EXTRACTION OF ANTIOXIDANT FROM KUNDUR PLANT

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EXTRACTION OF ANTIOXIDANT FROM KUNDUR PLANT

MUHAMMAD BILAL BIN MUSLIM

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

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JULY 2014

SUPERVISOR'S DECLARATION

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology).

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STUDENT'S DECLARATION

I hereby declare that this thesis entitled-"EXTRACTION OF ANTIOXIDANT FROM *KUNDUR* PLANT' is the result of 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.

Signature :
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Date :

Dedication

In the name of ALLAH, Most Gracious, Most Merciful

To my beloved parents

MUSLIM BIN AHMAD & ZALINA BINTI ZAINON

and to my lovely siblings

UMAIRAH, AINI KHALIDA & FATIHAH

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ABSTRACT

Kundur or its scientific name *Benincasa Hispida*, is a member of cucurbitaceae family and it is categorized as one of the more famous crops that is grown primarily for its fruits. These plants are usually recognized for their nutritional and medicinal properties. Commonly, the antioxidant from plant has been extracted by conventional extraction such as Soxhlet extraction and includes supercritical carbon dioxide extraction. However, those methods have a lot of disadvantages such as the process need high temperature and low pH which the result may come in acidic condition and the process is difficult to achieve high percentage of yield in the product. Therefore, another type of extraction must be conducted to replace the entire problem mentioned before. So, enzymatic-assisted extraction has been choosing to replace all the conventional extraction method. Mostly, enzymatic extraction gives a lot of advantage compared to conventional extraction such as the process will give more yields in the product.The present study is to investigate the effectiveness of two methods either enzymatic extraction or Soxhlet extraction in obtaining of antioxidants from the Kundur plant. Generally, there are three major parts in completing the researches on extraction of antioxidant from Kundur plant. The first part is the sample preparation. The sample consists of Kundur peels and leaves were grounded into fine powder in order to be used in enzymatic extraction. The second part is the extraction process by using conventional extraction. The conventional extraction that used in our researches is Soxhlet extraction. Firstly, the samples were prepared in the Soxhlet apparatus. The oil were obtained by removal the excess of solvent by using the rotary evaporator. The reading of absorbance was obtained by usingUV–vis spectrophotometer. The third part is the enzymatic extraction. For this part, the phenolic compound has been extracted in order to get the absorbance of the antioxidant inside of the sample. The sample was prepared in different temperature which is in the range from 20◦C to 60◦C and different in time which is from 3 until 10 hours in order to obtain the optimum parameter for the extraction process. The phenolic content of the samples was determined by using UV–vis spectrophotometer. The fourth part is the antioxidant determination. The fruit extract was reacted with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the absorbance was taken about 515 nm. The results shows that the kundur peels have antioxidant activity inside the sample. The reading of the recorded value of the absorbance shows that the linear range was lower than the standard curve. The standard curve was linear at 25 and 800 µM. These results suggest that it is possible to produce antioxidant from Kundur content, peel and seedand it can expands the usage of Kundur plant and it also can reduce the cost in production of antioxidant in Malaysia.

ABSTRAK

Kundur ataupun nama saintifiknya *Benincasa Hispida* adalah merupakan salah satu keluarga *cucurbitaceae* dan ianya telah dikategorikan sebagai salah satu tumbuhan yang terkenal di mana ditanam untuk mendapatkan buahnya. Tumbuhan ini dikenali hanya untuk kandungan nutrisi dan tujuan perubatan. Kebiasaanya, antioksida daripada tumbuhan ini telah diekstrakkan dengan menggunakan pengekstrakkan secara konvensional contohnya Soklet pengekstrakkan dan termasuk karbon dioksida pengekstrakkan. Walaubagaimanapun, cara tersebut mempunyai banyak kekurangan contohnya proses tersebut memerlukan suhu yang tinggi dan pH yang rendah dimana akan menyebabkan hasil mungkin berada dalam keadaan asid serta sukar untuk mendapatkan peratusan hasil yang tinggi di dalam produk. Oleh itu, kaedah pengekstrakkan yang lain hendaklah dilakukan untuk mengantikan masalah tersebut. Justeru, pengekstrakkan berasaskan enzim telah dipilih untuk menggantikan kaedah ekstrak secara konvensional. Pengekstrakkan berasaskan enzim memberikan lebih banyak kelebihan berbanding kaedah pengekstrakkan secara konvensional seperti produk yang terhasil lebih banyak. Objektif kajian dijalankan adalah untuk menyiasat keberkesanan dua kaedah tersebut sama ada pengekstrakkan berasaskan enzim atau pengekstrakkan secara konvensional dalam mendapatkan antioksida daripada pohon Kundur. Secara umumnya, terdapat tiga bahagian dalam melengkapi kajian ini berdasarkan pengekstrakkan antioksida daripada pohon Kundur. Bahagian pertama ialah penyediaan sampel. Sampel ini mengandungi kulit dan daun Kundur yang telah dihancurkan sehingga menjadi serbuk dan akan digunakan untuk proses pengekstrakan enzim. Bahagian seterusnya adalah proses pengekstrakan dengan menggunakan proses pengestrakan konvensional. Ekstrak konvensional yang digunakan dalam penyelidikan ini ialah pengekstrakan Soxhlet. Dalam proses pengekstrakan Soxhlet, perkara pertama yang akan dilakukan adalah penyediaan sampel menggunakan alatan Soxhlet. Hasil pengestrakan Soxhlet adalah didalam bentuk minyak yang dihasilkan melalui dengan mengeluarkan lebihan bahan pelarut menggunakan penyejat putar. Seterusnya, bacaan "absorbance" akan didapatkan menggunakan sistem UV-vis spektrofotometer.Bagi bahagian pengekstrakan enzim pula, sebatian phenolic telah diekstrak demi mendapatkan bacaan "absorbance" bagi kadar antioksida yang terdapat di dalam sampel tersebut. Sampel-sampel tersebut telah disediakan dengan kadar waktu yang berlainan iaitu didalam tempoh 3 hingga 10 jam dan juga kadar suhu yang berlainan, dari 20◦C hingga ke 60◦C untuk mendapatkan parameter yang optimum bagi proses pengekstrakan ini. Kadar kandungan phenolic yang tedapat didalam sampel tersebut telah diukur menggunakan "UV-Vis Spectrophotometer". Seterusnya pula, bagi bahagian penentuan kadar antioksida, ekstrak kundur tersebut telah ditindakbalaskan dengan "2,2-diphenyl-1-picrylhydrazyl (DPPH)". Kemudian, bacaan "absorbance: telah diambil pada 515nm dan keputusan tersebut menunjukkan bahawa terdapatnya kesan antioksida dalam ekstrak kundur tersebut. Nilai bacaan yang telah direkodkan menunjukkan bahawa nilai "absorbance" yang diperoleh dalam "linear range" adalah lebih rendah daripada apa yang direkodkan dalam "standard curve". Dalam "standard curve" bagi eksperimen ini, nilai linear adalah dari 25 hingga ke 800 µM. Hasil eksperimen in membuktikan bahawa ia adalah amat mungkin untuk menghasilkan antioksida dari daun dan kulit Kundur. Akhir sekali, dengan mengikut cara dan proses yang betul, penggunaan Kundur untuk menghasilkan antioksida boleh diperluaskan dan kos penghasilan antioksida dalam Malaysia juga boleh dikurangkan.

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1 INTRODUCTION

1.1 Background of Study

Curcurbitacea is one of the most genetically diverse groups of food plants whereas the families of this group family are frost sensitive and drought-tolerant. The example of *Cucurbit* family include; gourd, melon, cucumber, squash and pumpkin. The fruits from this group species are valued nutritional and medical purpose). *Benincasa hispida* is categorizing one of the *cucurbitacea* families. *Benincasa hispida* fruit is a good source of a lot of valuable nutrients such as organic acid, natural sugar, vitamins, amino acids and mineral elements. The fruit has been widely used for the treatment of ulcer, epilepsy, diabetic complications, hypertension, nervous disorders and Alzheimer disease in the traditional medicine system of Asian communities (Farooq *et al*., 2011). Several analysis and investigation focused on the biologically active components of benincasa species and it's proved that antioxidant activity on different tissues like liver and brain have been seen. (Madanna *et al.,* 2013). Currently, the uses of this plant are mostly due to its food and medicinal benefits. These types of plant contain a lot of antioxidant especially inside of its seed and peel. Many done investigation have be extract all the antioxidant from the Kundur plant in order to cure disease.

1.2 Motivation and Problem Statement

Free radical is a molecule that has an odd number of electrons. These free radicals are mostly caused by stress, illness, drugs and pollution. These free radicals can induce damage to cell, DNA, lipids and protein. It also play key role in cancer, cardiovascular disease and neurological disorders. To protect human body, and antioxidants are needed in order to against damages of free radicals due to their redox properties. (Madanna *et al*.,2013).Antioxidants are the compound that can delay or inhibit the oxidation of molecule and capable of stabilizing, or deactivating the free radicals before it can attack the cells. Phenolic compound is one of the stable antioxidant and it can be found in plants and fruits. The presences of flavonoid inside of the phenolic compound will

make the phenolic compound act as good cell cycle inhibitor. This makes flavonoids have beneficial implications in human health due to their antioxidant activities and free radical scavenging abilities. (Smith,2006).

Nowadays, there has been an interest by the industry and a desire by consumers to replace synthetic compounds with natural antioxidant alternatives. It can reduce the cost of industry, making antioxidant. We can use the natural alternatives sources of antioxidant for our health. We also can identify the sources by using laboratory equipment such as HPLC and also GC. Lastly, the extraction of ascorbic acid from Kundur can expands the usage of Kundur plant and it can reduce the cost in production of antioxidant in Malaysia.

Curcurbitacea is one of the most genetically diverse groups of food plants whereas the families of this group family are frost sensitive and drought-tolerant. The example of Cucurbit family include; gourd, melon, cucumber, squash and pumpkin. The fruits from this group species are valued nutritional and medical purpose). *Benincasa hispida* is categorizing one of the *cucurbitacea* families. *Benincasa hispida* fruit is a good source of a lot of valuable nutrients such as organic acid, natural sugar, vitamins, amino acids and mineral elements. The fruit has been widely used for the treatment of ulcer, epilepsy, diabetic complications, hypertension, nervous disorders and Alzheimer disease in the traditional medicine system of Asian communities (Farooq *et al*.,2011). Several analysis and investigation focused on the biologically active components of *benincasa* species and it's proved that antioxidant activity on different tissues like liver and brain have been seen. (Madanna *et al*.,2013). Currently, the uses of this plant are mostly due to its food and medicinal benefits. These types of plant contain a lot of antioxidant especially inside of its seed and peel. Many done investigation have be extract all the antioxidant from the Kundur plant in order to cure disease.

Numerous studies and researches have been done in order to find the total antioxidant activity and total phenolic content in Kundur plant such as Chopra et al.(1956) , Grover and Rathi,(1994), Grubben (2004), Yadav *et al*.(2005) and Zaini *et al*.(2011). All the previous studies basically used conventional extraction as their main extraction process. Although all the extraction process is understood, however there is no research has be performed by applying enzymatic extraction on Kundur plant. Therzadeh & Karimi (2007) stated that The advantages of enzymatic extraction are

can be undergo in mild condition of temperature and pH, high yield of hydrolysis and low toxicity of the hydrolyzates formed. In other study, it have stated that the conventional extraction are the process need to be carried out in high temperature and in lower pH. This will make the outcome product will be more in acidic and corrosive condition. Besides, during the process of conventional extraction, several inhibitor compound can be formed and the time for conventional extraction sometimes need to be carry out in long period of time. Therefore, the aim of this study is to apply the enzymatic extraction as the main extraction process rather than conventional extraction to Kundur plant. This paper aims for several objectives which are (1) to apply enzymatic extraction and determine the optimum parameter in enzymatic extraction (2) to analyze the antioxidant scavenging activity and total phenolic content from the Kundur plant (3) to compare the enzymatic extraction with conventional extraction.

1.3 Objective of the Research

1) To identify the antioxidants from the Kundur plant
2) To apply enzymatic extraction and determine the optimum parameter in enzymatic extraction
3) To compare the enzymatic extraction with conventional extraction.

1.4 Scope of the Research

1) Study the Soxhlet extraction and enzyme-assisted aqueous extraction process
2) Determination of phenolic content
3) Determination of Antioxidant activity by DPPH

1.5 Rational of Significant Study

1) To expands the usage of Kundur plant
2) Save the amount of solvent used
3) Reducing the effect to the environment
4) Minimize the extraction time

1.6 Organization of This Thesis

Chapter 2 provides a description of the characteristic and the content of *Benincasa Hispida*. A general description related to the uses and applications of this plant in industry are presented. This chapter also provides a brief discussion of the conventional extraction method and enzymatic hydrolysis extraction method. A summary of the previous experimental work on the optimum parameter of the extraction process which is time and temperature also provided.

Chapter 3 gives a review about the sample preparation of Kundur, Soxhlet and enzymatic extraction process and determination of antioxidant by using two different assay. The sample preparation of Kundur is prepared in different time and temperature to obtain the optimum parameter. Both of conventional and enzymatic extraction have been done to obtain the result of antioxidant in different type of extraction. The antioxidant activity is determined by using DPPH assay.

Chapter 4 shows the review of the result of antioxidant activity inside of the Kundur sample. A preliminary result about the standard curve of the ascorbic acid and gallic acid also provided. The result about the optimum parameter condition of enzymatic extraction, total phenolic content, activity of antioxidant activity and the comparison of enzymatic extraction also have been provided.

Chapter 5 shows the review of the overall conclusion of this study and the recommendation that can be taken in order to improve this study in the future.

2 LITERATURE REVIEW

2.1 Overview

This paper presents the experimental studies extraction of antioxidant from Kundur plant. In order to identify the antioxidant inside the Kundur plant, both of extraction which are conventional and enzymatic hydrolysis extraction process will be done. Basically, conventional extraction such as Soxhlet extraction use chemical solvent such as ethanol as their extraction medium while the enzymatic hydrolysis extraction use enzyme such as cellulase as their medium of extraction. Various temperature and time have been done in order to get the optimum parameter of the extraction. The optimum parameter for the extraction process time is 80 minutes according to Thoo et al, (2010) while the optimum temperature for the process is basically run in the lower temperature (Chan et al, 2009). The phenolic content inside of the Kundur was identified by using Folin reagent and the phenolic compound is 74. 83 ± 1.42 mg GAE/g extract weight (Noriham,2012). The total antioxidant that have been extracted is mostly abundant in enzymatic extraction rather than conventional extraction after the sample was tested with 2,2-diphenyl-2-picrylhdrazyl radical (DPPH assay).

2.2 Kundur- Introduction

Benincasa hispida is one of the species of cucurbitaceae family. According to Whitaker & Bohn, 1950, Cucurbitaceae (cucurbit) family is one of the most genetically diverse groups of food plants in the plant kingdom. The plants belonging to this family are frost-sensitive, drought-tolerant, and intolerant to wet and poorly drained soils. Some prominent cucurbit family members are gourd, melon, cucumber, squash and pumpkin (Robinson & Decker-Walters, 1999). This type of plant is grown primarily basically for its fruits. Table 2.1 shows the classification of Kundur plant according to the data from the United States Department of Agriculture (USDA, 2009). Kundur fruit is known as an important vegetable in India, China, Philippines and elsewhere in Asia.

Table 2.1 Classification of Kundur (Benincasa hispida).

Kingdom Plantae	Plants
Subkingdom	Tracheobionta — Vascular plants
Superdivision	Spermatophyta — Seed plants
Division	Magnoliophyta — Flowering plants
Class	Magnoliopsida — Dicotyledons
Subclass	Dilleniidae
Order	Violales
Family	Cucurbitaceae — Cucumber family
Genus	Benincas Savi — benincasa
Species	Benincasa hispida

This plant also has a great potential for food production. For commercial purposes in Malaysia, just two cultivar (round shape and elongated) of wax gourd are grown (Mohd Zaini, N.A et al, 2010).

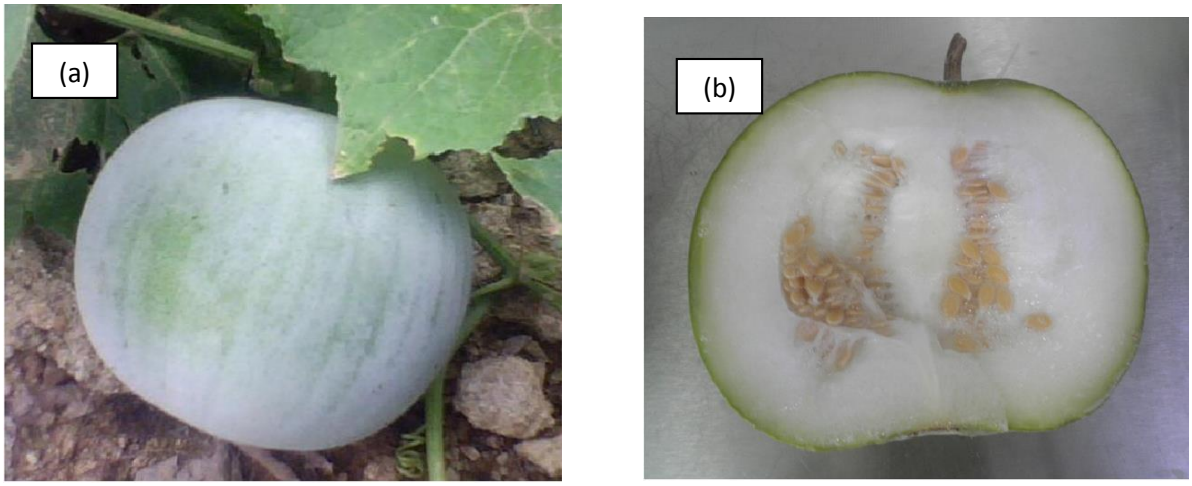

Figure 2.1 Whole (a) and half-cut (b) Kundur (Benincasa hispida) fruit

This plant is also known as Kundur (Malay), ash gourd or winter melon (English), Bhuru Kolu or Safed Kolu (Gujarati), Petha (Hindi), Kushmanda (Sanskrit), Dōngguā (Chinese) and Beligo (Indonesian). (Sew,C.C et al,2010). Mature winter melon can be identified when it's thickly deposited hairs with easily removable waxy bloom while the young fruit has succulent, fleshy and hairy attributes. The shape of the fruit can be cylindrical, globular or oblong and the flesh of the mature fruit is white, juicy and spongy. (Mohd Zaini, N.A et al,2011).

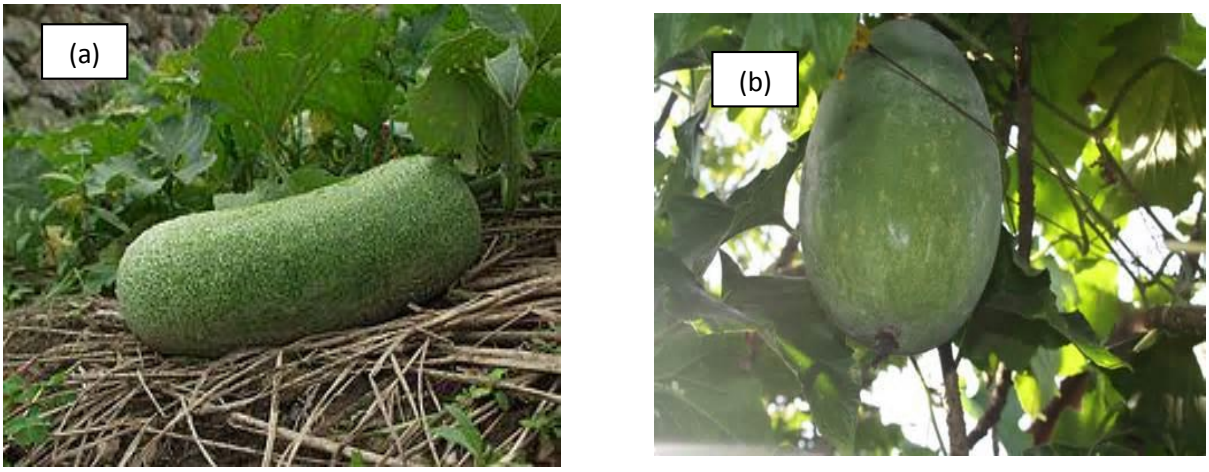

Figure 2.2 (a) Cylindrical Kundur fruit **(b)** globular Kundur fruit

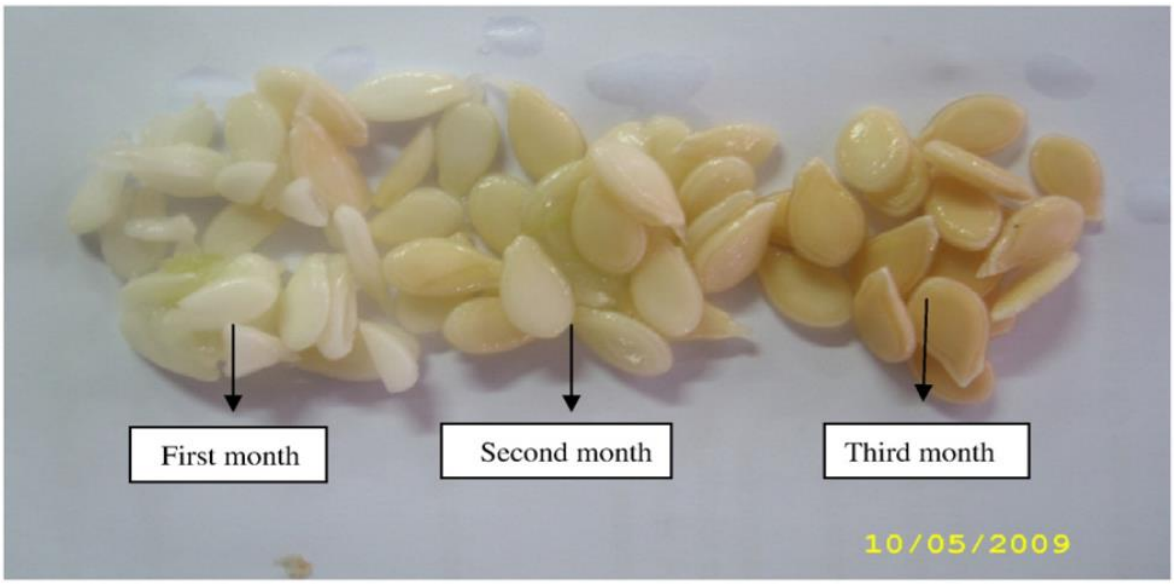

Figure 2.3 Color changes in Kundur (*Benincasa hispida*) fruit seed with maturity.

Bates & Robinson (1995) state that there are four recognized cultivars of winter melon fruit which are unrigged winter melon, ridged winter melon, fuzzy gourd and wax gourd. These types of winter melon are mainly characterized based on their size, shape, fuzziness, waxiness, and presence or absence of a dusty or ashy layer. In Malaysia, winter melon, with local name "Kundur" is mainly represented by two cultivars namely round and oval. However a hybrid-type round which the fruit is developed through breeding of the green winter melon genotype and fuzzy white gourd genotype is also have been grown widely in Malaysia (Zaini et al., 2011). Kundur fruit in Malaysia, although cultivated on a considerable area, is underutilized. Johor, Pahang, Perak Sabah, Kelantan and Selangor are some of the important states cultivating this fruit species mainly for vegetable purposes.

Almost all parts of *Benincasa hispida* (B.hispida) which are leaves, flower, fruit and seed have been used, either as food or as medicine. The young shoots, leaves and flowers can be used as vegetable. The immature as well as mature, large size fruits are often cooked as vegetable, stuffed and steamed or chopped into small blocks candied with sugar. Marr et al.(2007) stated that In China, India, Nepal, Cuba and Southeast Asian regions, the mature fruits are used in preparation of soups, while these are also sliced and eaten as cooked alone or with meat and as well as incorporated in the preparations of other dishes.

Many researches have been done in order to identify the uses of this plant. There have been research prove that Kundur fruit is a good source of valuable nutrients including organic acids, natural sugars, amino acids, vitamins and mineral elements. A number of biological and medicinal properties such as anti-obesity, anti-inflammatory, anti-diarrhoeal , anti-pyretic, anti-compulsive, antioxidant , anti-ulcer and diuretic have been ascribed Kundur (Grover et al., 2001; Rachchh & Jain, 2008).As a potential source of wide array of functional bioactive and therapeutics, the component inside of the plant such as phenolics, triterpenes, glycosides and sterols, the fruit has been widely used for the disease treatment. The disease such ulcer, epilepsy, diabetic complications, hypertension, nervous disorders and Alzheimer disease are mainly disease that can be treated by antioxidant inside of the Kundur plant.

