene (1422.55 µg per 100g edible), followed by Chimti shak (1115.9 µg per 100g edible), Orhor leave (994.81 µg per 100g edible) and Katanotey (962.09 µg per 100g edible) ,while leaves of Orange fleshed sweet potato (OFSP) contained a very satisfactory and extremely good amout (2732.52 µg per 100g edible);the lowest amount of β-carotene contained in Sada Koroi (5.62 µg per 100g edible), while lower quantity was also in Jolpai pata (7.20 µg per 100g edible). Lutein content was highest (2303.01µg per 100g edible) in Tetul pata, next in Kachuripana (566.33 µg per 100g edible) and lower in OFSP leaf(3.09 µg per 100g edible) or shetodhrone(4.63 µg per 100g edible) ;other hand in the present study Alpha-carotene, lycopene, β-cryptoxanthin contents were in the range of 8.0 (Shornalata) to 72.41 (orhor leave), 1.05 (venna pata) to 10.60 (Shetodhron), 2.13 (Shetodhron) to 36.70 (Tetul pata) µg per 100g edible respectively. It was seen that most of analyzed vegetables did not contained α- carotene, lycopene, β –cryptoxanthin or it were in so small amount that are not detectable with HPLC. However, like the root of orange flesh sweet potato (OFSP), its leaves also contains very rich amount of β -carotene, but lycopene and β cryptoxanthin was found in small amount. (Table3.1)

Among the non leafy unconventional vegetables of the present study, Mulachi contained all of the carotenes tested, of which leutin content was highest (165.51 µg per 100g edible) very small amount in Pahari kolar thor (7.76 µg per 100g edible); Orhor seed contained 106.26 µg β-carotene per 100g edible ,OFSP root contained rich quantity of beta carotene (691.14 µg per 100g edible), rich quantity as like OFSP leaves . In Mulach the amount of alpha carotene contained 45.61 µg per 100g edible and beta cryptoxanthin obtained 6.78 µg per 100g edible ;which values of carotene compound are not found in vegetables item or found in a small amount in carotene profile analysis, may be no detectable by HPLC.(Table: 3.1)

Table: 3.1 Carotenoids and Carotene profile of unconventional leafy and non leafy vegetables (µg per 100 gm edible)

Sl.	Sample Name	Total Carotenoids	β-carotene	α-carotene	Lutien	Lycopene	B-cryptoxanthin
	Leafy						
1	Shetoddhron	1568.73	1422.55	43.88	4.63	10.60	2.13
2	Shetokanchon	542.74	12.20	nd	113.80	nd	nd
3	Muktajhuri	569.23	344.09	10.15	nd	5.81	nd
4	Amrul	311.29	15.43	nd	13.55	nd	nd
5	Sada Koroi	544.10	5.62	nd	168.14	nd	nd
6	Chimti Shak	1519.43	1115.90	nd	nd	5.37	7.58
7	Mandar	794.15	51.68	14.13	163.96	3.13	7.35
8	Kachuripana	1531.24	32.97	nd	566.33	nd	nd
9	Tatul pata	5313.83	62.89	nd	2303.01	nd	36.70
10	Beth Gach	715.92	59.96	19.76	43.70	nd	15.70
11	Roktodrone	599.22	465.82	nd	nd	1.64	4.43
12	Shorno lota	424.37	16.38	8.00	36.67	2.85	3.19
13	Orhor leaves	1581.74	994.81	72.41	0.00	5.44	6.28
14	Telakucha*	72.05	nd	nd	nd	nd	nd
15	Parul	1015.80	10.99	nd	256.16	nd	nd
16	Jolpai pata	87.26	7.20	nd	nd	nd	nd
17	Bondhonia	416.29	225.60	nd	nd	nd	3.61
18	Helencha*	129.38	nd	nd	nd	nd	nd
19	kantanotey*	1125.16	962.09	33.09	nd	7.68	5.31
20	Venna pata	281.63	192.72	nd	nd	1.05	nd
21	OFSP Leaves	9696.82	2732.52	64.17	3.09	nd	nd
22	Holud pata	562.90	nd	nd	nd	nd	nd
23	Rakhal Sosha	165.18	12.41	nd	22.26	nd	nd
	Non-Leafy						
24	Mulachi	1202.38	27.81	45.61	165.51	12.53	6.78
25	Pahari kolar thor	615.15	15.50	11.33	7.76	0.83	nd
26	Orhor seeds	200.82	106.26	36.82	nd	1.29	nd
27	OFSP	776.56	691.14	nd	nd	16.72	3.74

*nd -not detectable

Chapter 4
Discussion and Conclusion

4 .1 Discussion

It is evident that unconventional vegetables grown in Bangladesh are rich in nutrient content. It can be explored more to avoid micronutrient as well as vitamin A deficiency in bearable scale. Ours is a developing country with a large number of population, there are food shortage remain whole year due our economical condition, sometimes for any natural disaster like, flood, storm ,land laid or now a day's earthquake. However, rich people of Bangladesh now having a good interest in consuming foreign vegetables, which are not even healthy and fresh, other hand there are also large number of people living in our country who cannot affords even single time meal a day, beside that there also have another group of people who cannot afford expensive food for their family ,so in a consideration of all the circumstances if mass population start having local foods, like vegetables and fruits, there may be stands contemporary solution for all group of people. Fruits and vegetables both are great source of vitamin A, dietary fiber, minerals. Vitamin A which is one of the vital cause of malnutrition, found in fruits, and in liver, egg. meat, which are very expensive for mass people, except vegetables.

Micronutrient like vitamin A deficiency is one of major problem in Bangladesh, now its became a public health matter. In development country like Bangladesh, young children and women of childbearing age are considered to be at greatest risk of vitamin A deficiency, more than 80% of the people suffering from malnutrition(BBS,2006)[11],in spite of being endowed with plenty of green around almost everywhere in the country. Vegetables act as antioxidant for human body, contained provitamin A carotenoids, good for health .There are lots of common and local vegetables grown and found, which are nutrient rich. Besides that, in a addition to those, there are some relatively unconventional varieties of vegetables, which carry a good quality of nutririve value. For a better and improvise human nutrition we need to explore their nutritive value to mass population,to make a well balanced diet in our daily menu.

Present study demonstrates carotenoids and carotene profile of twenty-seven unconventional vegetables including leafy. It has been evidence, that these vegetables contained carotene, can be provide a better choice of food.

The present study determined carotenoids and carotene profile of twenty-seven unconventional vegetables including leafy. It has been evidenced, that these vegetables contained carotene, that can provider of a better food choice also.

Among all the non vegetables , it was demonstrated that Mulachi contained (1202.38 µg of 100g edible) highest quantity of carotenoids and Orange Fleshed Sweet Potato (OFSP) contained, highest amount of Beta carotene, which are the excellent source. Mulachi is found in some specific area ,but OFSP is one of the versatile crop ,served as vegetable, called as “poor man's crop”,a cheap source of carotenoids.During the past few years, further evidence has been obtained regarding the potential impact of OFSP in young children vitamin-A status, obtained by jaarsveld et al,2005 [2]; it has been reported raw potato of OFSP was found to contain significantly increased amount of carotenoids,(Tumuhimbise ,et al 2009.)[118](Table 4.1)

Leaves of Orange Fleshed Sweet potato (OFSP), are a rich source of carotenoids as like root of OFSP, obtained 9696. .82 µg of carotenoids per 100g edible and Tatul pata contained 2732.32 µg per 100 g edible,these are the excellent source of leafy vegetables. OFSP leaves are consumed by people,have significant effect on Vitamin A impact .OFSP leaves are also consumed in many countries in SSA, bioavailability is certain too much lower than OFSP roots (Jan W Low et al); tetul pata is also a healthy food, skin, control diabetes, also have antioxidant activity.[185,196](Table 4.2)

Carrot and OFSP contain 1890 and 776.56 µg per 100 g edible of carotenoids.The value compared by the book of Nutritive value of of Indian Food and present study, both are the good source. The word carotene comes from carrot**;** promote good vision, especially night vision, and help combat health-damaging free radical activity. Although carrots are available throughout the year, locally grown carrots are in season in the summer and fall when they are the freshest and most flavorful. (Worlds healthiest food by Jorge mataligjan), the results of a new 10-year study from the Netherlands about carrot intake and risk of cardiovascular disease (CVD)—and those results are fascinating. Intake of fruits and vegetables in the study was categorized by color and focused on four color categories: green, orange/yellow, red/purple, and white. Out of these four categories, orange/yellow (and in particular, foods with deeper shades of orange and yellow) emerged as most protective against Cardiovascular disease (CVD).[8] And even more striking, carrots were determined to be the most prominent member of this dark orange/yellow food category. Much of the research on carrots has traditionally focused on carotenoids and their important antioxidant benefits. According to that journal issue and with the result value of carotenoids ,its has evidence, that OFSP, which is one of the cheap vegetable is much nearer to carrot than other vegetables, in consideration of color, shape or nutrient value.[183,187] (Table 4.3)

Regular Sweet potato is the cheapest vegetables to the poor household, which leaves has also a good impact on health, contained 7800 µg of 100 g edible of total carotenoids in Deshio Khaddodrobber Pushtiman (DKPM) , but OFSP leaves carotenoids has higher in higher rate 9696.82 µg of 100 g edible in present study ,has much nutritive value, Several recent studies have shown the superior ability of sweet potatoes its root and leaves to raise our blood levels of vitamin A. This benefit may be particularly true for children. [186] (Table 4.4)

Mulachi contained 1202 µg per 100g edible of carotenoids, analyzed in present study, is a uncommon food but looks similar to kachamorich ,but in the case of carotenoids, has the higher value, a good source, while kachamorich though contained 690 µg per 100g edible of level carotenoids (Nutritive value of Indian food) ,is a one of the most common, cheap vegetable consumed by people, which also known as a very good source of carotenes. This amount supplies nearly 30 percent of the Food and Nutrition Board's recommended daily allowance of vitamin A for an adult man and 38 percent for a woman. It is also necessary for proper growth and development and to support immune system health. Without adequate vitamin A, you may be more likely to develop cancer and vision disorders, obtained by Health eating green chili , Michel, Denmark media[189,190,196)].(Table 4.5)

It is noted that orhor seed contained 200.82 µg per 100g edible of carotenoids in the present study, while orhor seed contained higher amount of 200.82 µg carotenoids of 100 g edible. structurally orhor seed and motorshuti has similarity, A Mexico City-based study has shown that daily consumption of green peas along with other legumes lowers risk of stomach cancer (gastric cancer),has a strong antioxidant benefits, though orhor seeds are not so common, can refer replacement of motorshuti.(George Metaligian ,worlds healthiest Food) [1187](Table 4.6)

In this present study, parul contained 940 µg per 100g of carotenoids, while lal shak contained 11,940 µg per 100 g edible(DKPM),which is one of common,cheap vegetable,has strong antioxidant activity, high in carotenoids, other hand, parul is less known but similar to lal shak in view, Researchers reported that lal shak is one of the major sources of anthocyanins, which are directly involved in protecting use against cancer, diabetes, aging and oxidative damage, obtained in daily star journal in Bangladesh, so that parul can be taken some more quantity, may be it can be nearer to lal shak,though it is a colored vegetable contained carotenoids.[192,193](Table 4.7)

Sajne shak is common vegetable, carotenoids (6780 µg per 100 g edible) by DKPM and tetul pata which is also not so rare but normally not consumed by mass people except specific group of any locality, which carotenoids in the level of 5313.83 µg per 100 g edible found in this present study, nearer to sajne shak. few analysis has done previously, so that this carotene value of higher level considered a better plant food in daily food. menu.Sajne sak and tetul pata both are same category tree, nutritive value of carotene were quite similar also. [194]. (Table 4.8)

In the present study bathua sak contained 1740 µg per 100g edible of carotenoids, while its noted to chimti sak contained 1519 µg per 100g edible of carotenoids in Deshio Khaddodrobber Pushtman (DKPM).Bathua sak is known to people also consume regularly, carry aquality value of carotenoids, while chimti sak sak in not known to people, by involve it to our daily meal menu, could observed some carotenoids in human body. [193,194]((Table 4.9)

Present study investigated about the analysis of twenty seven unconventional vegetables consumed by the people of some selected area. Due to different geographical location, seasonal variation, physiological state and maturity, some value may be differ by other national or international value. Moreover, these foods are rare and also exact location of distribution was merely available. So it was very difficult to find any existing data available in national and International database, very few data were found in some International, national database and also in some study conducted by personal interest of some researcher.

Table: 4.1 :(Excellent source of carotenoids and beta carotene among non leafy vegetables (µg of 100g edible)

Name of Vegetables	Total Carotenoids	Beta-carotene
Mulachi	1202.38	27.81
OFSP	776.56	691.14

Table: 4.2 :(Excellent source of carotenoids and beta carotene among leafy vegetables (µg of 100g edible)

Name of Vegetables	Total Carotenoids	Beta-carotene
OFSP Leaves	9696.82	2732.52
Tatul Pata	5313.83	62.89

Table 4.3 :(Comparison of Carotenoids between present study and OFSP and Carrot /Nutritive Value of Indian Food, µg of 100g edible)

Name of Vegetables	Total Carotenoids
OFSP	776
Carrot	1890

Table 4.4 :(Comparison of Carotenoids between present study / leaves of OFSP and Regular Sweet Potato Leaf/ DKPM, µg of 100g edible)

Name of Vegetables	Total Carotenoids
OFSP Leaves	9696.82
Regular Sweet Potato Leaf	7800

Table 4.5: Comparison of Carotenoids content between present study /Mulachi and kacha morich /Nutritive Value of Indian Food, µg of 100g edible)

Name of Vegetables	Total Carotenoids	
Mulachi	9696.82	
Green Chilli (Kachamorich)	5313.83	

Table 4.6 :(Comparison of Carotenoids between present study /Orhor Seed and Motor shuti / the worlds healthiest food/George Mateljan Foundation, µg of 100g edible)

Name of Vegetables	Total Carotenoids	
Orhor Seed	200.82	
Motoshuti	110.34	

Table 4.7 :(Comparison of Carotenoids content between present study Parul sak And lal sak/DKPM, µg of 100g edible)

Name of Vegetables	Total Carotenoids	
Parul	1015.8	
Lal shak	11940	

Table 4.8 :(Comparison of Carotenoids between present stud/Tatul Pata and Sajne Sak/ DKPM,µg of 100g edible)

Name of Vegetables	Total Carotenoids	
Tatul Pata	5313.83	
Sajne Sak	6780	

Table 4.9 :(Comparison of Carotenoids between present study/ Chimti Sak and Batua Sak/DKPM ,µg of 100g edible)

Name of Vegetables	Total Carotenoids	
Chimti Sak	1519	
Batua Sak	1740	

Policy Recommendation

Based on the findings, the following policy recommendations have been suggested

- Lowering pressure on common food sources of vitamins and minerals and to achieve food security.
- Inform and aware people about the uncommon plants which may rich and cheap source of pro vitamin A.
- In order to maintain biodiversity, attempt to conserve these unconventional vegetables plants is to be taken to stop extinction of this vegetables source.
- The Govt. and NGOs need to take strong initiative for cultivation and conservation of ethnic foods in plane land and also in home gardens.
- The medicinal properties of the ethnic plants need to be explored to introduce into daily food menu to get health benefit.
- Encouraging researchers for further investigations on these plants for their effect on human health.

Key Findings: Carotenoids, Carotene Profile, Unconventional Vegetables.

Conclusions

The present study provides data on a general view of some selected unconventional vegetables including leafy and non-leafy. Due to socio-economic, physiological condition, proper initiative or environmental consequence, there is lackness of knowledge about this carotene (provitamin A) rich plant food to the mass population. The unconventional vegetables contained good amount of carotenoids, beta carotene, to some extent of also leutin. Other carotene contents are in trace level or absent. The result of the study suggested that regular intake of these vegetables may provide provitamin A. In this study, it was also proclaimed that, beside local common vegetables , relatively unconventional or uncommon varieties of vegetables also grown in our country, these contained good nutritive value, also cheap in rate, which is really a great advantage of mass population for improving health status, specially for poor community. Production and popularization of these plant foods need to be regularize to confirm food security in our country.

Limitation of the study

Every research has some limitations. And this study is not an exception.
Overall it was an expensive study which limited the various scopes along with the scope of extending the study area and increasing the number of samples.

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Appendix

Chromatogram of Carotene standard

Standard peak of alpha carotene

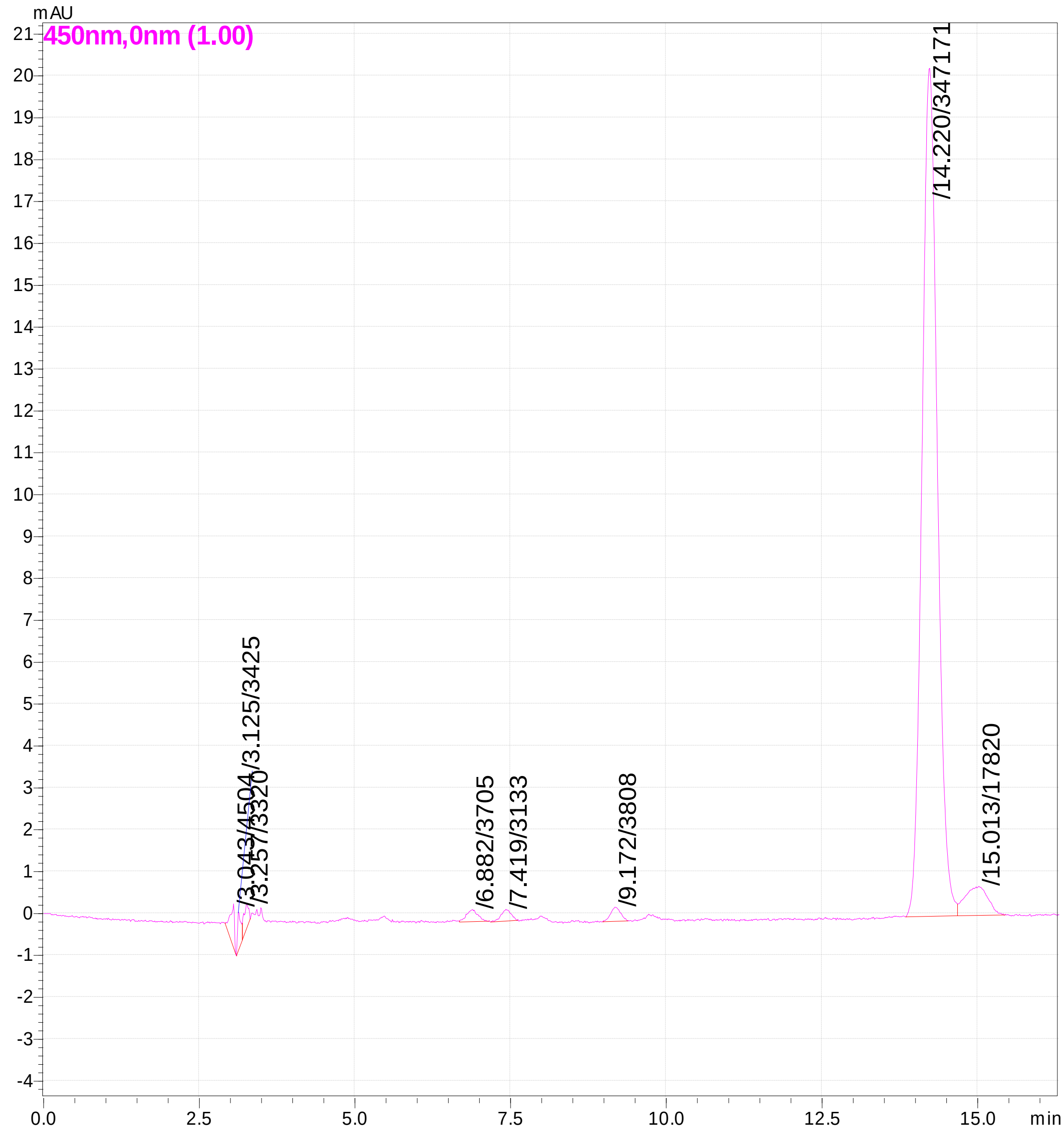


Fig.1: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks alpha-carotene in 14.22 minute

Standard peak of beta carotene

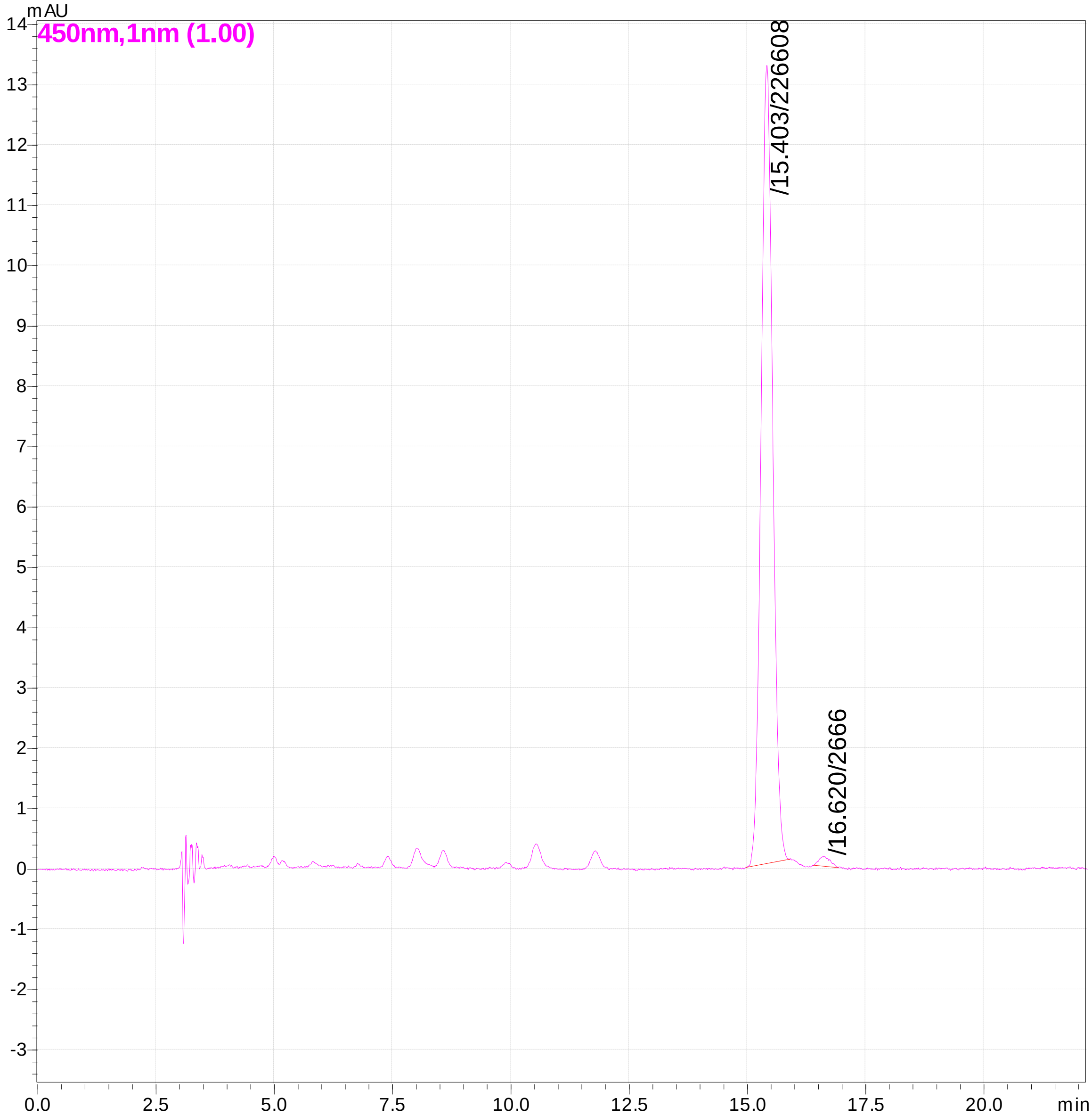


Fig.2: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene in 15.403 minute.

