EXTRACTION OF CAROTENE FROM *MORINGA OLEIFERA* LEAVES FOR COSMETICS USES

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Thesis submitted in partial fulfilment of the requirements
For the degree of
Bachelor of Chemical Engineering

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We hereby declare that we have checked this thesis and this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering.

Signature	:
Name of main supervisor	: DR. EMAN N. ALI
Position	: SENIOR LECTURER
Date	: 10 JANUARY 2017

STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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DEDICATION

To ALLAH, my parents and supervisors.

ACKNOWLEDGEMENT

Firstly, I would like to express my gratitude to my supervisor, DR. EMAN N. ALI for her continuous support of my study and for her patience, motivation and her tremendous knowledge on the topic. Her guidance has helped me in this study and the writing of this thesis. I could not have imagined finishing the experiments without her guidance. Her technical knowledge and expertise has helped me understand this study better. I would also like to take this opportunity to thank my friends and family for their continuous spiritual support.

ABSTRACT

In this research, carotene in the *Moringa Oeifera* leaves will be extracted by using Soxhlet extraction method. Carotene as pigment that naturally contained in most vegetables is important source of nutrients used for pharmaceutical and cosmetic. The main constituent of the carotene which is beta-carotene will be investigated. Two variables were chosen to investigate the effect of different solvent on the extraction and beta-carotene concentration. Samples with different solvent will be prepared which the solvents are acetone, petroleum ether and water respectively. After the extraction process, the mixture of carotene and solvent will be separated using rotary evaporator in order to get the extracted carotene. Then, the extracted carotene was analysed using HPLC to determine the beta-carotene concentration.The best type of solvent use to extract the beta carotene from *Moringa Oleifera* is petroleum ether and was proven from the HPLC analysis based on the area of peak.

ABSTRAK

Dalam penyelidikan ini, beta karotena akan diekstrak daripada daun yang berspesis *Moringa Oeifera* melalui kaedah Soxhlet pengekstrakan. Karotena merupakan suatu pigmen yang terhasil secara semulajadi di dalam kebanyakan sayuran. Disamping itu, beta carotene merupakan sumber utama bagi nutrient dan amat berguna bagi bidang farmasi dan kosmetik. Bahagian yang paling utama dalam karotena iaitu beta karotena akan dikaji dengan lebih mendalam bagi penyilidikan ini. Dua pemboleh ubah manipulasi akan dipilih untuk mengkaji kesan solven yang berbeza terhadap pengekstrakan beta karotena dan jumlah yang dapat dihasilkan. Sampel yang mempunyai solven yang berbeza akan disediakan, solven itu adalah acetone, petroleum ether dan air. Selepas selesai proses pengekstrakan campuran karotena dan solven akan dipisahkan dengan menggunakan rotary evaporator untuk mendapatkan penghasilan beta karotena yang lebih efektif. Beta karotena yang telah diekstrak akan dianalisis dengan menggunakan HPLC bagi menentukan kepekatan tersebut. Melalui pengkajian ini dapat disimpulkan bahawa petroleum ether merupakan ejen yang paling berkesan untuk mengekstrak beta karotena dan penyataan ini telah dibuktikan melalui hasil analisis daripada HPLC

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LIST OF ABBREVIATIONS

HPLC	**High Performance Liquid Chromatography**
CH_3CN	**Acetone nitrile**
CH_3OH	**Methanol**
CH_2Cl_2	**Dichloromethane**
C_3H_6O	**Acetone**
C_6H_{14}	**Petroleum ether**
H_2O	**Water**

CHAPTER 1

INTRODUCTION

1.1 Background, Motivation and statement of problem

1.1.1 Background and Motivation

Cosmetic products in the major markets of the world are becoming increasingly regulated. As this progresses, the needs of the cosmetic industry increase for raw materials that are suitable and are made under manufacturing conditions that assure consistent purity, free from unexpected and harm-ful contaminants, even in minute quantities. Cosmetics are becoming gradually popular with dermatologists, also known as make-up. They are care substances used to reverse the effects of aging, to improve the quality of the skin, to augment facial structures, and to improve the patient's appearance in general (Castle, 2006). They can also be beneficial for certain dermatoses. Cosmetics are generally mixtures of chemical compounds, some being derived from natural sources (such as *Moringa oleifera* oil) and some being synthetics, They are used for personal care and hygiene, to improve appearance, to protect the skin and to keep it in good condition. Not being drugs, cosmetics do not have curative effects. During their daily use, often without knowing it, mistakes are made that may result in discomfort, starting. There is a lot of raw material used for cosmetics products; most of them are natural which abstracted from animals, plants, insects and vegetables and fruit. For example fish scales are in nail polish and mascara, fish scales use in the formulation of bath products, cleansing products, fragrances, hair conditioners, lipsticks, nail products, shampoos and skin care products (Jenkins, 2012). Also Cochineal Beetles are tiny insects that feed on cactus plants, female cochineal beetles eat the red cactus berries, so when the beetles are crushed, a very powerful red dye is produced. Beside that many of plants used for beauty products such as Lavender in an oil base might moisturise your skin, Rose oil is, well, an oil, so it's bound to be

hydrating, and it should at least plump up skin temporarily. It's also high in vitamin C (Castle, 2006), and Calendula has a reputation for healing up skin and reducing redness (Gattis, 2014). From the vegetable and fruits there are cucumber, tomato, carrot, banana and apple and others which used for cosmetics. For an example of the vegetable that extract the cosmetics material is the Sweet Potato ,which is extract the beta carotene that used for the cosmetics products, Beta Carotene is a carotenoid compound responsible for giving fruits and vegetables their orange pigment (Top 10 Foods Highest in Beta Carotene, 2016). A powerful antioxidant, beta carotene has been found to help protect against cancer and aging. (However beta-carotene supplements can increase lung cancer risk for smokers). β-carotene is a fat soluble vitamin, so eating the following foods with a fat like olive oil or nuts can help absorption, therefor the beta carotene is used for cosmetics (Top 10 Foods Highest in Beta Carotene, 2016). There are some of sources of beta carotene such as Carrot, Spinach, Squash and Cantaloupe Melon. In this research, will be extracted the beta-carotene from *Moringa oleifera*. And finding out the yield of the beta-carotene and the types of carotene in the *Moringa oliefera*.