According to Huang et al. (2004), the antioxidant activity of Kundur fruit show that the seed has the higher capacity for inhibition of linoleic acid oxidation and scavenging 2,2 diphenyl-1-picrylhydrazyl (DPPH) radicals compared to the peel, pulp and core of the fruit. This is because

the total phenolic contents and superoxide dismutase (SOD) activity of the seeds is higher. The fruit also exhibits anti-carcinogenic effects in vivo. Kundur fruit also give positive potential antioxidant activity on the kidney (Bhalodia et al,2011). Kundur fruit can reduce renal damage after ischemia or reperfusion injury of the kidney. Ischemia or reperfusion of the kidney is the major cause of acute renal failure and may be involved in chronic renal problems. This finding also supported by Mingyu et al (1995) which show that Kundur fruit has significant protection and blocking effects upon the kidney injury caused by mercury chloride. This might be the presence of polyphenolics such as flavones (iso-vitexin) inside of the Kundur fruit.

One of antioxidant that can be found in Kundur plant is flavonoid C-glycoside. Flavonoids also known as Vitamin P and citrin. It also classified in class of plant secondary metabolites (William,2004). According to the IUPAC nomenclature, they can be classified into three which are flavonoids, isoflavonoids and neoflavonoids. Flavonoids are most commonly known for their antioxidant activity in vitro. Industrial manufacturing such as food and consumers manufacturers have become interested in flavonoids for their possible medicinal properties, especially their putative role in prevention of cardiovascular diseases and cancers (Cushnie, 2011). Although physiological evidence is not yet established, the beneficial effects of fruits, vegetables, and tea or even red wine have sometimes been attributed to flavonoid compounds rather than to known micronutrients, such as vitamins and dietary minerals. Flavonoids might induce mechanisms that affect cancer cells and inhibit tumor invasion. In preliminary studies, cancer researchers proposed that smokers who ate foods containing certain flavonoids, such as the flavan-3-ols (catechins) found in strawberries and green and black teas, kaempferol from brussel sprouts and apples, and quercetin from beans, onions and apples, may have reduced risk of developing lung cancer (Serafini,2003).

2.3 Extraction Method

Many extraction methods can be done to extract one component from another component. Extraction is a major step for the isolation, identification and use of valuable compounds from different plants. The examples for extraction method are Soxhlet extraction, Supercritical fluid extraction, ultrasound extraction and enzymatic-assisted extraction. The choice of extraction technique is basically decided upon based on initial cost, operating cost, simplicity of operation,

amount of organic solvent required and sample throughout (Saim,N. et al, 1998). The range of approaches currently available makes the selection of the most appropriate extraction technique difficult. Soxhlet extraction is one of solid-liquid extraction process. Originally, Soxhlet extraction is applying leaching technique in the extraction process and it is one of the oldest ways of solid sample pretreatment. Basically in conventional Soxhlet, this extraction method used for the determination the value of component inside of the sample. The sample is placed in a thimble-holder. During operation, the thimble-holder gradually filled with condensate fresh solvent from a distillation flask (see Figure 2.4). When the liquid reaches the overflow level, a siphon aspirates the solute of the thimble-holder and unloads it back into the distillation flask, carrying the extracted analytes into the bulk liquid. This operation is repeated until complete extraction is achieved. This performance makes Soxhlet a hybrid continuous and discontinuous technique. In as much as the solvent acts stepwise, the assembly can be considered as a batch system; however, since the solvent is recirculated through the sample, the system also bears a continuous character (Luque,1994).

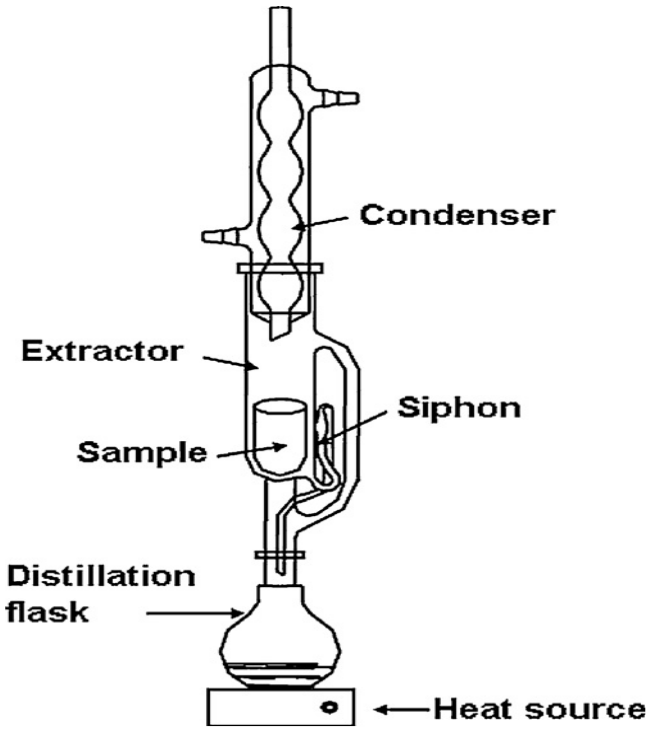

Figure 2.4 Conventional Soxhlet extractor.

According to Luque de Castro & Garcia-Ayuso (1998), Soxhlet extraction is one of the leaching techniques mostly used for a long time. According to Priego (2007), ultrasound assisted extraction is can improve the Soxhlet extraction process. Ultrasound-assisted methods are usually developed in a batch mode, discontinuous, and the shortening of the extraction time which is respect to that in the absence of ultrasounds, is due to an increase of both pressure and temperature (which improves solubility and diffusivity), both increasing the transport phenomena and displacing the partitioning equilibrium. The use of ultrasound-assisted extraction is advisable for thermolabile analytes which are altered under Soxhlet working conditions. In a number of comparisons, the efficiency of both the alternatives is similar but Soxhlet extraction provides better reproducibility .In other comparisons the efficiency of Soxhlet is higher than that of ultrasound-assisted extraction (Miller, 1988).

Enzyme-assisted extraction is a extraction method apply the study of metabolites releasing from biogenic materials. This type of extraction use enzyme to break down the linked-bond inside of the plant cell wall. Enzyme-assisted extraction has advantages of high efficiency, environmental friendship and easy operation process. It also been represented as an alternative way for natural product extraction (Fu et al., 2009). All the mechanism for enzyme-assisted extraction of phenolic compounds from residual sources is mostly based on the cell-wall degrading enzymes that can weaken or break down the cell wall. This will make the intracellular materials will more exposed for extraction. Enzyme-assisted extraction of natural functional compounds from plants is widely investigated in recent years for its advantages in high efficiency, easy operation, and environment friendship (Barzana,2002). Most of the works in this field utilize pectinase and cellulase to hydrolyze and degrade plant cell wall constituents in order to improve the release of intracellular contents. Another important factor in the extraction are the intrinsic property especially the solubility of the target compound, has seldom been concerned according to our knowledge. Low solubility of target compounds in the extractant leads to low extraction yield and require large amount of solvents, which largely impedes the economic efficiency in industry.

Supercritical fluid extraction (SFE) is the extraction process that involve of separating one component from another by using supercritical fluids as the extracting solvent. Besides, it also can be defined as fluid state of carbon dioxide where it is held at or above its critical temperature and critical pressure (Bimakr, 2012). Supercritical fluid extraction (SFE) has received considerable

attention as a promising alternative to conventional technology for separation of different valuable compounds from natural sources. According to Mitra et al, (2009), carbon dioxide (CO2) used as main supercritical fluid, sometimes modified by solvent such as methanol or ethanol. This gases also behaves as a gas in air at standard temperature and pressure (STP), or as a solid called dry ice when frozen. If the temperature and pressure are both increased from STP to be at or above the critical point for carbon dioxide, it can adopt properties midway between a gas and a liquid. More specifically, it behaves as a supercritical fluid above its critical temperature (304.25 K) and critical pressure (72.9 atm or 7.39 MPa), expanding to fill its container like a gas but with a density like that of a liquid. The suitable condition for supercritical CO2 extraction is above the critical temperature of 31°C and critical pressure of 74 bars (R.B. Johnson, 2003).Supercritical carbon dioxide (SC-CO2) has been the most commonly used solvent in the food and pharmaceutical industries, since it is non-toxic, non-flammable, chemically stable, inexpensive, environmentally acceptable and easily separated from the extract.

Ultrasound is acoustic (sound) energy in the form of waves having a frequency above the human hearing range. The highest frequency that the human ear can detect is approximately 20 thousand cycles per second (20 kHz). This is where the sonic range ends, and where the ultrasonic range begins. Ultrasound is used in electronic, navigational, industrial, and security applications. It is also used in medicine to view internal organs of the (Garcia & J.L., 2003).The enhancement of extraction efficiency of organic compounds by ultrasound is attributed to the phenomenon of cavitation produced in the solvent by the passage of an ultrasonic wave. Cavitation bubbles are produced and compressed during the application of ultrasound. The increase in the pressure and temperature caused by the compression leads to the collapse of the bubble. With the collapse of bubble, a resultant ‘‘shock wave’ ’ passes through the solvent enhancing the mixing. Ultrasound also exerts a mechanical effect, allowing greater penetration of solvent into the sample conmatrix, increasing the contact surface area between solid and liquid phase. This coupled with the enhanced mass transfer and significant cell disruption of cells via cavitation bubble collapse, increases the release of intracellular components into the bulk medium. The use of higher temperatures in UAE can increase the efficiency of the extraction process due to the increase in the number of cavitation bubbles formed (M.A., Palma, & Barroso, 2003). The application of ultrasound waves have also been reported (Suslick, 1998)to result in the formation of free radicals as a result of very short term local temperature and pressure increases, which may partially consume the antioxidants released

from the matrix. Therefore, the optimum duration of ultrasonication may be anywhere between very short and very long times. UAE of various analyses from a variety of organic and inorganic samples using different types of solvents have been reported in the literature. Ultrasonic bath and ultrasonic probe systems are the two most common devices used in ultrasound-assisted extraction. The UAE is carried out in three ways; indirect or direct sonication using an ultrasonic bath and direct sonication using an ultrasonic probe (Vinatoru, et al., 1997). Direct sonication is more effective on extracting solvent and solid. During indirect sonication, effects of ultrasound waves may be buffered due to the presence of a layer around material. Longer sonication times can be needed to get same extraction efficiencies as those obtained by indirect sonication. Wu, Lin, & Chau (2001) compared direct and indirect ultrasonication with Soxhlet extraction of ginseng saponins from ginseng roots and cultured ginseng cells. It was found that UAE was about three times faster than the traditional extraction method and direct sonication by the probe could provide much higher ultrasound energy to the samples than indirect sonication by the cleaning bath.

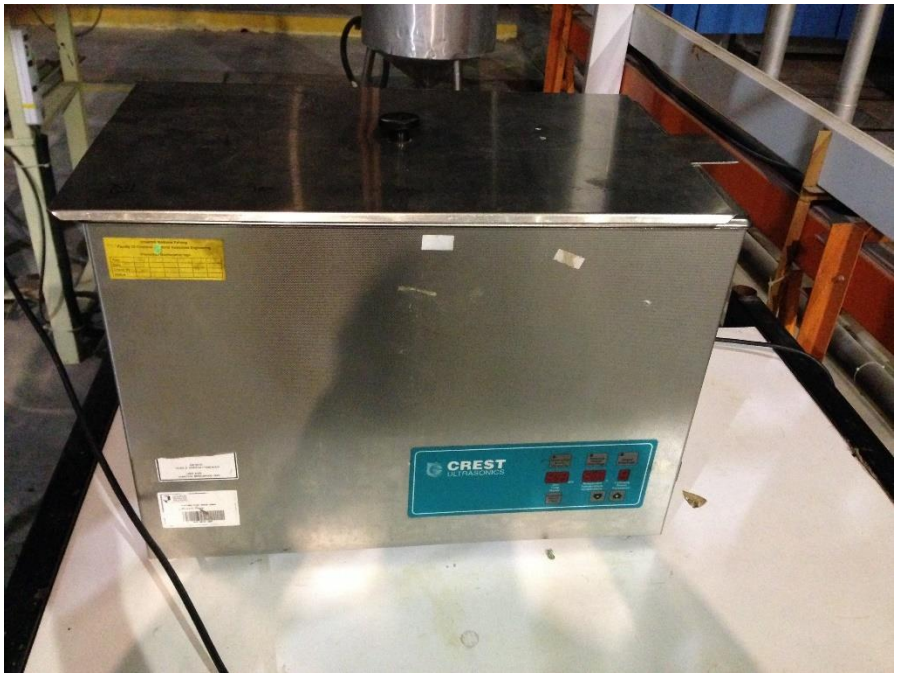

Figure 2.5 Ultrasonic Assisted Extractor

2.4 Enzyme-Assisted Extraction Advantages

Enzyme-based extraction of bioactive compounds from plants is a potential alternative to conventional solvent based extraction methods. Basically, solvent extraction and conventional extraction has more disadvantages rather than enzymatic-assisted extraction. Enzymatic hydrolysis can be carried out under mild conditions, whereas acid hydrolysis requires high temperature and low pH, which results in corrosive conditions. Besides, acid hydrolysis is difficult to achieve high yield of cellulose hydrolysis while enzymatic extraction can obtain almost 100% (Ogier et al. 1999). During enzymatic hydrolysis, the inhibitor compound did not formed while for the acid hydrolysis extraction, several inhibitory compounds are formed during the extraction process.

Enzymes are ideal catalysts to assist in the extraction, modification or synthesis of complex bioactive compounds of natural origin. Enzyme-based extraction is based on the inherent ability of enzymes to catalyze reactions with exquisite specificity, regioselectivity and an ability to function under mild processing conditions in aqueous solutions (Gardosi:, 2009). According to Meyer (2010), this method also offers the possibility of greener chemistry as pressure mounts on the food industry and even pharmaceutical companies to identify cleaner routes for the extraction of new compounds. Besides, a studies that have been done by Pinelo (2009) stated that enzymes have the ability to degrade or disrupt cell walls and membranes, thus enabling better release and more efficient extraction of bioactives. Enzyme-assisted extraction methods are gaining more attention because of the need for eco-friendly extraction technologies. A quantitative characteristic of enzymatic processing in industry is represented in the literature by relatively few enzyme applications. These include laccase applied in bleaching in the pulp and paper industry , protease/ lipase applied in leather making , lipase applied in the production of skin care products (Veit, 2004), and phospholipase applied in degumming of soybean oil (DeMaria, 2007). A particularly useful application of enzymes increases the effect of solvent pre-treatment and either reduces the amount of solvent needed for extraction or increases the yield of extractable compounds. Enzymes such as pectinases, cellulases and hemicellulases are widely used in juice processing and beer clarification to degrade cell walls and improve juice extractability. The disruption of the cell wall matrix also releases components such as phenolic compounds into the juice, thus improving product quality. Moreover, Barzana (2002) revealed that enzyme-assisted extraction methods have been shown to achieve high extraction yields for compounds including polysaccharides, oils,

natural pigments, flavours and medicinal compounds. Recent studies on enzyme assisted extraction have shown faster extraction, higher recovery, reduced solvent usage and lower energy consumption when compared to non-enzymatic methods. In this review, we provide a brief description of quantitative screening of enzyme applications, comparing the overall energy consumption of systems involving enzymatic processing to systems involving conventional chemical processing.

For Soxhlet extraction, a large consumption needs to be done as well as a long sample treatment (Luque de Castro & Garcia- Ayuso, 1998). Large amount used will cause a lot of solvent wasted which not only expensive to dispose and also can cause additional environmental problems. Supercritical fluid extraction also has several disadvantages such as essential oils from plant undergo incomplete extraction, high operating temperatures with the consequent breakdown of thermally labile components, the hydration reactions of chemical constituents will be promoted, and need post extraction process to remove water (Chyau et al. (2007). Another disadvantages of this extraction process are the process itself required elevated pressure, compression of solvent requires elaborate recycling measures to reduce energy costs and high capital investment for equipment. While, for ultrasonic baths, although it is more widely used devices, they have two main drawbacks that considerably decrease experimental repeatability and reproducibility (Luque-Garcia and Luque de Castro, 2003):

- Lack of uniformity in distribution of ultrasound energy (only a small fraction of total liquid volume in the immediate vicinity of the ultrasound source experiences cavitation)
- Decline of power with time, so the energy supplied to bath is wasted.

2.5 Effect of Temperature and Concentration

The enzymatic extraction need to be run in suitable temperature. According to Chan et al. (2009), at higher extraction temperature, the loss in antioxidant capacities of plant extracts was due to degradation of phenolic compounds that mobilized at low temperature. So, if the phenolic compound undergoes extraction process at high temperature, the amount of antioxidant activity

will be lowered compared to those which were extracted under low temperature. The antioxidant capacity also depends on the structure and interaction between extracted phenolic compounds (Huang et al., 2005). Therefore, further study on identification of phenolic compound in Kundur plant which is extracted at different temperature with respect to their antioxidant mechanism should be carried out. Another parameter that affects the extraction yield is extraction time. The cost can be saved by not wasting time in order to gain the yield of extraction. A study was carried out by Silva et al., (2007) show that the increasing of excess extraction time will reduce the amount of yield of extraction compound. The optimum extraction time for antioxidant compounds is varies with antioxidant capacity. Thoo et al, (2010) show that the best extraction time in order to get antioxidant capacity is 80 minutes. From the study by Maisuthisakui and Pongsawatmanit, (2004), the yield of the extract and total phenolic content were almost constant after 3 hours of extraction time at room temperature. However, Chew K.K, (2011) gives another results which is the extraction was the best at 300 minutes. Therefore, in order to get the exact time for enzymatic-assisted extraction time for Kundur sample must be done.

2.6 Structure of the Cell Wall and Composition of Lignocellulose

The structure of plant cell wall (Figure 2.6) is a highly ordered structure formed, mainly, by cellulose, hemicelluloses and lignin, a phenolic polymer. Exact identity and relative abundances of each of these polymers vary even within the same plant, depending on tissue, ageand growth stage (YH & Toward, 2004).

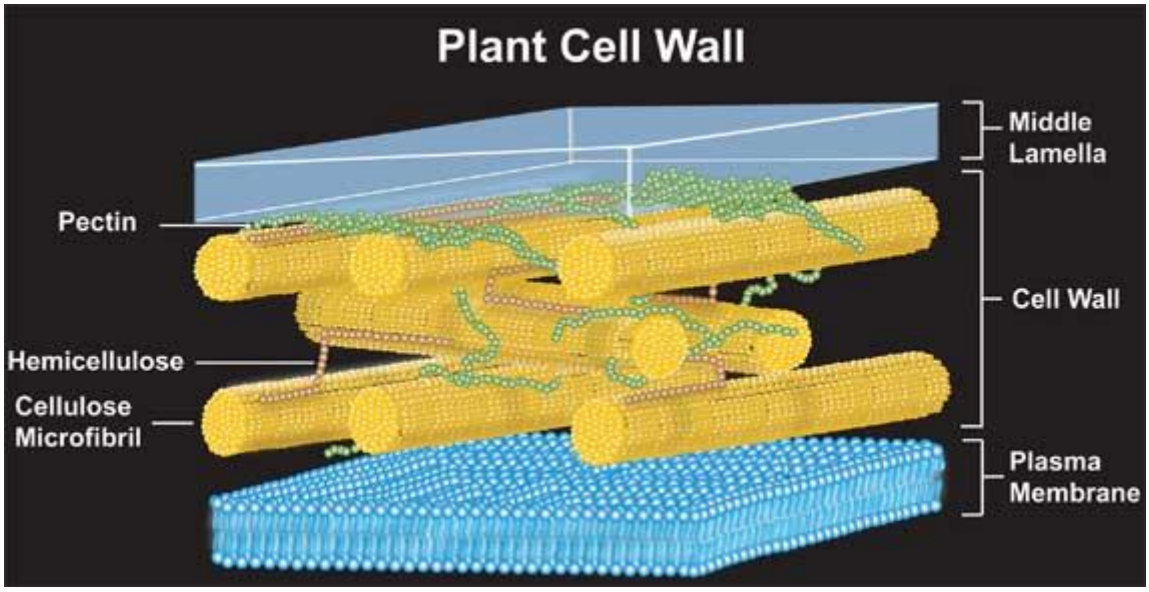

Figure 2.6 The structure of plant cell wall

Cell walls are structured so as to enable them to play a wide array of disparate, sometimes opposing roles. They provide resistance to shape the cell, mechanical stress and protect it against many pathogens; at the same time, they must be reasonably flexible to withstand shear forces, and permeable enough to allow the passage of signaling molecules into the cell (Levy I., Shani Z., & O., 2002).

Cellulose, the most abundant natural polymer, is highly stable and insoluble in water. It constitutes the principal component of plant cell walls, accounting for 50% of the dry weight of wood and its degree of polymerization varies according to its origin, and may range from 2000 to 25,000 monomers (Hildén L. & G., 2004). According to Pérez J, Muñoz-Dorado J, de la Rubia T, & J., (2002), cellulose is formed by D-glucose monomers condensed together through β-1,4 glycosidic

bonds, forming cellobiose molecules (β-1,4-linked glucose dimer) that are, in turn, linked together into straight, non-branched chains . In nature cellulose is seldom found as single, isolated chains; forming filaments instead from the very moment it is synthesized. These filaments, denominated microfibrils, may contain from 36 to over 1200 cellulose chains, and have diameters of 5 to 15 nm (Levy I., Shani Z., & O., 2002). Cellulose chains are held together in the microfibril through hydrogen bonds and Van der Waals forces, forming a crystalline, organized structure that is refractory to hydrolysis in certain areas of the microfibril (Pérez J, Muñoz-Dorado J, de la Rubia T, & J., 2002). Highly ordered, crystalline regions are interspersed with regions containing disorganized or amorphous cellulose, which constitute 5 to 20% of the microfibril. Amorphous regions are more susceptible to enzymatic degradation (Atalla R., 1993). Cellulose is a highly resistant substrate that is, in turn, tightly associated with hemicelluloses and lignin, forming a structure that is very resistant to degradation. Degrading lignin, therefore, is a feat accomplished only by a few cellulolytic organisms (Hildén L. & G., 2004).

Hemicellulose is a complex heteropolysaccharide composed mainly of pentoses (D-xylose and L-arabinose) and hexoses (D-mannose, D-glucose and D-galactose), usually acetylated and forming branched chains, in addition to D-galacturonic, 4-O-methylglucuronic and D-glucuronic acids, condensed through β-1,4 and, occasionally, β-1,3 glycosidic linkages (Pérez J, Muñoz-Dorado J, de la Rubia T, & J., 2002). These short lateral branches, formed by different sugars, make hemicellulose less refractory to a number of treatments (Martínez AT., et al., 2005). The components of hemicellulose are also classified as xylans, xyloglucans, mannans, glucomannans and glucans, bonded together through β-1,3 or β-1,4 linkages. The mannans and galactomannans of hemicellulose have a core structure of β-1,4-linked mannose residues, which is randomly branched with mannose and glucose residues in glucomannans. There are structural differences between hemicelluloses from different species and even different cell types in the same individuals (Kumar R., Singh S., & Singh OV., 2008). Thus, the most important role of hemicellulose is to bond together lignin and cellulose fibers, thus providing rigidity to the cellulose-hemicellulose-lignin mesh. Lignin and hemicellulose are linked together mainly by ester bonds between arabinose residues in hemicellulose and hydroxyl groups in lignin residues, whereas cellulose binds to hemicellulose through hydrogen bonds (Laureano-Perez L., Teymouri F., Alizadeh H., & BE., 2005).

Lignin is one of the most abundant polymers in nature after cellulose and hemicellulose. It is highly resistant to chemical or biological degradation, providing structural support to the cell wall, decreasing its permeability and conferring resistance to the attack of microorganisms. Together, lignin and hemicellulose form an amorphous matrix imbibing cellulose fibers to protect them from degradation (Sánchez C, 2009).Structurally, lignin is an irregular, water-insoluble, branched heteropolymer formed through the polymerization of three phenylpropane-type aromatic alcohols (coniferyl, coumaryl, and sinapyl alcohols) through C-C bonds and esters involving the aromatic rings. This polymer, with constitutes 20 to 30% of wood by protects, weight and confers rigidity to the structural polysaccharides (cellulose and hemicellulose) (Cunningham RE & GD, 1994).The main constituent of lignin in soft woods is coniferyl alcohol; in hard woods, this place is occupied by coumaric and sinapinic acids instead (Martínez AT., et al., 2005). Lignin is the component of lignocellulosic material exhibiting the highest resistance to degradation, which limits its application and that of the polysaccharides it protects. The number of microorganisms able to mineralize this substance is really small (Cunningham RE & GD, 1994).