Standard peak of lycopene

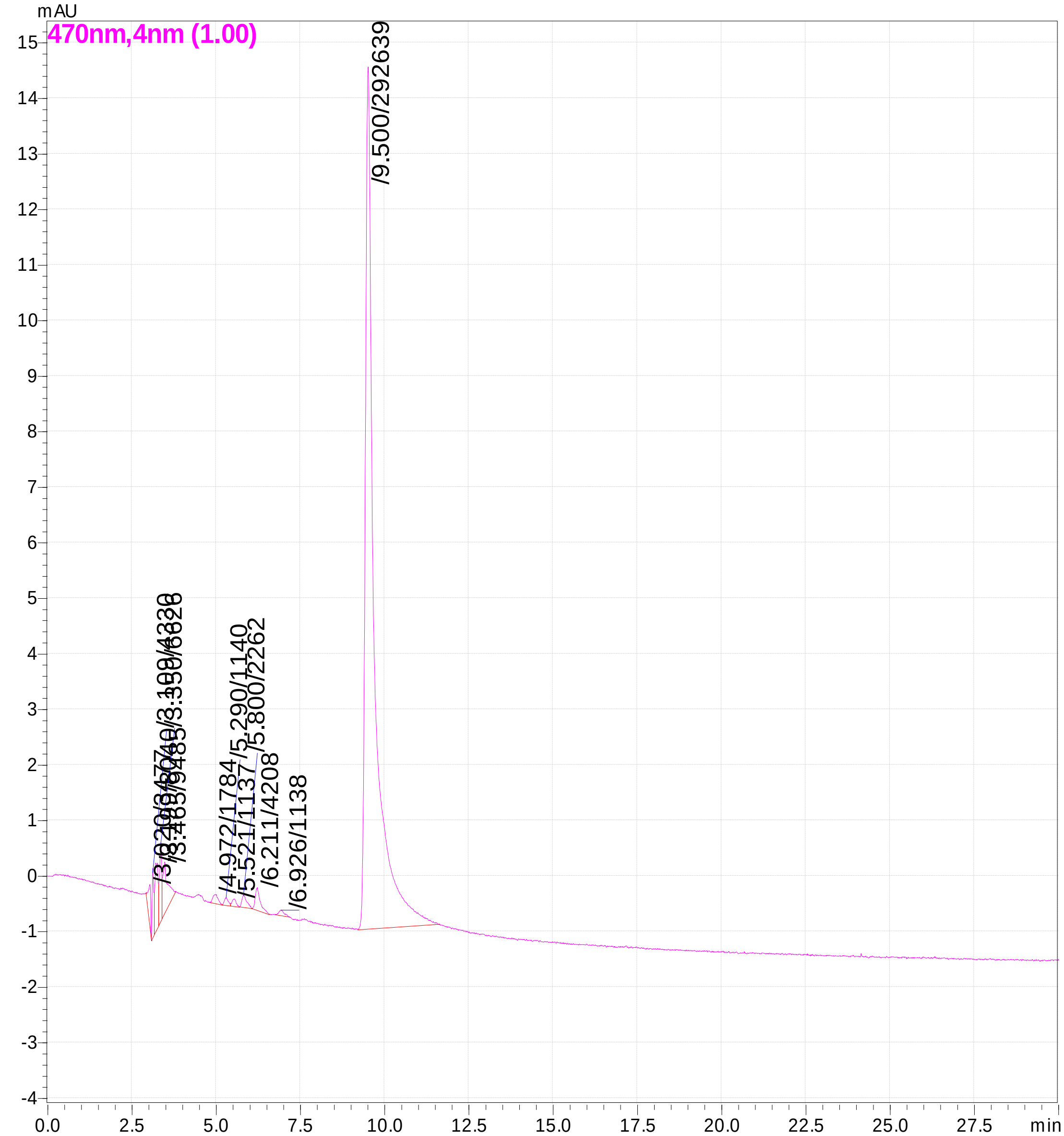


Fig.3: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 470 nm, Peaks lycopene in 9.500 minute

Standard peak of lutein

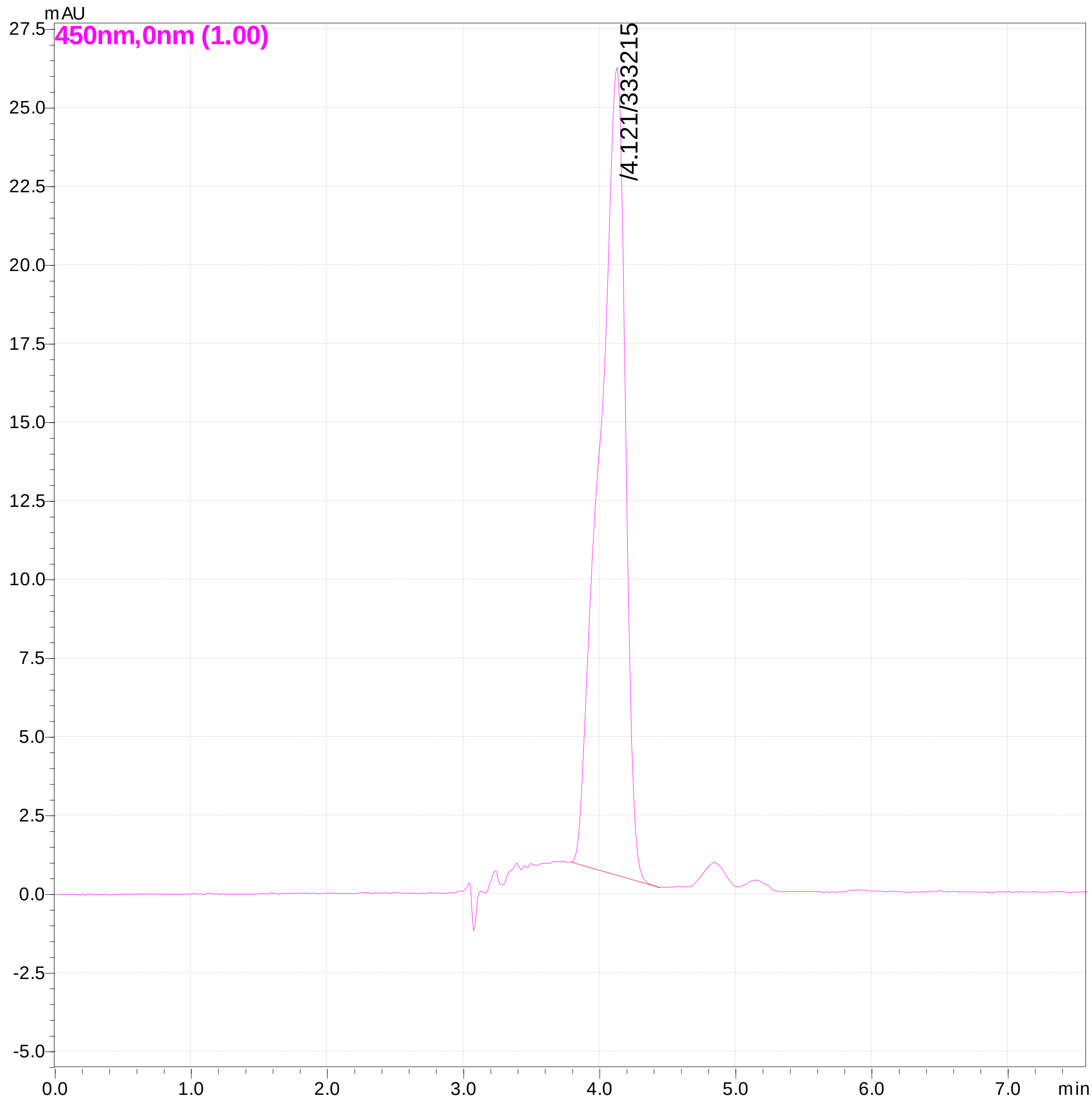


Fig.4: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks lutein in 4.121 minute

Standard peak of beta-cryptoxathin

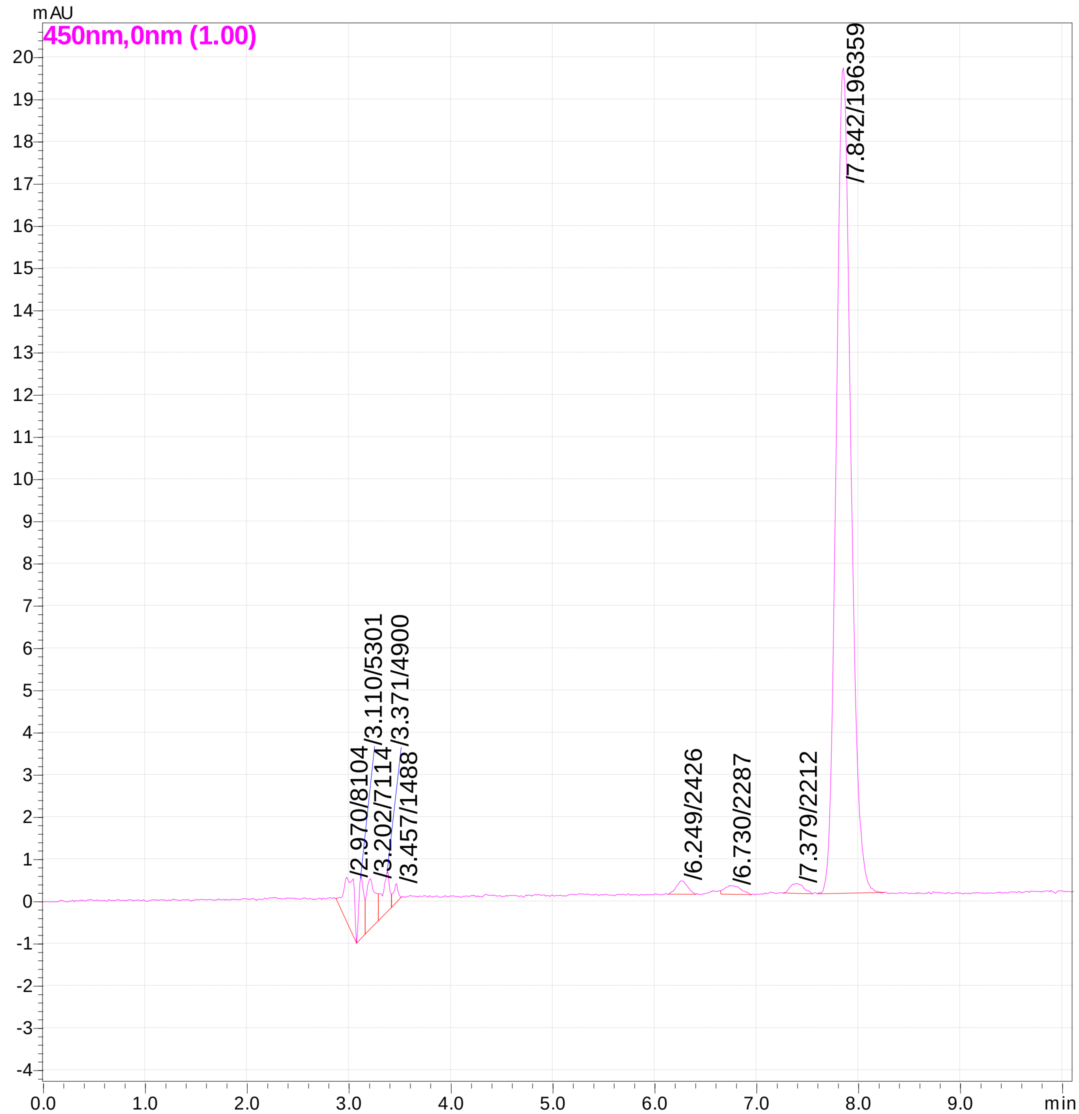


Fig.5: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks beta-cryptoxanthin 7.842 minute.

Mix standard peak at 450 nm

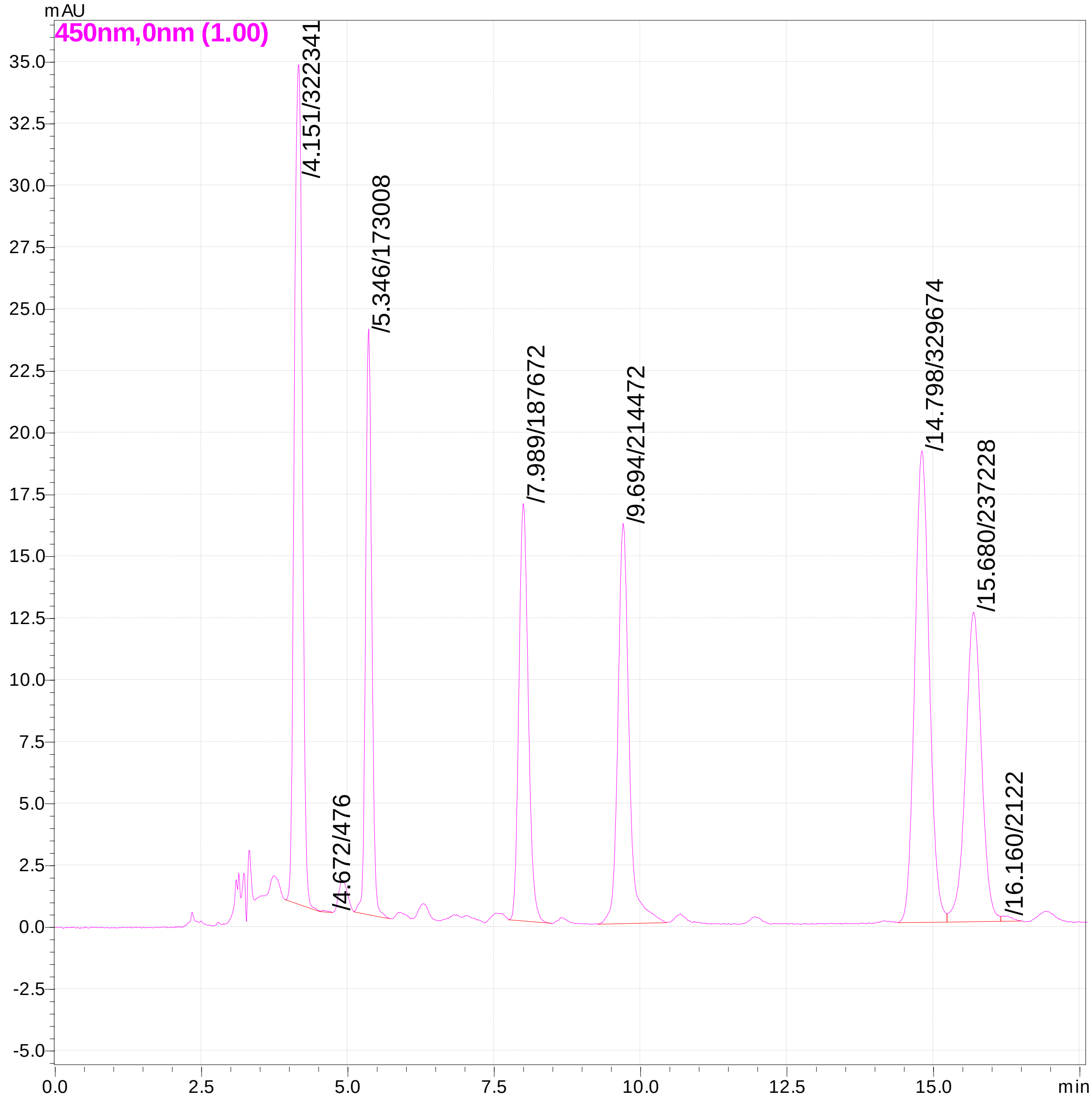


Fig.6: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks alpha carotene in 14.798 min, beta carotene in 15.680 min, lutein in 4.151 min , beta-cryptoxanthin in 7.982 minute

Mix standard peak at 470 nm

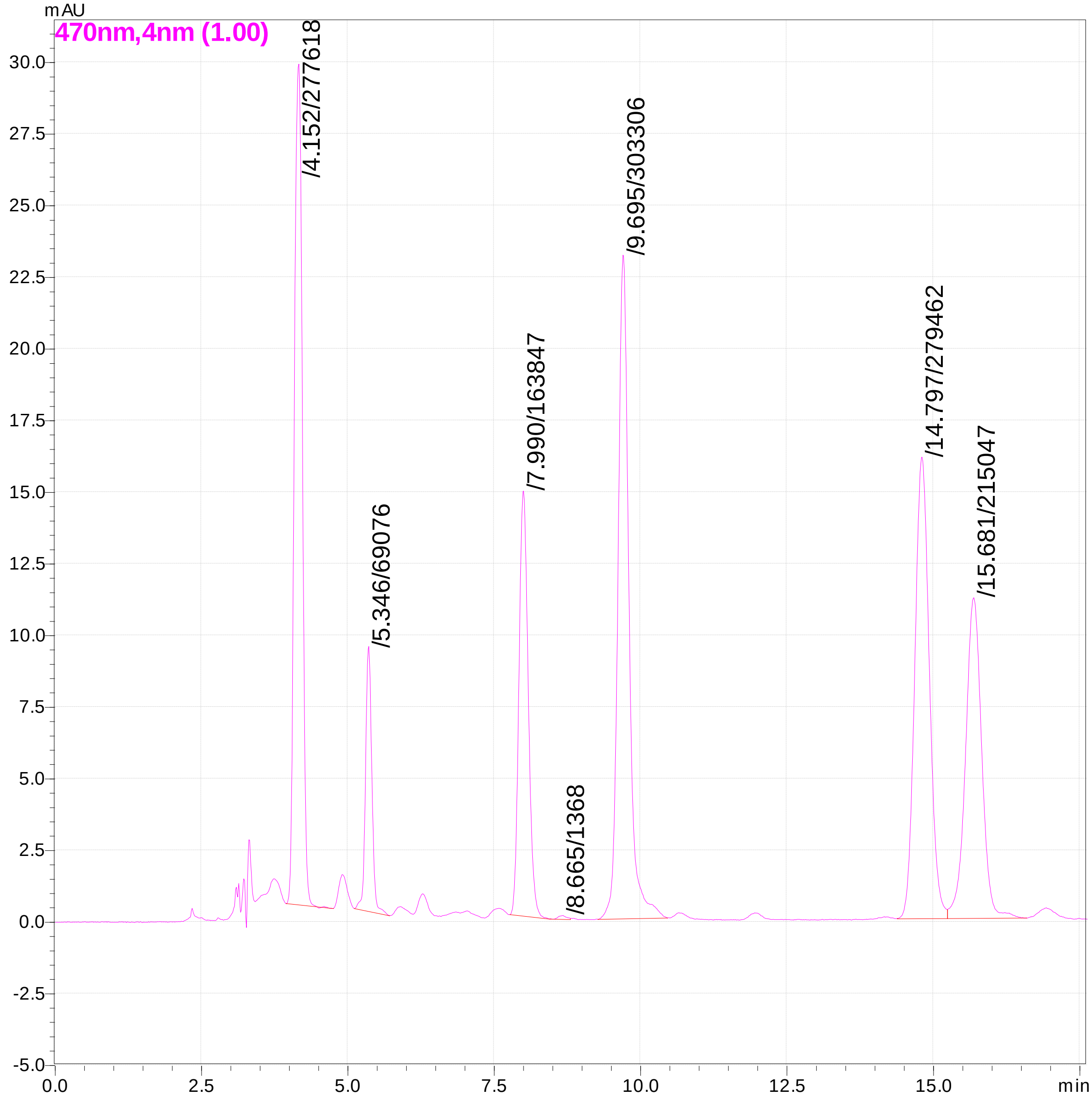
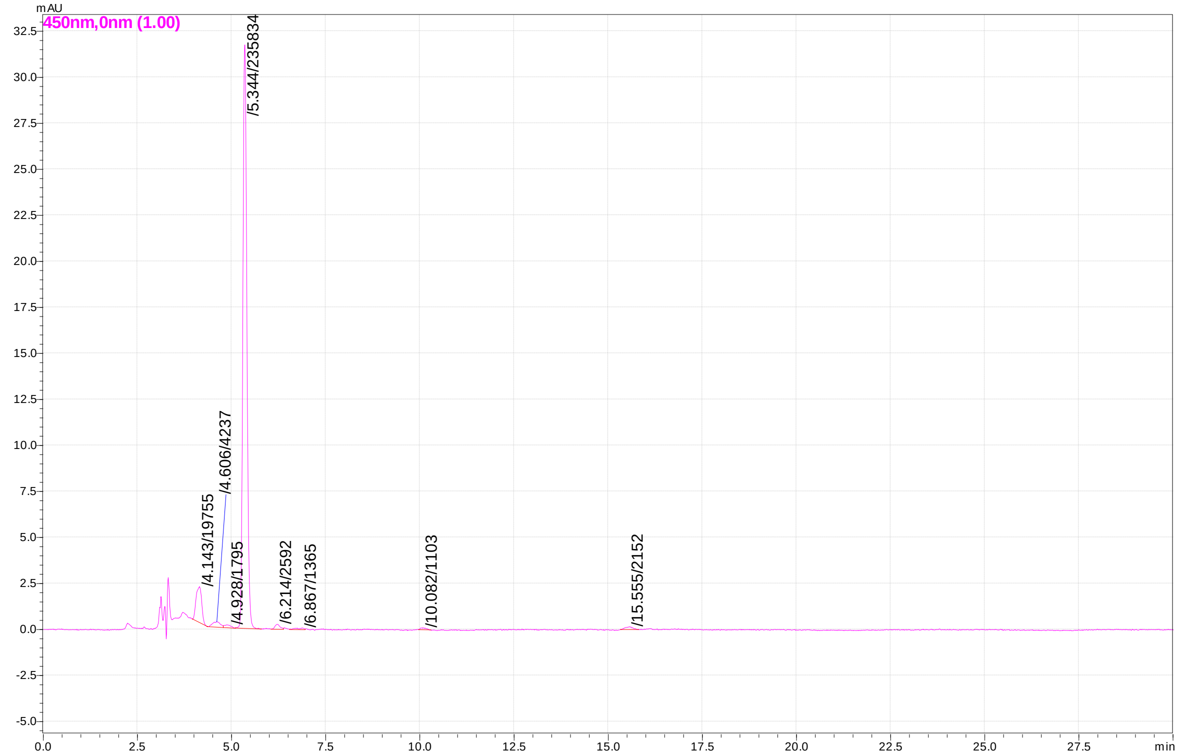