1.1-2 Problem Statement

One of the major public health nutritional problems in Malaysia as a developing country is vitamin A inadequacy. This is because most people in developing countries do not really know the function of carotenoids. They rarely include fruits and vegetables into their diet. Preventable blindness is responsible due to deficiency of Vitamin A. According to (V.S.Ekamet al., 2006), diseases such heart diseases, cancer, cataracts, and macular degeneration can be minimized if sufficient carotenoids are taken into the diet.

Carotenoids had known of it’s attributed to health benefits when consumed as part of human diet .Carotenoids consumptions can reduced the risks of cancers , variety of diseases,eyedisease(cataract),andage-related mascular degeneration(luteinlab.unh.edu). So,it can be said that carotenoids is very useful as it can act as diseases prevention.This project proposes to extract carotenoids from carrot. Fortunately, carotenoids is believed to have derived their name from the fact that they constitute the major source the major pigment in the *Moringa oleifera*, Daucus Carola (Tee, 1995). *Moringa oleifera* is one of

the major source of carotenoids. In Malaysia, these kinds of leaves are can be easily obtain .But the problem is people do not know the use of carotenoids and the importance of it . From the early discovery based on *Moringa oleifera*, it is worth to extract the carotenoids from *Moringa oleifera* that is very useful for human health in term of the role carotenoids play as provitamim A, antioxidant, and food colourant and disease prevention.

Due to the multifunctioning of carotenoids, another method of extraction process was being developed such as supercritical fluid extraction (SFE). Actually, there are many method of extraction process available such as supercritical fluid extraction (SFE), hydrodistillation, and microwave-assisted distillation. However, there are still lack of detail information regarding this process, especially for the purpose of beta- carotene extraction. Thus, Soxhlet extraction has been identified as one of the most economical method and is widely since it required simple apparatus and safe.

1.2 Objectives

There are two main objectives that need to be fulfilled in this study;

1) To extract the carotene form *Moringa Oleifera* leaves.

2) To analyse the extraction of *Moringa Oleifera* leaves and find the yield of carotene extracted from *Moringa Oleifera* leaves.

1.3 Scope of this research

There are some important tasks to be carried out in order to achieve the objective of this project.

i. Extraction of carotenoids via Soxhlet extraction from *Moringa oleifera* leaves
ii. Study the effect of different solvents (Acetone, petroleum ether and water) on extraction yields.

1.4 Rational and Significant

Currently, there are lot of disease contributes to the lack of vitamin A in body. With high level of carotenoids in *Moringa Oleifera* which means high vitamin A, carotenoids can be used as an alternatives to medicine that are need to consume by patients who suffer diseases caused by malnutrition of vitamin A .This is useful to human as medicine from hospital usually contain drugs where carotenoids on the other hand are natural, provitamin A.Other than that, carotenoids have the anti-cancer activity that can be used to fight cancer.

Based on the knowledge gain from this project, it will enable to obtain the best condition for obtaining the highest concentration of carotenoids. Thus, the knowledge gain will enable for the development and technology transfer to the local producers.

1.5 **Organisation of this thesis**

The structure of the thesis is out as followed:

Chapter 2 provides a description of the raw materials which is Moringa. A general description on the nutritions contain in the *Moringa oleifera* are presented. This chapter also provides a brief discussion of the carotenoids are also presented. Three stages of experimental work which are Soxhlet extraction, rotary evaporation and analyzed by HPLC is also discussed in general.

Chapter 3 gives a review of the procedure for this experiment. This includes the extraction of beta-carotene from *Moringa oleifera* by Soxhlet extractor, separation of solvent from beta-carotene by rotary evaporation and analyzed beta-carotene contained in the solution by HPLC. Apart from that, the pre-treatment method for the sample preparation is also presented.

Chapter 4 contains result related to this experiment. From this result, the best solvent and extraction time can be discovered. The result data is tabulated in table and graph from. Other than that, discussion also been made regarding the result obtain.

Chapter 5 provides anything possible that can be done in order to improve this experiment.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

This chapter presents the previous work on the carotene extraction process based on Soxhlet extraction technique. Beta-carotene content that available in *Moringa Oleifera* is discussed regarding to their leaves. This review on beta-carotene is made including their chemical and physical properties, advantages and disadvantage. Soxhlet extraction method is one of the simplest technique used in this carotene extraction. This technique is most suitable for studying purpose about the extraction under laboratory scale. The general operation of the Soxhlet extractor outlined in order to know the basic mechanism in conduction the equipment. Besides, the outstanding advantages for using this technique are highlight and some comparison is made with other conventional extraction method. The factors of drying time and solid-to-solvent ratio that affecting extraction yield are discussed according to pervious research. In this case, the effect of drying time is explained on water content in the sample and long drying time can destroy some of the antioxidant compounds. Conversely, the solid-to-solvent ratio is discussed in cutest of diffusion rate of that compound from solid to solvent. Lastly, the high –performance liquid chromatography (HPLC) that used in this research is reviewed including its principles and analysis the data.

2.2. Beta-carotene

β-carotene is a natural yellow-red coloured pigment with the chemical structure C40H56, (Figure 7). It occurs mainly in plants, fruit and vegetables. It belongs to the group of carotenes that together with xanthophyll belongs to the upper-level grouping of carotenoids (Bergmann, 2004). Chemically β-carotene is classified as tetraterpene (Koskinen, 2012). Carotenoids are divided in oxygen containing molecules (xantophylls) and non-oxygen containing molecules (carotene) (Domke et al., 2004), Figure 2. The lack of hydroxyl groups makes β-carotene hydrophobic. Due to its two cyclic rings at each end of the molecule chain, β-carotene is a dicyclic compound, composed of 8 isoprene-units (C5H8). The high amount of conjugated double bonds is called chromophor and is responsible for the colour impression (Bergmann, 2004). β-carotene absorbs light of the wavelength 450 nm of the visible part of the spectrum (Bauernfeind,1981).