2.7 Phenolic Contents

In organic chemistry, phenols, sometimes called phenolics, are including in a class of chemical compounds consisting of a hydroxyl group (-OH) attached to an 17 aromatic hydrocarbon group. The simplest molecule of the class is phenol (C_6H_5OH). Although phenols is similar to alcohols, it have unique properties and are not classified as alcohols (since the hydroxyl group is not bonded to a saturated carbon atom). They have relatively higher acidities due to the aromatic ring's tight coupling with the oxygen and a relatively loose bond between the hydrogen and oxygen. Polyphenols are a group of chemical substances found in plants, characterized by the presence of more than one phenol group per molecule. According to (Kandaswami C. & E., 1994), the polyphenols are generally further subdivided into hydrolysable tannins, which are gallic acid esters of glucose and other sugars; and phenylpropanoids, such as lignins, flavonoids, and condensed tannins. They are primary antioxidants and singlet oxygen quenchers. They are also very powerful metal chelating agents and they can trap free radicals and break chain initiation reactions. Flavonoids have structural variations in carbon ring that identifies the different types, namely, flavones, flavones, isoflavones, flavonol, flavonones and anthocyanins (Shi J., Yu J., Pohorly J.E., & Kakuda Y., 2003).Polyphenols are reducing agents, and together with other dietary reducing

agents, such as vitamin C, vitamin E and carotenoids, they protect the body tissues against oxidative stress. Commonly referred to as antioxidants, they may prevent various diseases associated with oxidative stress, such as, cardiovascular diseases, cancers inflammation and others (Scalbert and Williamson 2000).

2.8 Summary

Currently, the uses of winter melon (*B. hispida*) fruit are mostly due to its food and medicinal benefits, rather than due to production of byproducts such as seed oil and waxes or other value-added products. As winter melon plant produce large amount of peels and leaves, often discarded as agro waste, therefore the peels and leaves can be explored as a potential source of oil for value-addition. Regardless of the high nutritional and medicinal potential, availability and cultivar diversity in plant and fruit characters, no efforts have yet been made on studying full physicochemical characteristics of seed oils from different cultivars of winter melon fruit. The present study was under taken with the main objective to identify the antioxidant activity inside of the Kundur plant.

3.0 METHODOLOGY

3.1 Overview of Researches Methodology

Generally, there are four major parts in completing the researches on extraction of antioxidant from Kundur plant. The first part is the sample preparation. The sample consists of Kundur peels and leaves were grounded into fine powder in order to be used in enzymatic extraction. The second part is the extraction process by using conventional extraction. The conventional extraction that used in our researches is Soxhlet extraction. Firstly, the samples were prepared in the Soxhlet apparatus. The oil were obtained by removal the excess of solvent by using the rotary evaporator. The reading of absorbance was obtained by using High-performance liquid chromatography (HPLC). The third part is the enzymatic extraction. For this part, the phenolic compound has been extracted in order to get the absorbance of the antioxidant inside of the sample. The sample was prepared in different time which is in the range from 20◦C to 60◦C and different in time which is from 3 until 10 hours in order to obtain the optimum parameter for the extraction process. The phenolic content of the samples was determined by using UV–vis spectrophotometer. The fourth part is the antioxidant determination. The fruit extract was reacted with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the absorbance was taken about 515 nm. The entire chemicals used were of analytical grade.

3.2 Material used

3.2.1 Plant material

The kundur peels and leaves were purchased at local market in Kuantan, Pahang, Malaysia. The samples were washed to remove any dirt. The samples were stored at 0- 4°C until the extraction process.

3.2.2 Chemicals and Reagent

All the chemicals and reagents used in this study were analytical grade. All chemicals and reagents were 2,2-diphenyl-1-picrylhydrazyl, sodium carbonate, sodium acetate trihydrate, glacial acetic acid, Folin–Ciocalteu reagent, anyhydrous sodium sulphate, methanol and enzyme (*Cellulase from Aspergillus niger*), ultrapure water. All these chemicals and reagent can be obtained from laboratory.

3.3 Extraction Preparation

3.3.1 *Sample preparation*

The fruits were peeled to get the fresh content, peel and seed samples. All samples are stored in cool condition about 4 ◦C before any further. Then, it was ground into a fine powder using a pre-chilled mortar and pestle. In order to achieve the standard size of particle, the ground material was sieved through 1mm metal sieve. The larger part of that remaining on the metal sieve was grounded again. The process was repeated until all the material passed through the sieve.

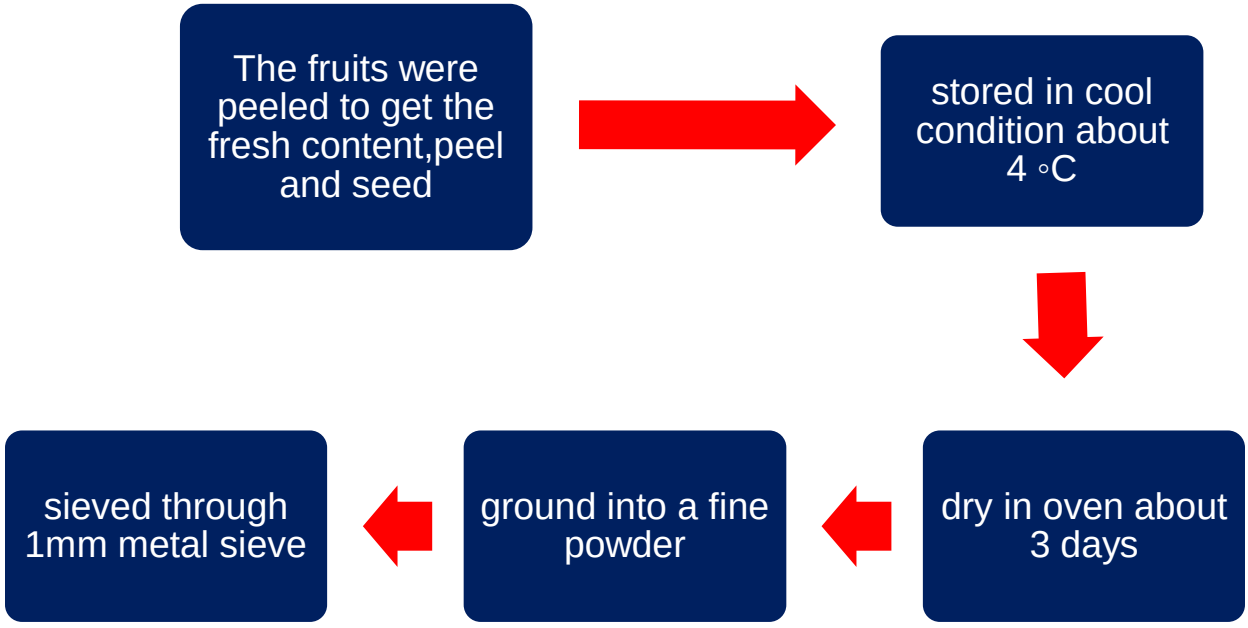

Figure 3.1 Research method for sample preparation of *Benincasa Hispida* sample

3.4 Extraction Procedure

3.4.1 Soxhlet Extraction

All the sample (content, peel and seed) were dissolve with 150 mL methanol ether by using Soxhlet apparatus. The samples were extracted about 3h on water bath. After extraction, the excess of the solvent was totally removed under reduced pressure by using a rotary vacuum evaporator. A small amount of anhydrous sodium sulphate was mixing together with the recovered oil in order to free the recovered oil from any trace of moisture. The oil then was filtered. The oil was capped in a dark brown Schott bottle and stored below temperature of 4°C until used for further analysis (Bimakr, et al., 2012).

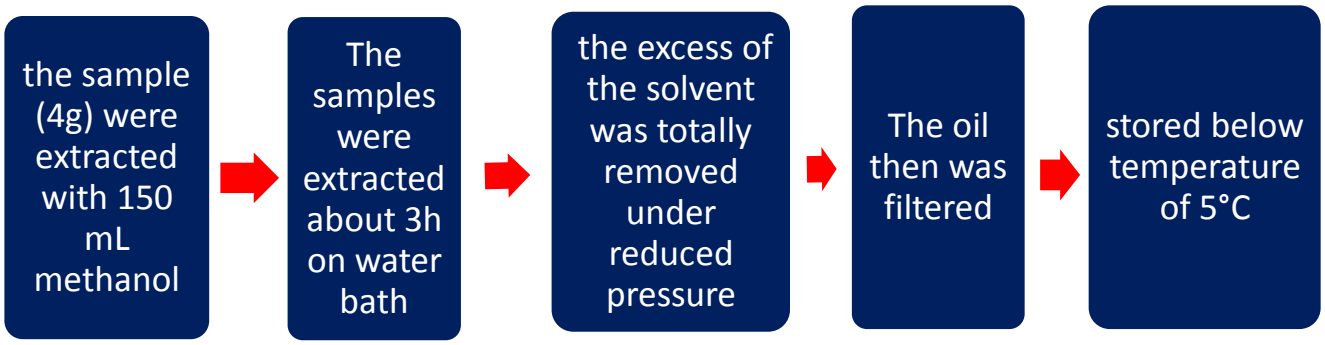

Figure 3.2 Research method for Soxhlet extraction

3.4.2 Extraction of Phenolic

An enzyme-assisted aqueous extraction was tried and compared with the previously obtained results of solvent extraction. The frozen Content sample powder (4g) was placed in a 50 mL centrifuge tube and aqueous enzyme solution (16 mL) was added at specific desired concentration. The preparation was left and run for different temperature (varies from 30 to 70 ◦C) and different time (3 to 12 hours). Then, the mixture of the solution were centrifuged by using a Mistral 10000 centrifuge for 10 minutes at -4◦C (R.R. Selvendran & Ryden, 1990). Then, the supernatant from the centrifuge were filtered through vacuum filtration process. Next, 10 mL aliquot of the filtrate was taken and concentrated by evaporation of the solvent, using a rotary evaporator (see Figure 3.4) under partial vacuum at 40 ◦C until less than 1ml of filtrate remained. The extract was then re-dissolved in 10 ml of Milli-Q water and stored at temperature of 4 ◦C prior to purification step (up to 1 day). All the extracts were prepared in triplicate. The method was repeated by using peel and seed.(B.B. Li, B. Smith, & Hossain, 2006).

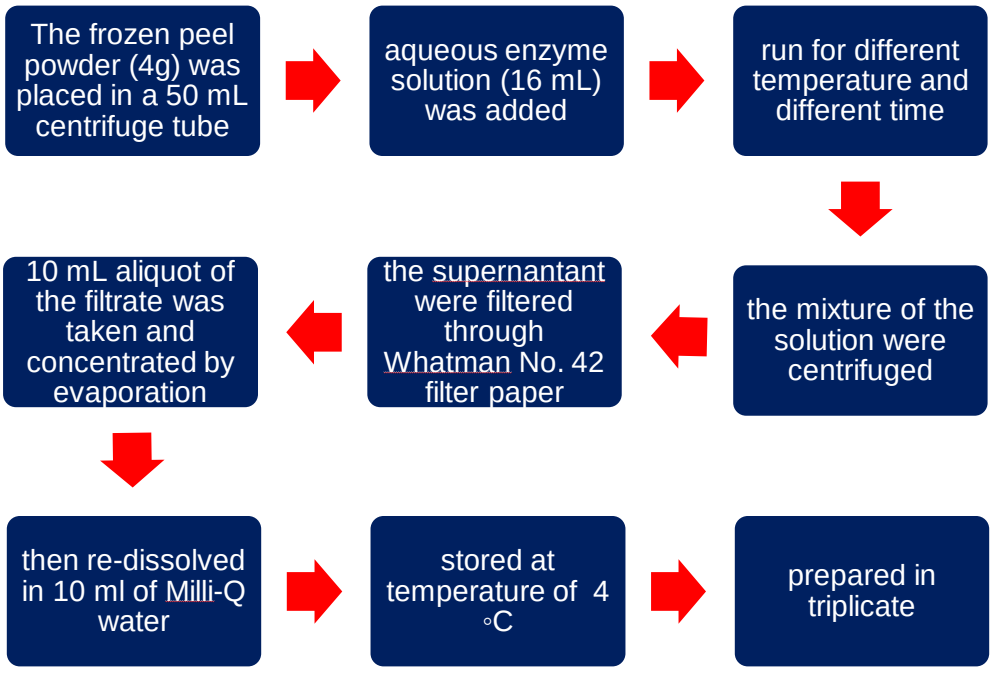

Figure 3.3 Research method phenolic content extraction

Figure 3.4 Rotary evaporator (Brand: Heidolph)

3.4.3 Determination of Phenolic Content

Total phenolic contents in citrus extract were evaluated using the Folin–Ciocalteu assay, which was adapted from (Li. *et al*, 2006) with some modifications. 1 mL of Kundur extract samples which is in triplicate and Milli-Q water which considered as blank were placed in a separate 25mL volumetric flask. Then, 15 ml Milli-Q water and 1.25 ml Folin–Ciocalteu reagent were immediately added into the flask. All the content were swirled to mix and then allowed to stand 5 to 8 minutes at room temperature. After that, 3.75 mL of sodium carbonate solution was added. The solution was ensure perfectly mix and stand for 2h at room temperature before measurement of the absorbance at 765 nm using UV–vis spectrophotometer. The amount of the total phenolic content was expressed in mg of gallic acid equivalent using the standard curve.

3.5 Antioxidant determination

3.5.1 Antioxidant Activity Determination By DPPH

25 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was dissolve in 100 mL methanol to prepare the stock solution. Then, the solution was stored at -20 ◦C until needed. The working solution was obtained by mixing 10mL stock solution with 45 mL methanol in order to obtain an absorbance of 1.67 ± 0.02 units at 515 nm using the spectrophotometer (see Figure 3.5). 150 µL of fruit extract were allowed to react with 3 mL of the DPPH solution for 24h in the dark condition. The absorbance was taken at 515 nm. All the results are expressed in mg EC50/g fresh mass (Braca, et al., 2001). The percentage of scavenging activity was measured after all the absorbance was taken.

Figure 3.5 Uv-vis spectrophotometer (Brand: Hitachi)

4.0 RESULT AND DISCUSSION

This chapter will discusses the outcomes of this study that related to the objectives and scopes of the research. The topic cover in this chapter is the analysis of sample of *Benincasa Hispida* based on all the parameters set which are the extraction method (Soxhlet extraction and enzymatic hydrolysis extraction), extraction temperature and time. The total phenolic content (TPC) and scavenging activity of antioxidant analysis was determined based on the absorbance reading and comparing the value with standard curve. The antioxidant scavenging analysis was done based on the total of phenolic content from each extraction method with the highest antioxidant activity.

4.1 DPPH (EC_{50}) Calibration Curve

In the antioxidant scavenging activity, the standard curve of DPPH was plotted in order to determine the inhibition in *Benincasa Hispida* extracts. According to Villano et al. (2007), DPPH assay is the popular and one of the common method for the study of natural antioxidant. DPPH free radical compound has been widely used to test the free radical scavenging ability of various food samples; the antioxidant present neutralizes the DPPH by the transfer of an electron or hydrogen atom. The reduction capacity of DPPH could be determined by color changes from purple to yellow by read at 515 nm. The antiradical activity of tested compounds is basically indicate as EC50 (concentration of a compound decreasing the absorbance of a DPPH solution by 50%). The concentration of DPPH was set at 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm by diluting the DPPH with distilled water. For each concentration, the absorbance were monitored at 515 nm by using Uv-vis spectrophotometer. Control sample contained all the reagents except the extract. By using equation 1, the percentage inhibition was calculated. Based on the study that have been done by Peiyuan et al.(2010), in order to find the scavenging activity, the percentage of inhibition was calculated by using equation 1.All the data were presented as mean values ± standard deviation (n = 3).

$$\% \text{ inhibition} = 100 \times \frac{(A_{blank} - A_{sample})}{A_{blank}} \qquad \text{Equation- (1)}$$

Where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of antioxidant in sample. Figure 1 shows the relationship between absorbance unit and concentration of DPPH was directly proportional with equation y=0.2512x+0.0186 and with R squared value of 0.9995.

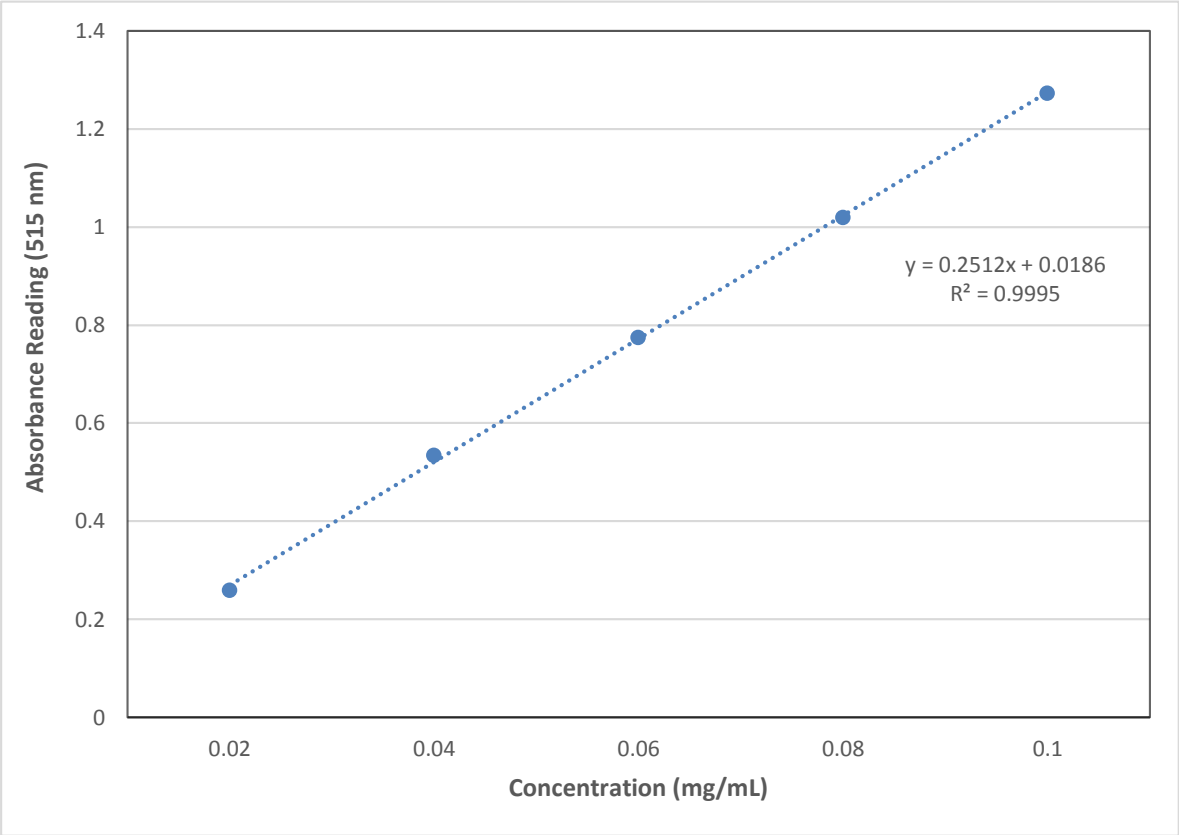

Figure 4.1 DPPH Standard Curve

4.2 Gallic Acid Standard Curve

The total phenolic content (TPC) was determined by Folin-Ciocalteu reagent as method described by Singleton & Rossi (1965) with some modification by using gallic acid as standard. Phenolic

compounds known as the secondary metabolites in plants which included as one of the most widely occurring groups of phytochemicals (Middleton.K.,*et al*, 2000) . Due to the presence of the hydroxyl groups and conjugated ring structures; many phenolic compounds have the potential to function as antioxidants by stabilizing or scavenging free radicals involved in oxidative processes through hydrogenation. In the gallic acid content analysis, the standard curve of gallic acid was plotted in order to determine the concentration of *Benincasa Hispida* phenolic compound. The concentrations of gallic acid was set at 20, 40, 60, 80 and 100 ppm by dilution with distilled water. Each of the ascorbic acid concentration absorbance then was monitored at 765 nm using Uv-vis Spectrophotometer. Figure 2 shows the relationship between absorbance unit and concentration of Gallic acid was directly proportional with equation y=0.1168x-0.0057 and with R squared value of 0.9976.

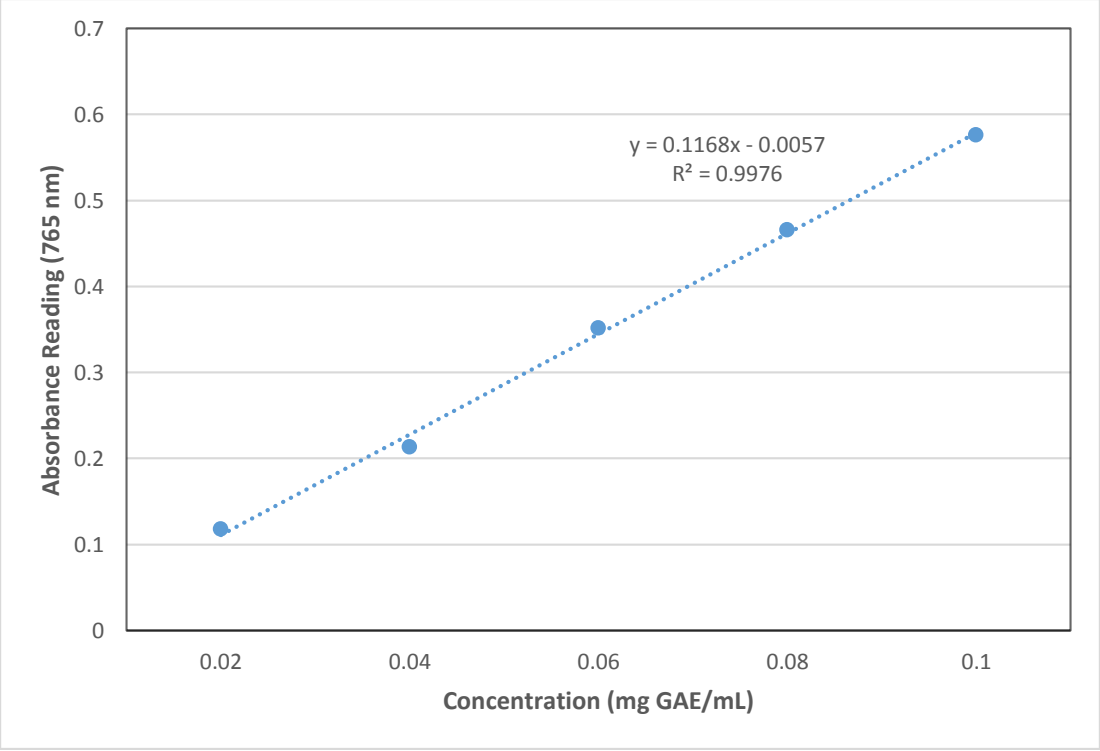

Figure 4.2 Gallic Acid Standard Curve

4.3 Extraction Yield

The yields of the selected Kundur parts are presented in Table 4.1. The seed contributed to the highest yield, amounting 19 %, followed by the peel with the total yield of 15 %, while the content has the lowest yield which have 11 %.

Table 4.1 Data of extraction yield

Sample	Weight of sample (g)		Yield [a]
	Before drying	After drying	(%)
Content	**100**	**11**	**11**
Peel	**100**	**15**	**15**
Seed	**100**	**19**	**19**

(%)Yield = Weight after drying / Weight before drying x 100%.

4.4 Effect of Temperature on Enzymatic Extraction

One of parameter that have been investigated in this paper is temperature. According to Jaradat, Dawagreh, Ababneh, & Saadoun (2008), temperature will give impact and effect on the enzymatic scavenging activity and the total phenolic content released from the sample. After all the methodology of extraction based on parameter of temperature completely done, all the data analysis of DPPH (EC_{50}) Scavenging Activity and TPC have been recorded. Figure 3 shows the result of analysis on effect of the temperature to the scavenging activity to each sample. Based on Table 4.2, the lowest temperature of scavenging activity for the sample to take place is at 50 °C. Within this temperature, the seed sample provide the lowest scavenging activity with 54.79% followed by peel sample (56.64%) and lastly content sample (70.73%). According to Azizah *et al*. (2007), the lowest value of EC_{50} reveal the strongest ability of the extracts to act as DPPH scavengers.

Table 4.2 Data analysis of DPPH by different temperature

Temperature(°C)	DPPH (EC_{50}) Scavenging Activity (%)		
	Content	Peel	Seed
30	78.06 ± 0.01	60.81 ± 0.02	54.03 ± 0.01
50	70.73 ± 0.01	56.64 ± 0.01	54.79 ± 0.02
70	71.29 ± 0.01	71.23 ± 0.01	51.03 ± 0.01

*Each value is the average of three analyses ± standard deviation

While from the Figure 4.3, seed show the lowest amount of the scavenging activity in all temperature compared to content and peel. This result support the previous studies from Noriham,A.,*et al.* (2012) which state that seed provide the lowest scavenging activity compared to the other parts in a Kundur fruit.