Fig.7: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 470 nm, Peaks lycopene in 9.695 min

Chromatogram of unconventional vegetable sample

Sample Name: Shetokanchon



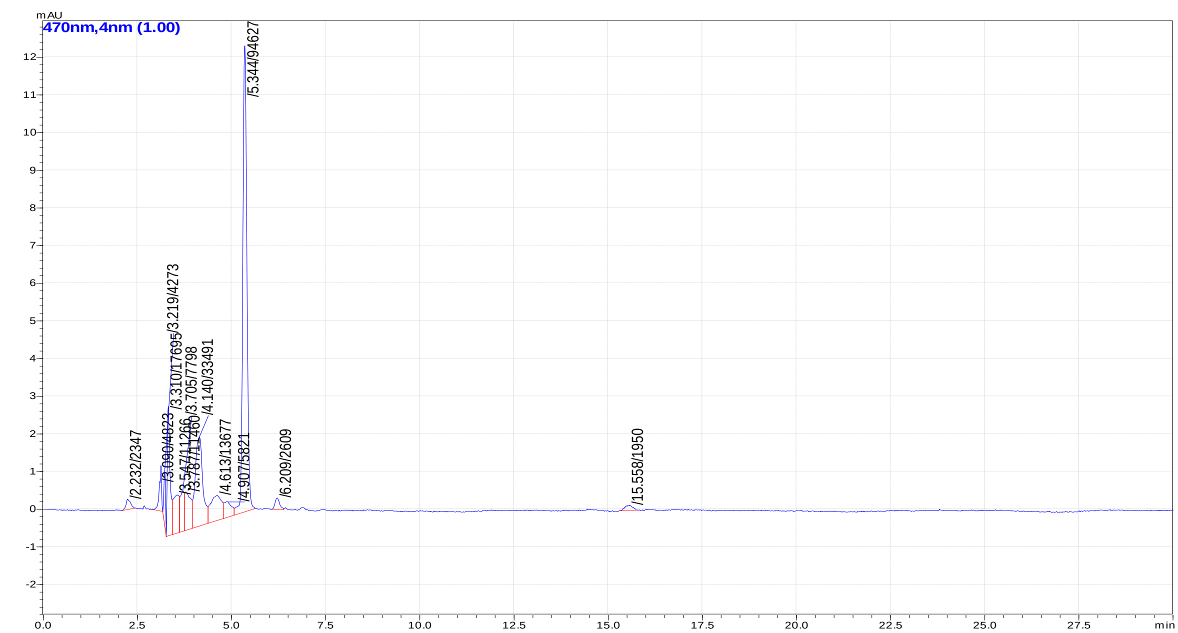
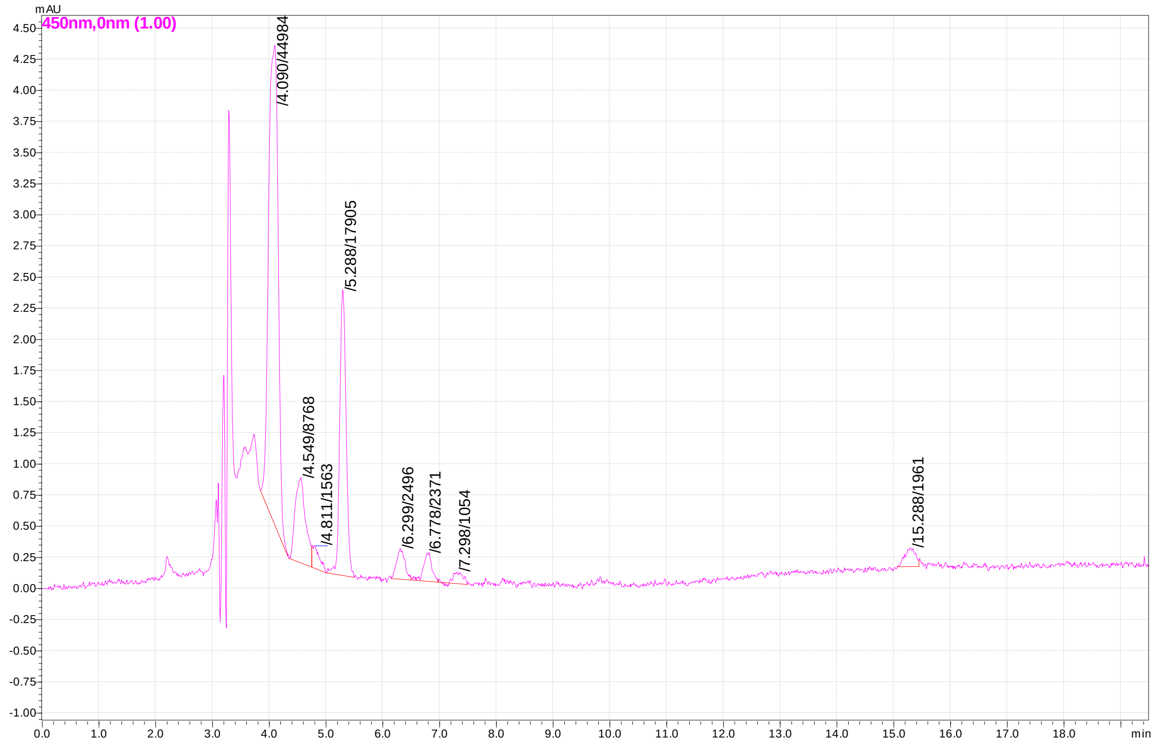


Fig.1: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.55min, leutin 4.6 min, α-carotene and β-cryptoxanthin not detected and at 470 nm Lycopene not detected.

Sample Name: Parul



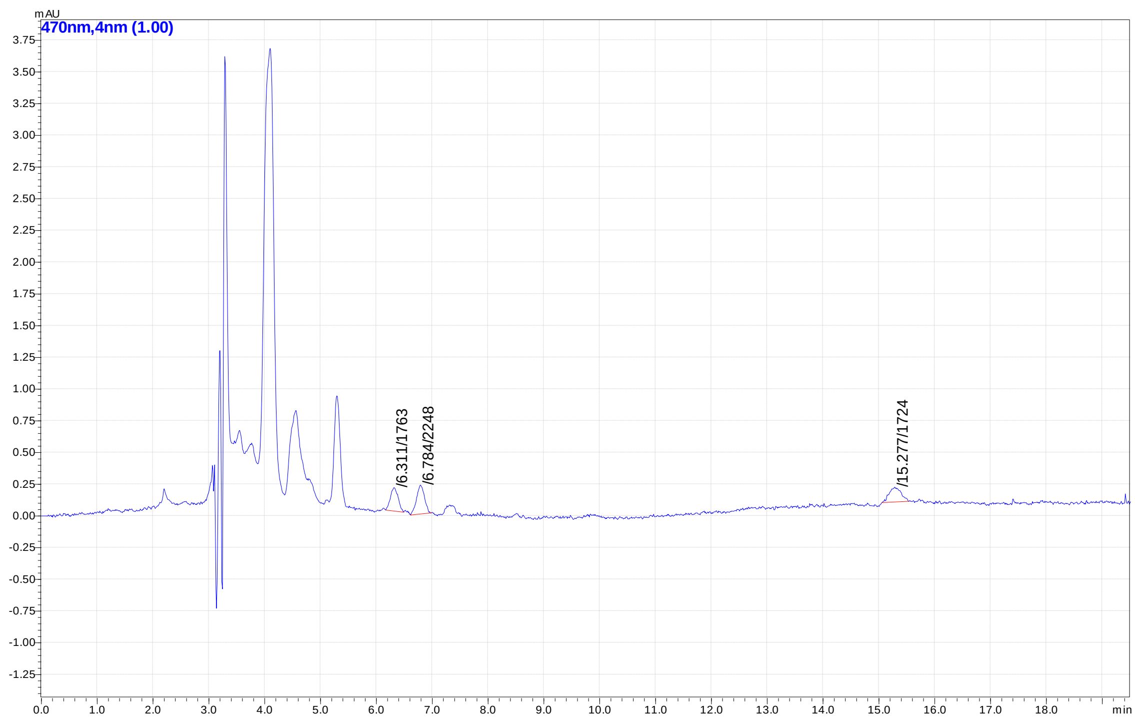


Fig.2: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.29min, leutin 4.54 min, α-carotene and β-cryptoxanthin not detected and at 470 nm Lycopene not detected.

Sample Name: Helencha

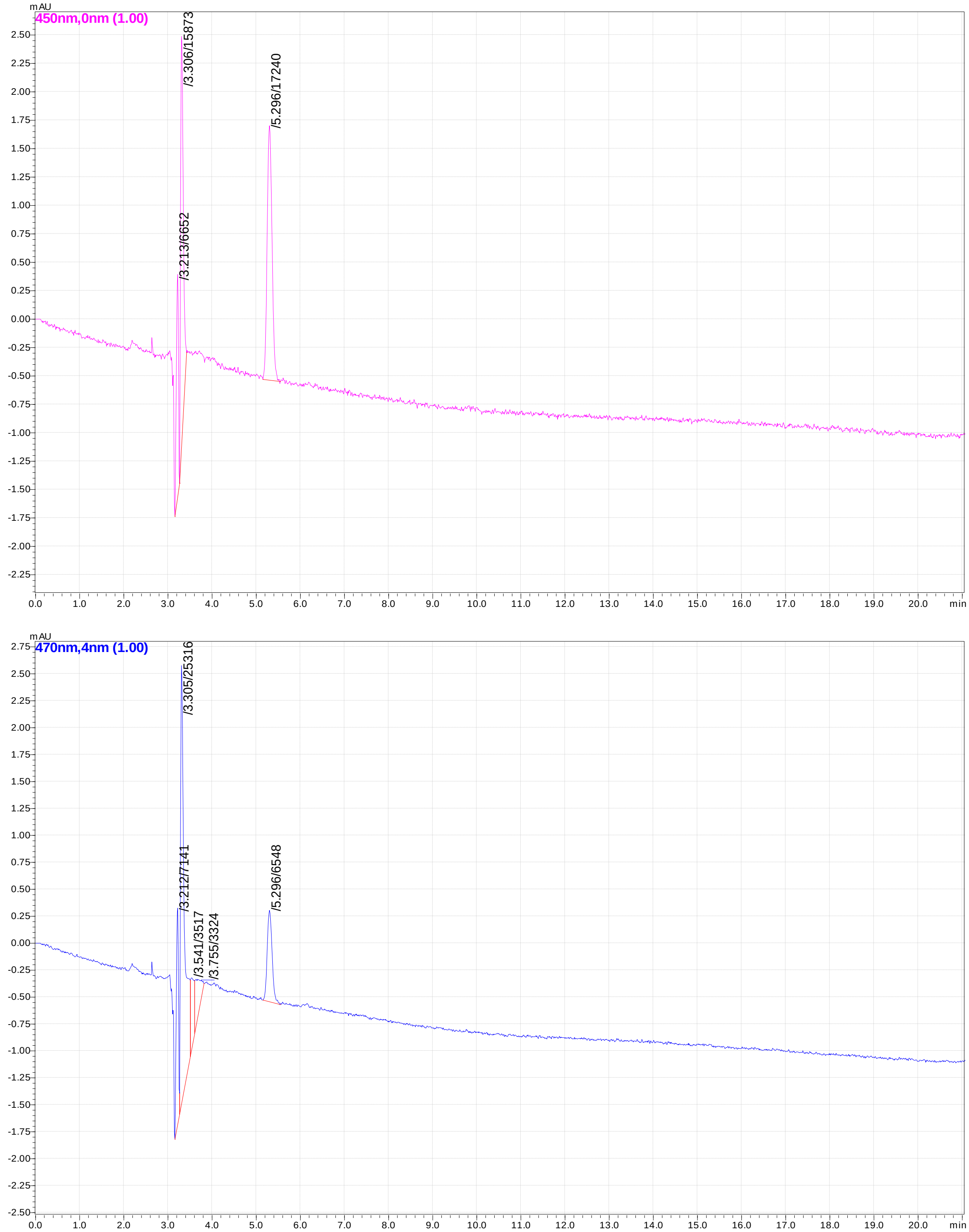


Fig.3: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene, leutin , α-carotene, β-cryptoxanthin not detected and at 470 nm Lycopene not detected.

Sample Name: Amrul

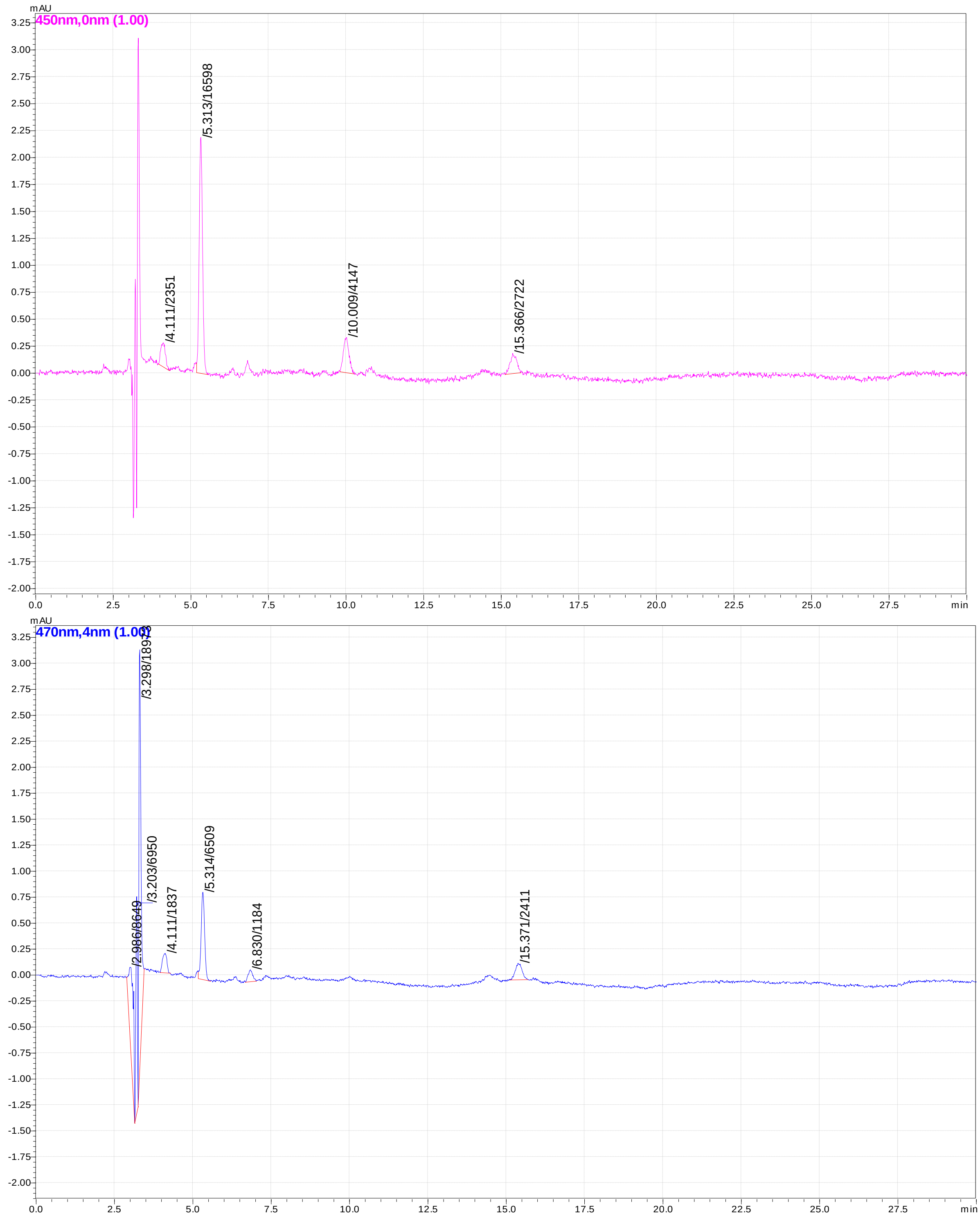
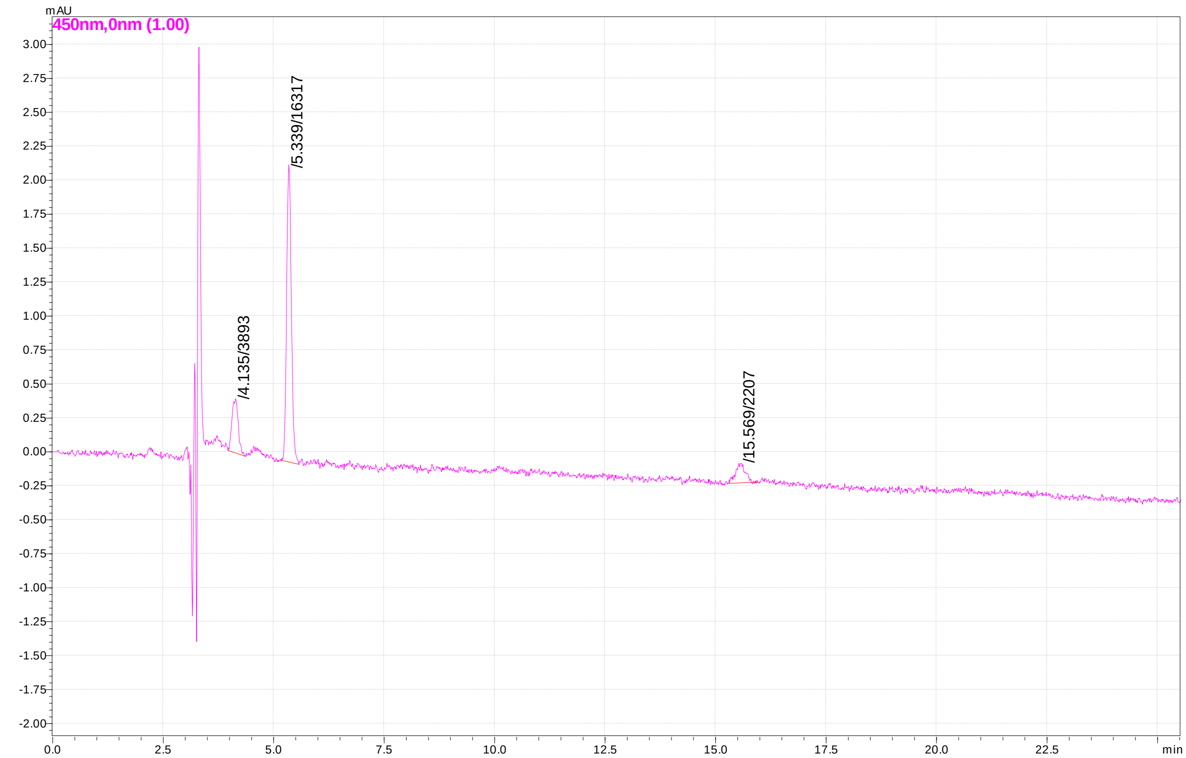


Fig.4: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.36min, leutin 4.11 min, α-carotene and β-cryptoxanthin not detected and at 470 nm Lycopene not detected.