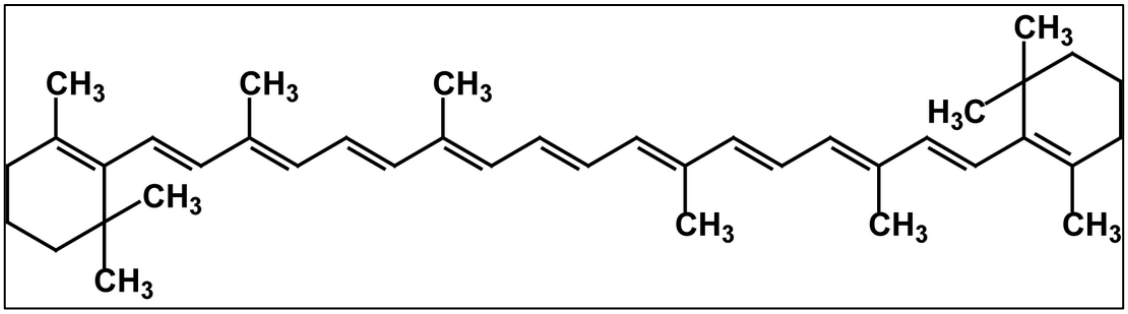


Figure2.2: All-transβ-carotene

In the nature carotenoids have an indispensable protective role for chlorophyll and the human eyes by absorbing and dissipating excessive light energy that would damage them (Campbell, 2003). β-carotene is the most common carotene (Schlieper, 2005). β-carotene is very sensitive to light, heat and oxygen. It can change its chemical structure due to oxidation, degradation or isomerization. The latter doesn't have any effect on the colour impression of β-carotene because the double bonds do not break (Liaaen-Jensen, 1989). The handling of carrots and orange peel residue for the β-carotene pigment extraction and the later dyeing process therefore has to be handled with care. Too high exposures to light, heat and oxygen have to be avoided. The storing is recommended

under frozen conditions. According to Qian et al. (2012) the β-carotene stability against degradation is higher at a pH between 4-8. Natural β-carotene occurs in trans- and cis-isomers whereas synthetically produced β- carotene is mostly all-trans-form, due to its higher absorbance for the human body.

The β-carotene synthesis is either produced by employing a Wittig reaction or a Grignard reaction. The following reaction (Figure 3) is by Wittig. It shows a transselective Wittig olefination of aldehydes II—synthesis of β-carotene from a dialdehyde.

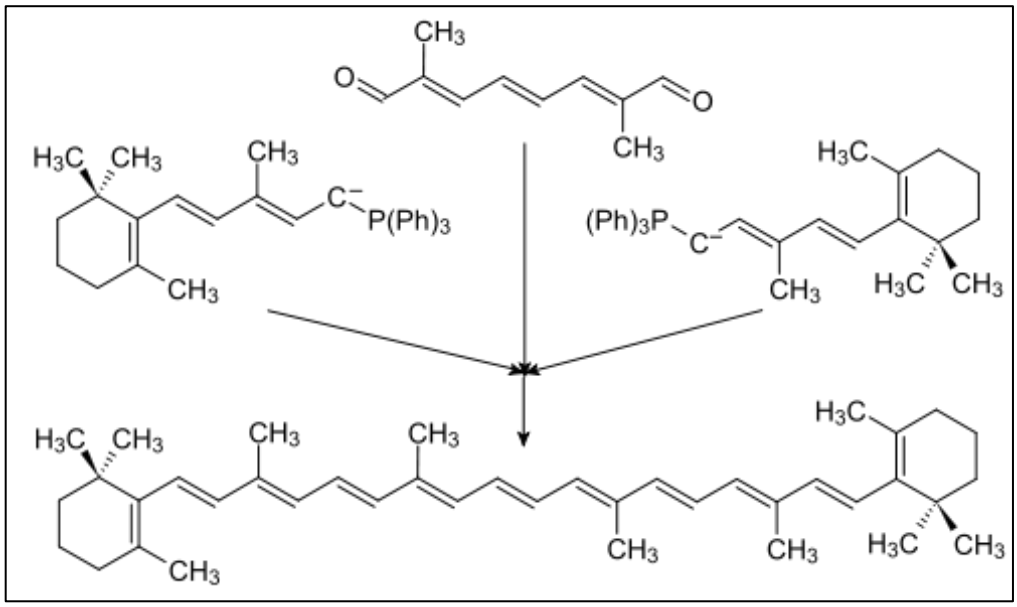


Figure2.3: β-carotene synthesis by Wittig Reaction

As vitamin A precursor, synthetically produced β-carotene is especially important for the use in the food industry as completion to the natural, in the food existing β-carotene. As food additive with the purpose of a food colorant it is known under the numbers E160a-f.(Domke.*etal*.,2004)

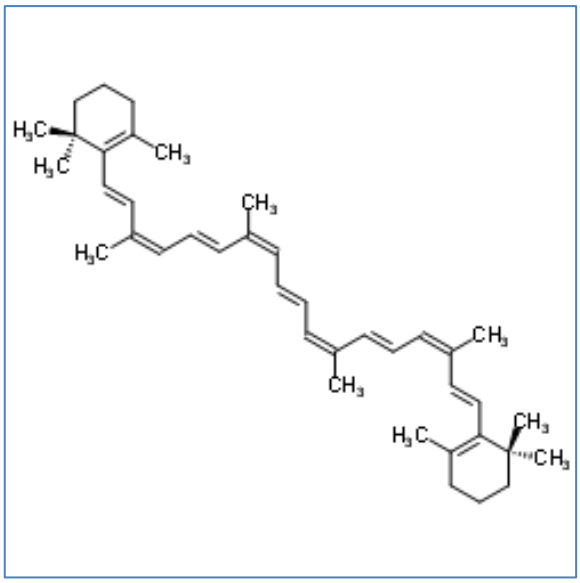


Figure 2.4-cis-β-carotene

The following literature review gives a look into the physical and chemical characteristics of β- carotene, its synthesis and uses today. It gives an overview on the existing research in the field of extraction of β-carotene and other natural pigments and their appliance in different dyeing methods. It is shown how much β-carotene other researchers could extract from Moringa Oleifera. An investigation on different solvents and their suitability for β- carotene extraction was done. Different dyeing methods used for water insoluble dyes and on cotton and the usage of mordents is described as well (Heckers,2014).

2.3 Soxhlet Extraction Method

Soxhlet extraction method is one of the simplest extraction techniques and mostly used for a long time (Luque de Castro & Garcia- Ayuso, 1998). This solvent extraction is commonly known as solid–liquid extraction where it is a process of removing solute from a solid by using of liquid solvent. The general operation of Soxhlet extraction is the solvent will condensed by heating the boiling flask and is allowed to drip back onto the thimble. The liquid condense that drips out onto the sample perform the extraction which then passes through the container and back into boiling flask. The cycle is repeated continuously as long as needed. As it progress, the extracts are concentrated in the flask. This technique is adequate for both initial and bulk extraction.