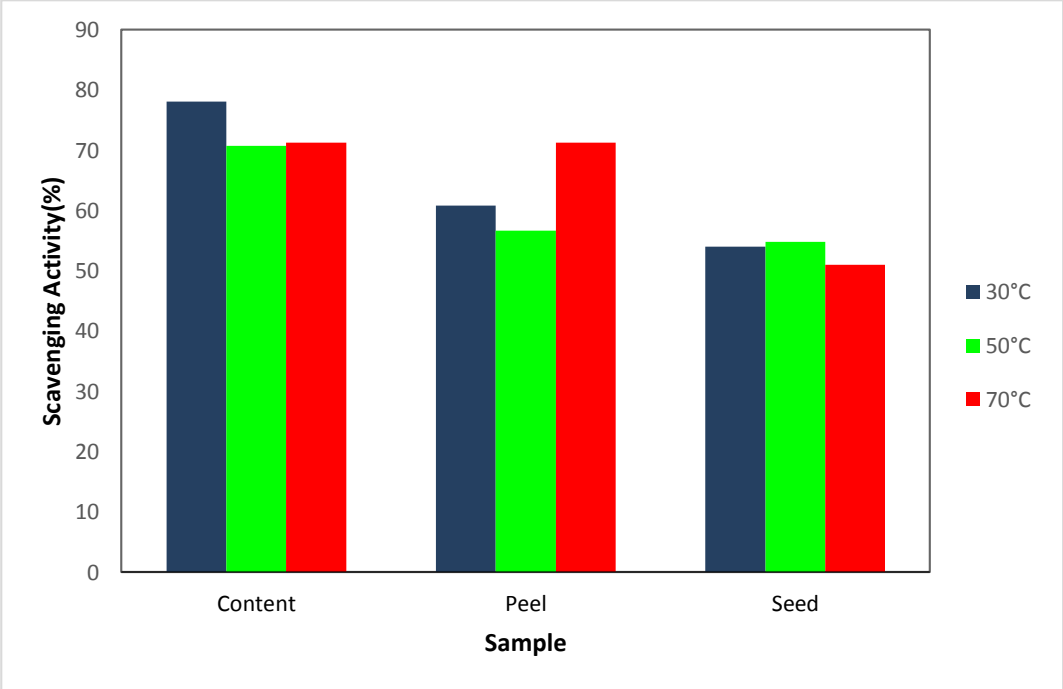

Figure 4.3 Effect of temperature on DPPH scavenging activity based on different sample

As for the total phenolic content (TPC) analysis, all the data was calculated and recorded as shown in Table 4.3 in order to gain the concentration of TPC. Based on Table 4.2 and Figure 4.4, it reveal that the median for highest concentration of total phenolic content in every sample is at 50 °C. However, the presence of TPC at 70°C is almost similar to the amount presence at 50°C. According to Xing-hua Li *et al*., (2010), the highest TPC content could be found at 50°C, although some journals, such as B.B. Li *et al*. (2006), mentioned that the TPC contents are available from 50 to 70°C. From the experimental data, the highest TPC could be found at a temperature of 50°C, while the TPC at 70°C comes a close second. Some studies such as Eriksen & Goksoyr (1976) reported that the optimum temperature for enzymatic extraction to take place are in range 50 °C to 70 °C. However, at high temperature (70 °C), the effect of enzyme cannot be fully considered due to the presences of hot water while conducting the analysis. This is fully supported by B.B. Li *et al.*(2006), which reveal that some of the phenols extracted by enzymes in hot water, possibly though by degradation or extraction of the cell wall polysaccharides with some additional assistance from enzymes at the lower temperatures. It can be said that endogenous enzymes have good activity under warm temperature, but are inactivated at high temperature. Therefore, due to this statement, it conclude that the optimum parameter for temperature on enzymatic extraction is 50 °C. This result also was confirmed by Rai*et al.* (2012) which state that the effective temperature for cellulase activity for enzymatic extraction was 50 °C.

Table 4.3 Data analysis of TPC by different temperature

Temperature(°C)	Concentration (mg GAE /4 g sample)		
	Content	Peel	Seed
30	1.32 ± 0.01	1.97 ± 0.02	2.44 ± 0.01
50	2.23 ± 0.03	2.43 ± 0.01	2.41 ± 0.01
70	1.34 ± 0.01	1.56 ± 0.01	4.54 ± 0.01

*Each value is the average of three analyses ± standard deviation

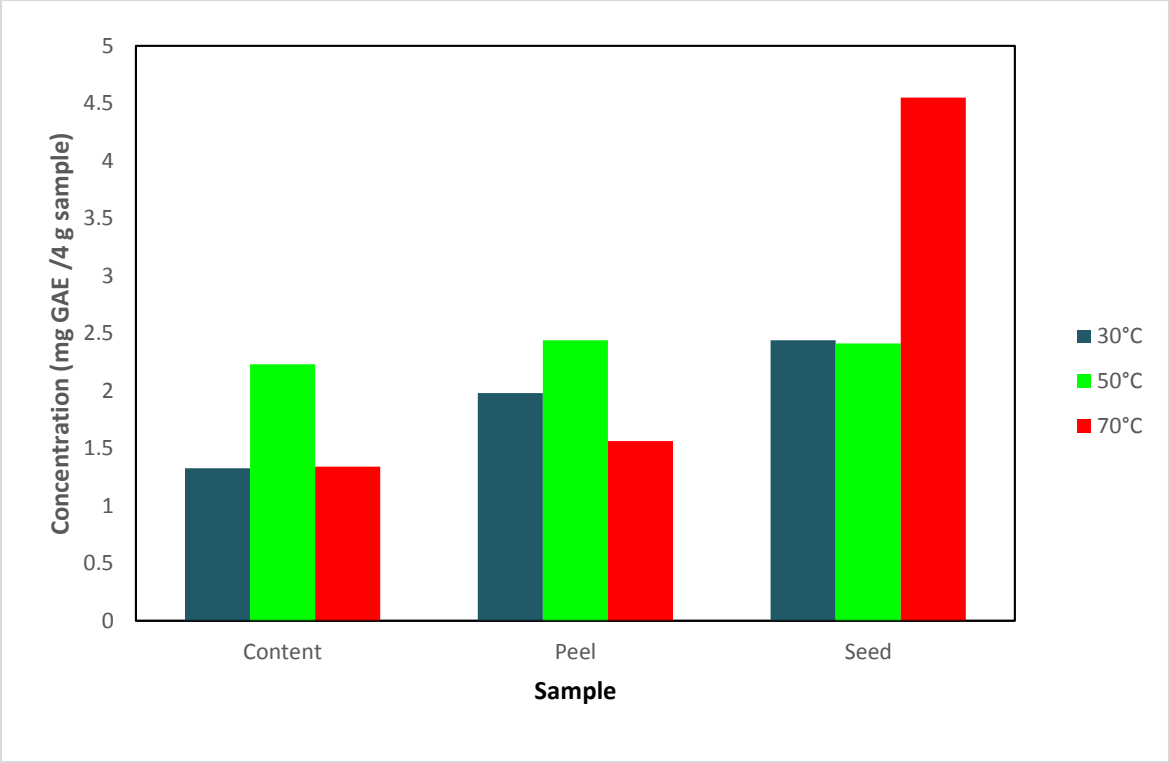

Figure 4.4 Effect of temperature on TPC based on different sample

4.5 Effect of Time on Enzymatic Extraction

Based on Table 4.4 and Figure 4.5, the data revealed that the scavenging activity was the lowest at 12 hours for all samples. The data also indicate that the seed have the lowest scavenging activity which was 50.93% compared to content (80.09%) and peel (54.61%). According to Samuagam L., Akowuah G.A., & P.N., (2013), the lower EC_{50} value is associated with a stronger DPPH scavenging capacity.The presence of cellulase enzyme inside of the extraction process will provide more enzyme complex that breaks down the sample cellulose to beta-glucose. Previous study explained that, the increasing of time will cause more cellulose and glycosidic bond to be broke down by cellulase. Hence, more concentration of phenolic will be release out and scavenge the radical element. Therefore, the scavenging activity by DPPH will be low according to the presences of antioxidant. Apart from that, the data analysis also showed that the increasing of time will decrease the scavenging activity. This statement was fully supported by (Mahmoudreza

Ovissipour, Abdolmohammad Abedian, & Ali Motamedzadegan, 2009) which stated that the high degree of hydrolysis can be achieved when longer time was taken in extraction process.

Table 4.4 Data analysis of DPPH by different time

Time (hour)	DPPH (EC_{50}) Scavenging Activity (%)		
	Content	Peel	Seed
3	83.47 ± 0.01	57.12 ± 0.01	55.323 ± 0.01
6	80.21	63.27 ± 0.01	51.11 ± 0.01
12	80.09 ± 0.01	54.61 ± 0.01	50.93 ± 0.05

*Each value is the average of three analyses ± standard deviation

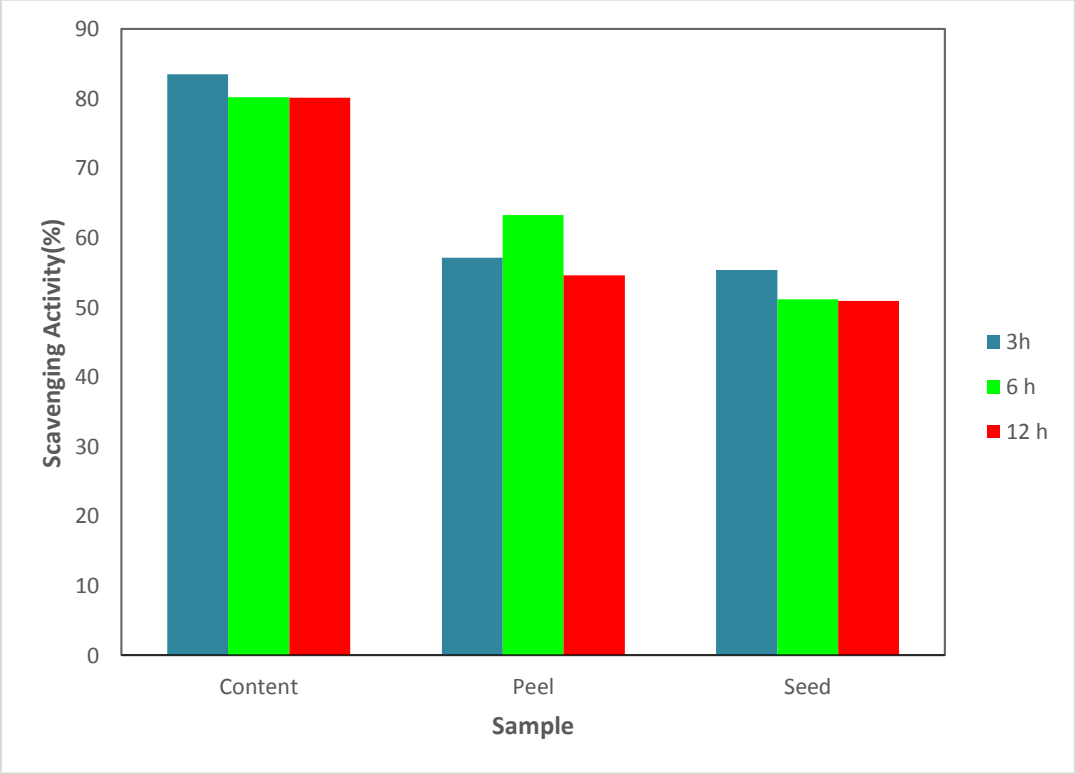

Figure 4.5 Effect of time on DPPH based on different sample

Table 4.5 Data analysis of TPC by different time

Time (hour)	Concentration (mg GAE /4 g sample)		
	Content	Peel	Seed
3	0.26 ± 0.27	2.43 ± 0.01	2.44 ± 0.01
6	0.27 ± 0.27	2.48 ± 0.01	2.46 ± 0.02
12	0.29 ± 0.31	2.70 ± 0.01	2.48 ± 0.01

*Each value is the average of three analyses ± standard deviation

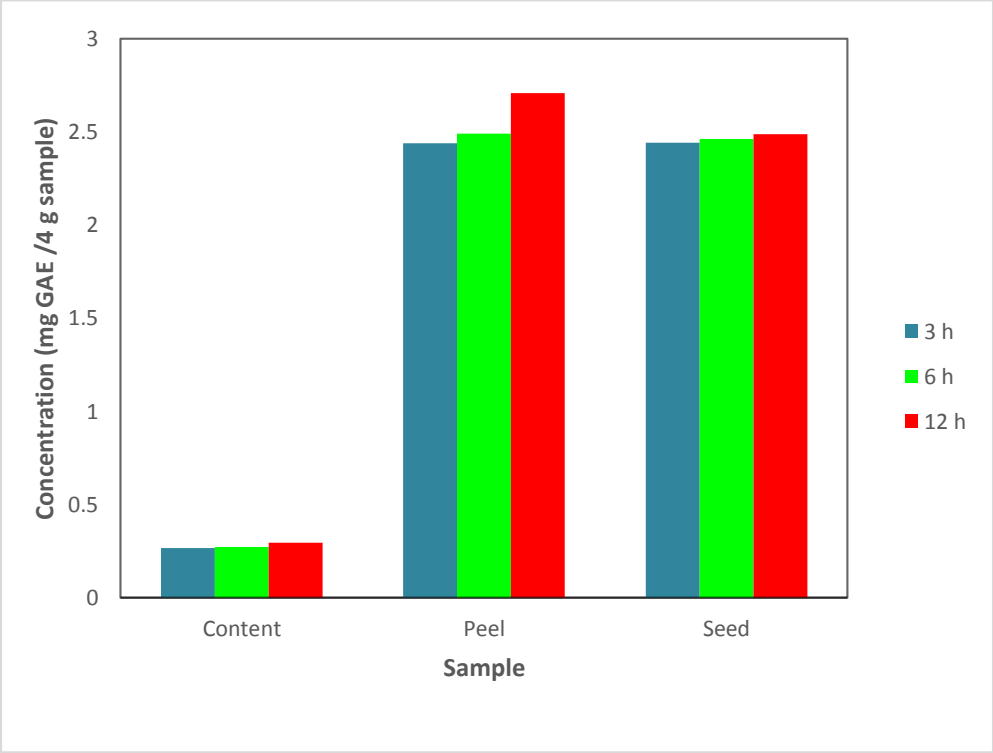

Figure 4.6 Effect of time on TPC based on different sample

Table 4.5 and Figure 4.6 proves that the 12 hour is the optimum time for enzymatic extraction to occur. It is because the amount of phenolic content concentration was the highest on that time compared to 3 hour and 6 hour. Guerard, Guimas, & Binet (2002) speculated that the increasing

of time will increase the hydrolysis rate and this may increase in concentration of peptides bonds that available for hydrolysis, enzyme inhibition and enzyme deactivation. Table 3 and Table 4 also show that there have a linear correlation between total phenolic content and the antioxidant activity of plant extract. This result is supporting the studies that have been done by Milan S. (2010) which said that when there have a significant linear correlation between the values of the concentration of phenolic compounds and antioxidant activity, it indicate that the sample will contribute to the strong antioxidant activity.

4.6 Effect of Extraction System on the Antioxidant Activity

All the data from previous parameter was selected in order to compare the effectiveness between to different extraction system. The two extraction system which are enzymatic assisted extraction and conventional extraction. The conventional extraction in this study was Soxhlet extraction. Based on the Table 4.6 and the Figure 4.7, the data analysis present that all the samples give the lowest scavenging activity in enzymatic assisted extraction compared to Soxhlet extraction. This may due to the several inhibitory compounds are formed during acid hydrolysis in Soxhlet extraction, whereas this problem is not so severe for enzymatic hydrolysis(Taherzadeh & Karimi, 2007).Moreover, during the conventional extraction process, the possibility of thermal decomposition of the target compounds cannot be ignored as the extraction usually occurs at the boiling point of the solvent for a long time(Castro, D., & Garcia-Ayuso, 1998). This may affect the amount of the target compound within all the sample.

Table 4.6 Data analysis of DPPH by different extraction system

Sample	**DPPH (EC50) Scavenging Activity (%)**	
	Enzymatic Extraction	**Soxhlet Extraction**
Content	70.74	84.69
Peel	56.65	83.29
Seed	54.79	90.56

*Each value is the average of three analyses ± standard deviation

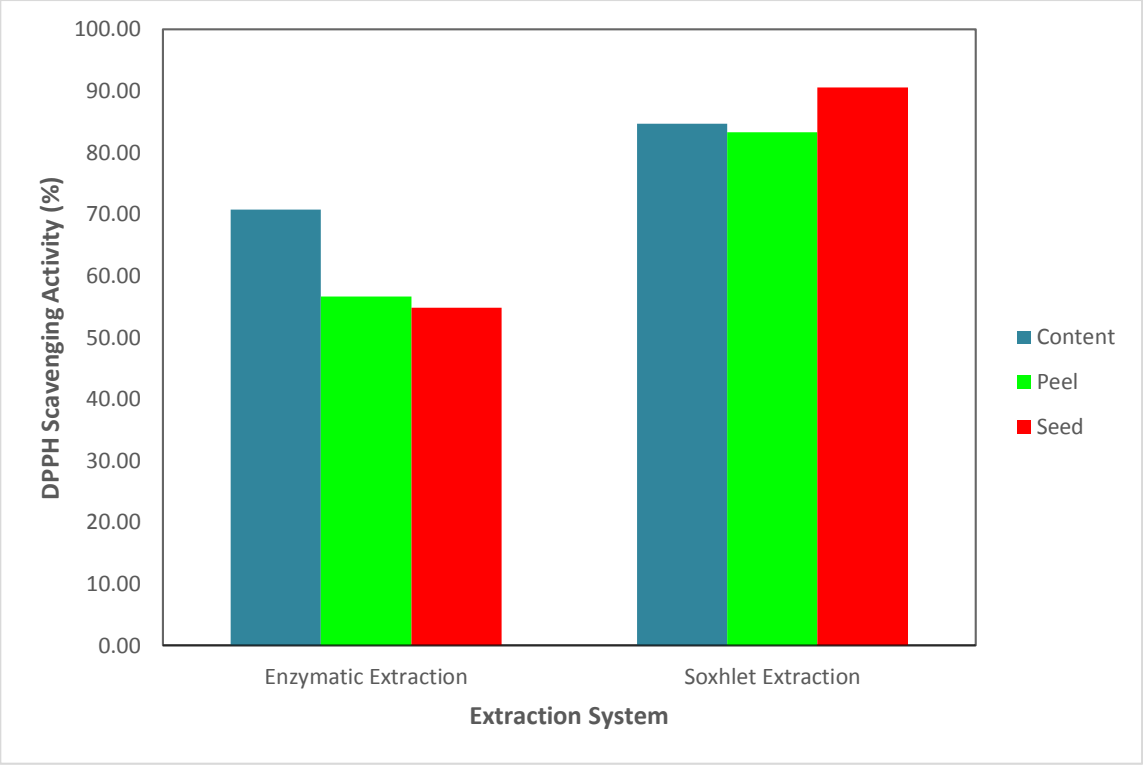

Figure 4.7 Effect of extraction system on DPPH based on different samples

Table 4.7 Data analysis of TPC by different extraction system

Sample	Concentration (mg GAE /4 g sample)	
	Enzymatic Extraction	Soxhlet Extraction
Content	2.23	1.15
Peel	2.44	1.21
Seed	2.41	1.06

*Each value is the average of three analyses ± standard deviation

The data analysis in Table 4.7 listed all the amount of the total phenolic content (TPC) revealed after the extraction process between enzymatic assisted extraction and Soxhlet extraction. In overall, enzymatic extraction give more concentration of TPC compared to Soxhlet extraction.

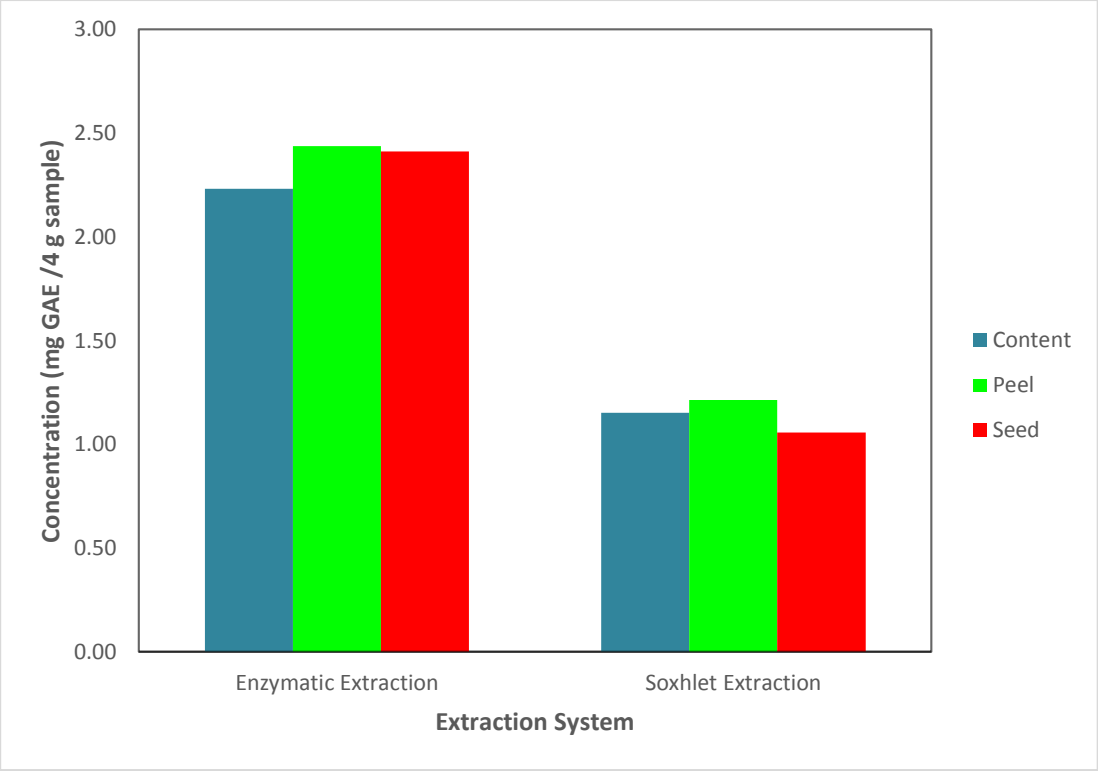

Figure 4.8 Effect of extraction system on TPC based on different sample

The pattern shown in Figure 4.8 prove that the enzymatic extraction have higher amount of phenolic content compared to Soxhlet extraction. This will contribute to increasing of antioxidant since there have linear correlation the phenolic content and antioxidant activity. Carpita & McCann, (2000) mentioned that all the plant cells are surrounded by a rigid, semi-permeable cell wall. The cell wall is comprised mainly polysaccharides with some proteins and lipids. The three main polysaccharide components of the cell wall are cellulose, an unbranched polymers of β-(1-4)-D-glycopyranosyl units associated in microfibril bundles. According to Li et al. (2006), cellulase inside of the enzymatic extraction system will disrupt the integrity of the cell wall during the extraction process; as a result, the extraction is more efficient. This statement also have been proved by Puri, Sharma, & Barrow (2012) by stated that the enzymes such as cellulases, pectinases and hemicellulase are often required to disrupt the structural integrity of the plant cell wall, thereby enhancing the extraction of bioactives from plants. These enzymes hydrolyzecell wall components thereby increasing cell wall permeability, which results in higher extraction yields of bioactives. Apart from that, the effect of cellulase enzyme on Kundur sample also produced an improvement in the yield of phenols and reduced sugar production and antioxidant capacity(A.K. & A.S.,

2001).Moreover, during process of conventional extraction system, some several inhibitory compounds will formed that will affect the scavenging activity of the antioxidant, whereas this problem is not so severe for enzymatic hydrolysis (Lee et al. 1999; Taherzadeh 1999; Wyman 1996).

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, the total phenolic content and antioxidant activities are depending on the extraction methods and the extraction parameter (temperature and time). All the extraction method in this studies which are enzymatic assisted extraction and Soxhlet extraction were functioning to identify the total phenolic content and antioxidant activity inside of the component of *Benincasa Hispida*. Based on the results obtained, the seed contributed to the highest extraction yield, amounting 19 %, followed by the peel with the total extraction yield of 15 %, while the content has the lowest extraction yield which have 11 %. For the first parameter which is temperature, the lowest temperature of scavenging activity for the sample to take place is at 50 °C. Within this temperature, the seed sample provide the lowest scavenging activity with 54.79% followed by peel sample (56.64%) and lastly content sample (70.73%). At this temperature also, it was founded that the total phenolic content was the highest compared to another temperature. For the second parameter which is time, the scavenging activity was the lowest at 12 hours for all samples. The seed also again have been founded as having the lowest scavenging activity which was 50.93% compared to content (80.09%) and peel (54.61%). The total phenolic content analysis also found that the higher phenolic content can be achieved at 12 hour. Hence, from all the data, it can be concluded that, the optimum temperature for enzymatic assisted extraction was 50 °C and the optimum time for enzymatic assisted extraction was and 12 hour. Moreover, data analysis also proved that all the samples in *Benincasa Hispida* give the lowest scavenging activity in enzymatic assisted extraction compared to Soxhlet extraction. Besides that, all the samples also give the highest total phenolic content in enzymatic assisted extraction compared to Soxhlet extraction. Therefore, this concluded that the enzymatic assisted extraction was found to be the most efficient and promising method where lower scavenging activity and highest amount of total phenolic content can be achieved. In consequence, antioxidant capacity of *Benincasa Hispida* was greatly influenced by extraction method used. Apart from that, this extraction system have a lot of advantages and can be considered as a feasible technology to extract phenolic contents from plant materials.