Sample Name: Rakhal Shosha



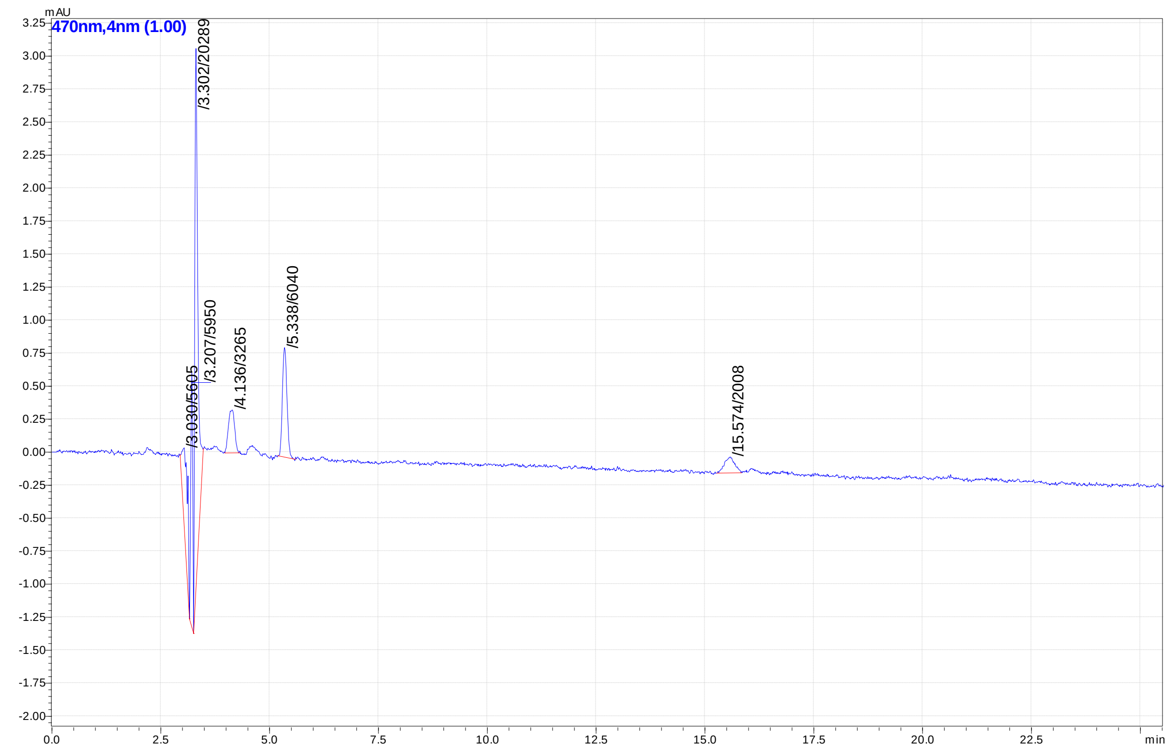
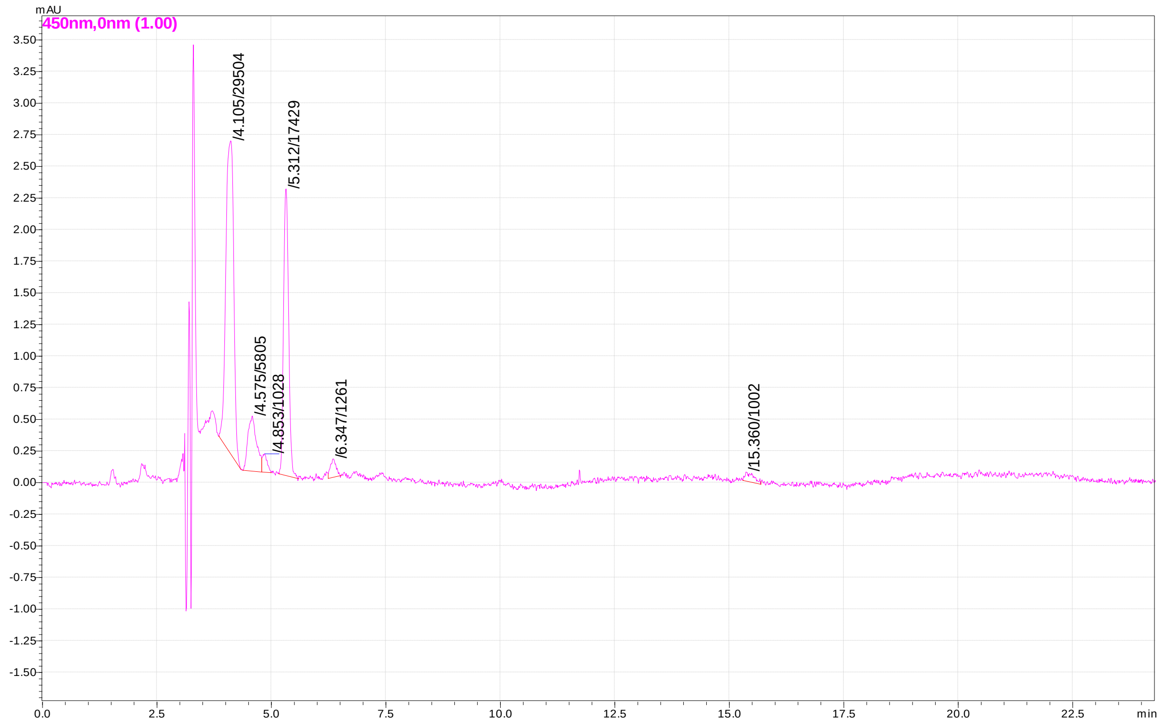


Fig.5: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.56min, leutin 4.13 min, α-carotene and β-cryptoxanthin not detected and at 470 nm Lycopene not detected.

Sample Name: Sada koroi



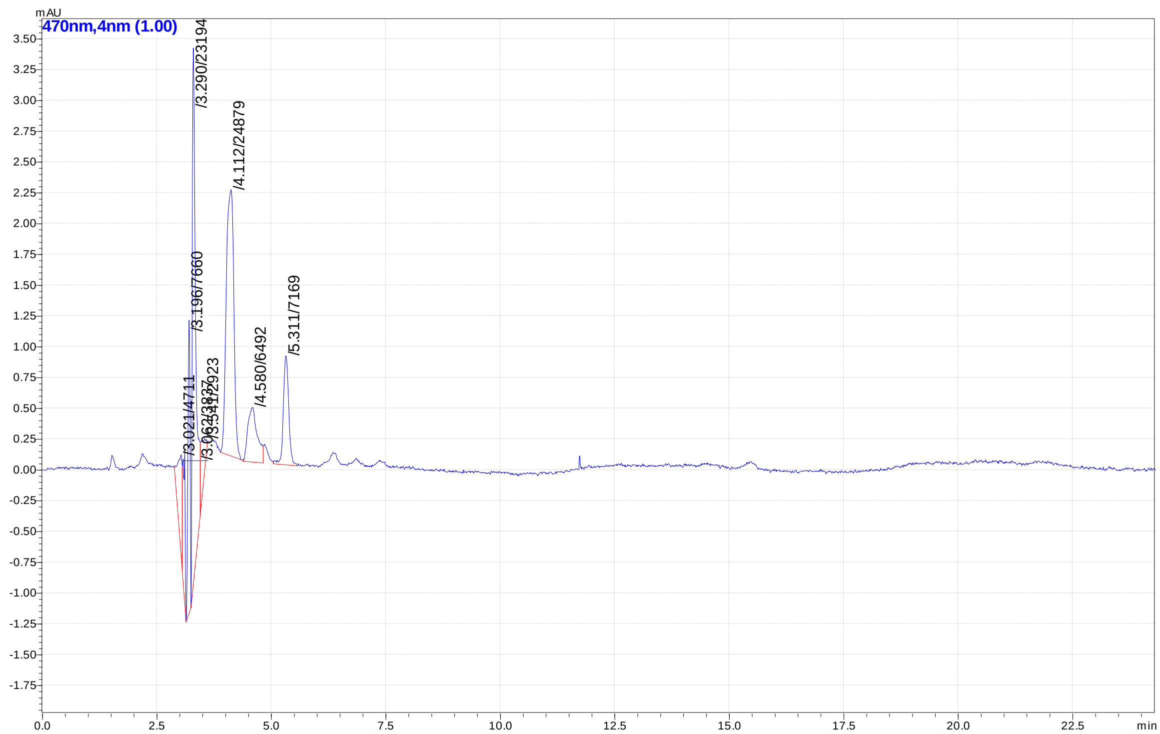
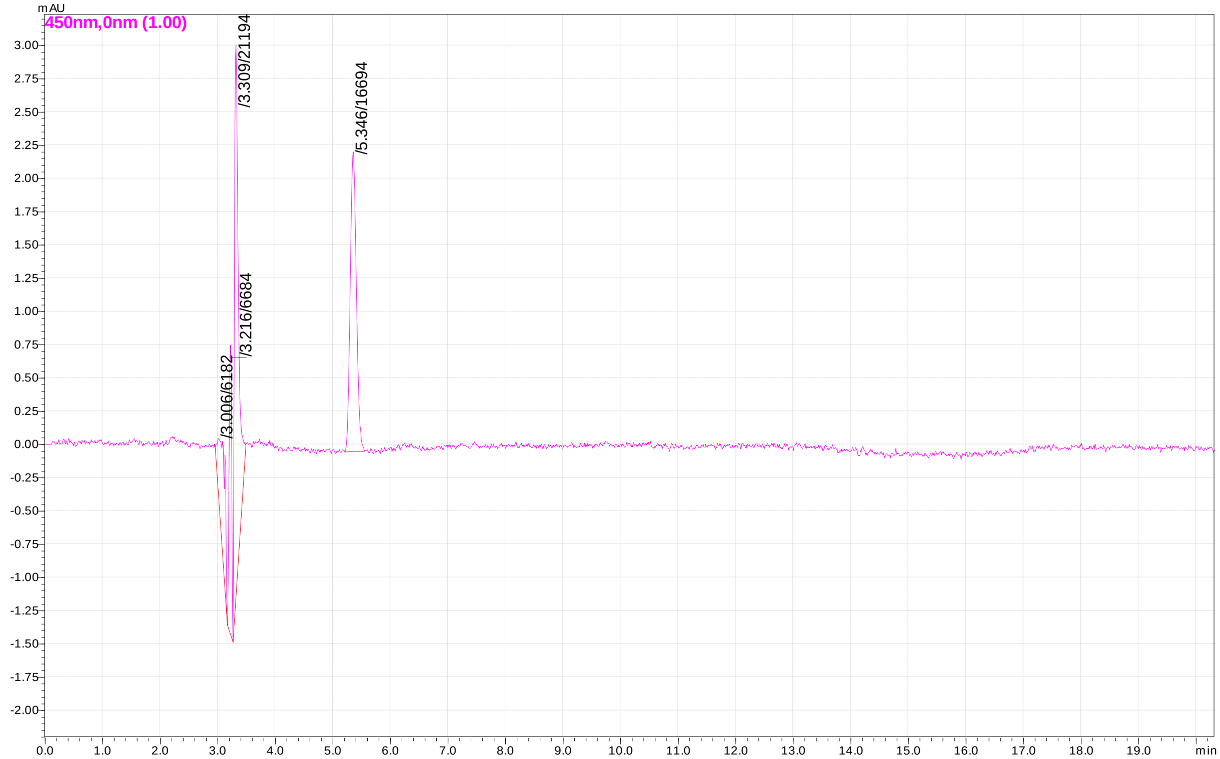


Fig.6: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.36min, leutin 4.57 min, α-carotene and β-cryptoxanthin not detected and at 470 nm Lycopene not detected.

Sample Name: Telakucha



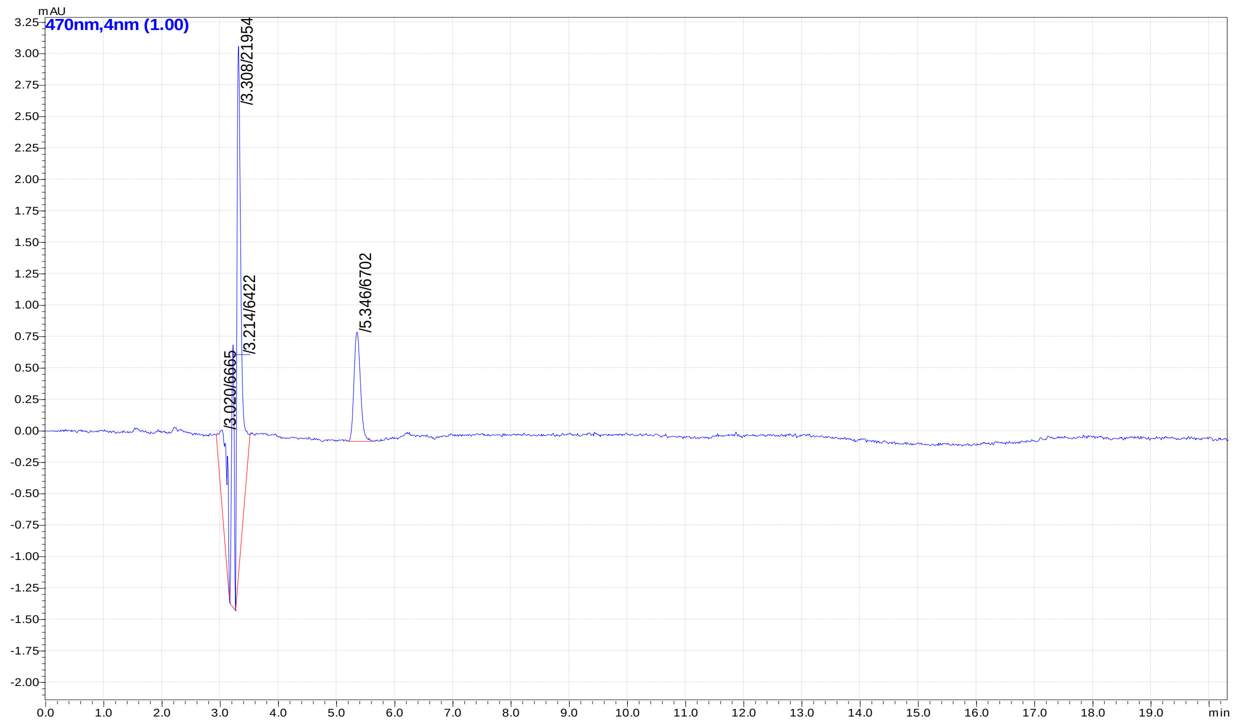
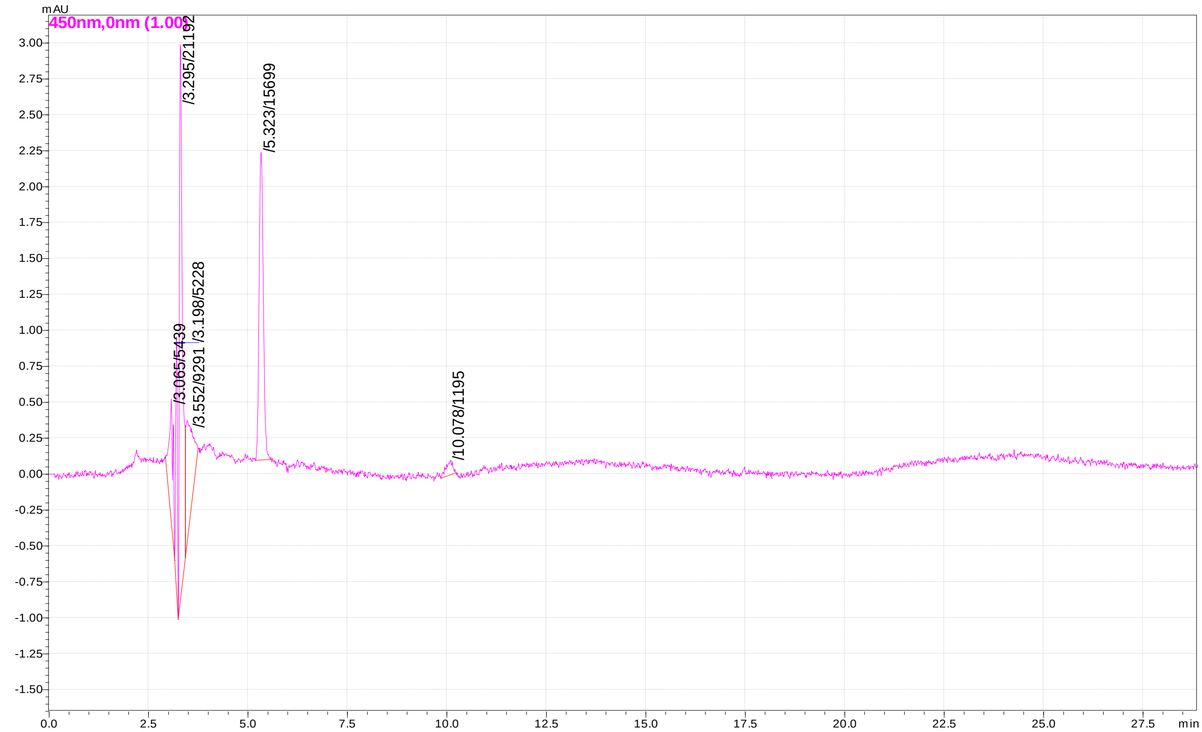


Fig.7:Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene, leutin , α-carotene, β-cryptoxanthin not detected and at 470 nm Lycopene not detected.

Sample Name: Holud pata



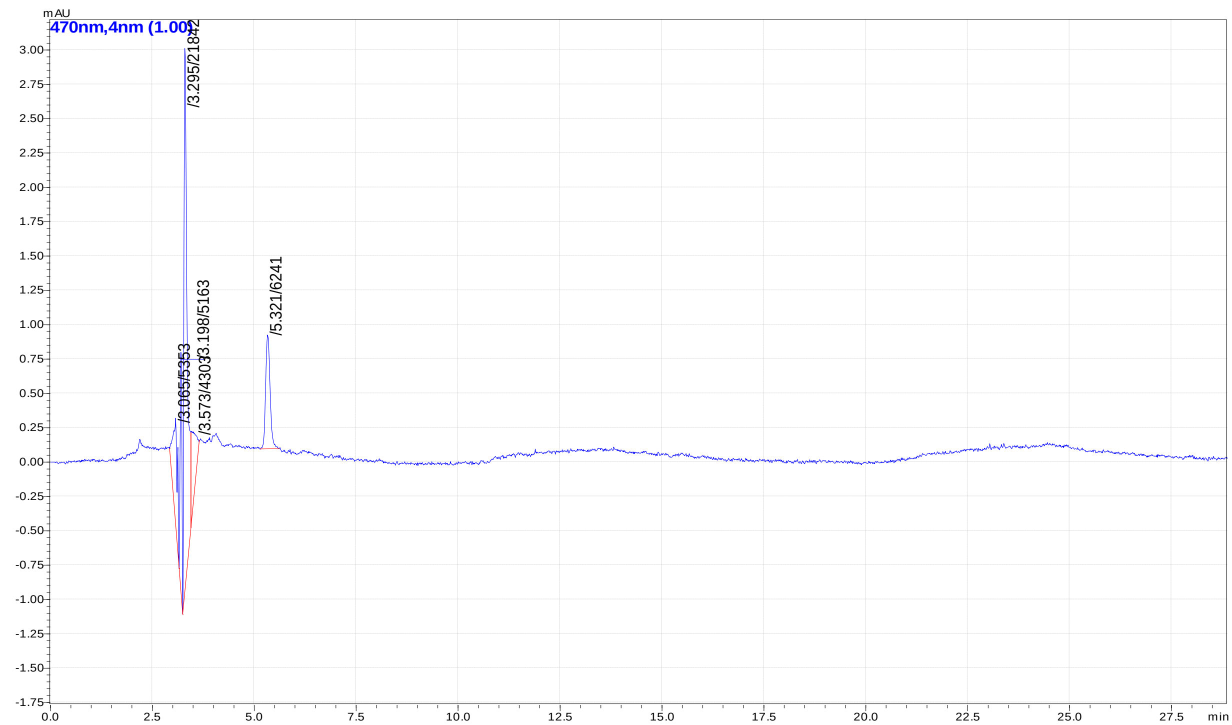
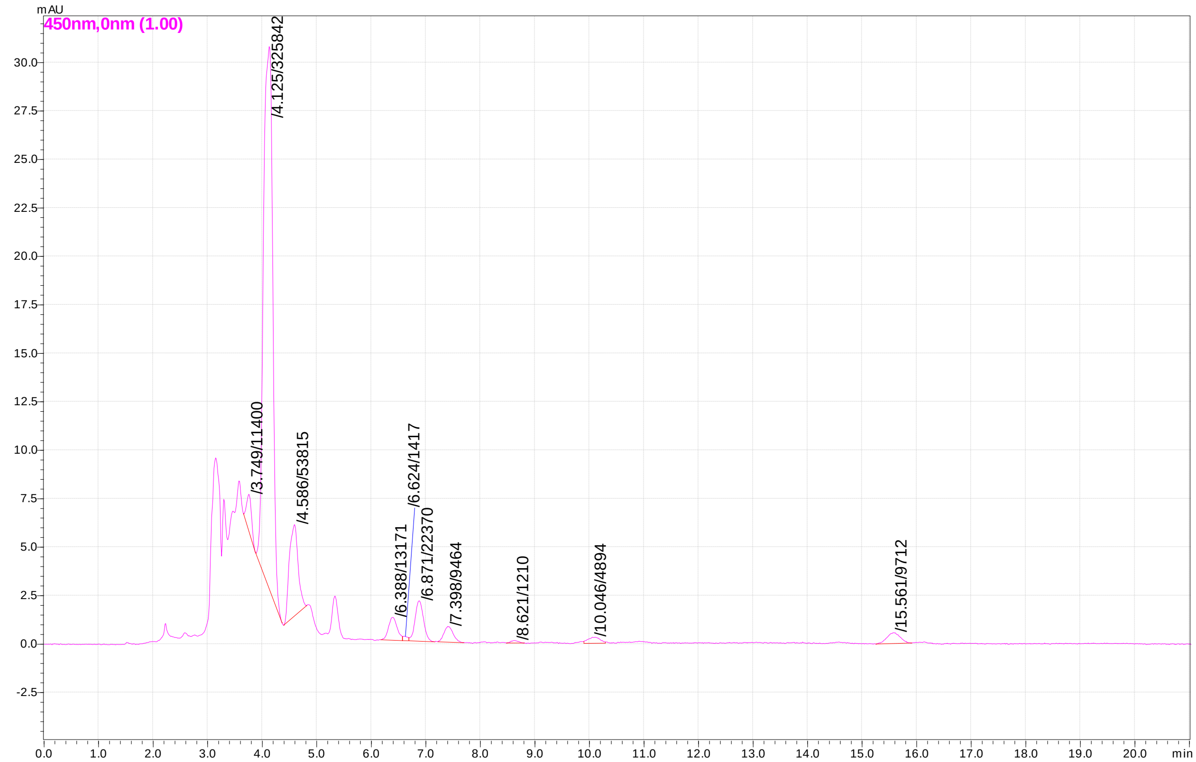


Fig.8: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene, leutin , α-carotene, β-cryptoxanthin not detected and at 470 nm Lycopene not detected.