The concept of the Soxhlet extraction is organic compound are extracted by repeated washing with an organic solvent under reflux in special glassware as shown in Figure 2.2. Generally, the setup consists of the round bottom flask containing the solvent, an extraction chamber and a condenser. The solid used are the consistency of small particle like powder or soil. It is stated in several extraction studies, the raw materials are grounded before the extraction can be preceded (Barriada-Pereira, 2002). The smaller the size of particles will increase the contact area between the solid and solvent. Hence, it will increase the mass transfer of active component into the solvent

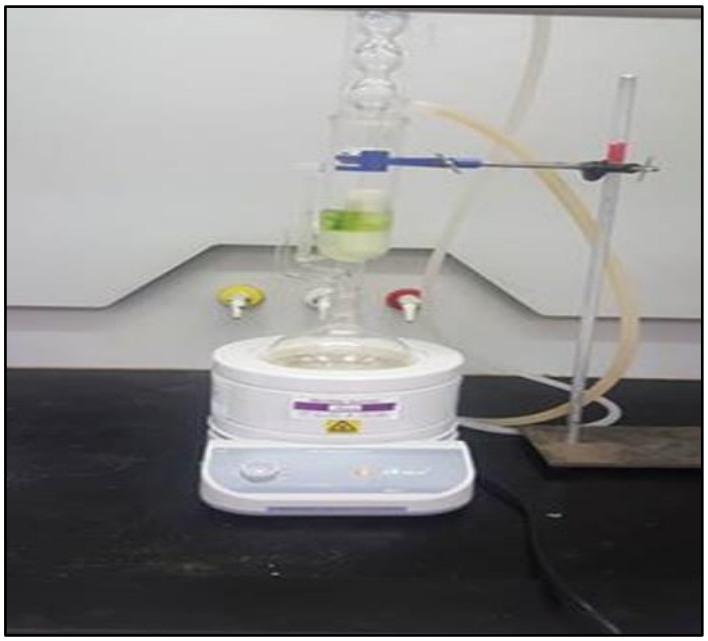


Figure 2.5 Soxhlet extractor

According to previous studies on the Soxhlet extraction method, there are most outstanding advantages of this conventional extraction method. In the Soxhlet extraction, sample is repeatedly brought into contact with fresh solvent, thereby helping to displace the transfer equilibrium. The temperature of the system remains high since the heat applied to the distillation flask reaches the extraction capacity to some extent. Furthermore, no filtration is required after the extraction process. The Soxhlet extraction method also is a very simple methodology which needs little specialized training for the basic equipment is inexpensive. This conventional method also has the possibility to extract more sample mass compare to other methods like microwave extraction. Based on the advantages of the Soxhlet extraction, this conventional method has been a standard leaching technique in the extraction process.

2.4 High Performance liquid Chromatography (HPLC)

High Performance liquid Chromatography (HPLC) is a type of chromatography technique which used to separate, identify and quantify compounds that dissolved in a solution. Theoretically, this technique involves a liquid sample being passed through a solid adsorbent material packed into a column using a flow of liquid solvent. The compounds of the sample are separated from each other due to their different degrees of interaction with the adsorbent particles. These interactions are physical in nature, such as hydrophobic dispersive, dipole-dipole and ionic, most often a combination the both. Each compound in the sample interacts slightly differently with the adsorbent material, thus retarding the flow of time, and if the interaction is weak, the compound flow off the column in a short amount of time, and if the interaction is strong, then the elution time is long.

In HPLC, a sample is injected into a mobile liquid phase and it passes along a stationary phase. Although manual injection of samples is still possible, most HPLCs are fully automated and controlled by a PC, allowing up to 200 or more samples to be injected. The stationary phase comprises a column which is usually stainless steel and packed with silica particles bonded with alkyl chains. The length of the chain depends on the type of molecule being analysed. For example, for large protein molecules a C4 column could be used but for smaller molecules C8 or even C18 may be more appropriate.

2.5 Summary

In this chapter, the explanation on carotene extraction process. Soxhlet extraction method, factors affecting extraction process and HPLC principles are reviewed based on the previous research. All these information and methods applied in the previous research have been used as a reference in this research, especially for discussion on the factors affecting on the carotene extraction and HPLC analysis. Based on this literature review, it is shown that research on beta-carotene had been done for a long time ago and every improvement with respect to the extraction process is made to ensure compentency

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The chemicals that were used in this study are *Moringa Oleifera* leaves as sample in the extraction and the solvent used are acetone (C_3H_6O), petroleum ether (C_6H_{14})and water (H_2O). For mobile phase preparation, chemical used are acetonitrile(CH_3CN), dichloromethane (CH_2Cl_2) and methanol (CH_3OH).

3.2 Experimental Method

3.2.1 Extraction

20g of *Moringa oleifera* was measured then transfered into porous thimble and placed in extraction chamber. Three type of solvents was prepared in the amount of 400ml. for each of the solvent prepared, 20g of *Moringa oleifera* will be needed respectively. After that the 400ml of solvent was transfered into round bottom flask. Next the flask was put into the heater which is the compartment of Soxhlet extraction equipment. The temperature was set at 70 ^{o}c. The experiment was carried for three cycles which each cycle need duration of 40 minutes. To be precise each solvent will undergone the the extraction process for two times. The total amount of 40g *Moringa oleifera* leaves and and 800ml of solvent will be needed for the extraction process.

3.2.2 Sample Preparation

First of all, 0.33g of beta carotene was homogenized in 3ml of acetone. Then this resulting extract was filtered through Buchnar's funnel. After that the residue produced was washed with 5ml of acetone until it become colourless. The residue was discarded and the filtrated was combined with 2ml of water. The extract was transferred

into 100ml volumetric flask. The next procedure was preparing 3 different type of concentration. For first concentration preparation, 10ml of the extract was taken and mixed with 40ml acetone. Second concentration, 20ml of extract mixed with 30ml of acetone whereas for the third concentration 30ml of extract mixed with 20ml acetone. Next step is for dilution, in which the same method of dilution proposed in standard solution preparation was carried in this step as well. First dilution was prepared by diluting 5ml from the first concentration into 50ml acetone. Second dilution , 5ml was taken from second concentration than diluted with 50ml acetone. For the last dilution, 5ml of the third concentration diluted in 50ml acetone as well.