5.2 Recommendation

For future study, it is recommended that High Performance Liquid Chromatography (HPLC) should be applied and practicing during the analyzing process of the each extraction sample in order to get more accurate result. HPLC equipment known as one of technique in analytic chemistry used to separate the components in a mixture, to identify each component, and to quantify each component. This equipment allows the uses of very small particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past. This allows a much better separation of the components of the mixture. Besides, it is suggested that scanning electron microscope (SEM) observation is carry out in the future in order to fully understand how each extraction method disrupt plant matrix in extracting out bioactive compounds. Besides that, using other extraction method such as ultrasound-assisted extraction and microwave-assisted extraction in extraction of *Benincasa hispida* is suggested for further extraction method comparison. Moreover, instead of cellulase, another enzyme such as Kleerase and Celluzyme MX can be applied in order to extract the component inside of the *Benincasa Hispida*. Finally, by looking enzymatic extraction method efficiency in this study, analysis of another type of antioxidant compound such as Vitamin A is proposed for further study in extracting bioactive compound from *Benincasa Hispida*.

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APPENDICES

Physical Properties Solvents used

Table A.1.1 Properties of DPPH

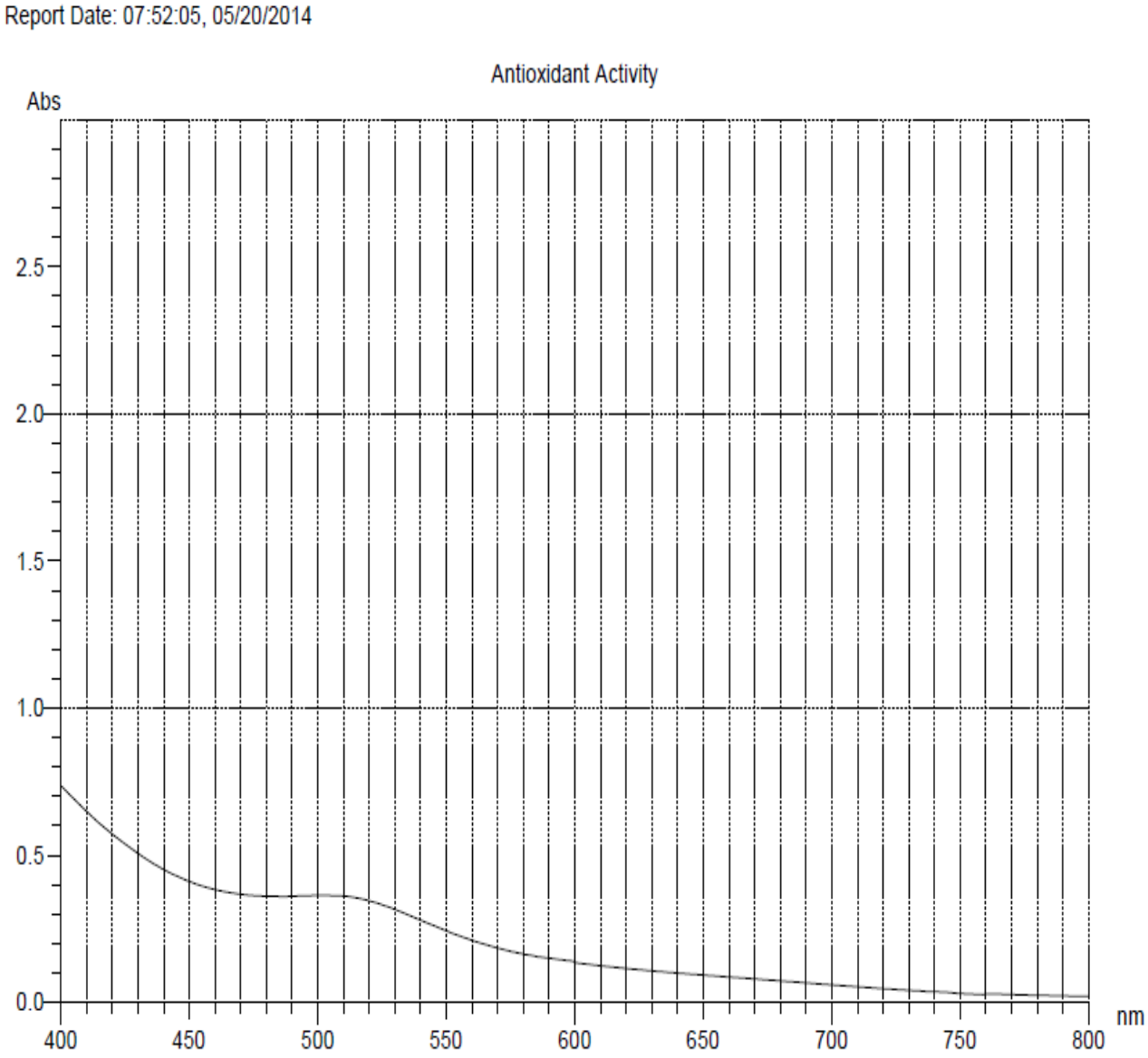
Properties	
Molecular formula	$C_{18}H_{12}N_5O_6$
Molar mass	394.32 g/mol
Appearance	Black to green powder, purple in solution
Density	1.4 g/cm^3
Melting point	135 °C, 408 K, 275 °F (decomposes)
Solubility in water	insoluble

Table A.1.2 Properties of methanol

Properties	
Molecular formula	CH_4O
Molar mass	32.04 g mol−1
Appearance	Colourless liquid
Density	0.7918 g/cm^3
Melting point	-97.6 °C, 176 K, -144 °F
Boiling point	64.7 °C, 338 K, 148 °F
log P	-0.69
Vapor pressure	13.02 kPa (at 20 °C)
Acidity (pKa)	15.5
Viscosity	5.9×10−4 Pa.s (at 20 °C)
Dipole moment	1.69 D

Analysis of DPPH scavenging activity using UV-Vis Spectrophotometer

i) Enzymatic–assisted Extraction (Content, Temperature=50°C)



Sample:	Antioxidant Activity
File name:	s_6_h002.UDS
Run Date:	07:50:25, 05/20/2014
Operator:	KUKTEM
Comment:	enzymatic extraction (content 50 C)

Instrument

Model:	U-1800 Spectrophotometer
Serial Number:	
ROM Version:	2500 11

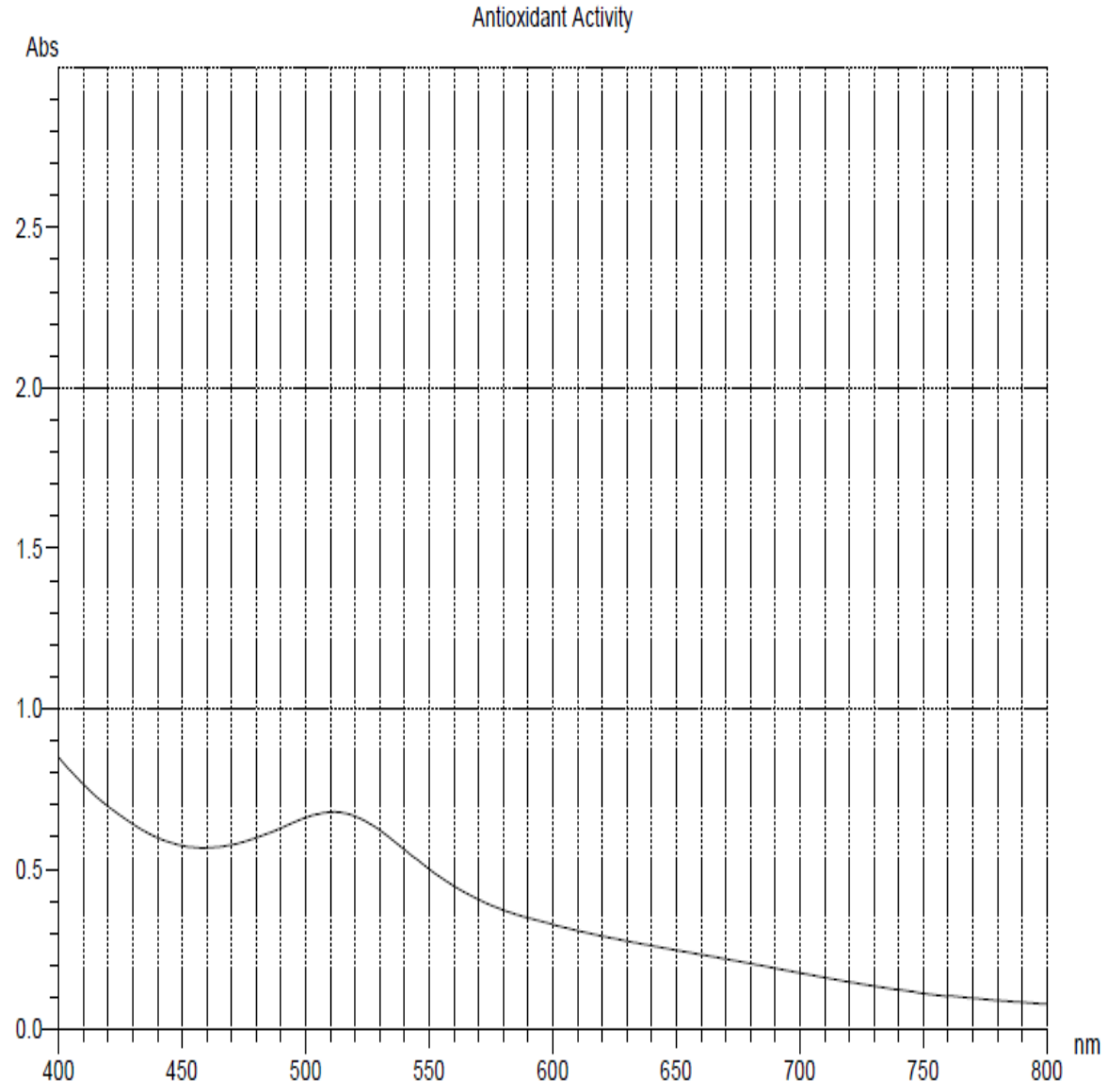
Instrument Parameters

Measurement Type:	Wavelength Scan
Data Mode:	Abs
Starting Wavelength:	800.0 nm
Ending Wavelength:	400.0 nm
Scan Speed:	400 nm/min
Sampling Interval:	0.5 nm
Slit Width:	4.00 nm
Lamp change mode:	Auto
Auto change wavelength:	340.0 nm
Baseline Correction:	User
Path Length:	10.0 mm

Data Points

nm	Abs	nm	Abs
800.0	0.021	790.0	0.021
780.0	0.024	770.0	0.026
760.0	0.028	750.0	0.030
740.0	0.036	730.0	0.041
720.0	0.046	710.0	0.053
700.0	0.059	690.0	0.066
680.0	0.072	670.0	0.079
660.0	0.086	650.0	0.092
640.0	0.099	630.0	0.107
620.0	0.115	610.0	0.124
600.0	0.136	590.0	0.149
580.0	0.163	570.0	0.184
560.0	0.210	550.0	0.243
540.0	0.279	530.0	0.315
520.0	0.344	510.0	0.361
500.0	0.363	490.0	0.360
480.0	0.360	470.0	0.367
460.0	0.383	450.0	0.410
440.0	0.451	430.0	0.506
420.0	0.571	410.0	0.649
400.0	0.735		

ii) Enzymatic–assisted Extraction (Peel, Temperature=50°C)



Sample:	Antioxidant Activity
File name:	s_6_h001.UDS
Run Date:	07:47:35, 05/20/2014
Operator:	KUKTEM
Comment:	enzymatic extraction (peel 50 C)

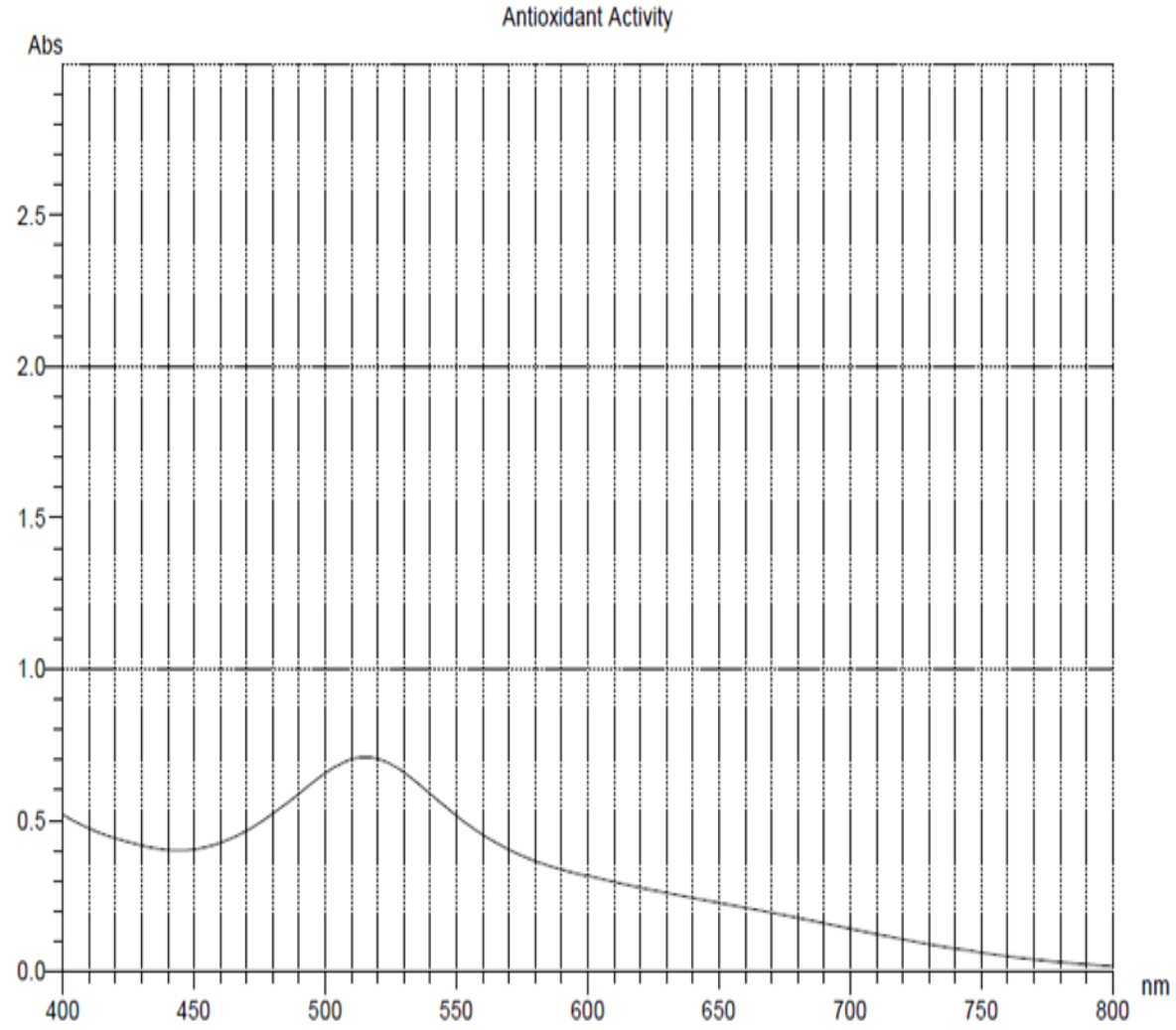
Peaks

Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	800.0	511.5	458.5	0.678	112.622	458.5	0.566

Data Points

nm	Abs	nm	Abs
800.0	0.080	790.0	0.084
780.0	0.090	770.0	0.097
760.0	0.104	750.0	0.112
740.0	0.124	730.0	0.135
720.0	0.148	710.0	0.162
700.0	0.176	690.0	0.191
680.0	0.205	670.0	0.219
660.0	0.233	650.0	0.247
640.0	0.261	630.0	0.275
620.0	0.291	610.0	0.308
600.0	0.327	590.0	0.347
580.0	0.371	570.0	0.405
560.0	0.447	550.0	0.499
540.0	0.560	530.0	0.621
520.0	0.664	510.0	0.677
500.0	0.660	490.0	0.627
480.0	0.597	470.0	0.575
460.0	0.566	450.0	0.573
440.0	0.598	430.0	0.640
420.0	0.695	410.0	0.764
400.0	0.847		

iii) Enzymatic–assisted Extraction (Seed, Temperature=50°C)



Sample: Antioxidant Activity
File name: s_6_h003.UDS
Run Date: 07:53:18, 05/20/2014
Operator: KUKTEM
Comment: enzymatic extraction (seed 50 C)

Peak Integration

Method:	Rectangular
Sensitivity:	1
Threshold:	0.0100

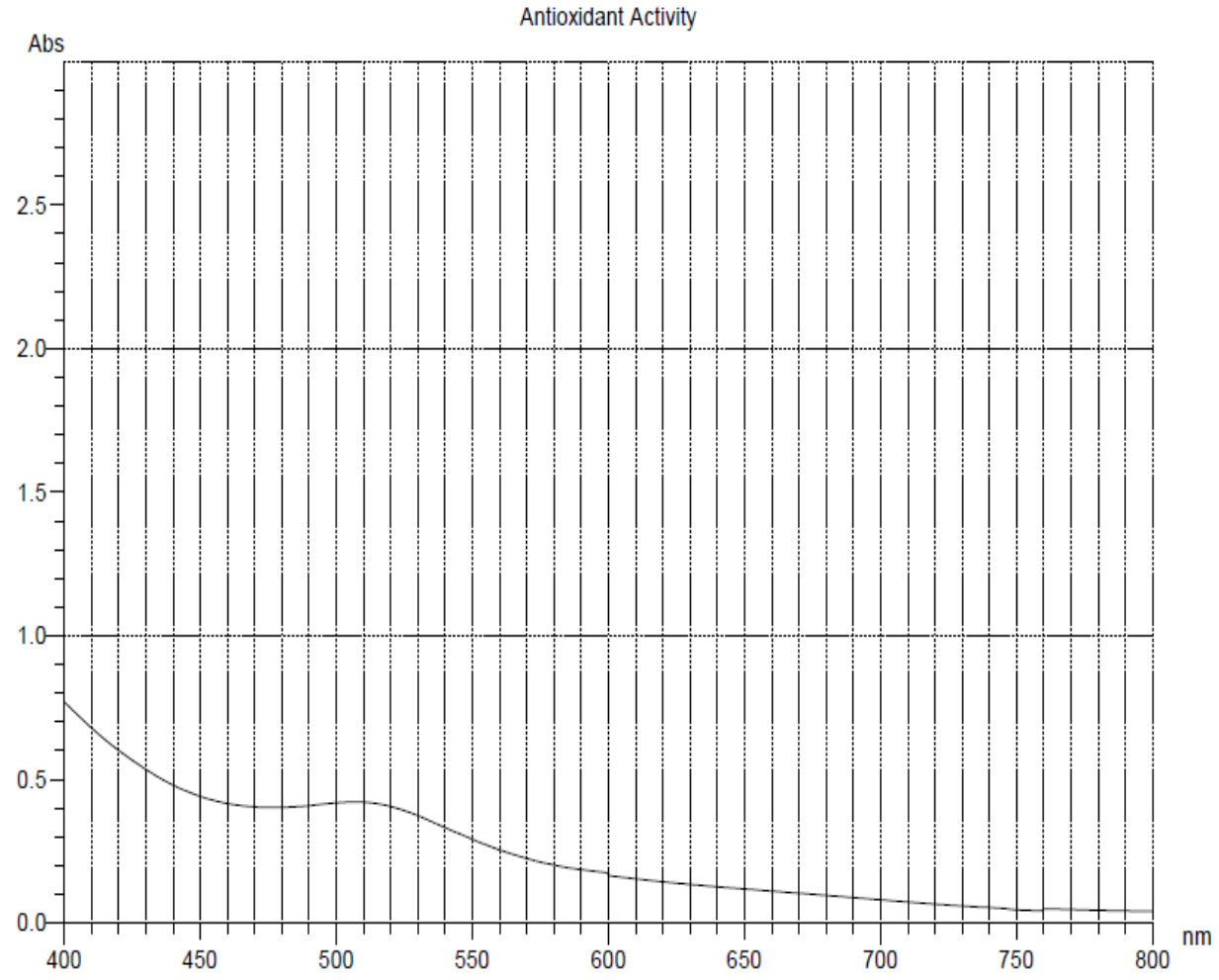
Peaks

Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	800.0	515.0	443.5	0.709	109.689	443.5	0.400

Data Points

nm	Abs	nm	Abs
800.0	0.018	790.0	0.024
780.0	0.031	770.0	0.040
760.0	0.050	750.0	0.062
740.0	0.076	730.0	0.090
720.0	0.106	710.0	0.124
700.0	0.142	690.0	0.160
680.0	0.177	670.0	0.194
660.0	0.211	650.0	0.227
640.0	0.243	630.0	0.260
620.0	0.277	610.0	0.296
600.0	0.317	590.0	0.337
580.0	0.364	570.0	0.403
560.0	0.452	550.0	0.515
540.0	0.587	530.0	0.658
520.0	0.702	510.0	0.702
500.0	0.656	490.0	0.588
480.0	0.522	470.0	0.466
460.0	0.426	450.0	0.404
440.0	0.402	430.0	0.417
420.0	0.441	410.0	0.473
400.0	0.518		

iv) Enzymatic–assisted Extraction (Content, Temperature=30°C)



Sample: Antioxidant Activity
File name: s_6_h003.UDS
Run Date: 08:27:51, 05/20/2014
Operator: KUKTEM
Comment: enzymatic extraction (content 30 C)

Peak Integration

Method:	Rectangular
Sensitivity:	1
Threshold:	0.0100

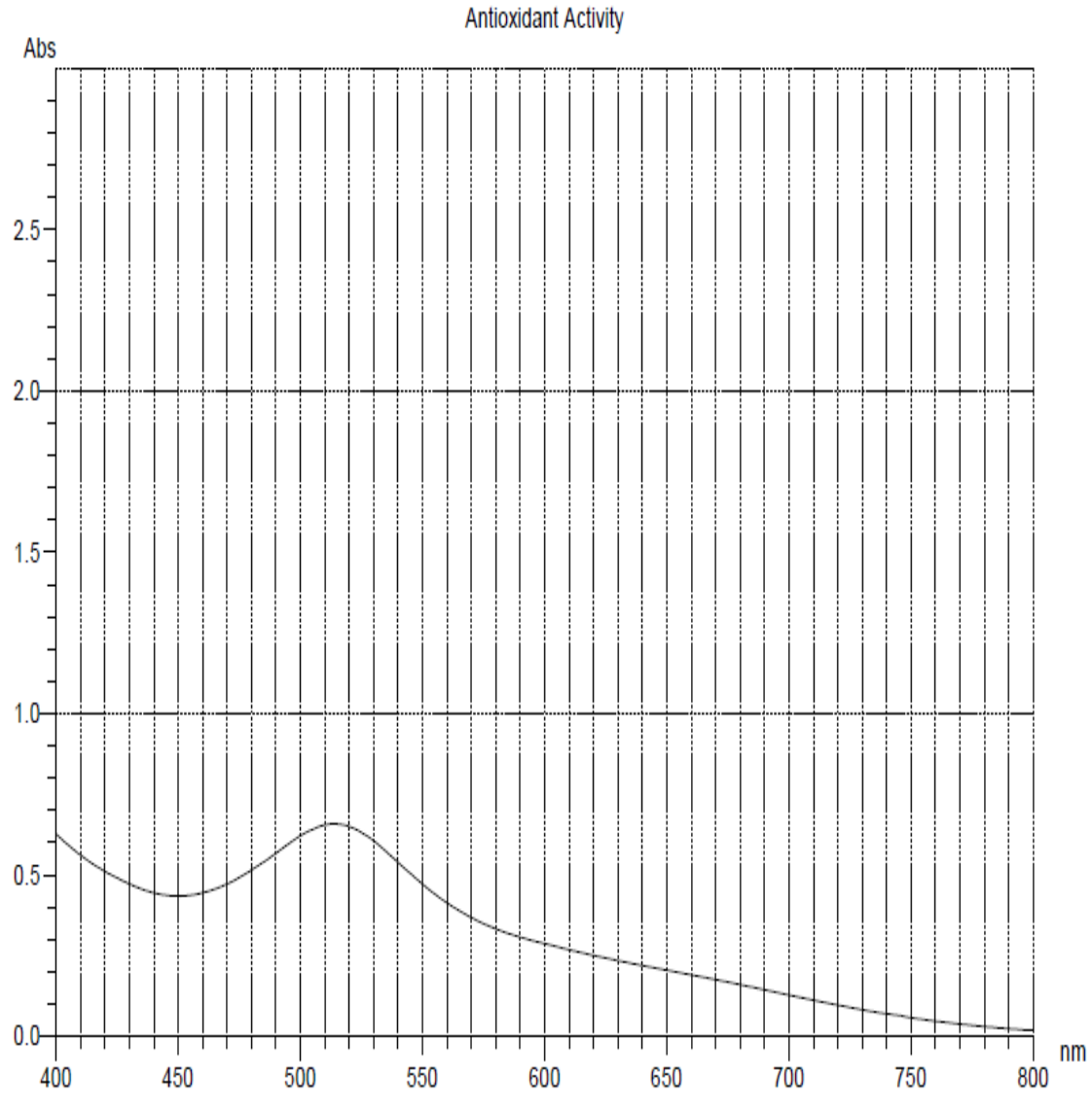
Peaks

Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	800.0	508.5	477.0	0.421	56.558	477.0	0.402