Sample Name: Kochuripana



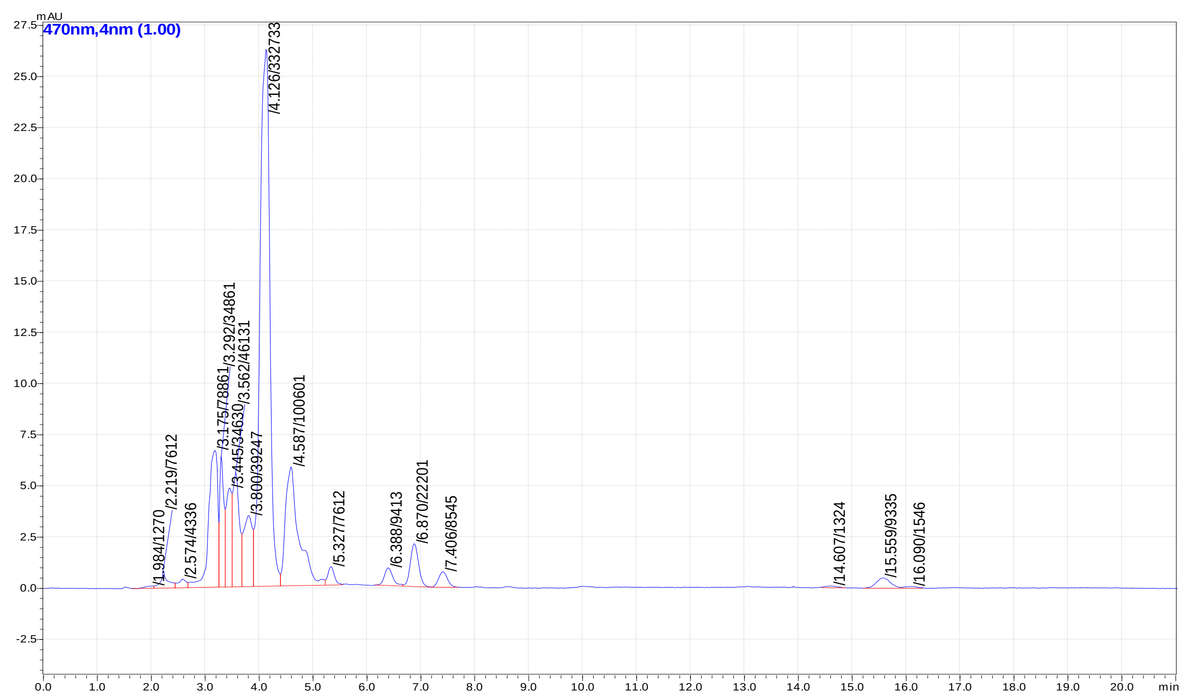


Fig.9: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.56min, leutin 4.58 min, α-carotene and β-cryptoxanthin not detected and at 470 nm

Sample Name: Tetul patan

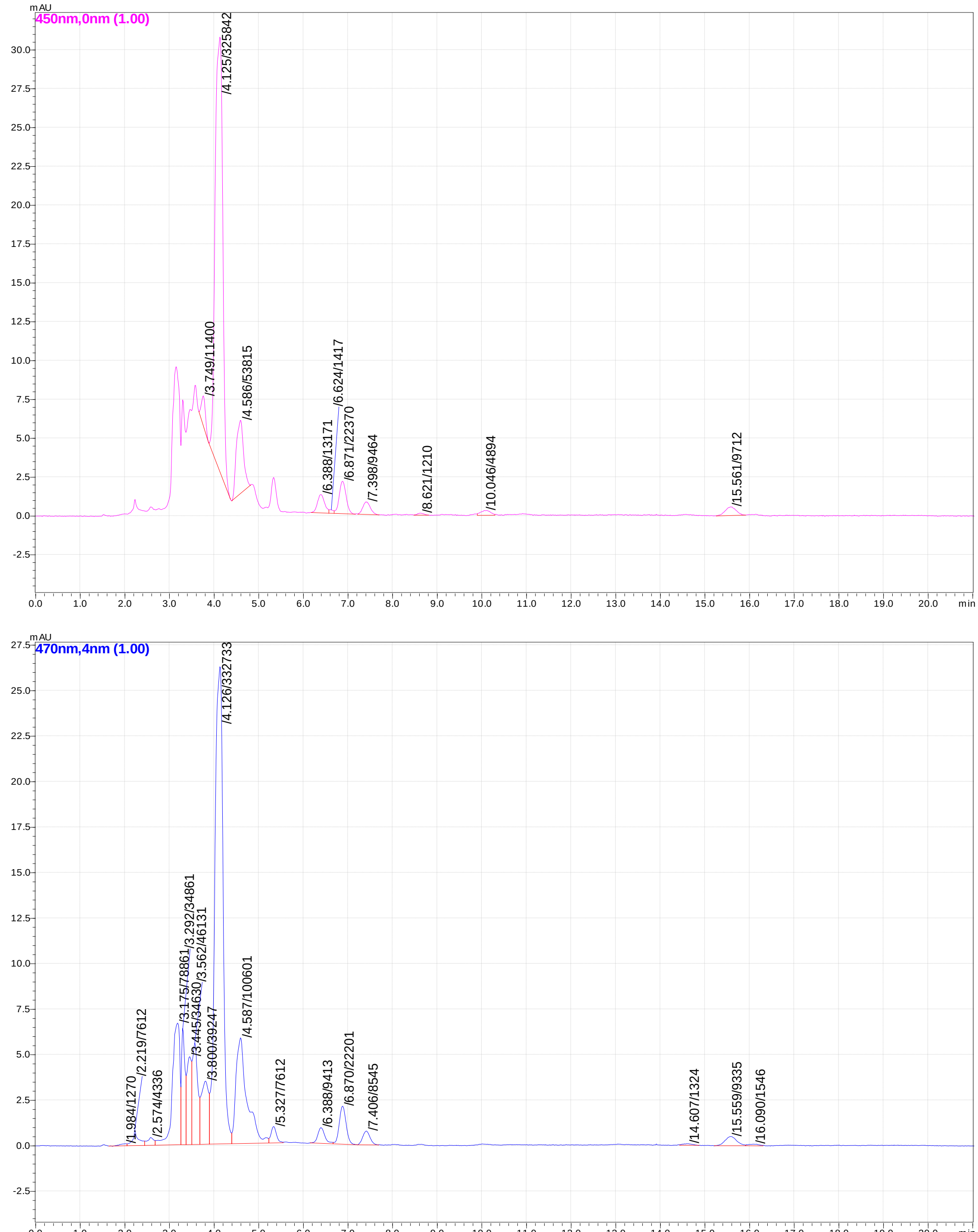
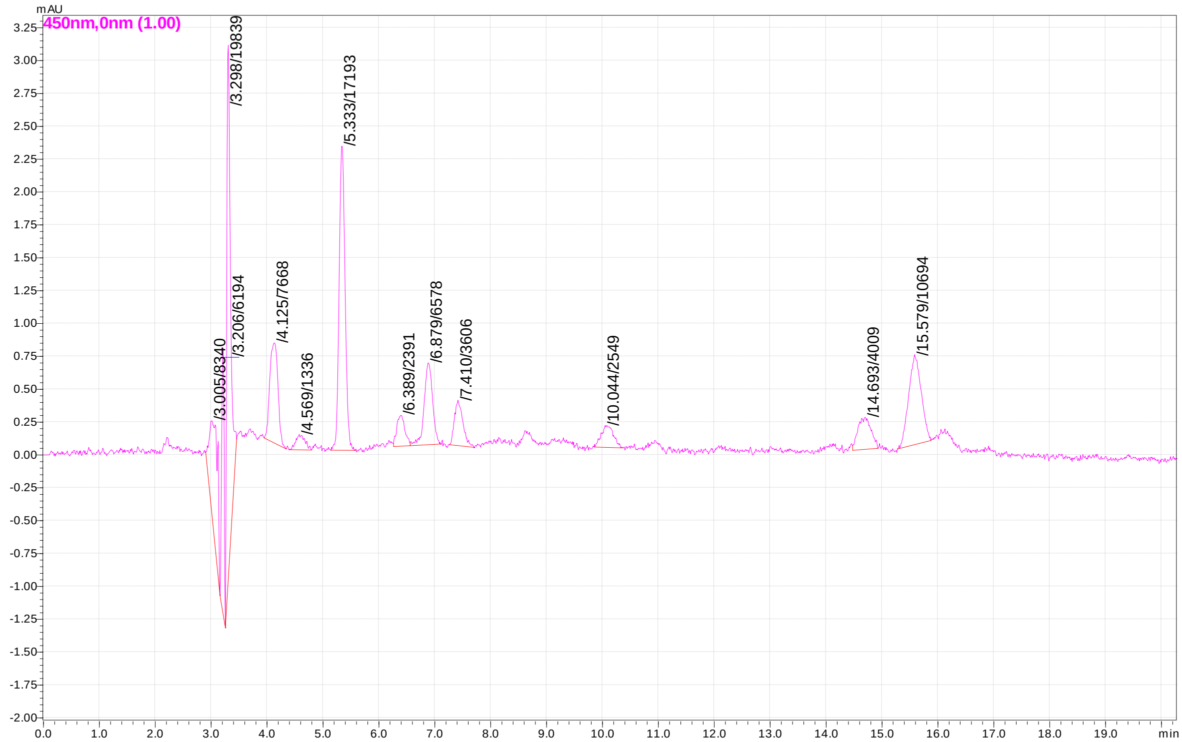


Fig.10: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.56min, leutin 4.58 min, α-carotene not detected and β-cryptoxanthin 7.66 min and at 470 nm Lycopene not detected.

Sample Name: Beth Gach



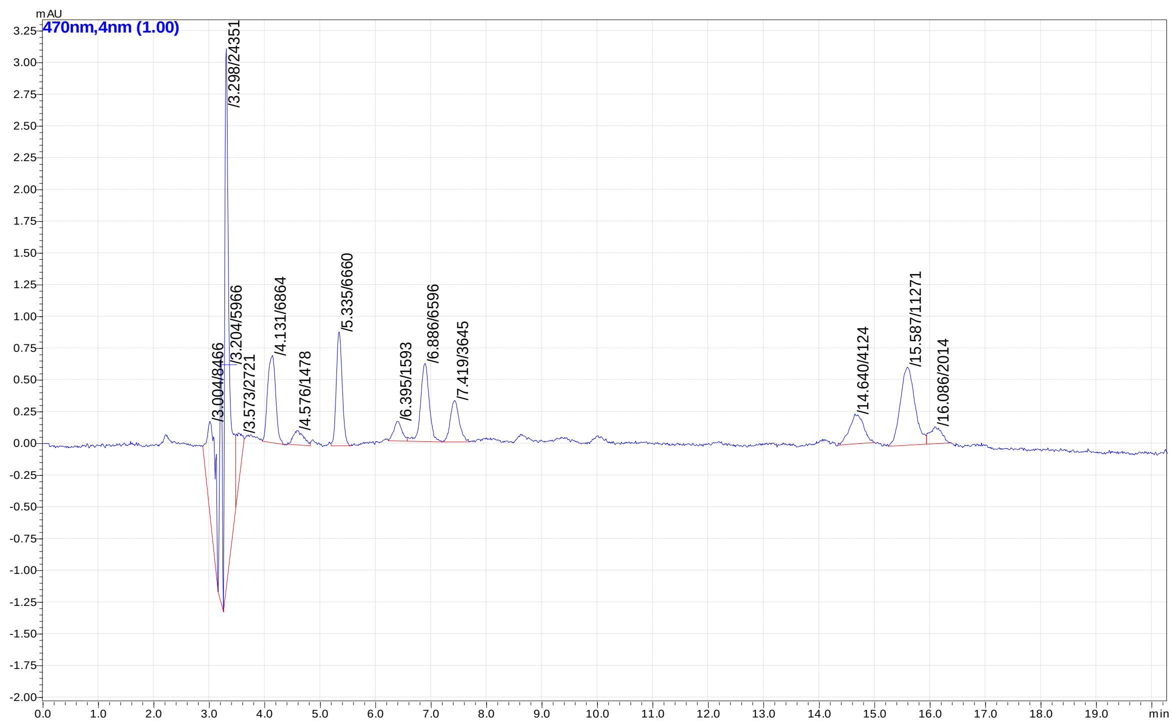


Fig.11: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.57min, α-carotene 14.69, leutin 4.56 min, β-cryptoxanthin 7.41min and at 470 nm Lycopene not detected.

Sample Name: Muktajhuri

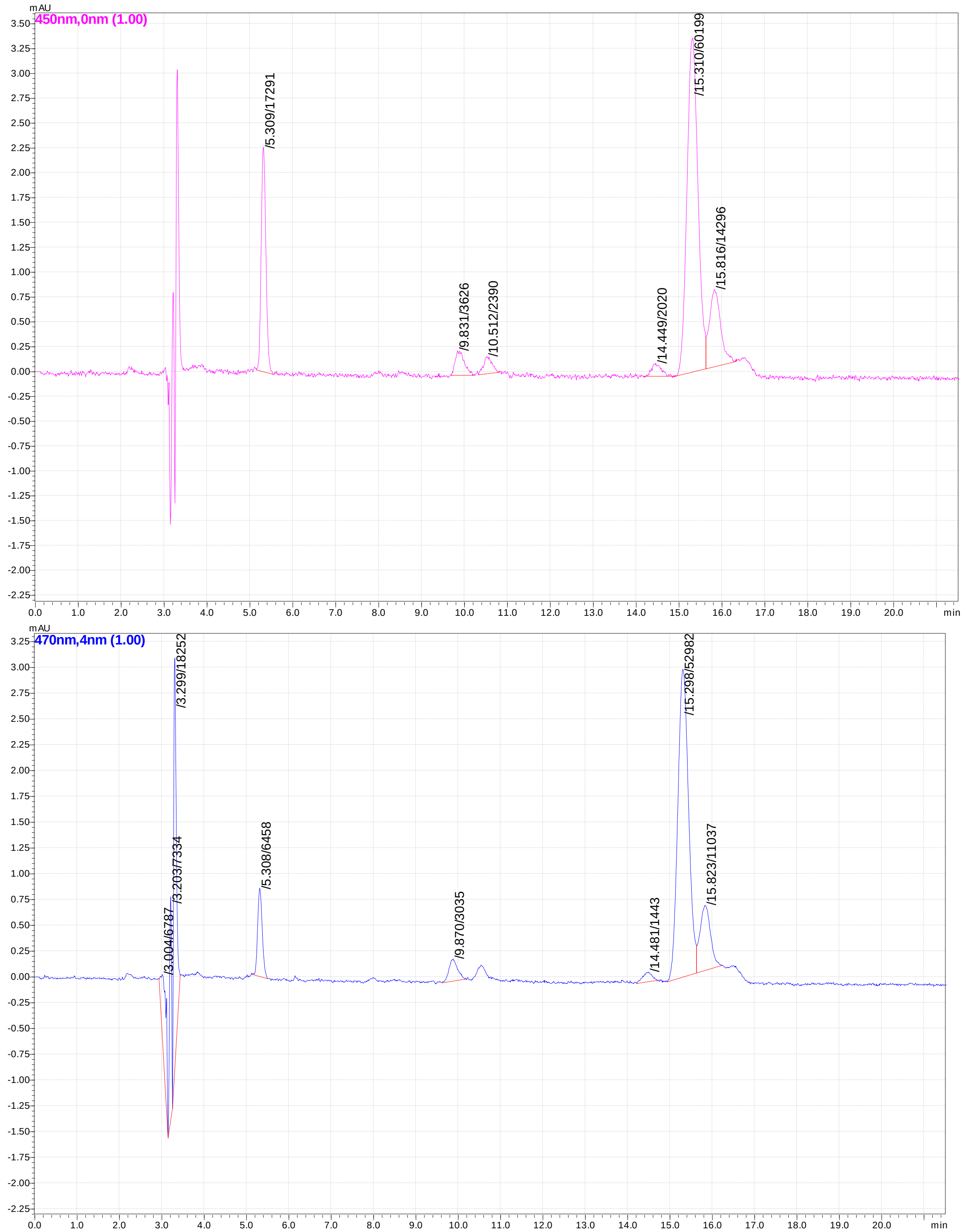
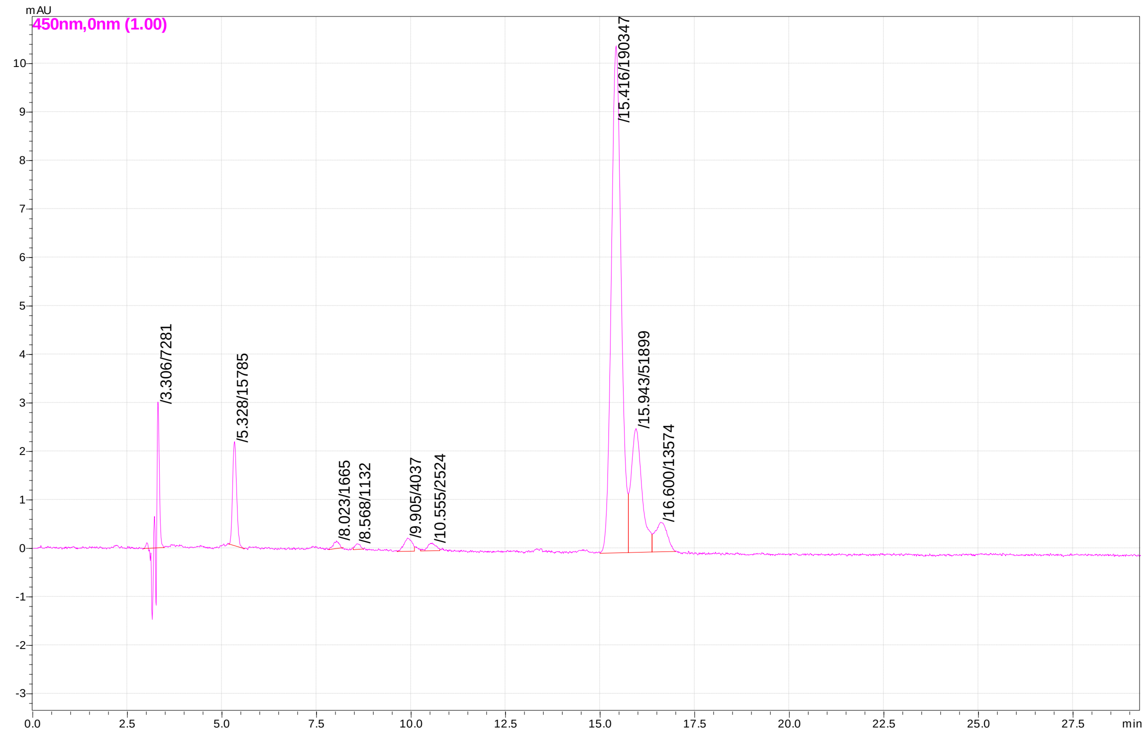


Fig.12: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.31min, α-carotene 14.44 min, leutin,, β-cryptoxanthin not detected and at 470 nm Lycopene in 9.87 min

Sample Name: Chimti Sak



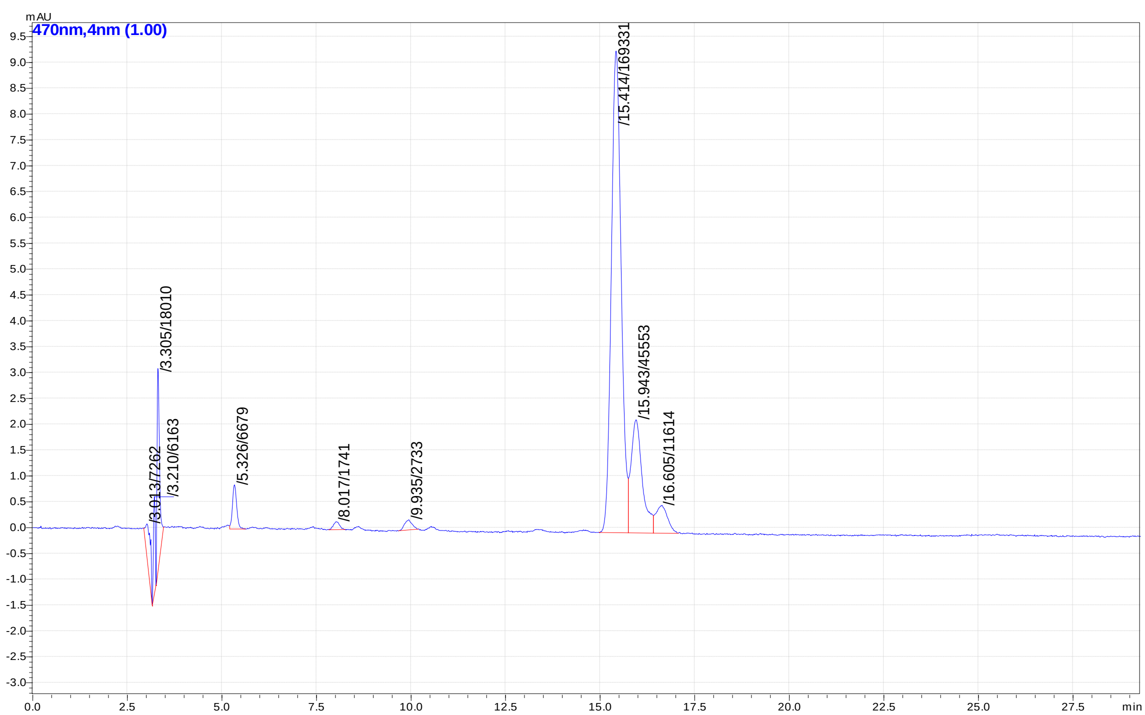


Fig.13: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.41min, α-caroten,, leutin,, β-cryptoxanthin not detected and at 470 nm Lycopene in 9.80min .