3.2.3 Standard Solution Preparation

For the preparation of standard solution, beta carotene of 10mg weight was dissolved in 100ml of n-hexane. Then this solution were transferred into 3 volumetric flask measuring 50ml which for every solution were having different amount of concentration accordingly. For first concentration, 10ml of the solution were mixed with 40ml of n-hexane, then for second concentration, 20ml of solution were mixed with 30ml of n-hexane. The third concentration was prepared by mixing the 30ml of the solution with the 20ml of n-hexane. After that, this 3 different concentration was diluted. For the dilution, 5ml was taken from each 3 type of concentration respectively and diluted with 50ml n-hexane.

3.3 Analysis Method

3.3.1 Mobile phase preparation

For the mobile phase preparation, 3 solvents were needed which are acetonitrile, dichloromethane and methanol. For the HPLC analysis these solvents were prepared according to the ratio which is 70:20:10 respectively at the rate of 2ml per minute. Before proceeding for the HPLC analysis, as the precaution step, all of these solvents were put inside ultrasonic. This step is needed because to ensure that all the bubbles were removed.

3.3.2 HPLC Analysis

The mobile phase was added into HPLC accordingly which are acetonitrile, dichlomethane and methanol by ratio of 70:20:10 respectively. This step is needed to clean the system and the duration taken was 40 minutes. After that, 1 µL of blank solution was used in HPLC and the time taken for the analysis was 8 minutes. Next, 1 µL of standard solution was used and the duration took is the same which is 40 minutes, and the result showed the peak reached during 16 minutes of duration. Then the rest of sample and standard solution will took 30 minutes for each one for analyzing. The condition needed for HPLC which the wave length was fixed at 452nm and the pressure of the column was kept between the range 1800-2000 PSI.

CHAPTER 4

RESULT AND DISCUSSION

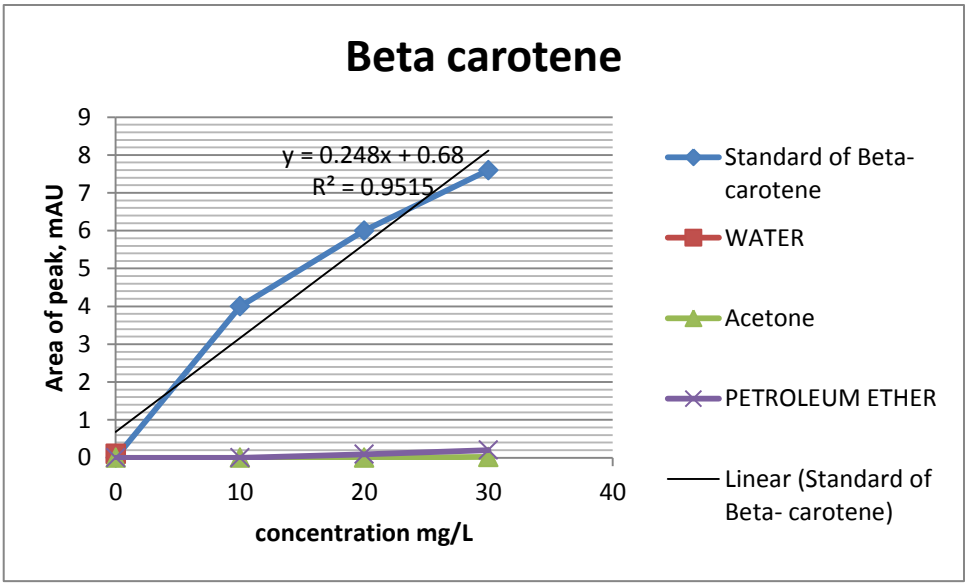
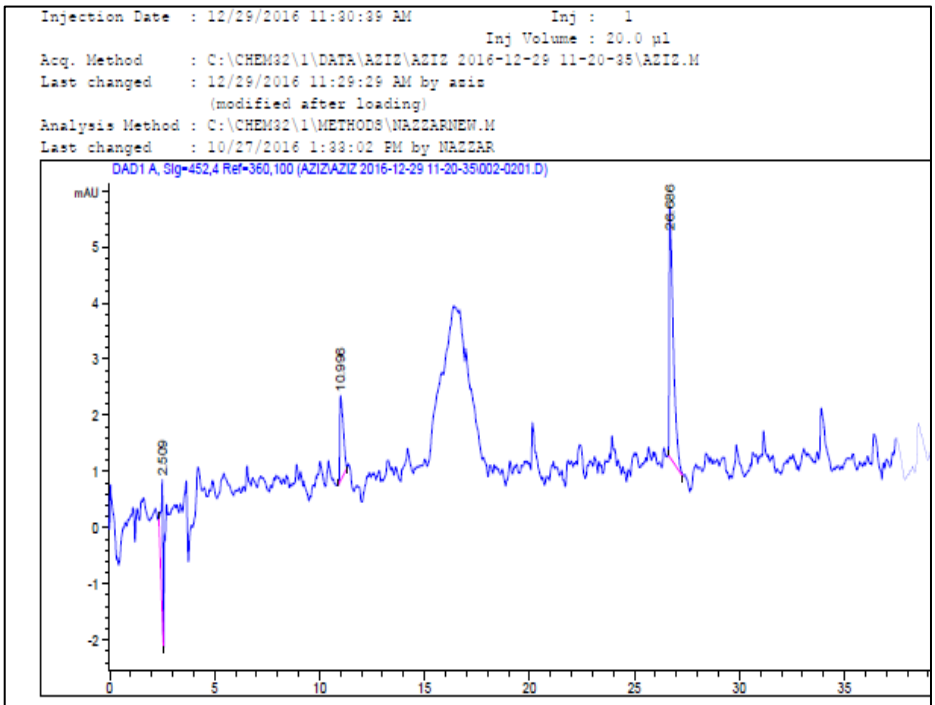