Data Points

nm	Abs	nm	Abs
800.0	0.041	790.0	0.041
780.0	0.044	770.0	0.046
760.0	0.047	750.0	0.045
740.0	0.054	730.0	0.059
720.0	0.065	710.0	0.073
700.0	0.080	690.0	0.088
680.0	0.096	670.0	0.103
660.0	0.110	650.0	0.118
640.0	0.126	630.0	0.134
620.0	0.143	610.0	0.153
600.0	0.167	590.0	0.185
580.0	0.201	570.0	0.224
560.0	0.254	550.0	0.291
540.0	0.331	530.0	0.373
520.0	0.405	510.0	0.420
500.0	0.418	490.0	0.408
480.0	0.402	470.0	0.404
460.0	0.415	450.0	0.440
440.0	0.480	430.0	0.534
420.0	0.600	410.0	0.679
400.0	0.769		

v) Enzymatic–assisted Extraction (Peel, Temperature=30°C)



Sample: Antioxidant Activity
File name: s_6_h004.UDS
Run Date: 08:30:13, 05/20/2014
Operator: KUKTEM
Comment: enzymatic extraction (peel 30 C)

Peak Integration

Method:	Rectangular
Sensitivity:	1
Threshold:	0.0100

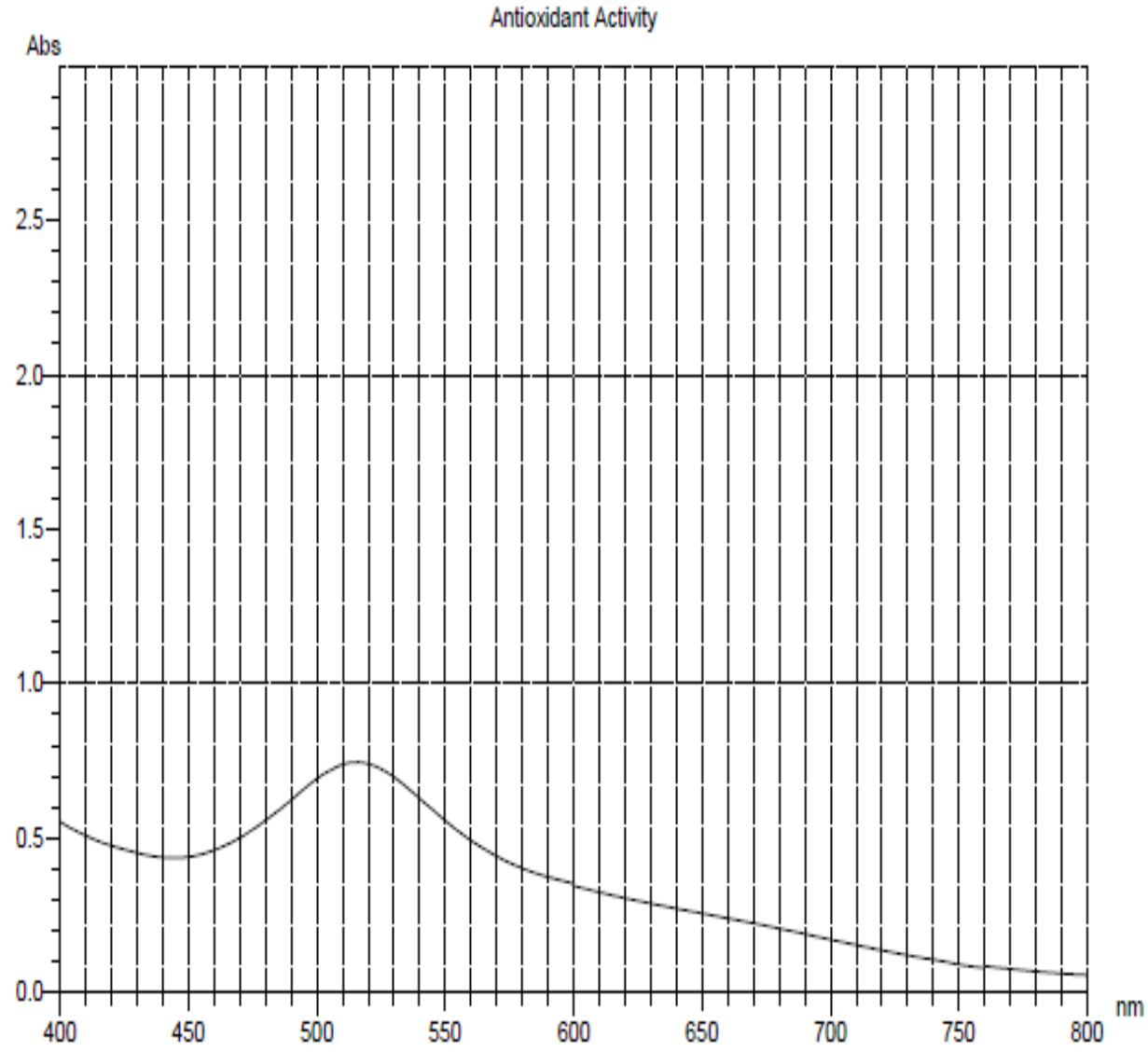
Peaks

Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	800.0	514.0	451.0	0.658	99.593	451.0	0.434

Data Points

nm	Abs	nm	Abs
800.0	0.019	790.0	0.023
780.0	0.030	770.0	0.037
760.0	0.046	750.0	0.057
740.0	0.069	730.0	0.082
720.0	0.096	710.0	0.111
700.0	0.127	690.0	0.143
680.0	0.160	670.0	0.175
660.0	0.190	650.0	0.204
640.0	0.219	630.0	0.234
620.0	0.250	610.0	0.268
600.0	0.287	590.0	0.307
580.0	0.332	570.0	0.367
560.0	0.413	550.0	0.471
540.0	0.538	530.0	0.605
520.0	0.649	510.0	0.655
500.0	0.621	490.0	0.567
480.0	0.515	470.0	0.472
460.0	0.444	450.0	0.434
440.0	0.444	430.0	0.472
420.0	0.510	410.0	0.561
400.0	0.626		

vi) Enzymatic–assisted Extraction (Seed, Temperature=30°C)



Sample: Antioxidant Activity
File name: s_6_h005.UDS
Run Date: 08:32:34, 05/20/2014
Operator: KUKTEM
Comment: enzymatic extraction (seed 30 C)

Peak Integration

Method:	Rectangular
Sensitivity:	1
Threshold:	0.0100

Peaks

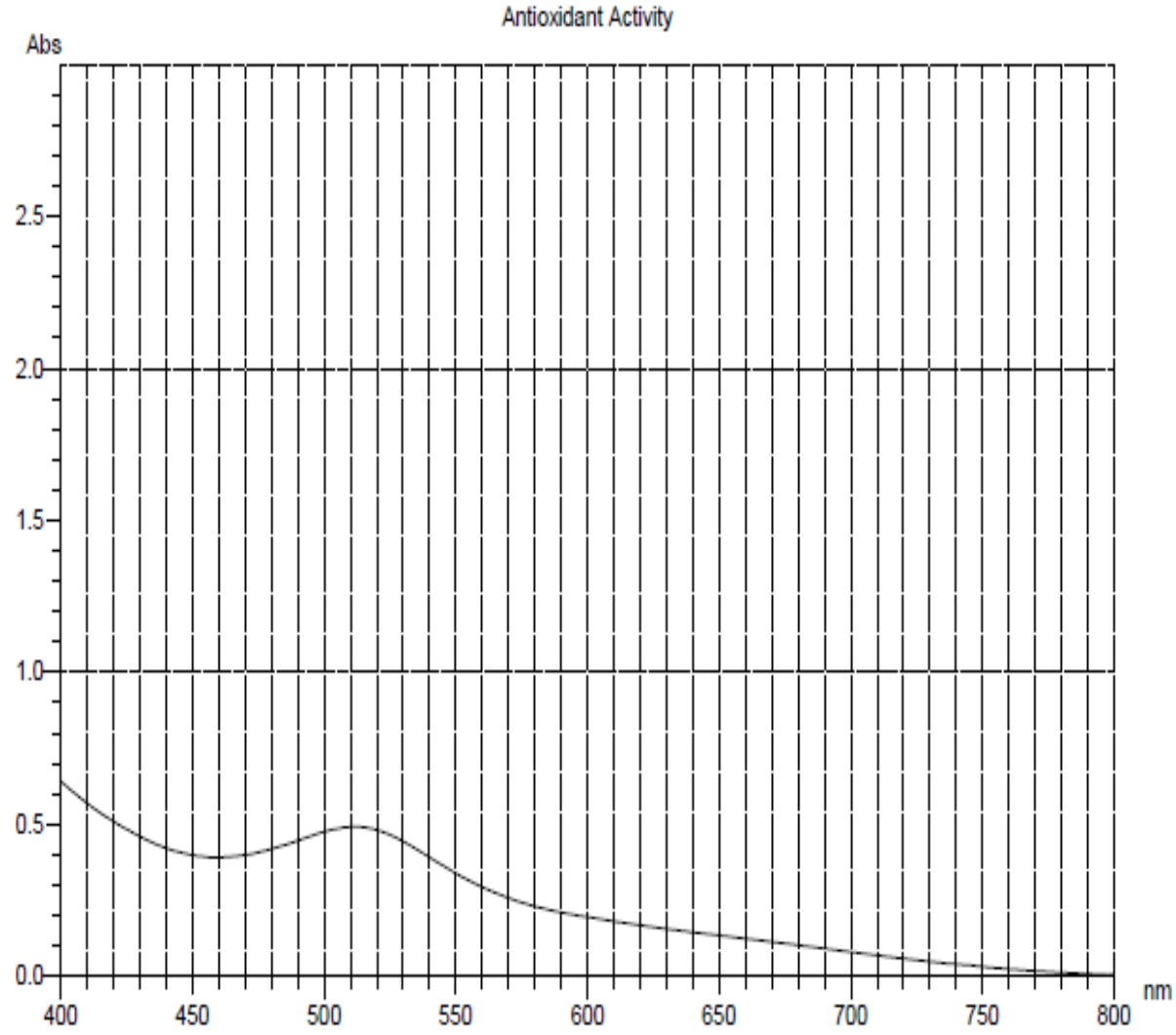
Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	800.0	516.0	443.5	0.745	121.522	443.5	0.435

Data Points

nm	Abs	nm	Abs
800.0	0.056	790.0	0.061
780.0	0.067	770.0	0.076
760.0	0.083	750.0	0.090
740.0	0.106	730.0	0.120
720.0	0.136	710.0	0.153
700.0	0.171	690.0	0.189
680.0	0.206	670.0	0.224
660.0	0.239	650.0	0.255
640.0	0.271	630.0	0.288
620.0	0.305	610.0	0.324
600.0	0.347	590.0	0.373
580.0	0.401	570.0	0.441
560.0	0.491	550.0	0.556
540.0	0.628	530.0	0.697
520.0	0.739	510.0	0.738
500.0	0.692	490.0	0.623
480.0	0.557	470.0	0.501
460.0	0.461	450.0	0.438
440.0	0.437	430.0	0.451
420.0	0.475	410.0	0.507
400.0	0.551		

vii) Enzymatic–assisted Extraction (Content, Temperature=70°C)

Report Date: 07:58:13, 05/20/2014



Sample: Antioxidant Activity
File name: s_6_h.UDS
Run Date: 07:56:33, 05/20/2014
Operator: KUKTEM
Comment: enzymatic extraction (content 70 C)

Instrument
Model: U-1800 Spectrophotometer
Serial Number:
ROM Version: 2500 11

Peak Integration

Method:	Rectangular
Sensitivity:	1
Threshold:	0.0100

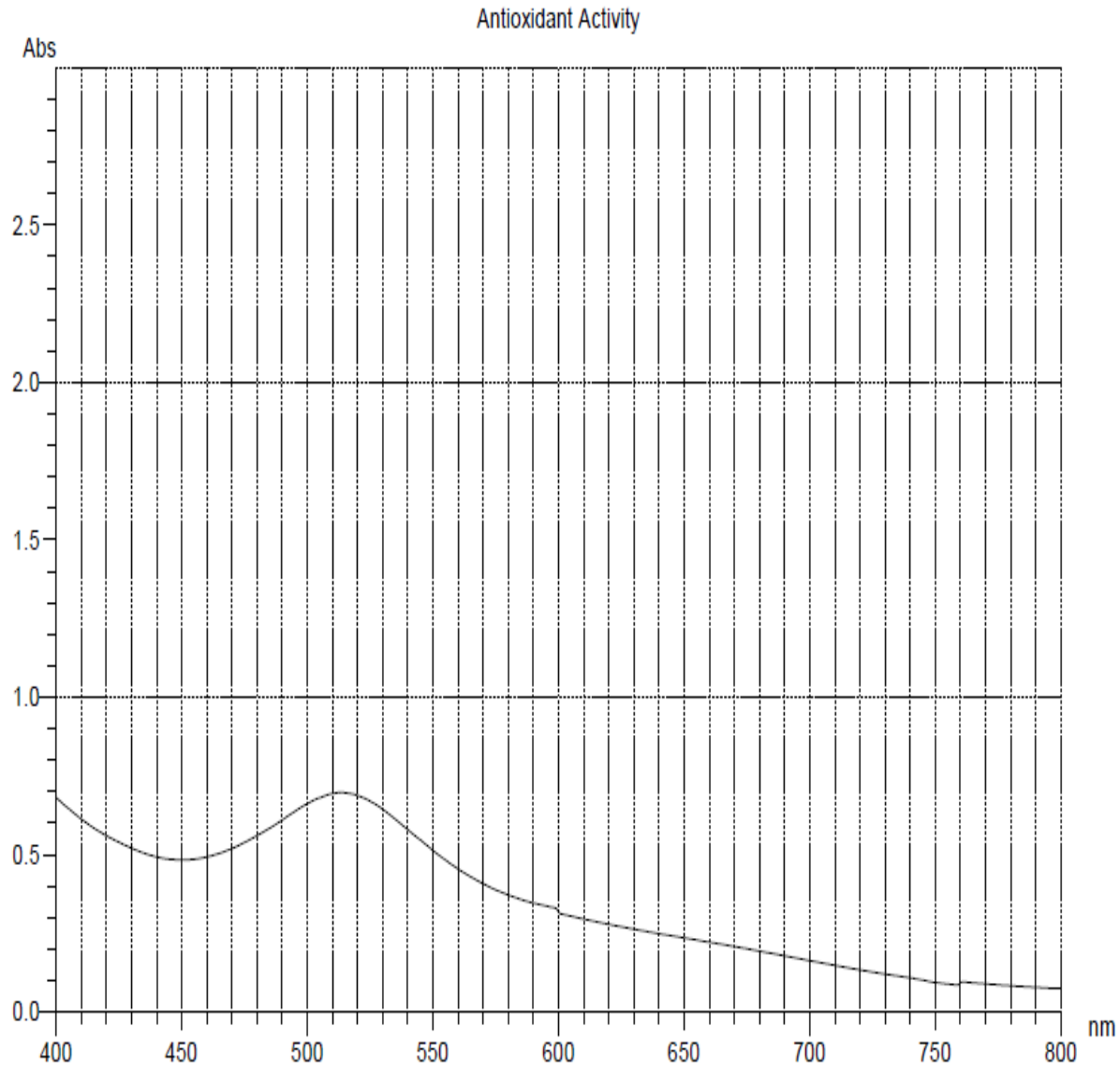
Peaks

Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	800.0	512.0	459.0	0.492	69.020	459.0	0.391

Data Points

nm	Abs	nm	Abs
800.0	0.005	790.0	0.007
780.0	0.012	770.0	0.017
760.0	0.023	750.0	0.031
740.0	0.039	730.0	0.048
720.0	0.058	710.0	0.068
700.0	0.079	690.0	0.090
680.0	0.102	670.0	0.113
660.0	0.123	650.0	0.134
640.0	0.144	630.0	0.155
620.0	0.167	610.0	0.180
600.0	0.195	590.0	0.209
580.0	0.229	570.0	0.257
560.0	0.293	550.0	0.338
540.0	0.391	530.0	0.443
520.0	0.480	510.0	0.491
500.0	0.475	490.0	0.446
480.0	0.418	470.0	0.399
460.0	0.391	450.0	0.398
440.0	0.421	430.0	0.459
420.0	0.508	410.0	0.568
400.0	0.640		

viii) Enzymatic–assisted Extraction (Peel, Temperature=70°C)



Sample: Antioxidant Activity
File name: s_6_h001.UDS
Run Date: 07:58:55, 05/20/2014
Operator: KUKTEM
Comment: enzymatic extraction (peel 70 C)

Peak Integration

Method:	Rectangular
Sensitivity:	1
Threshold:	0.0100

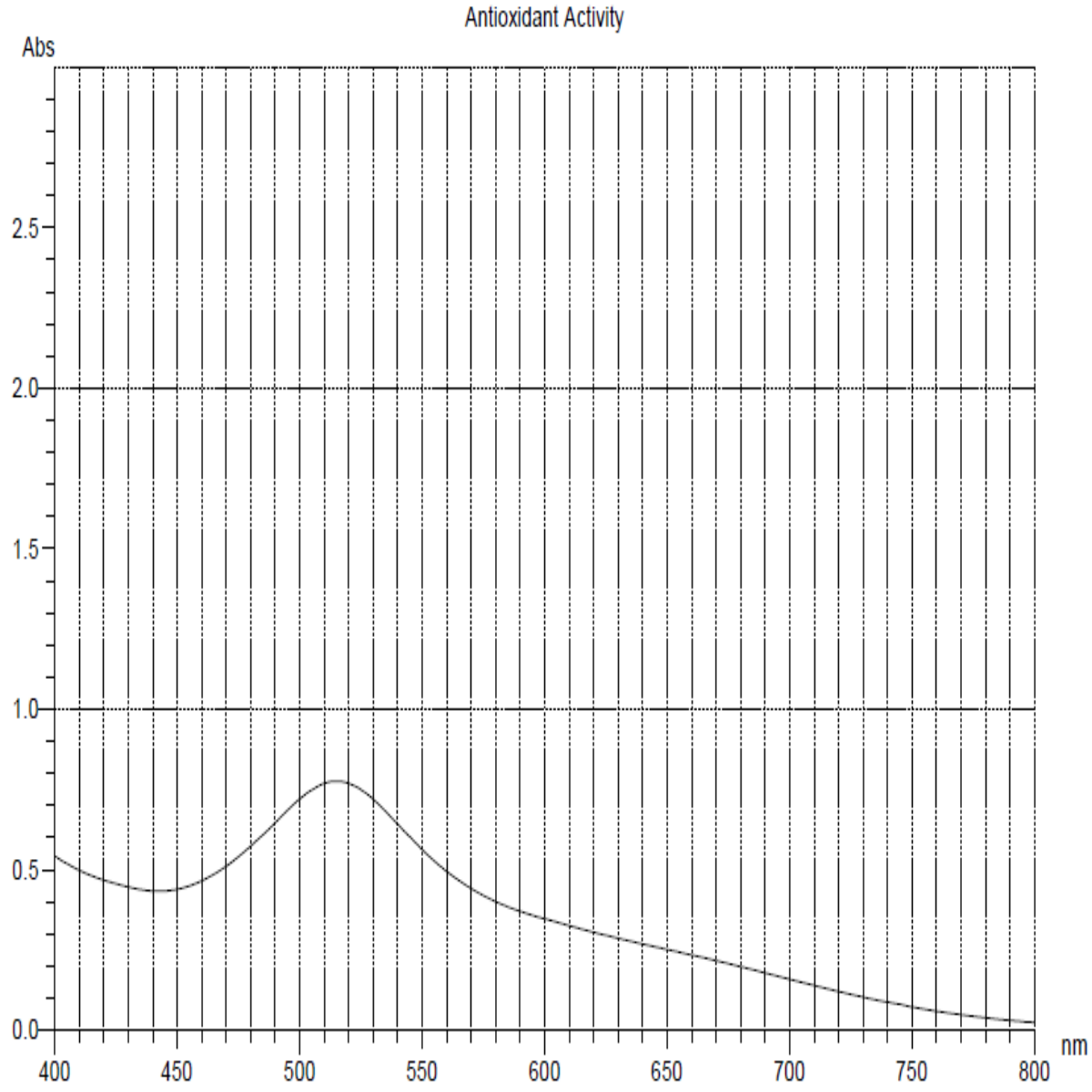
Peaks

Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	800.0	514.5	451.5	0.697	112.993	451.5	0.482

Data Points

nm	Abs	nm	Abs
800.0	0.075	790.0	0.078
780.0	0.083	770.0	0.089
760.0	0.093	750.0	0.093
740.0	0.108	730.0	0.120
720.0	0.133	710.0	0.148
700.0	0.163	690.0	0.178
680.0	0.193	670.0	0.207
660.0	0.221	650.0	0.235
640.0	0.248	630.0	0.263
620.0	0.278	610.0	0.295
600.0	0.317	590.0	0.346
580.0	0.371	570.0	0.407
560.0	0.454	550.0	0.513
540.0	0.579	530.0	0.644
520.0	0.687	510.0	0.693
500.0	0.661	490.0	0.610
480.0	0.560	470.0	0.519
460.0	0.492	450.0	0.482
440.0	0.492	430.0	0.520
420.0	0.560	410.0	0.612
400.0	0.680		

ix) Enzymatic–assisted Extraction (Seed, Temperature=70°C)



Sample: Antioxidant Activity
File name: s_6_h002.UDS
Run Date: 08:01:19, 05/20/2014
Operator: KUKTEM
Comment: enzymatic extraction (seed 70 C)

Peak Integration

Method:	Rectangular
Sensitivity:	1
Threshold:	0.0100

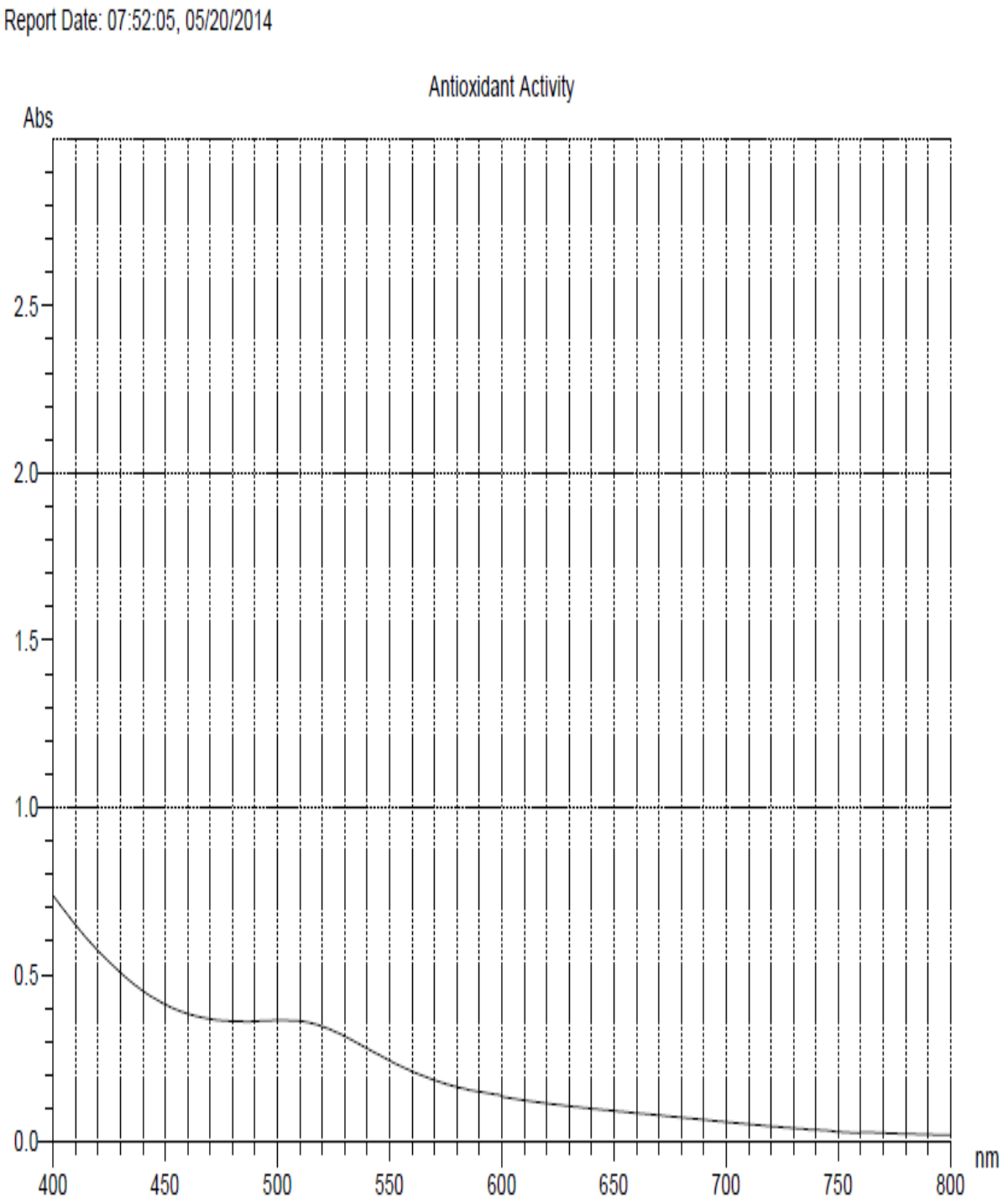
Peaks

Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	800.0	515.0	443.0	0.775	120.900	443.0	0.433