Sample Name: Pahari kolar thor

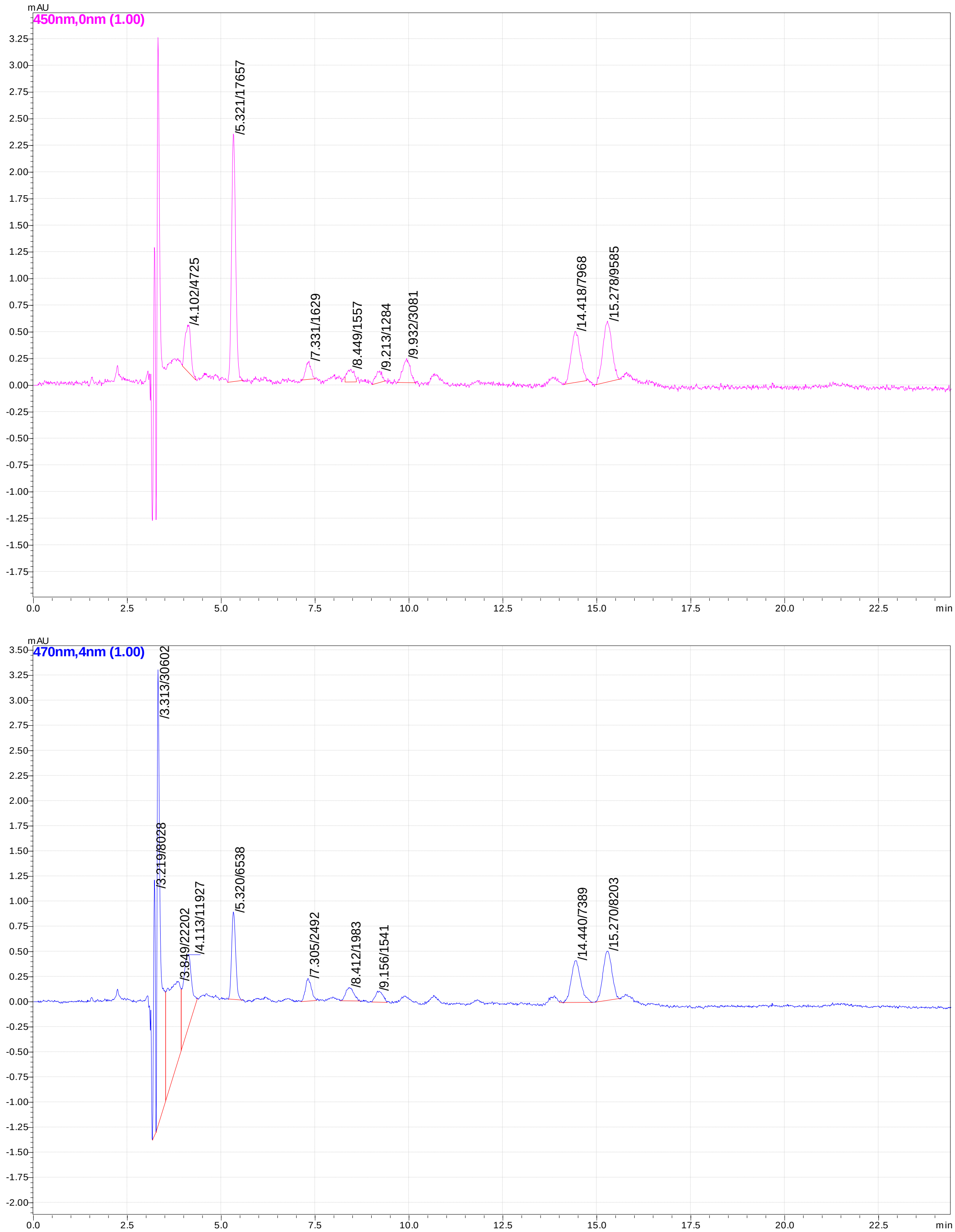


Fig.14: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.27min, α-carotene 14.41min, leutin 4.10,β-cryptoxanthin not detected and at 470 nm Lycopene in 9.15 min.

Sample Name: Roktrodrone

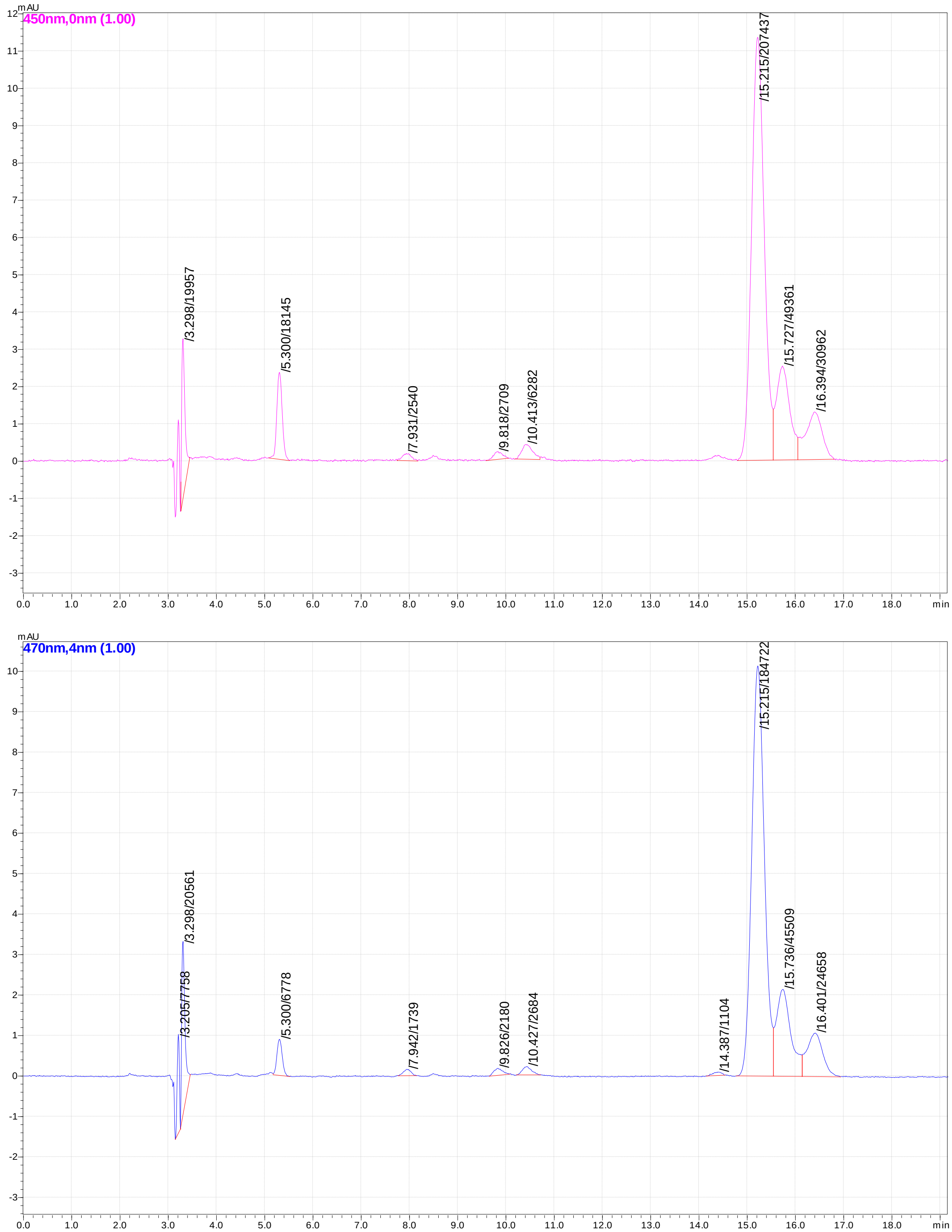


Fig.15: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.27min, α-carotene, leutin not detected,β-cryptoxanthin 7.931 min and at 470 nm Lycopene in 9.826 min.

Sample Name: Mulachi

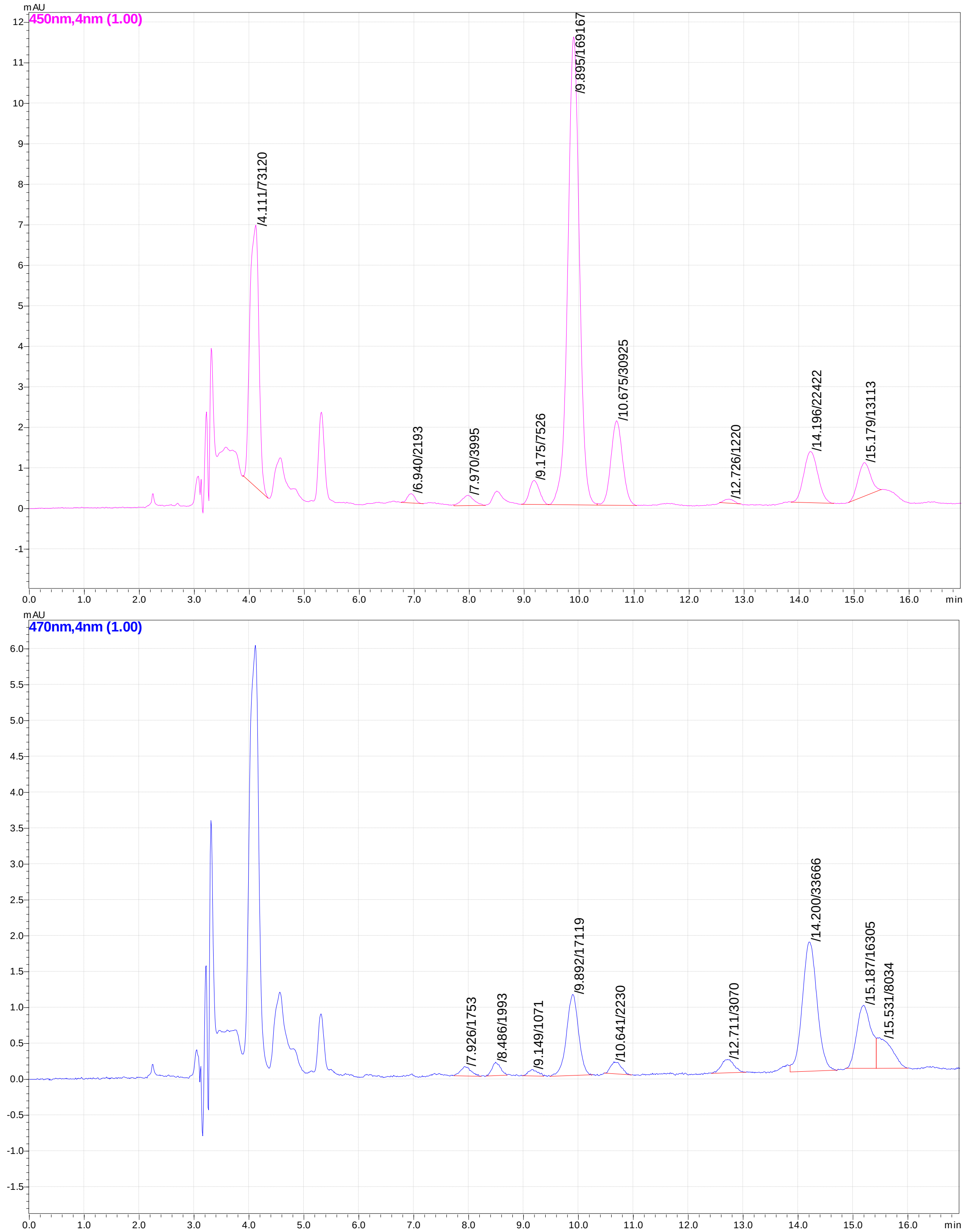


Fig.16: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.17min, α-carotene 14.196 min, leutin 4.11 min ,β-cryptoxanthin 7.92 min and at 470 nm Lycopene in 9.83 min.

Sample Name: Shornolota

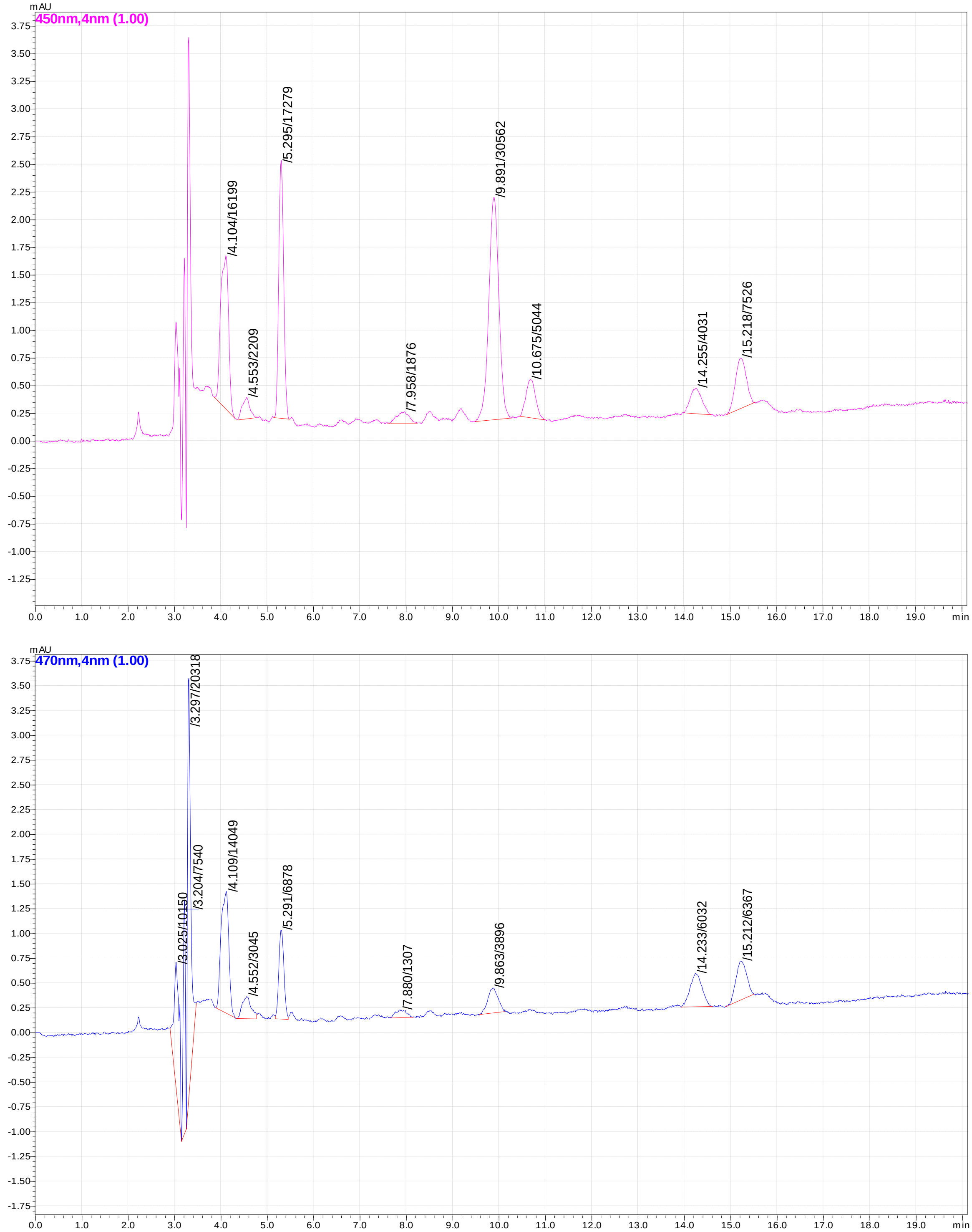
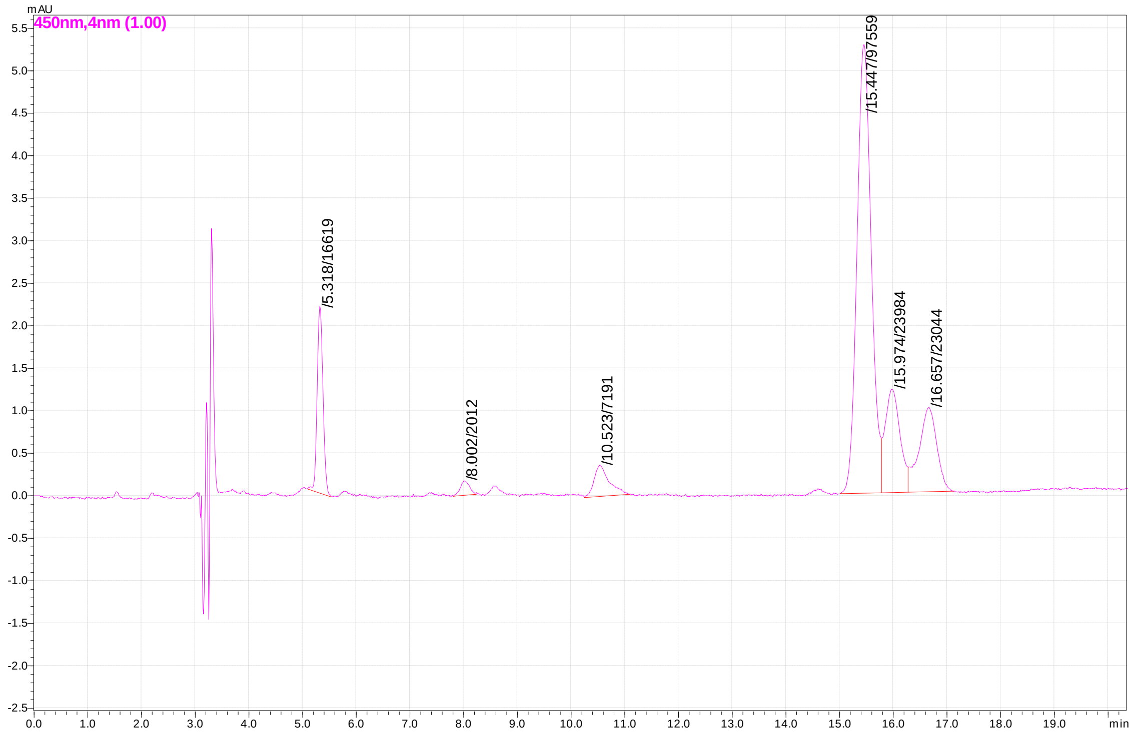


Fig.17: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.21min, α-carotene 14.255 min, leutin 4.55 min ,β-cryptoxanthin 7.95 min and at 470 nm Lycopene in 9.60min.

Sample Name: Bondhone



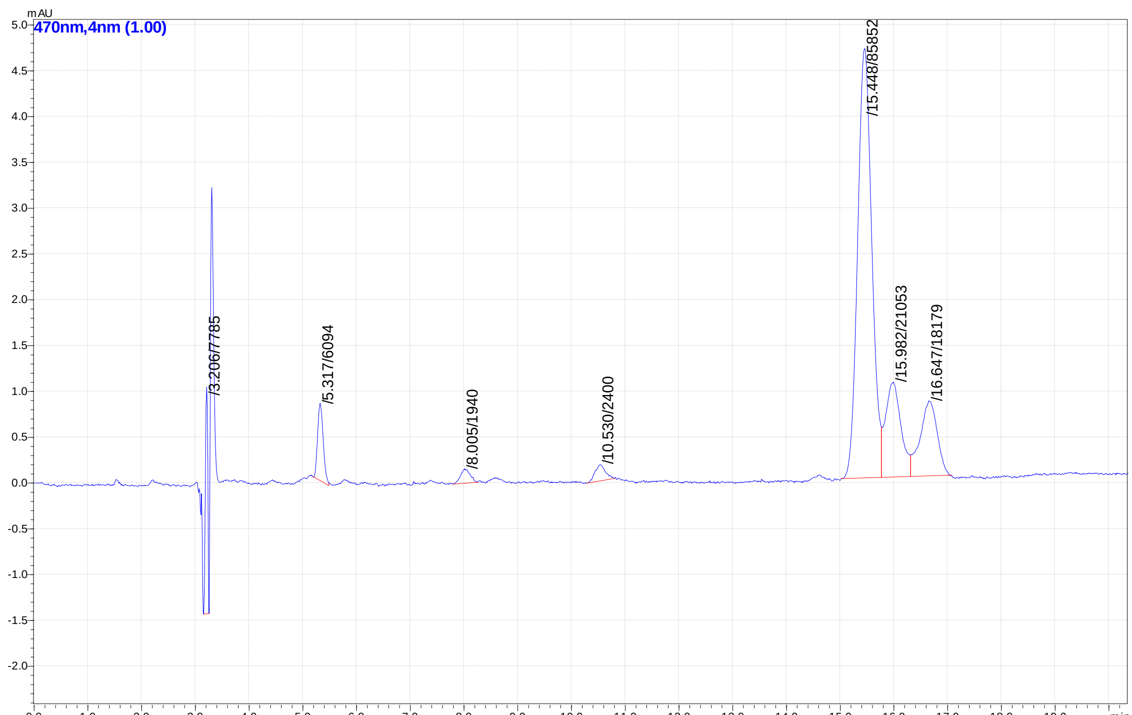
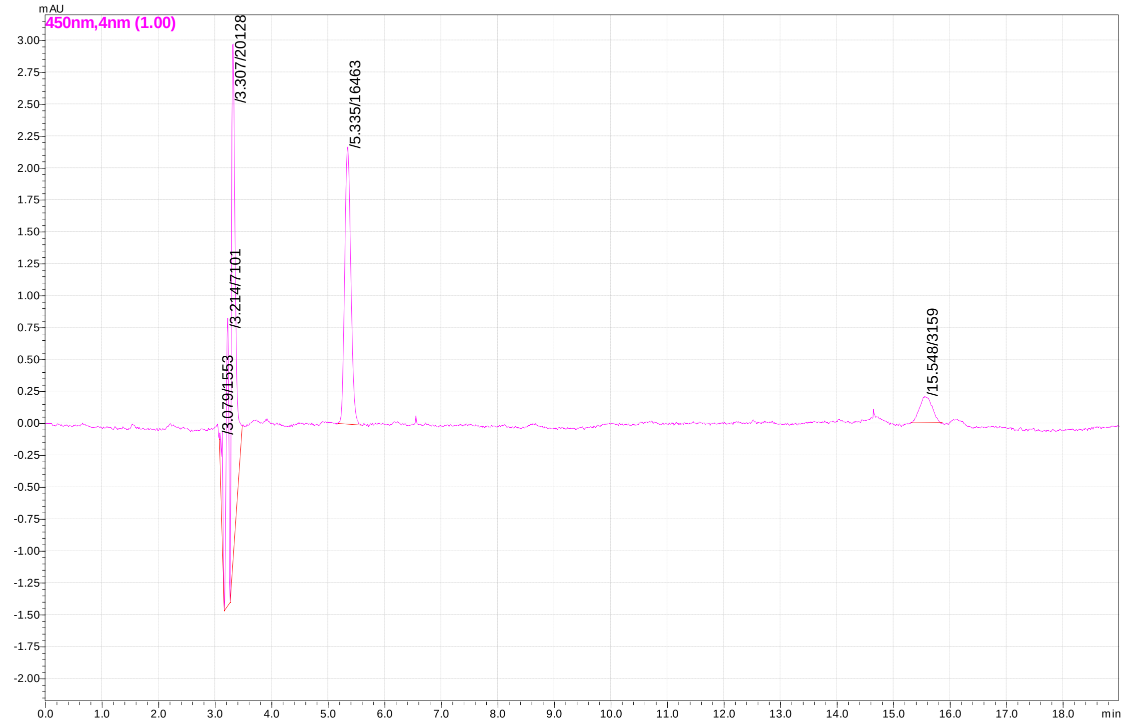


Fig.18: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.21min, α-carotene, leutin ,β-cryptoxanthin not detected and at 470 nm Lycopene in 10.53 min.