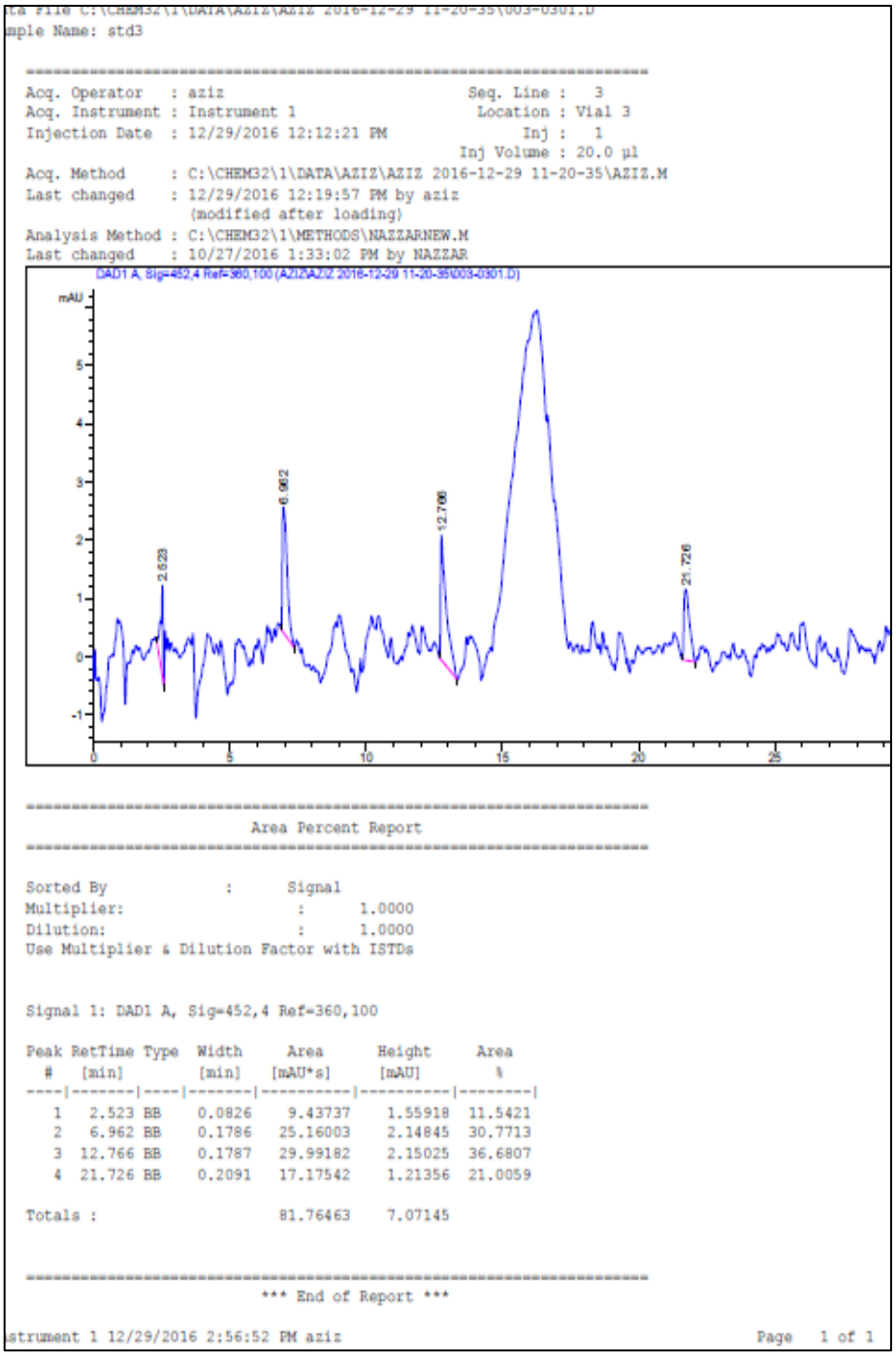
Figure 2.6 : Graph Of The Standard

From the graph above,for every each of the highest peak obtained, the duration of 16 min showed the result of the peak. During the concentration of 10mg/L , the HPLC analysis graph shown the highest peak at the area reading showing 4 mAU/S. For the 20 mg/L of concentration, the area of the highest peak obtained is 6 mAU/S and for the last concentration which is 30 mg/L , the area of the highest peak showing the reading of 7.6 mAU/S. The first solvent used is water, and from the results that obtained, the reading of the area of the peak shown zero reading. From this result it is proven that the water does not extract the beta carotene from the *Moringa Oleifeira Leaves.* The efficiency of water to act as solvent is the lowest. Next for second solvent which is acetone, the reading shows only small significant amount of area of peak, this result is better a bit from the previous solvent which is water. Petroleum ether act as the most effective

solvent, the result obtained from analyzing and comparing the amount of peak for 3 different solvents proven that petroleum ether behave the best as a solvent.



Result of analysis for standard 10ml beta carotene



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Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=452,4 Ref=360,100

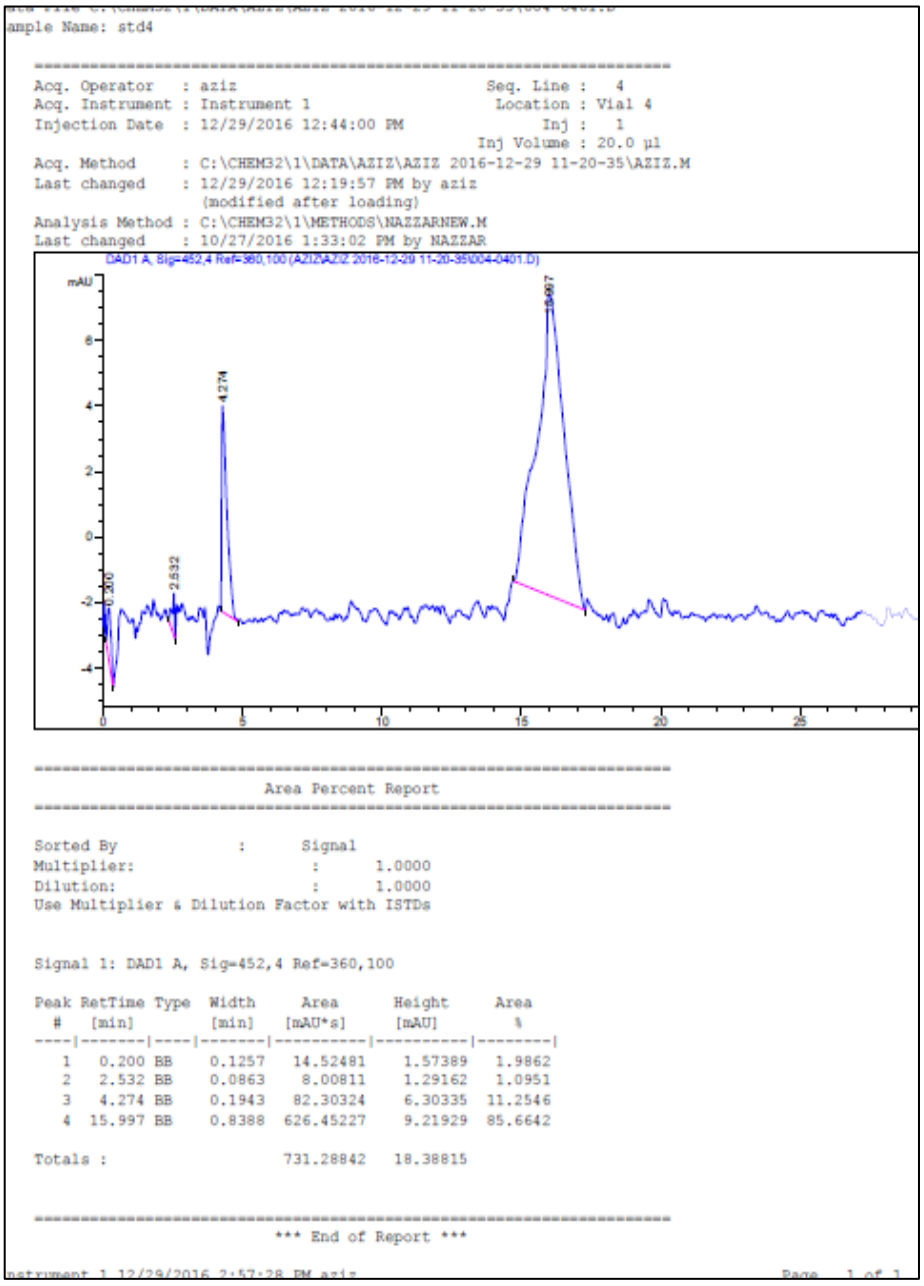
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.523 BB    0.0826    9.43737    1.55918  11.5421
   2   6.962 BB    0.1786   25.16003    2.14845  30.7713
   3  12.766 BB    0.1787   29.99182    2.15025  36.6807
   4  21.726 BB    0.2091   17.17542    1.21356  21.0059