Data Points

nm	Abs	nm	Abs
800.0	0.024	790.0	0.030
780.0	0.038	770.0	0.048
760.0	0.059	750.0	0.072
740.0	0.087	730.0	0.103
720.0	0.120	710.0	0.139
700.0	0.158	690.0	0.178
680.0	0.197	670.0	0.216
660.0	0.234	650.0	0.251
640.0	0.268	630.0	0.286
620.0	0.305	610.0	0.325
600.0	0.347	590.0	0.370
580.0	0.400	570.0	0.441
560.0	0.494	550.0	0.562
540.0	0.641	530.0	0.719
520.0	0.768	510.0	0.769
500.0	0.720	490.0	0.646
480.0	0.573	470.0	0.511
460.0	0.465	450.0	0.439
440.0	0.433	430.0	0.446
420.0	0.467	410.0	0.498
400.0	0.541		

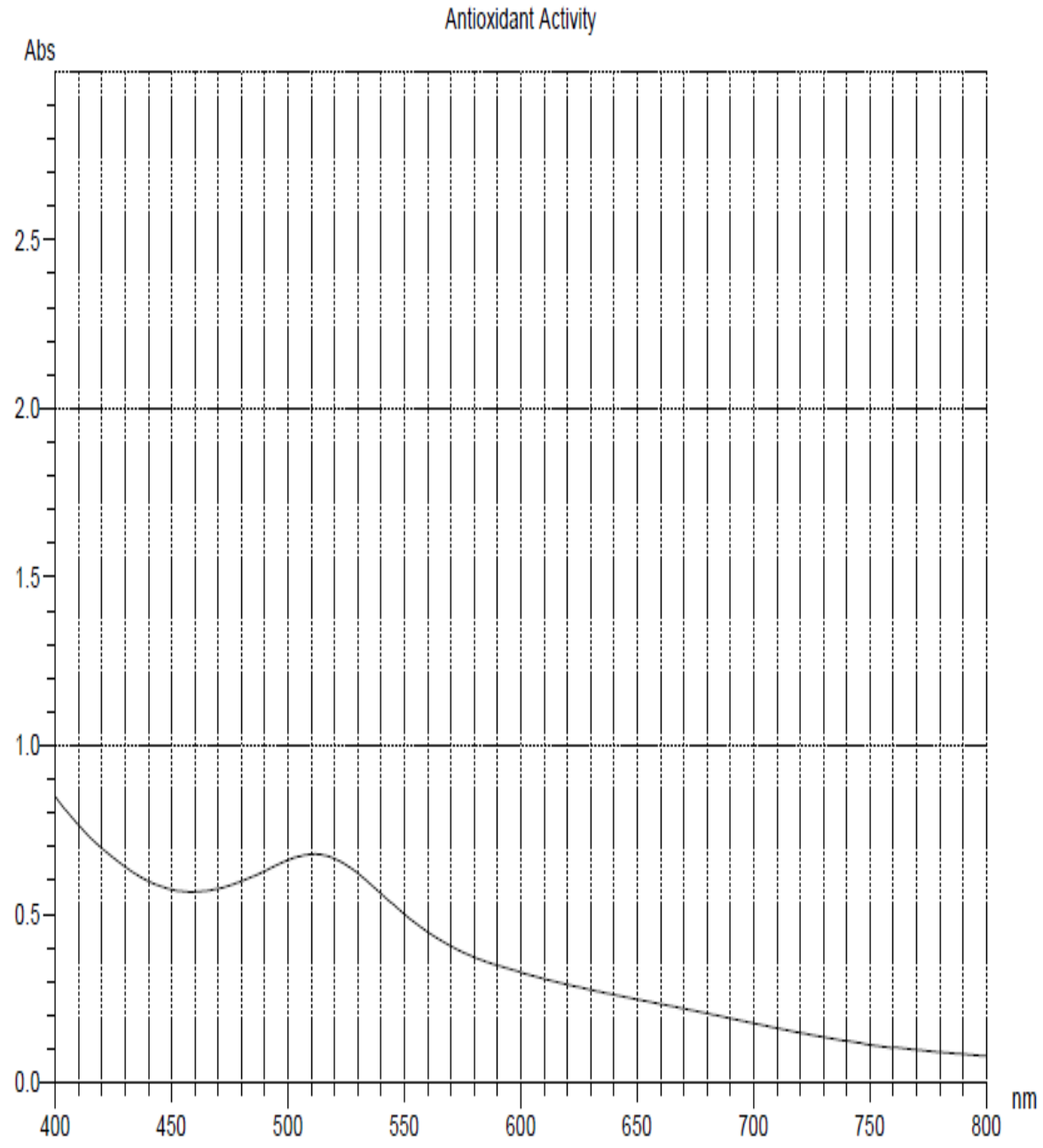
x) Enzymatic–assisted Extraction (Content, Time= 3 hour)



Data Points

nm	Abs	nm	Abs
800.0	0.021	790.0	0.021
780.0	0.024	770.0	0.026
760.0	0.028	750.0	0.030
740.0	0.036	730.0	0.041
720.0	0.046	710.0	0.053
700.0	0.059	690.0	0.066
680.0	0.072	670.0	0.079
660.0	0.086	650.0	0.092
640.0	0.099	630.0	0.107
620.0	0.115	610.0	0.124
600.0	0.136	590.0	0.149
580.0	0.163	570.0	0.184
560.0	0.210	550.0	0.243
540.0	0.279	530.0	0.315
520.0	0.344	510.0	0.361
500.0	0.363	490.0	0.360
480.0	0.360	470.0	0.367
460.0	0.383	450.0	0.410
440.0	0.451	430.0	0.506
420.0	0.571	410.0	0.649
400.0	0.735		

xi) Enzymatic–assisted Extraction (Peel, Time= 3 hour)



Sample: Antioxidant Activity
File name: s_6_h001.UDS
Run Date: 07:47:35, 05/20/2014
Operator: KUKTEM
Comment: enzymatic extraction (peel 50 C)

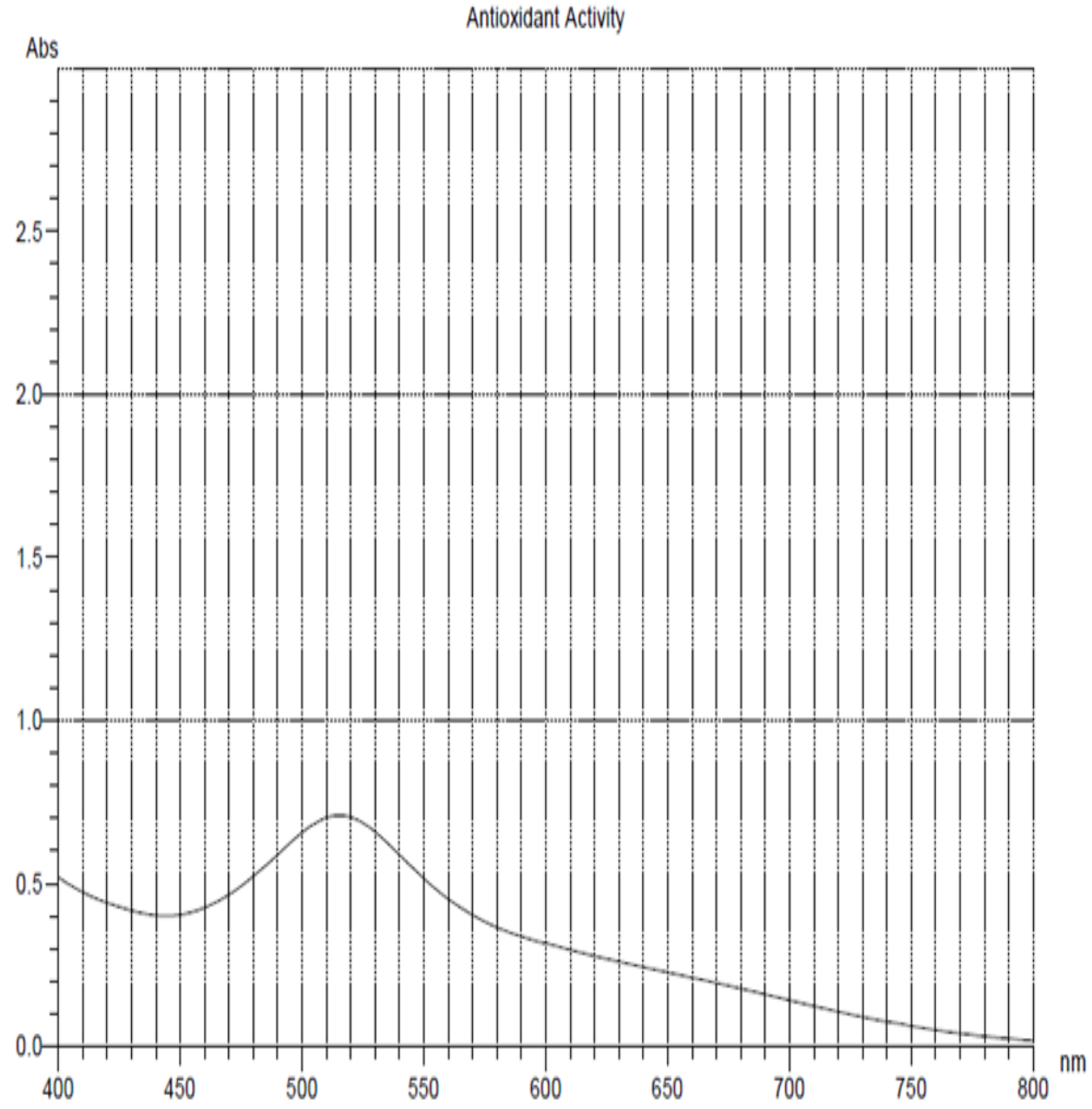
Peaks

Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	800.0	511.5	458.5	0.678	112.622	458.5	0.566

Data Points

nm	Abs	nm	Abs
800.0	0.080	790.0	0.084
780.0	0.090	770.0	0.097
760.0	0.104	750.0	0.112
740.0	0.124	730.0	0.135
720.0	0.148	710.0	0.162
700.0	0.176	690.0	0.191
680.0	0.205	670.0	0.219
660.0	0.233	650.0	0.247
640.0	0.261	630.0	0.275
620.0	0.291	610.0	0.308
600.0	0.327	590.0	0.347
580.0	0.371	570.0	0.405
560.0	0.447	550.0	0.499
540.0	0.560	530.0	0.621
520.0	0.664	510.0	0.677
500.0	0.660	490.0	0.627
480.0	0.597	470.0	0.575
460.0	0.566	450.0	0.573
440.0	0.598	430.0	0.640
420.0	0.695	410.0	0.764
400.0	0.847		

xii) Enzymatic–assisted Extraction (Seed, Time= 3 hour)



Sample: Antioxidant Activity
File name: s_6_h003.UDS
Run Date: 07:53:18, 05/20/2014
Operator: KUKTEM
Comment: enzymatic extraction (seed 50 C)

Peak Integration

Method:	Rectangular
Sensitivity:	1
Threshold:	0.0100

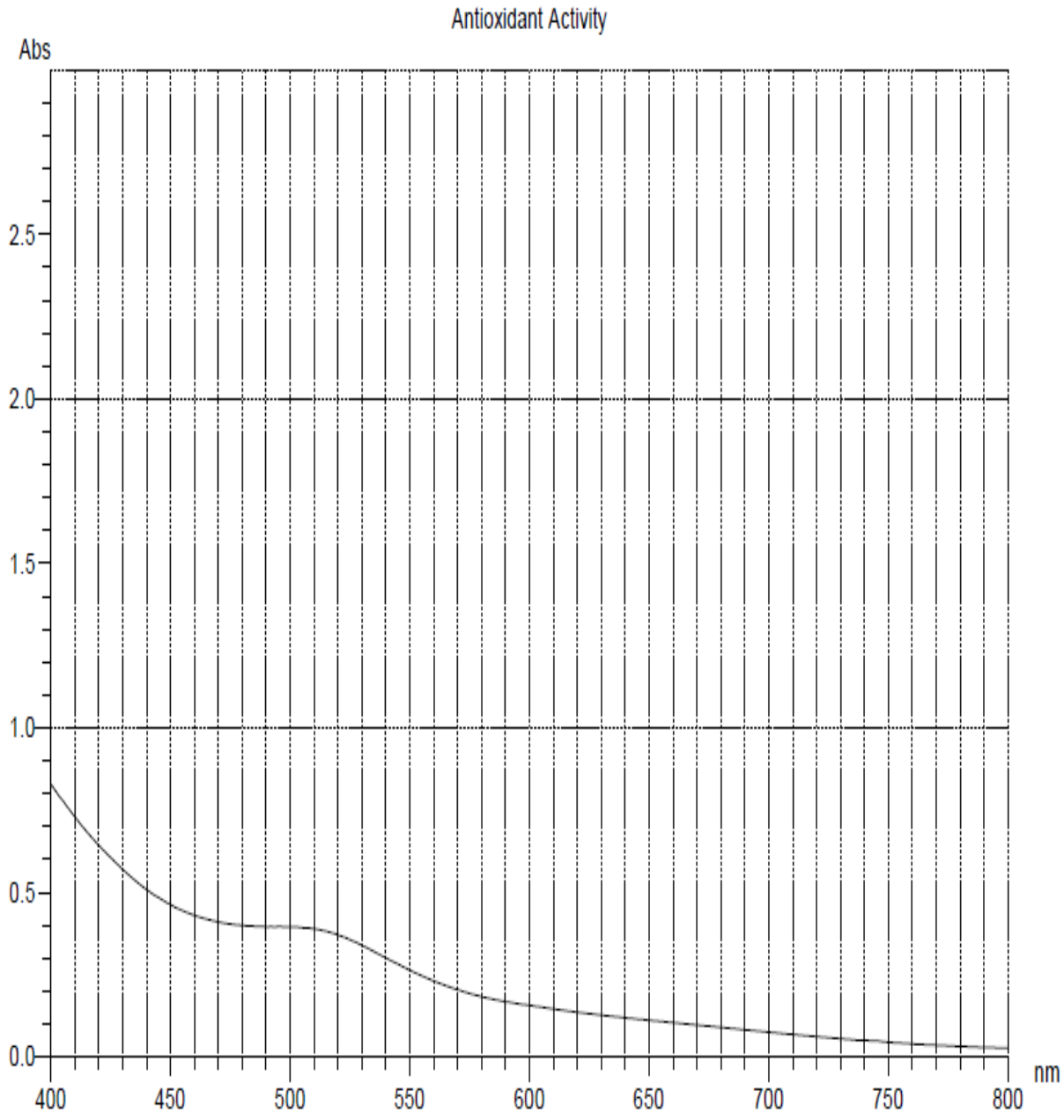
Peaks

Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	800.0	515.0	443.5	0.709	109.689	443.5	0.400

Data Points

nm	Abs	nm	Abs
800.0	0.018	790.0	0.024
780.0	0.031	770.0	0.040
760.0	0.050	750.0	0.062
740.0	0.076	730.0	0.090
720.0	0.106	710.0	0.124
700.0	0.142	690.0	0.160
680.0	0.177	670.0	0.194
660.0	0.211	650.0	0.227
640.0	0.243	630.0	0.260
620.0	0.277	610.0	0.296
600.0	0.317	590.0	0.337
580.0	0.364	570.0	0.403
560.0	0.452	550.0	0.515
540.0	0.587	530.0	0.658
520.0	0.702	510.0	0.702
500.0	0.656	490.0	0.588
480.0	0.522	470.0	0.466
460.0	0.426	450.0	0.404
440.0	0.402	430.0	0.417
420.0	0.441	410.0	0.473
400.0	0.518		

xiii) Enzymatic–assisted Extraction (Content, Time=6 hour)

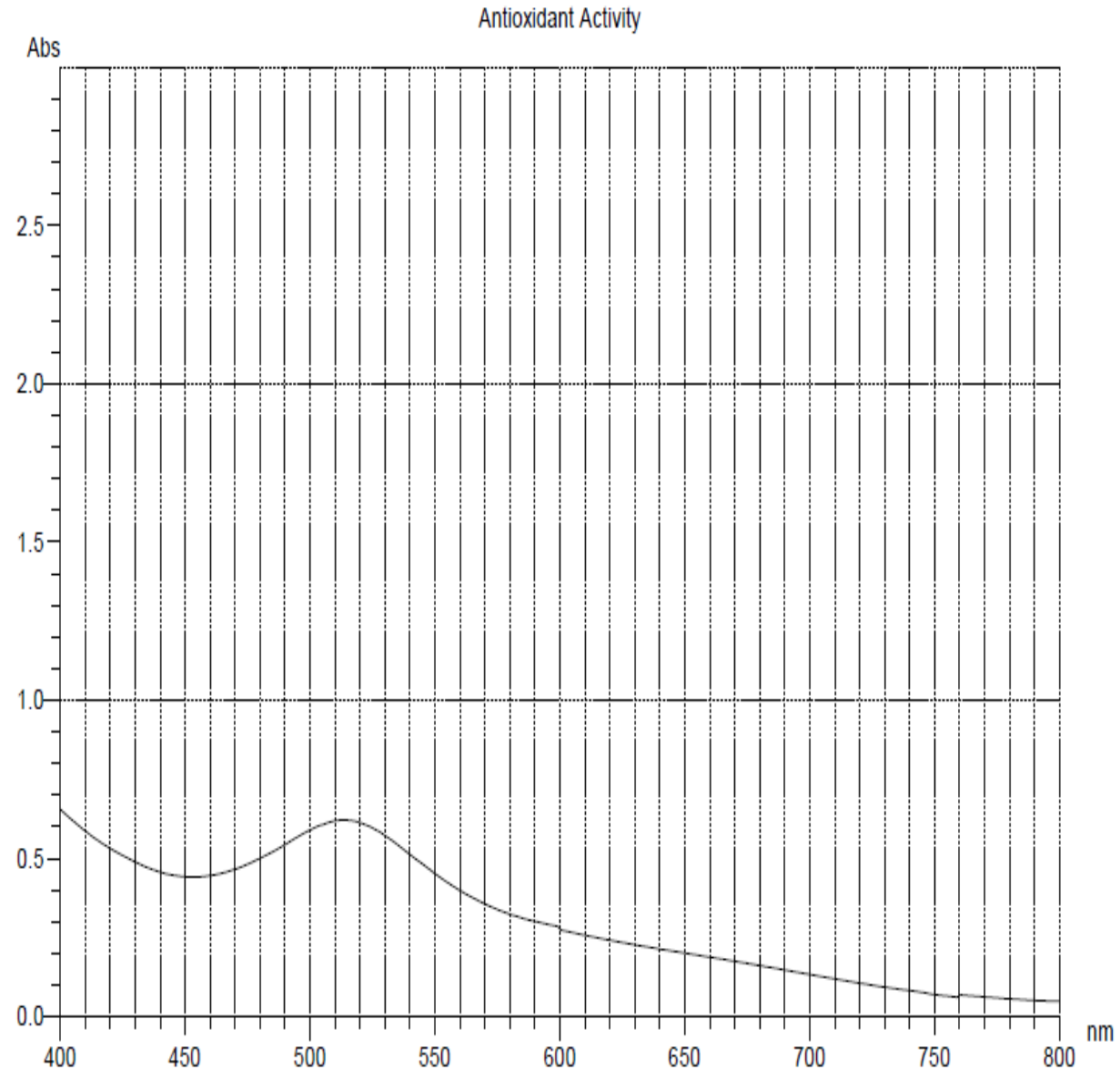


Sample:	Antioxidant Activity
File name:	s_6_h003.UDS
Run Date:	08:04:05, 05/20/2014
Operator:	KUKTEM
Comment:	enzymatic extraction (content 6 hour)

Data Points

nm	Abs	nm	Abs
800.0	0.027	790.0	0.028
780.0	0.032	770.0	0.035
760.0	0.039	750.0	0.044
740.0	0.050	730.0	0.055
720.0	0.061	710.0	0.068
700.0	0.075	690.0	0.082
680.0	0.089	670.0	0.096
660.0	0.103	650.0	0.111
640.0	0.118	630.0	0.126
620.0	0.135	610.0	0.145
600.0	0.156	590.0	0.167
580.0	0.182	570.0	0.204
560.0	0.230	550.0	0.263
540.0	0.300	530.0	0.339
520.0	0.370	510.0	0.389
500.0	0.394	490.0	0.395
480.0	0.399	470.0	0.410
460.0	0.430	450.0	0.462
440.0	0.508	430.0	0.570
420.0	0.643	410.0	0.730
400.0	0.828		

xiv) Enzymatic–assisted Extraction (Peel, Time=6 hour)



Sample: Antioxidant Activity
File name: s_6_h005.UDS
Run Date: 08:09:25, 05/20/2014
Operator: KUKTEM
Comment: enzymatic extraction (peel 6 hour)

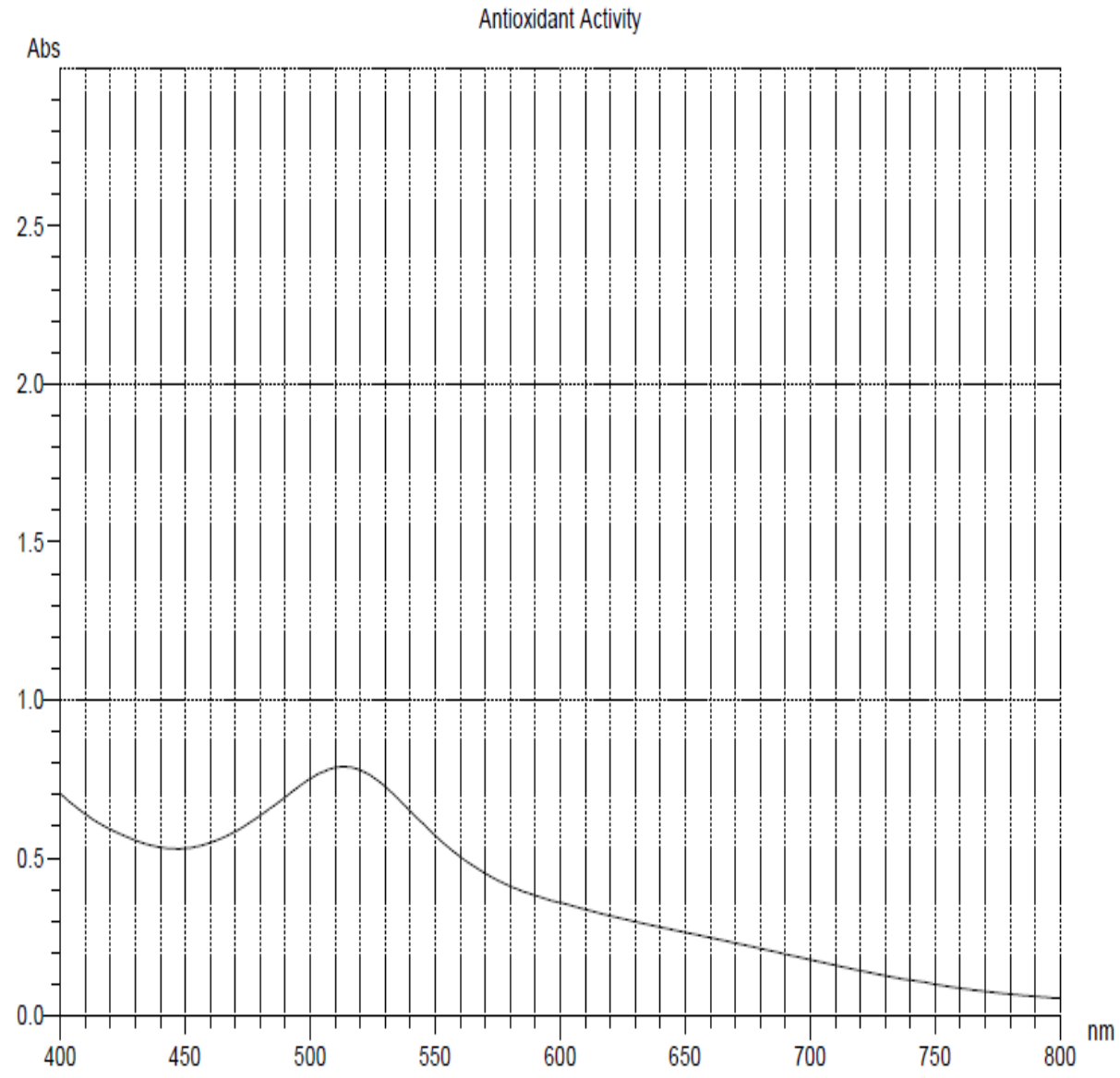
Peaks

Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	800.0	513.5	452.5	0.621	97.553	452.5	0.440