Sample Name: Jolpai Pata



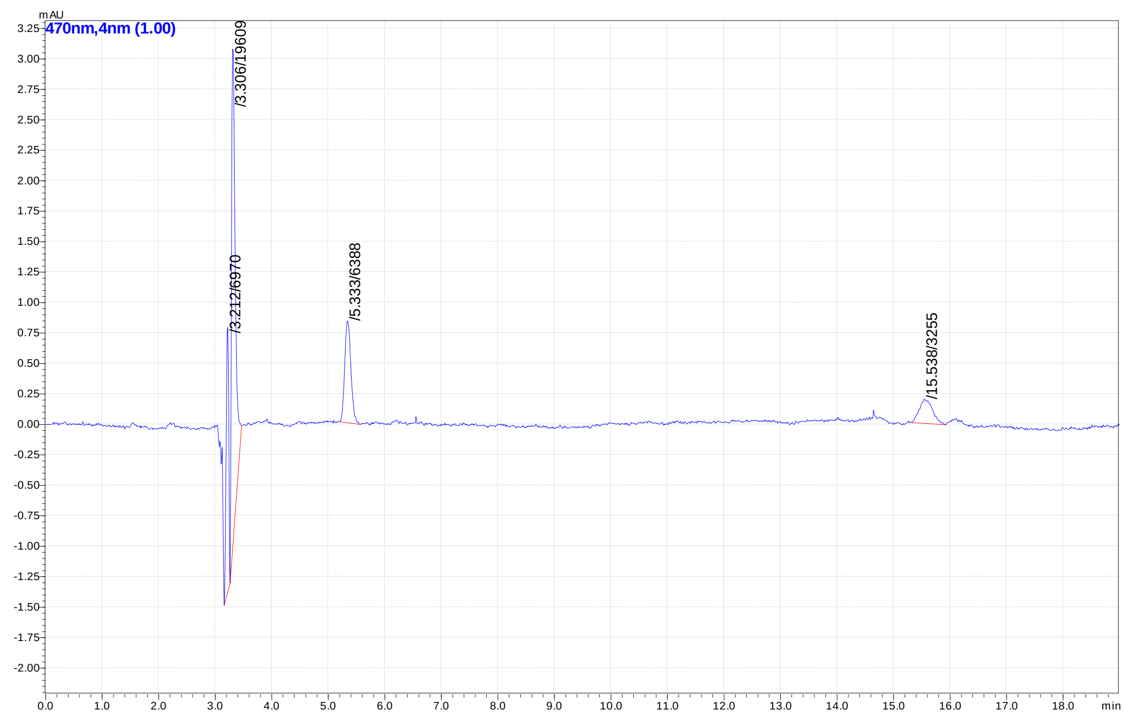
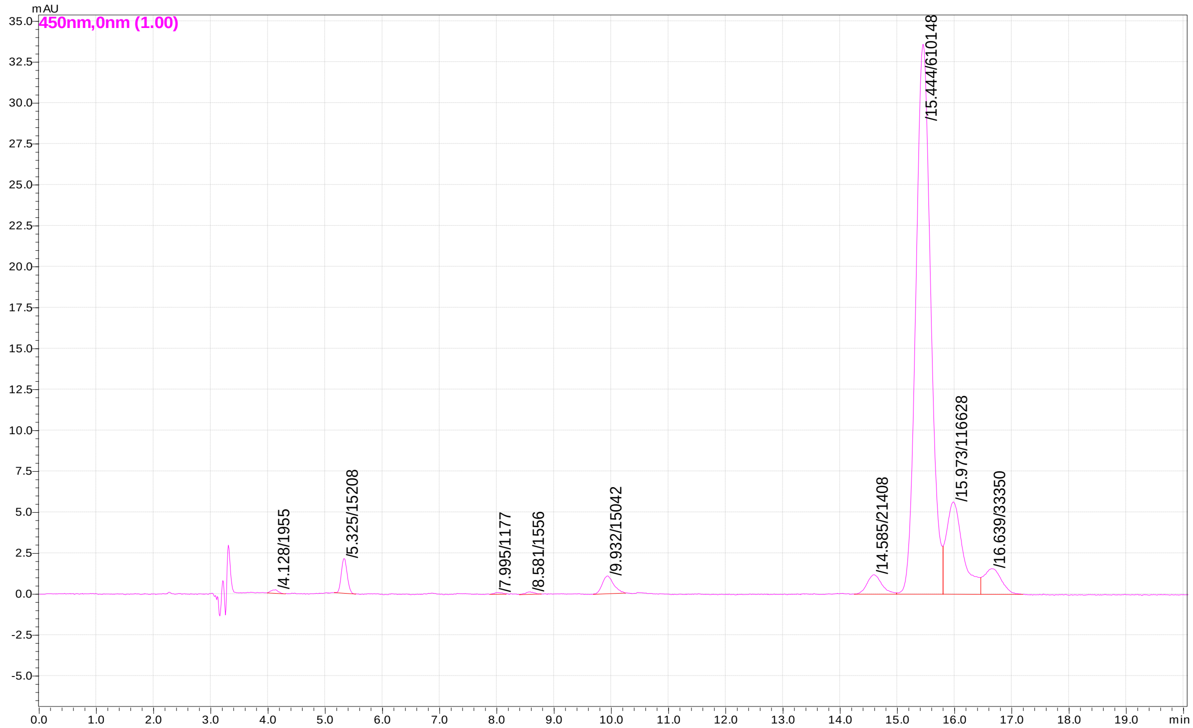


Fig.19: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.54 min, α-carotene, leutin ,β-cryptoxanthin not detected and at 470 nm Lycopene not detected.

Sample Name: Shetodhrone



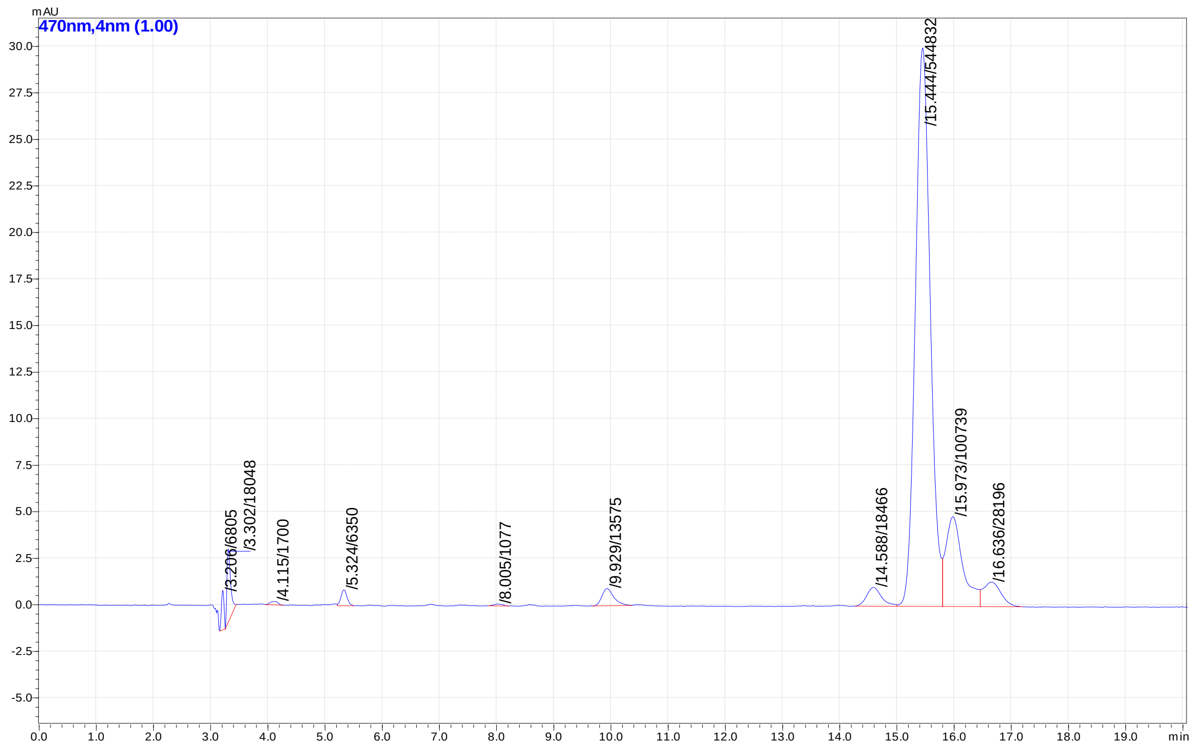


Fig.20: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rating at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.97 min, α-carotene 14.58min, leutin4.583 min ,β-cryptoxanthin 7.91 min and at 470 nm Lycopene 9.92 min

Sample Name: katanotey

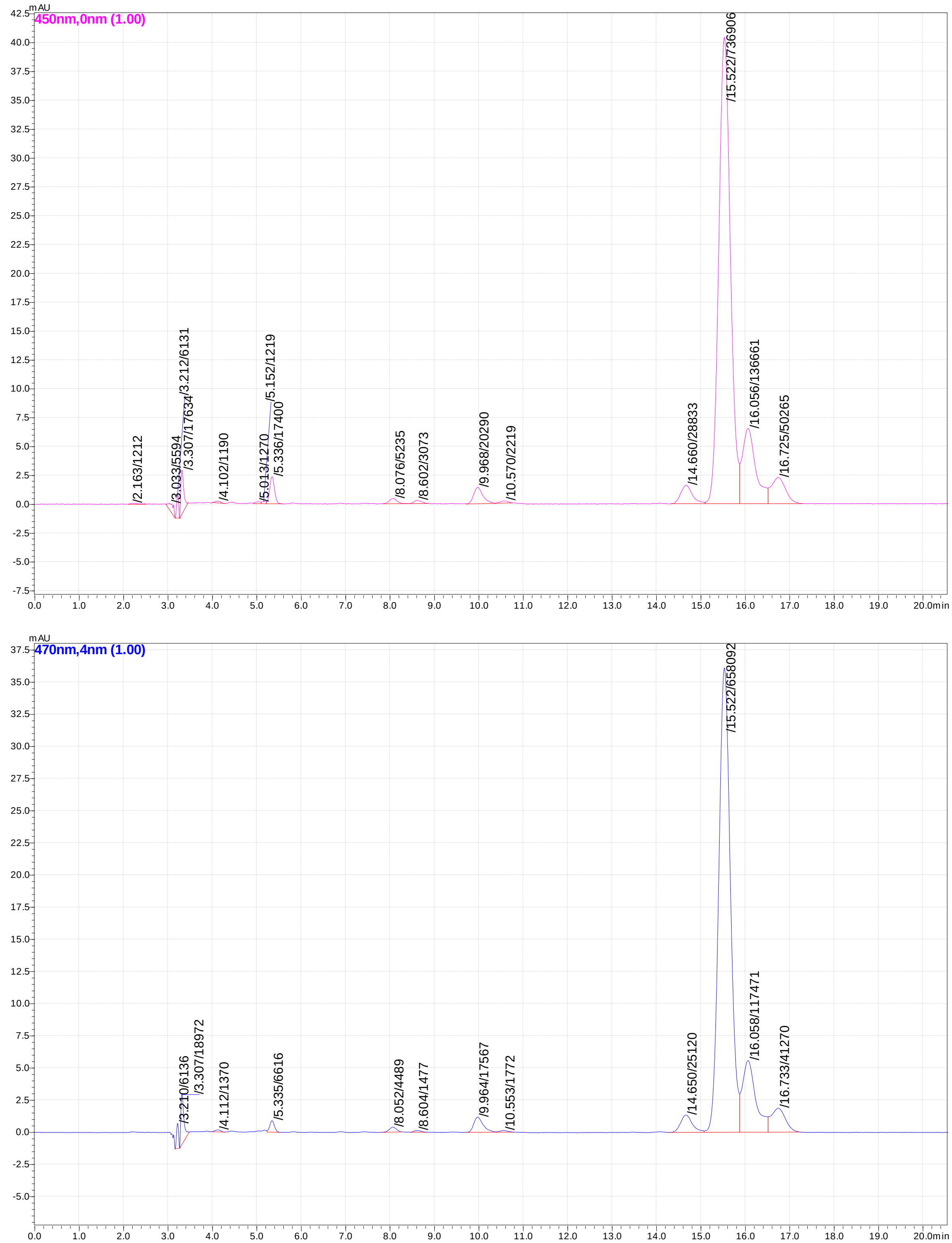
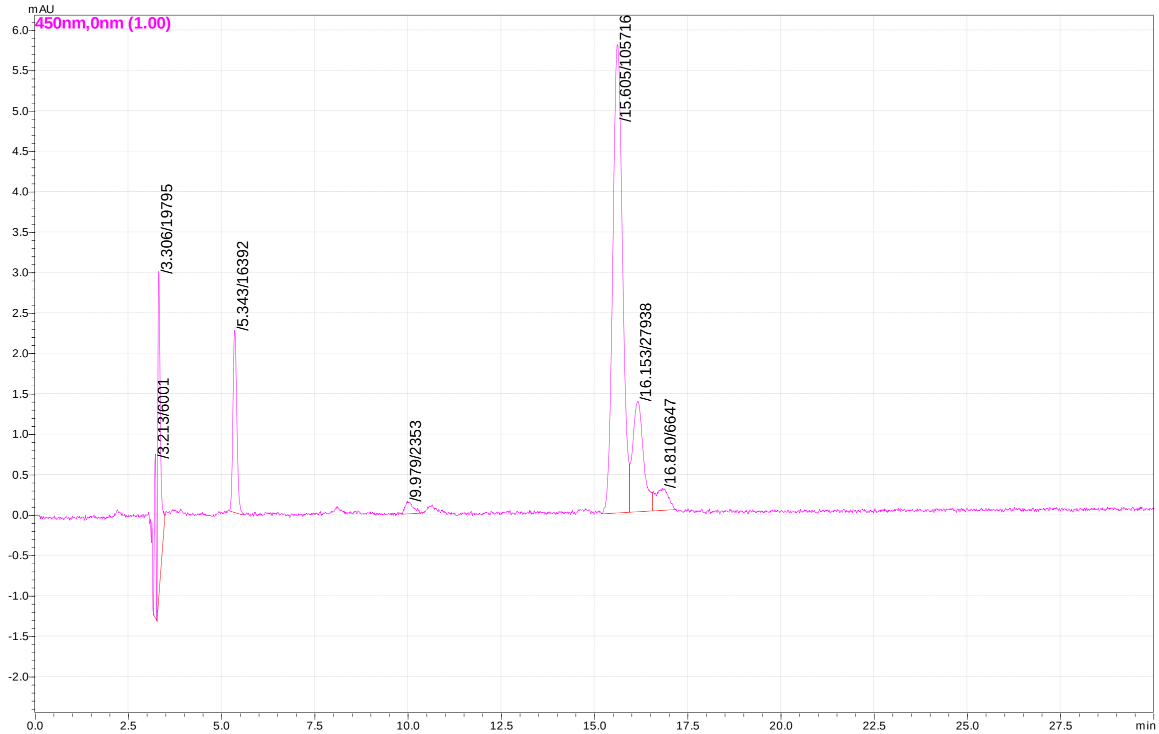


Fig.21: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.522 min, α-carotene 14.66min, leutin4.not detected ,β-cryptoxanthin 8.07 min and at 470 nm Lycopene 9.96 min.

Sample Name: Venna Pata



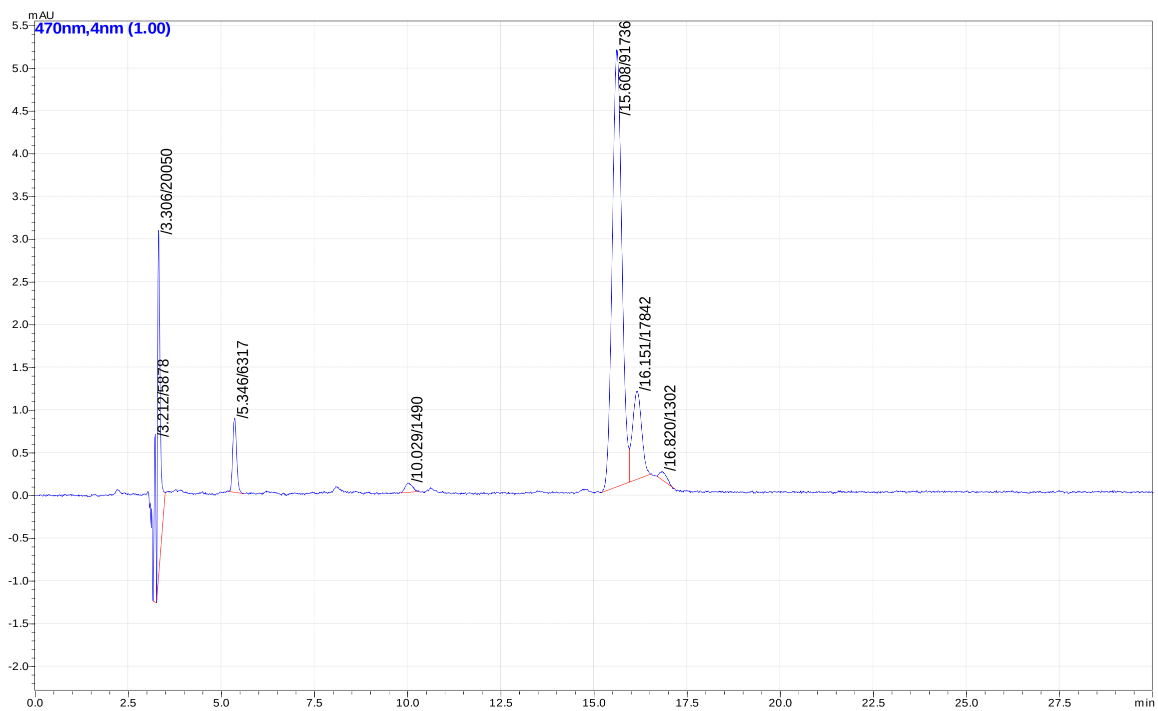


Fig.22: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.60 min, α-carotene, leutin,β-cryptoxanthin not detected and at 470 nm Lycopene 10.02 min.