Totals :                    81.76463    7.07145

==================================================================
                          *** End of Report ***

strument 1 12/29/2016 2:56:52 PM aziz                    Page   1 of 1
```

Result of analysis for standard 20ml beta carotene



ample Name: std4

Acq. Operator : aziz
Seq. Line : 4
Acq. Instrument : Instrument 1
Location : Vial 4
Injection Date : 12/29/2016 12:44:00 PM
Inj : 1
Inj Volume : 20.0 µl
Acq. Method : C:\CHEM32\1\DATA\AZIZ\AZIZ 2016-12-29 11-20-35\AZIZ.M
Last changed : 12/29/2016 12:19:57 PM by aziz
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NAZZARNEW.M
Last changed : 10/27/2016 1:33:02 PM by NAZZAR

Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=452,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.200	BB	0.1257	14.52481	1.57389	1.9862
2	2.532	BB	0.0863	8.00811	1.29162	1.0951
3	4.274	BB	0.1943	82.30324	6.30335	11.2546
4	15.997	BB	0.8388	626.45227	9.21929	85.6642
Totals :				731.28842	18.38815	

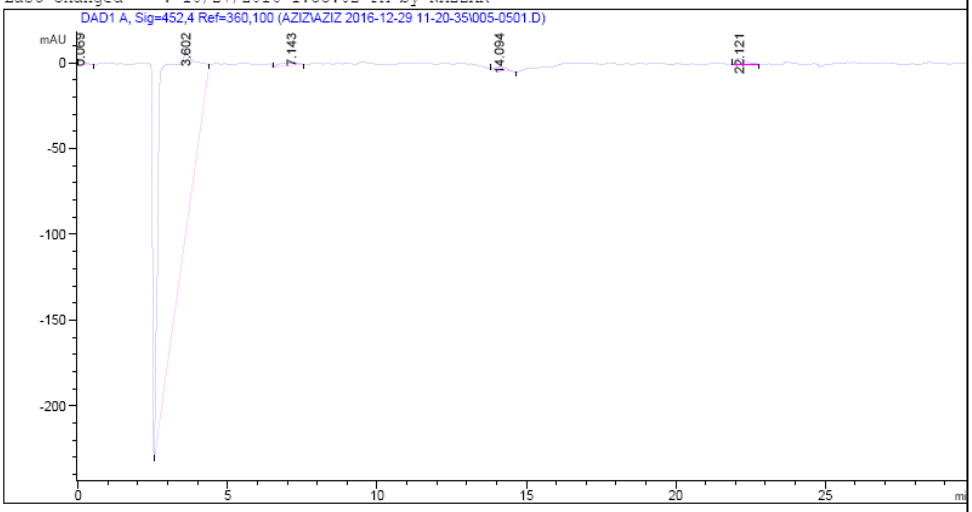
*** End of Report ***

nstrument 1 12/29/2016 2:57:28 PM aziz Page 1 of 1

Result of analysis for standard 30ml beta carotene

Data File C:\CHEM32\1\DATA\AZIZ\AZIZ 2016-12-29 11-20-35\005-0501.D
Sample Name: H2O

```
==================================================================
Acq. Operator   : aziz                          Seq. Line :   5
Acq. Instrument : Instrument 1                   Location : Vial 5
Injection Date  : 12/29/2016 1:15:38 PM               Inj :   1
                                                Inj Volume : 20.0 µl
Acq. Method     : C:\CHEM32\1\DATA\AZIZ\AZIZ 2016-12-29 11-20-35\AZIZ.M
Last changed    : 12/29/2016 12:19:57 PM by aziz
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NAZZARNEW.M
Last changed    : 10/27/2016 1:33:02 PM by NAZZAR
```




```
==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=452,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.069 BB    0.1612   35.47010    2.93075   0.3039
   2   3.602 BB    1.2963 1.14447e4   105.60540  98.0541
   3   7.143 BB    0.3975   76.38481    2.32380   0.6544
   4  14.094 BB    0.3544   53.98388    1.95370   0.4625
   5  22.121 BB    0.2294   61.27944    3.44674   0.5250