Data Points

nm	Abs	nm	Abs
800.0	0.048	790.0	0.051
780.0	0.056	770.0	0.062
760.0	0.067	750.0	0.069
740.0	0.082	730.0	0.093
720.0	0.105	710.0	0.119
700.0	0.133	690.0	0.147
680.0	0.161	670.0	0.175
660.0	0.187	650.0	0.200
640.0	0.213	630.0	0.227
620.0	0.241	610.0	0.257
600.0	0.276	590.0	0.300
580.0	0.323	570.0	0.356
560.0	0.398	550.0	0.452
540.0	0.512	530.0	0.572
520.0	0.612	510.0	0.618
500.0	0.589	490.0	0.543
480.0	0.500	470.0	0.465
460.0	0.445	450.0	0.442
440.0	0.457	430.0	0.488
420.0	0.531	410.0	0.586
400.0	0.654		

xv) Enzymatic–assisted Extraction (Seed, Time= 6 hour)



Sample: Antioxidant Activity
File name: s_6_h006.UDS
Run Date: 08:11:51, 05/20/2014
Operator: KUKTEM
Comment: enzymatic extraction (seed 6 hour)

Peak Integration

Method:	Rectangular
Sensitivity:	1
Threshold:	0.0100

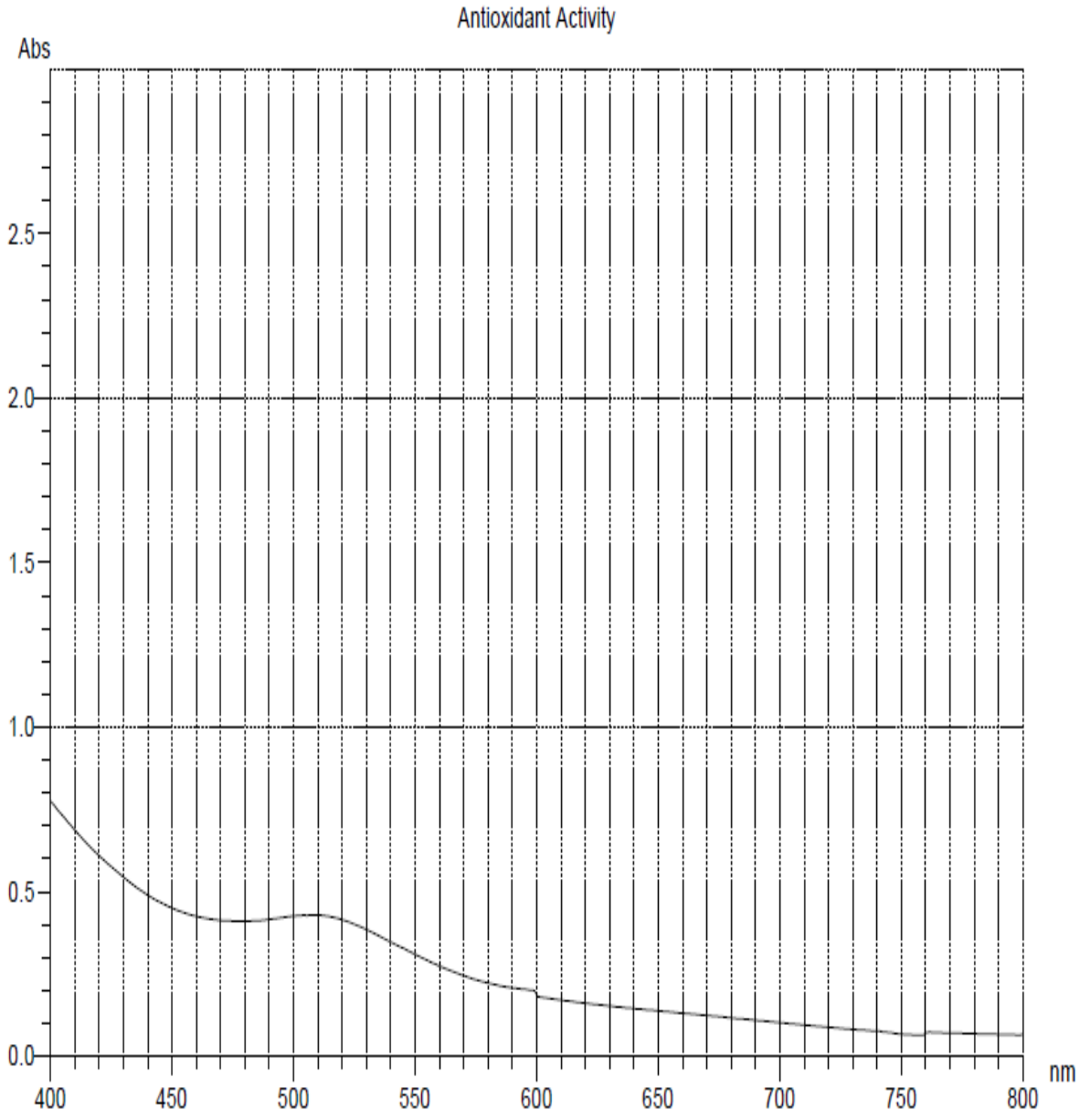
Peaks

Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	800.0	513.5	447.0	0.789	127.799	447.0	0.529

Data Points

nm	Abs	nm	Abs
800.0	0.056	790.0	0.061
780.0	0.068	770.0	0.077
760.0	0.087	750.0	0.100
740.0	0.113	730.0	0.127
720.0	0.143	710.0	0.160
700.0	0.177	690.0	0.195
680.0	0.213	670.0	0.230
660.0	0.247	650.0	0.264
640.0	0.281	630.0	0.298
620.0	0.316	610.0	0.337
600.0	0.358	590.0	0.380
580.0	0.410	570.0	0.451
560.0	0.503	550.0	0.570
540.0	0.648	530.0	0.725
520.0	0.777	510.0	0.786
500.0	0.751	490.0	0.692
480.0	0.634	470.0	0.583
460.0	0.548	450.0	0.530
440.0	0.533	430.0	0.555
420.0	0.590	410.0	0.638
400.0	0.702		

xvi) Enzymatic–assisted Extraction (Content, Time= 12 hour)



Sample:	Antioxidant Activity
File name:	s_6_h.UDS
Run Date:	08:18:23, 05/20/2014
Operator:	KUKTEM
Comment:	enzymatic extraction (content 12 hour)

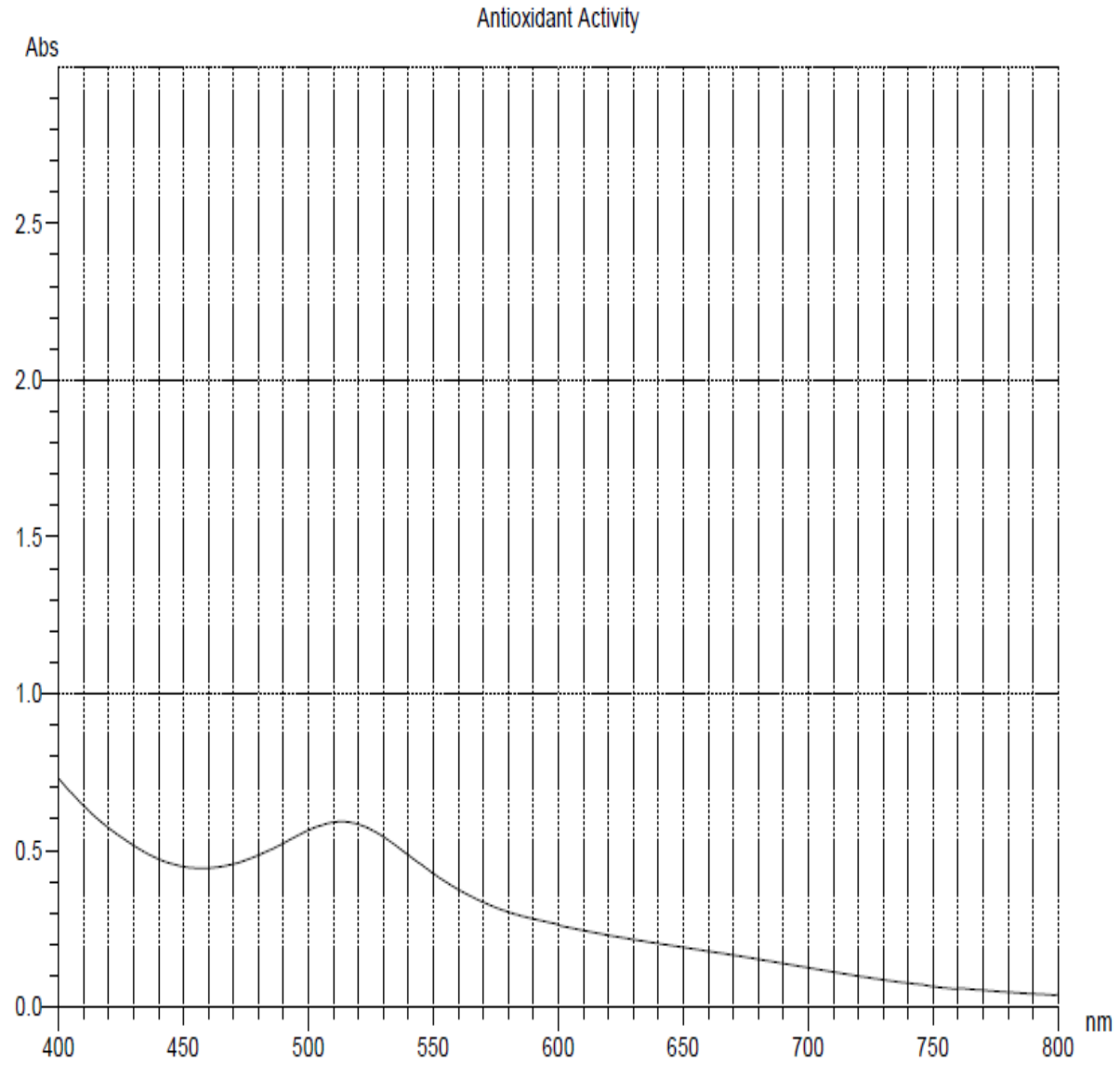
Peaks

Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	800.0	508.0	479.5	0.429	61.536	479.5	0.410

Data Points

nm	Abs	nm	Abs
800.0	0.065	790.0	0.066
780.0	0.068	770.0	0.070
760.0	0.070	750.0	0.067
740.0	0.076	730.0	0.081
720.0	0.088	710.0	0.094
700.0	0.101	690.0	0.109
680.0	0.116	670.0	0.123
660.0	0.130	650.0	0.137
640.0	0.144	630.0	0.152
620.0	0.161	610.0	0.170
600.0	0.185	590.0	0.207
580.0	0.222	570.0	0.245
560.0	0.274	550.0	0.309
540.0	0.347	530.0	0.384
520.0	0.414	510.0	0.428
500.0	0.425	490.0	0.415
480.0	0.410	470.0	0.413
460.0	0.425	450.0	0.450
440.0	0.490	430.0	0.544
420.0	0.609	410.0	0.687
400.0	0.775		

xvii) Enzymatic–assisted Extraction (Peel, Time= 12 hour)



Sample:	Antioxidant Activity
File name:	s_6_h001.UDS
Run Date:	08:20:41, 05/20/2014
Operator:	KUKTEM
Comment:	enzymatic extraction (peel 12 hour)

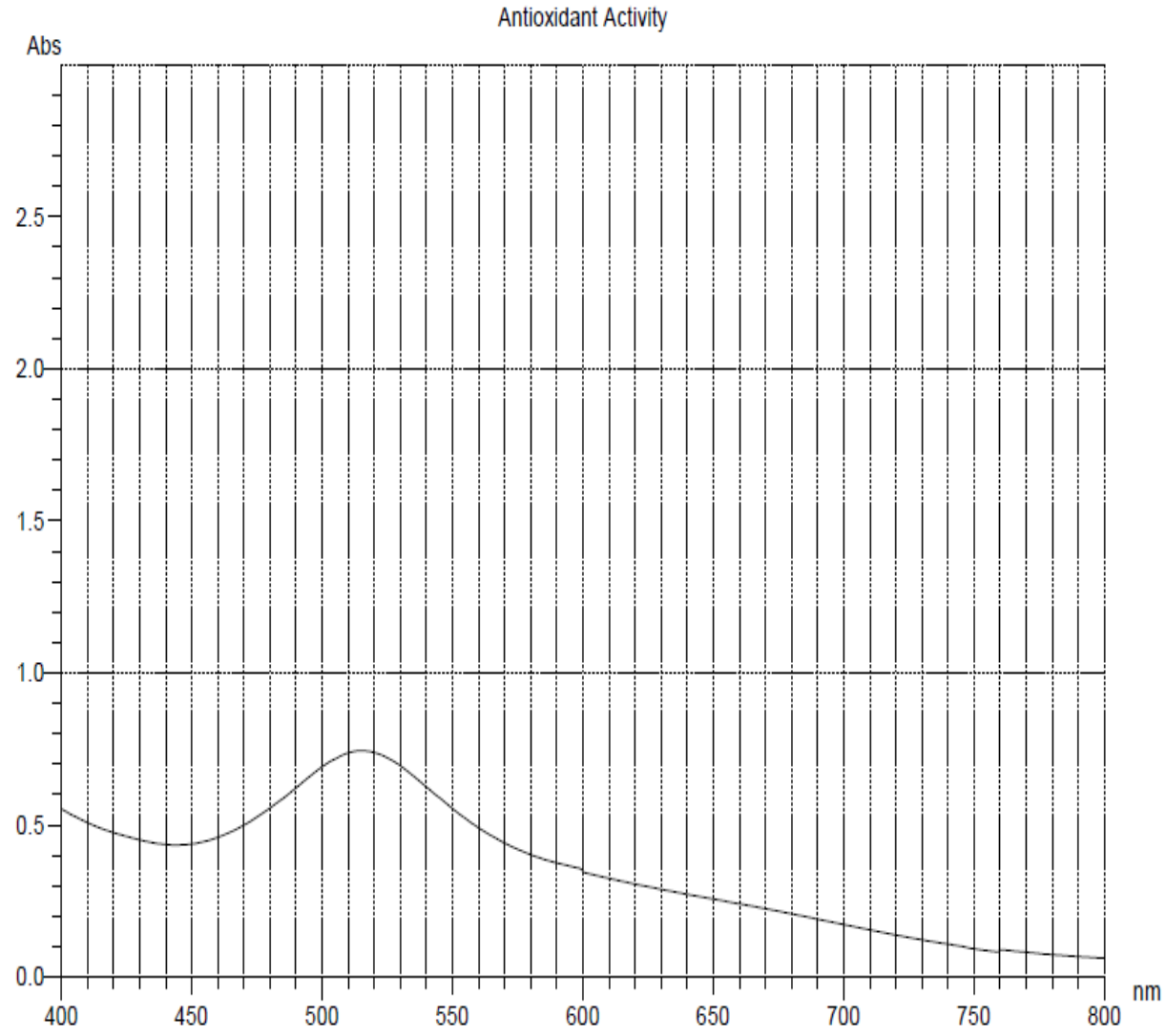
Peaks

Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	800.0	514.0	456.5	0.591	90.905	456.5	0.442

Data Points

nm	Abs	nm	Abs
800.0	0.039	790.0	0.042
780.0	0.047	770.0	0.053
760.0	0.059	750.0	0.065
740.0	0.076	730.0	0.087
720.0	0.098	710.0	0.112
700.0	0.125	690.0	0.139
680.0	0.152	670.0	0.165
660.0	0.178	650.0	0.190
640.0	0.202	630.0	0.215
620.0	0.229	610.0	0.244
600.0	0.261	590.0	0.280
580.0	0.303	570.0	0.334
560.0	0.374	550.0	0.426
540.0	0.484	530.0	0.543
520.0	0.582	510.0	0.589
500.0	0.564	490.0	0.522
480.0	0.484	470.0	0.456
460.0	0.443	450.0	0.448
440.0	0.472	430.0	0.515
420.0	0.571	410.0	0.642
400.0	0.727		

xviii) Enzymatic–assisted Extraction (Seed, Time= 12 hour)



Sample: Antioxidant Activity
File name: s_6_h002.UDS
Run Date: 08:23:11, 05/20/2014
Operator: KUKTEM
Comment: enzymatic extraction (seed 12 hour)

Peaks

Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	800.0	515.5	444.0	0.744	121.549	444.0	0.434

Data Points

nm	Abs	nm	Abs
800.0	0.063	790.0	0.067
780.0	0.073	770.0	0.081
760.0	0.088	750.0	0.092
740.0	0.108	730.0	0.122
720.0	0.137	710.0	0.155
700.0	0.172	690.0	0.190
680.0	0.207	670.0	0.224
660.0	0.240	650.0	0.256
640.0	0.272	630.0	0.288
620.0	0.305	610.0	0.324
600.0	0.347	590.0	0.375
580.0	0.402	570.0	0.440
560.0	0.490	550.0	0.554
540.0	0.625	530.0	0.694
520.0	0.738	510.0	0.737
500.0	0.691	490.0	0.622
480.0	0.556	470.0	0.499
460.0	0.459	450.0	0.437
440.0	0.436	430.0	0.451
420.0	0.474	410.0	0.507
400.0	0.552		

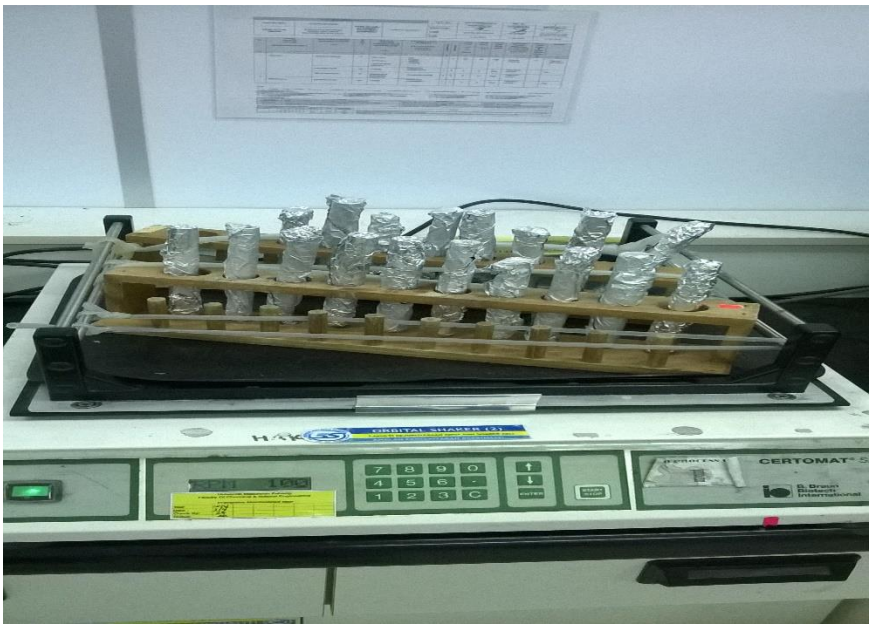


Figure A.3.1 Incubation of the Kundur sample with Folin–Ciocalteu reagent

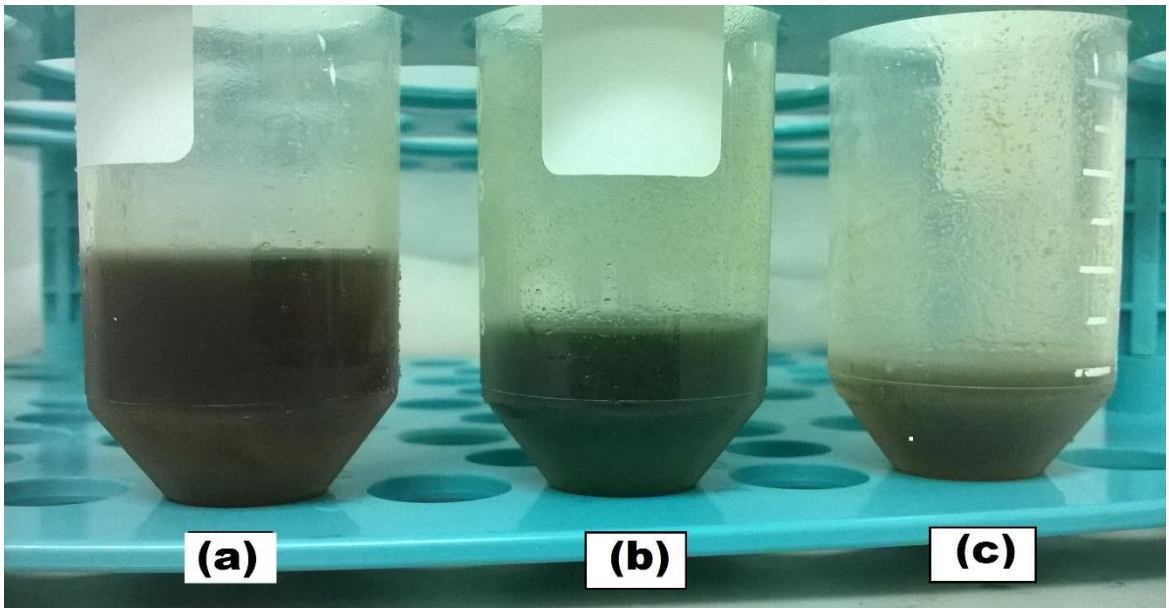


Figure A.3.2 Kundur extract oil from Soxhlet extraction (a) Content (b) peel (c) seed

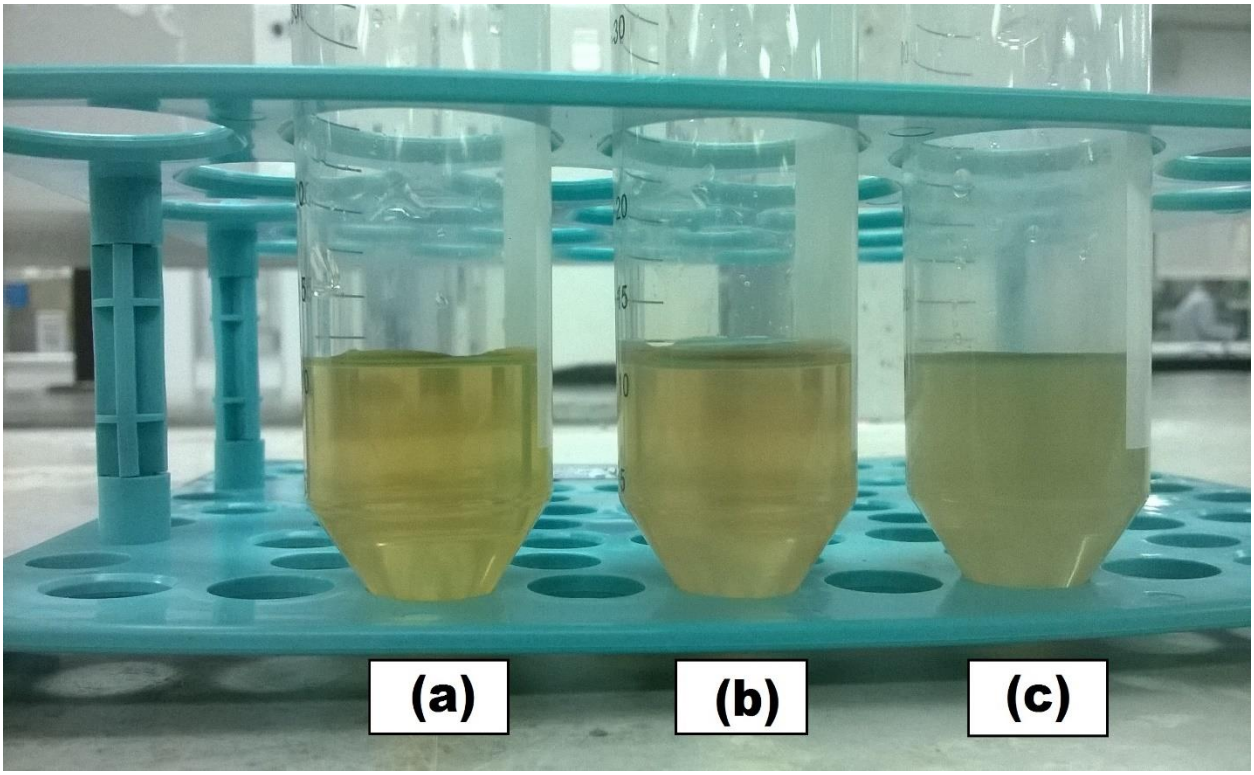


Figure A.3.3 Kundur extract oil from enzymatic extraction (a) Content (b) Peel (c) Seed

Figure A.3.4 Vacuum Filtration