Sample Name: Orhor seeds

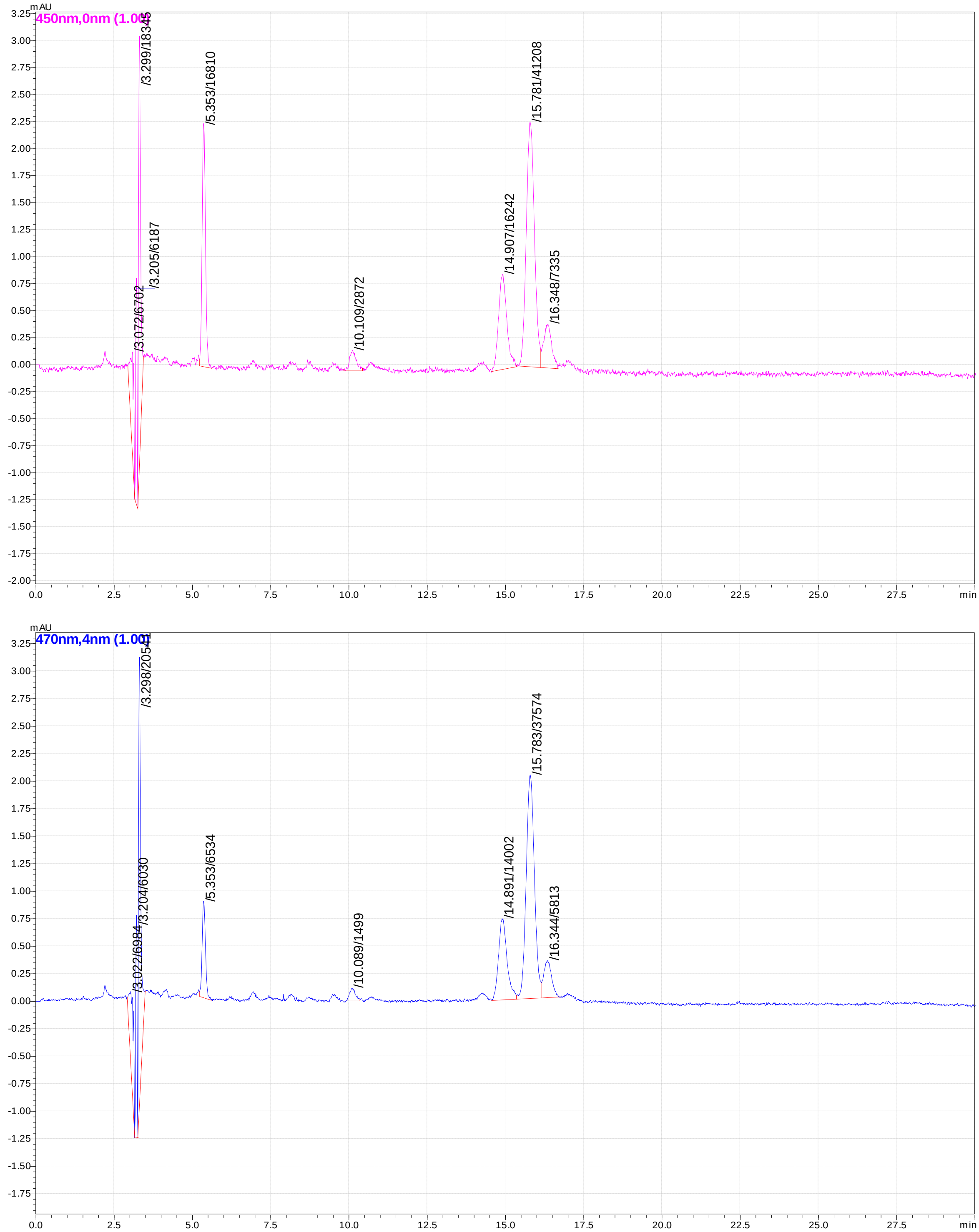
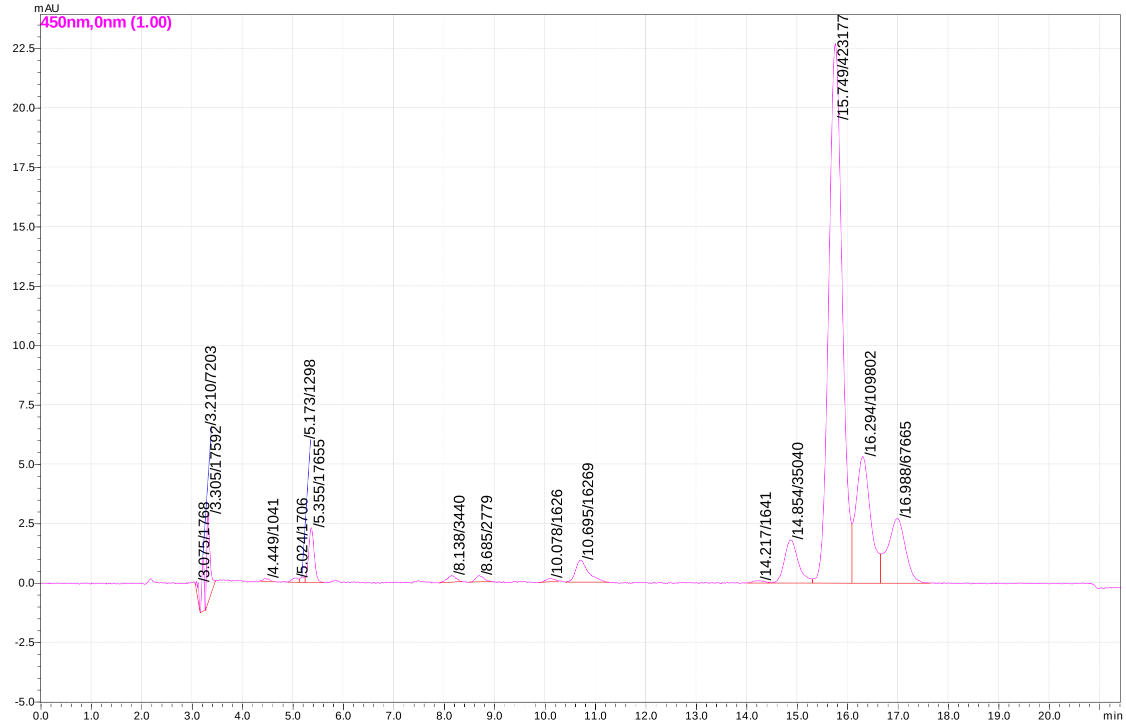


Fig.23: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15..78 min, α-carotene 14.90 min, leutin,β-cryptoxanthin not detected and at 470 nm Lycopene 10.03 min.

Sample Name: Orhor leaves



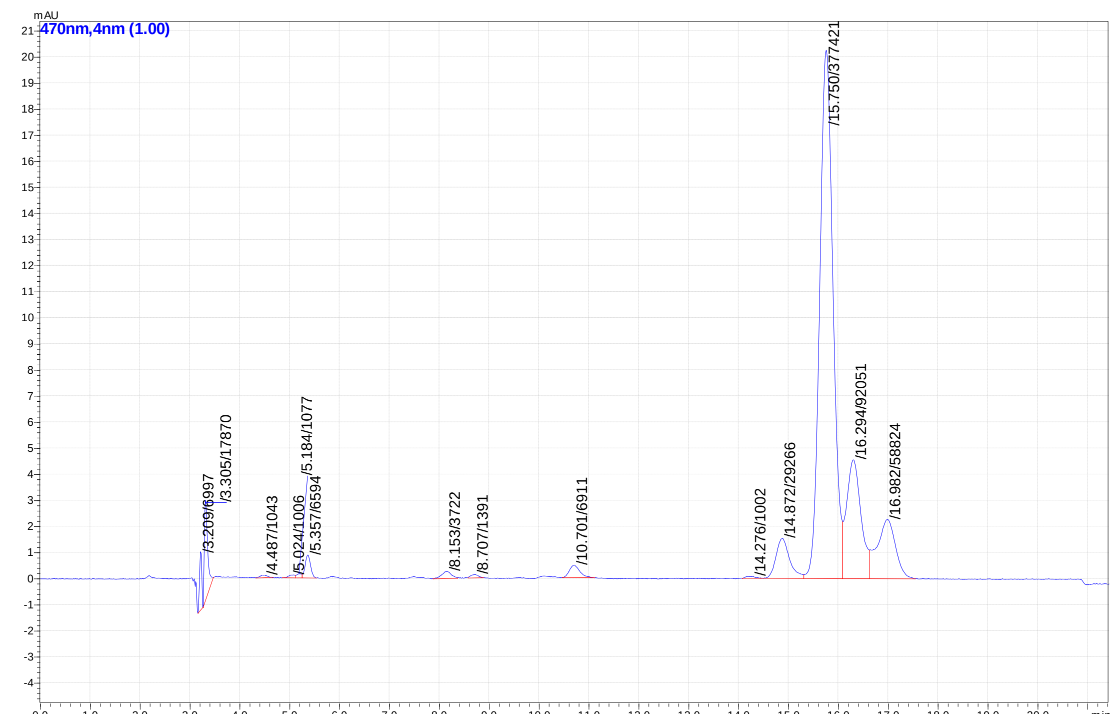
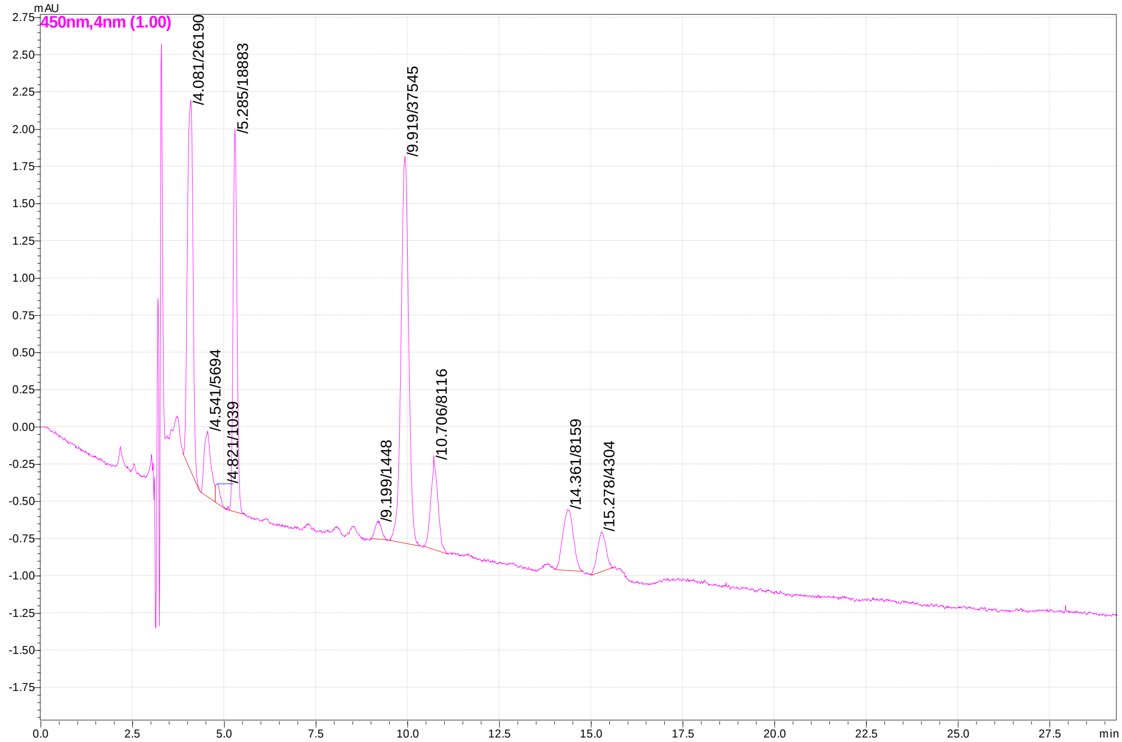


Fig.24: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.74 min, α-carotene 14.85 min, leutin not detected ,β-cryptoxanthin 8.13 min and at 470 nm Lycopene 10.7 min.

Sample Name: OFSP Leaves/Mishti Aloo pata



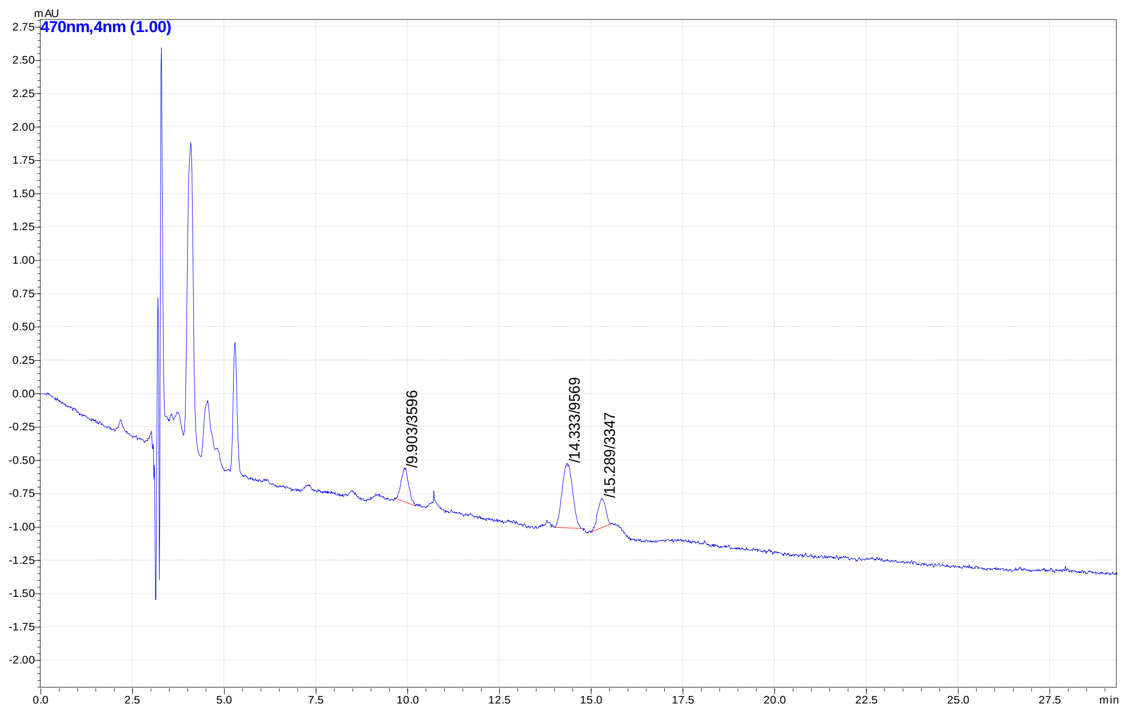
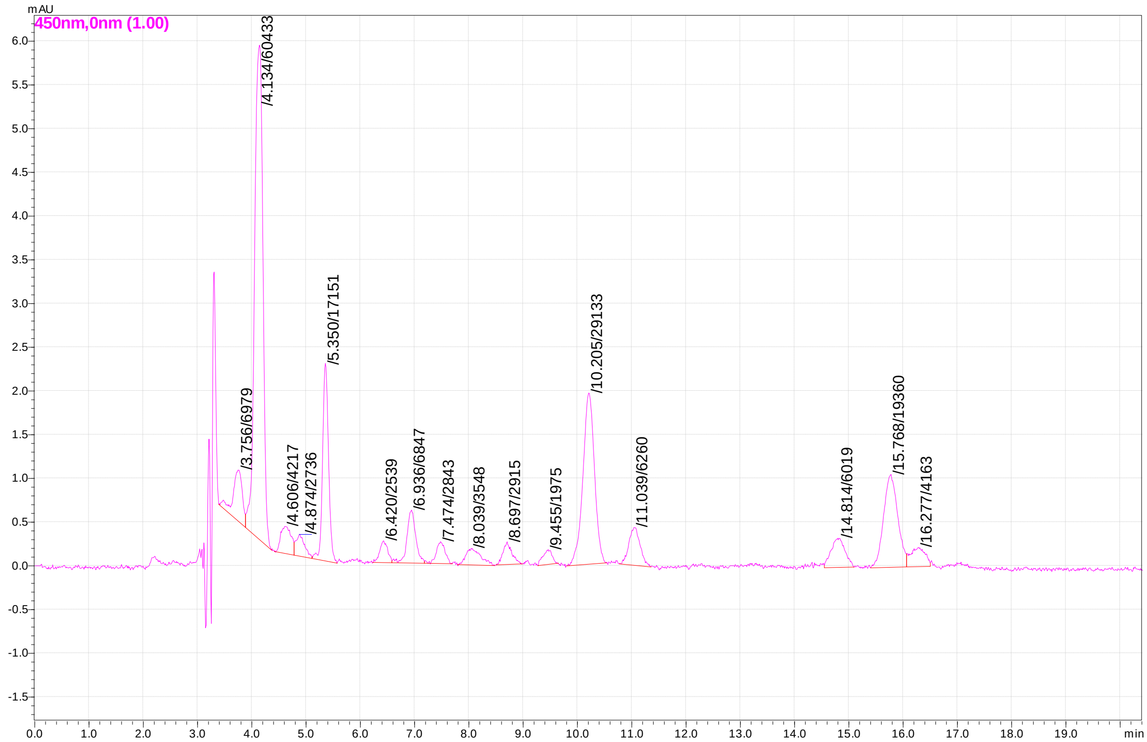


Fig.25 : Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.278min, α-carotene 14.361 min, leutin 4.80 min ,β-cryptoxanthin not detected and at 470 nm Lycopene not detected.

Sample Name: Mandar



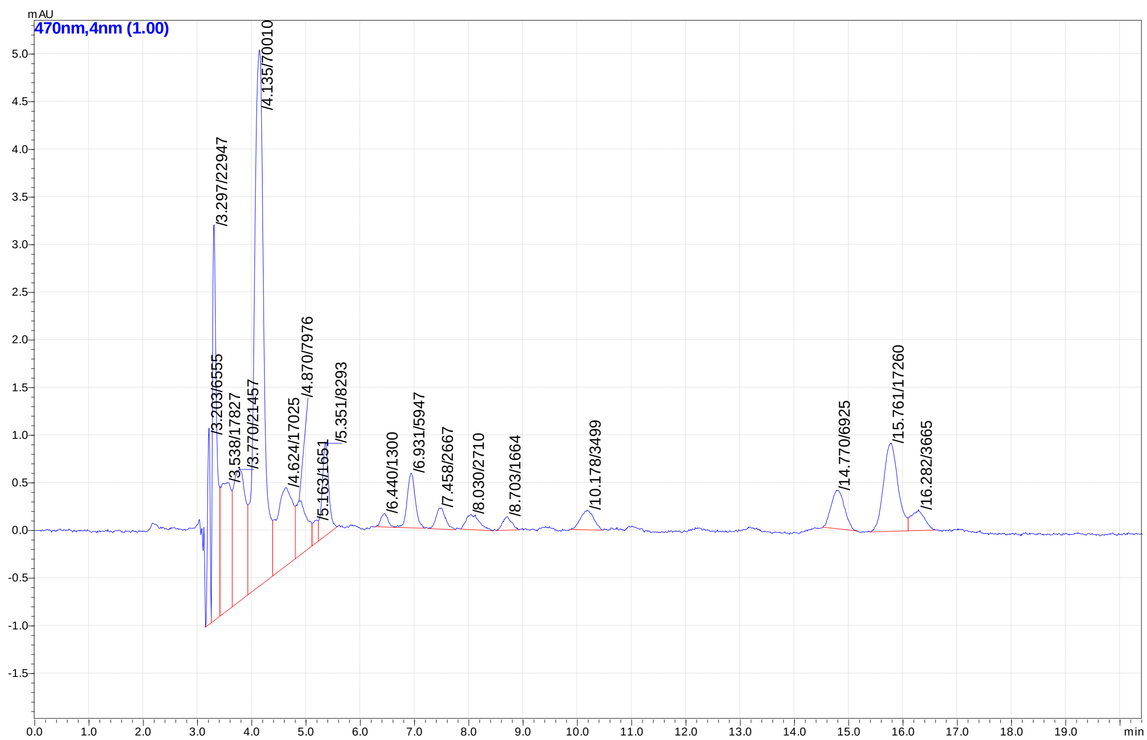


Fig.26: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.76min, α-carotene 14.81 min, leutin 4.60 min ,β-cryptoxanthin 7.47 min and at 470 nm Lycopene 10.17 min.

Sample Name: OFSP

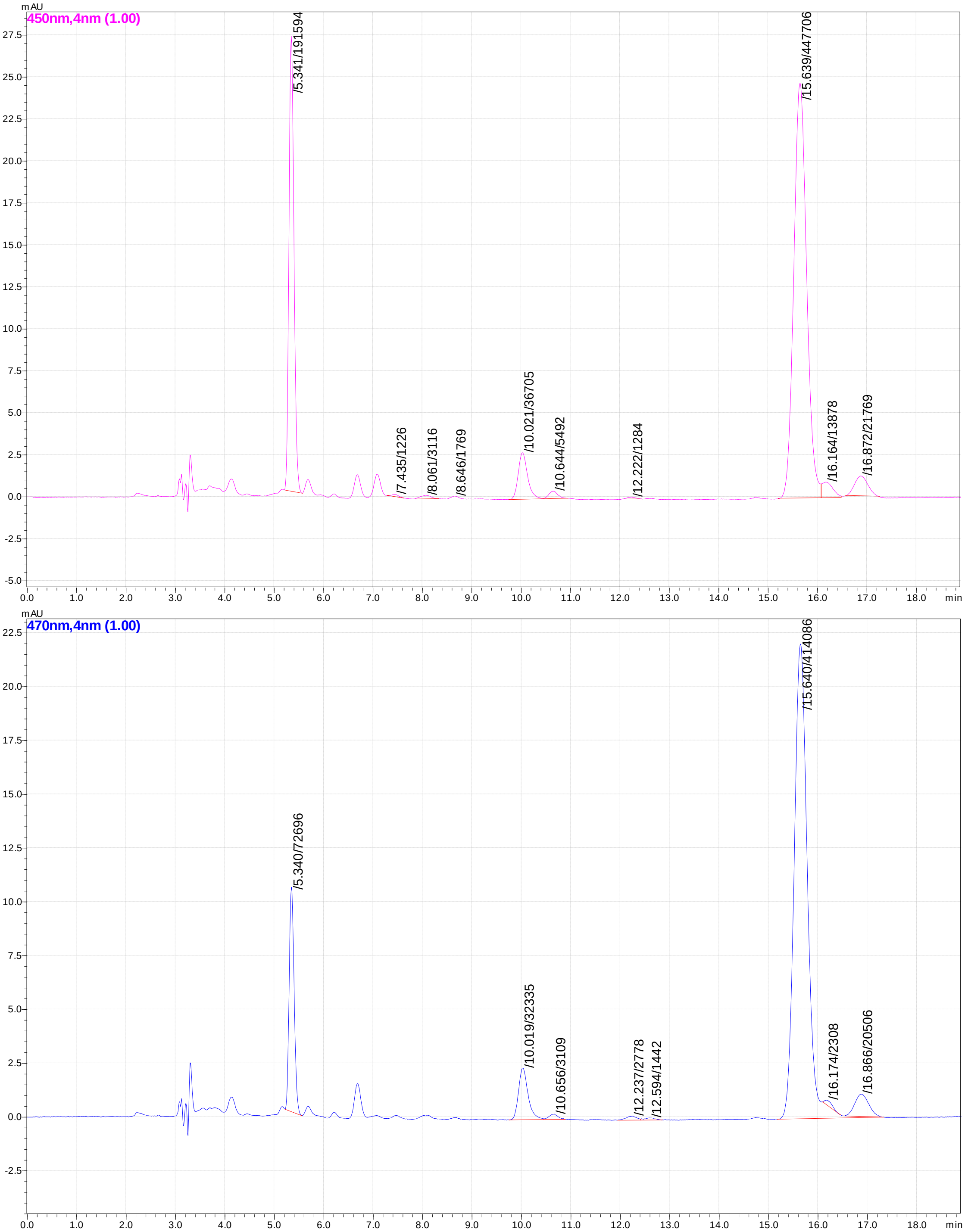


Fig.27: Analysis of Carotene profile by HPLC(High Performance Liquid Chromatography), 25µl reconstituted sample was injected into the reverse phase C_{18} column using the mobile phase (acetonitrile: methanol: 2-propanol 85:15:33 with 0.01% ammonium acetate) , flowing rate at 1.7 ml/min. HPLC separation of Carotene at 450 nm, Peaks β-carotene 15.639min, α-carotene, leutin not detected ,β-cryptoxanthin 10.02 min and at 470 nm Lycopene 10.01 min.