Totals :                  1.16719e4   116.26040

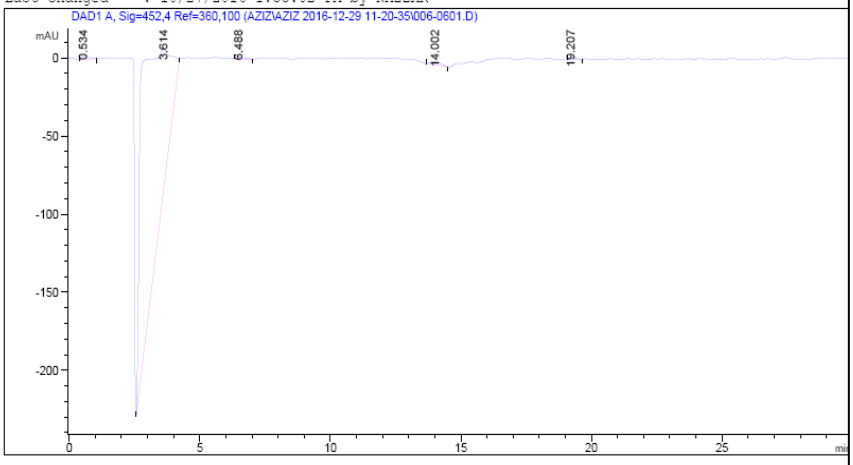

==================================================================
```

Instrument 1 12/29/2016 2:57:57 PM aziz Page 1 of 1

Result of analysis for water

Data File C:\CHEM32\1\DATA\AZIZ\AZIZ 2016-12-29 11-20-35\006-0601.D
Sample Name: PETROLEUM ETHER

```
Acq. Operator   : aziz                          Seq. Line :   6
Acq. Instrument : Instrument 1                   Location : Vial 6
Injection Date  : 12/29/2016 1:47:17 PM               Inj :   1
                                                Inj Volume : 20.0 µl
Acq. Method     : C:\CHEM32\1\DATA\AZIZ\AZIZ 2016-12-29 11-20-35\AZIZ.M
Last changed    : 12/29/2016 12:19:57 PM by aziz
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NAZZARNEW.M
Last changed    : 10/27/2016 1:33:02 PM by NAZZAR
```




```
                          Area Percent Report

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=452,4 Ref=360,100
```

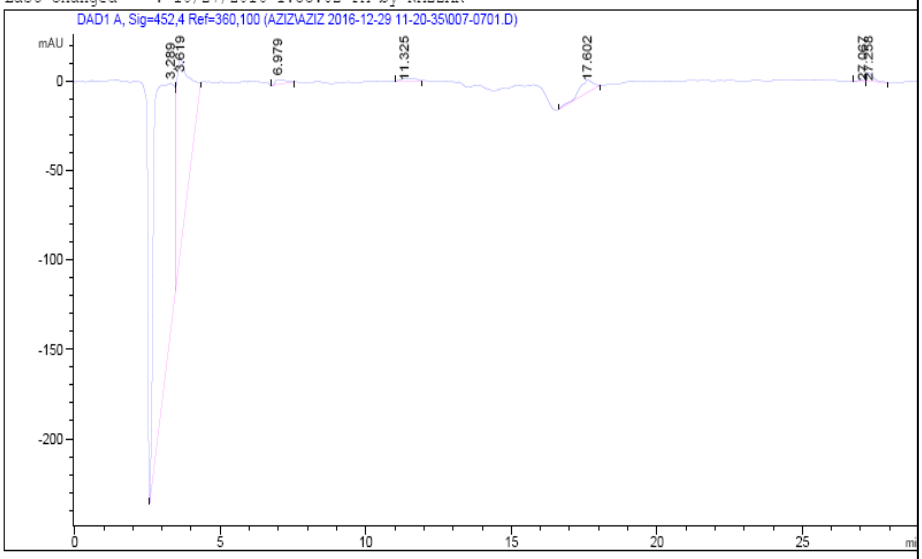
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.534	BB	0.2658	43.48854	2.37254	0.4261
2	3.614	BB	1.3225	1.00663e4	91.42882	98.6232
3	6.488	BB	0.1654	22.12238	1.70523	0.2167
4	14.002	BB	0.3476	57.68547	2.26132	0.5652
5	19.207	BB	0.2002	17.23509	1.11559	0.1689
Totals :				1.02068e4	98.88349	

```
Instrument 1 12/29/2016 2:58:13 PM aziz                                  Page   1 of 1
```

Result of analysis for acetone

Data File C:\CHEM32\1\DATA\AZIZ\AZIZ 2016-12-29 11-20-35\007-0701.D
Sample Name: ACETONE

```
    ======================================================================
    Acq. Operator   : aziz                          Seq. Line :   7
    Acq. Instrument : Instrument 1                  Location : Vial 7
    Injection Date  : 12/29/2016 2:18:58 PM              Inj :   1
                                                   Inj Volume : 20.0 µl
    Acq. Method     : C:\CHEM32\1\DATA\AZIZ\AZIZ 2016-12-29 11-20-35\AZIZ.M
    Last changed    : 12/29/2016 12:19:57 PM by aziz
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\NAZZARNEW.M
    Last changed    : 10/27/2016 1:33:02 PM by NAZZAR
```




```
    ======================================================================
                            Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier:                   :      1.0000
    Dilution:                     :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=452,4 Ref=360,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   3.289 BV    0.6980 7761.81348  139.32219  67.6642
       2   3.619 VB    0.3829 3272.39941  109.43576  28.5274
       3   6.979 BB    0.3243   61.78670    2.57194   0.5386
       4  11.325 BB    0.4187   51.27096    1.50023   0.4470
       5  17.602 BB    0.3920  231.45576    7.37712   2.0177
       6  27.067 BV    0.2112   20.53916    1.21543   0.1791
       7  27.258 VB    0.2077   71.81390    5.05574   0.6260

    Totals :                1.14711e4   266.47841


Instrument 1 12/29/2016 2:58:29 PM aziz                          Page   1 of 2
```

Result of analysis for petroleum ether

From the graph above,for every each of the highest peak obtained, the duration of 16 min showed the result of the peak. During the concentration of 10mg/L , the HPLC analysis graph shown the highest peak at the area reading showing 4 mAU/S. For the 20 mg/L of concentration, the area of the highest peak obtained is 6 mAU/S and for the last concentration which is 30 mg/L , the area of the highest peak showing the reading of 7.6 mAU/S. The first solvent used is water, and from the results that obtained, the reading of the area of the peak shown zero reading

CHAPTER 5

CONCLUSION AND RECOMMENDATION

The best type of solvent use to extract the beta carotene from *Moringa oleifera* is petroleum ether and was proven from the HPLC analysis based on the area of peak. It is suggested to use petroleum ether as a solvent as it can help to achieve higher yield of extraction of beta carotene via Soxhlet extraction from *Moringa oleifera* leaves.

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