EXTRACTION OF NIGELLA SATIVA USING MODERN HYDRO DISTILLATION TECHNIQUE

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EXTRACTION OF NIGELLA SATIVA USING MODERN HYDRO DISTILLATION TECHNIQUE

MOHD AIZUDIN BIN ABD AZIZ

A thesis submitted in fulfillment of the requirements for the award of the degree of Bachelor of Chemical Engineering

Faculty of Chemical and Natural Resources Engineering Technology
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NOVEMBER 2006

DECLARATION

"I declare that this thesis is the result of my own research except as cited references.
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In the memory of my dear mother Siti Rahani Bt. Amat

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ABSTRACT

Nigella Sativa (Jintan Hitam) can produce essential oil that is important in medicine and also important in developing our agriculture sector nowadays. To obtain *Nigella Sativa* essential oil, entrepreneur and researcher nowadays use hydro distillation method. So the objective of this study is to obtain essential oil from *Nigella Sativa* plant source using modern hydro distillation technique because of it market values, by investigating and understanding hydro distillation extraction process. For this research, the rotary evaporator will be use and the parameters which are expected to dominate in producing high yield of *Nigella Sativa* oil was the effect of time and the surface area of the Nigella Sativa seeds expose to the extraction process. The temperature for the extraction process is maintained at 100 ^{o}C and 1 bar for the pressure. It is expected that the optimum operating time will be establish from the experimental result. The main compound of the essential oil is Thymoquinone, which are around 50% from overall compounds.Therefore, the presence this compound should take as a characteristic for the essential oil and the essential oil will analyze using High Performance Liquid Chromatography (HPLC).

ABSTRAK

Jintan Hitam (*Nigella Sativa*) dapat menghasilkan pati minyak yang amat penting dalam bidang perubatan dan dalam bidang agrikultur pada hari ini. Untuk mendapatkan pati minyak tersebut, para usahawan dan penyelidik pada hari ini telah menggunakan kaedah penyulingan berair. Objektif kajian ini ialah untuk mendapatkan pati minyak disebabkan oleh harga pasaran yang tinggi. Ini dapat dilakukan dengan mengenalpasti dan memahami kaedah penyulingan berair. Untuk menjalankan eksperimen pada kali ini, alat yang akan digunakan ialah Peruap Berputar (rotary evaporator). Parameter yang dijangka akan mendominasi proses ini ialah pengaruh masa dan pengaruh luas permuakan biji yang terdedah kepada proses pengekstrakan. Suhu dan tekanan dikekalkan kepada 100 ^{o}C and 1 bar, dan masa optima untuk proses pengekstrakan akan dapat dikenalpasti daripada eksperimen ini. Komposisi utama minyak ini ialah Thymoquinone iaitu sebanyak 50 % daripada jumlah keseluruhan komposisi minyak. Oleh yang demikian, kewujudan komposisi ini boleh dikatogorikan sebagai karaktor utama kepada minyak yang akan dianalisa menggunakan Cecair Kromatogarm Keupayaan Tinggi (HPLC).

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CHAPTER 1

INTRODUCTION

1.0 Extraction

Extraction is phenomenon that can be defined as the process of separating desired components from a material. There are many types of extraction such as solid-liquid extraction and liquid-liquid. There are also a lot of extraction methods. Some of them are steam distillation, hydro distillation, solvent extraction, carbon dioxide extraction and cold pressing. The main objective in the extraction process is use to extract the essential oil. The main resources of essential oils are plants. In this research, the extraction will be conduct by using hydro distillation technique to extract essential oil from *Nigella Sativa seeds*.

1.1 Essential Oil

Essential oils or Volatile oils are the odorous principles found in various plant parts. It can be found in the bark of the plant, the flower of the plant or even in the seeds of the plant. Essential oils are oils that are found in bags inside these plants cell. These oils can be free from the bags and extracted using some of methods mentioned above. When they are exposed to air at ordinary temperatures they evaporate, therefore they are

called volatile oils, ethereal oils, or essential oils. The last term is applied because the oils represent the "essences" or odor constituents of the plants.

As a result, they are usually colorless, particularly when fresh, but with age they may oxidize and resinify, thus becoming darker, but sometimes the color of the oil is same with the material color. Therefore, storage should be in a cool, dry place, tightly stoppered, preferably full in amber glass containers. These essential oils have many uses. Most essential oils are produced for the perfume industry and minute amounts are used for flavors prepackaged foods. Besides that some essential oils that are produced from plants have high medical values in them like *Nigella Sativa* seeds.

1.2 Research Background / Problem Statement

In this research, Hydro Distillation Unit was used to get the *Nigella Sativa* essential oil. To obtain a series of high quality extraction from *Nigella Sativa*, the factors that influence the rate of extraction was study to get high quality of essential oil.

It is appropriate to improve the traditional hydro distillation method because of the energy wasting. The extraction using traditional hydro distillation method cannot give the highest purity and quality of Nigella Sativa Essential Oil. It is hard to determine the exact amount of the solvent. So the aiming of the research is to get the essential oil by using Rotary Evaporator representative the modern hydro distillation method. Figure 1.1 and 1.2 below show the traditional and modern hydro distillation technique[11] .

Figure 1.1: The Traditional Hydro Distillation Unit

Figure 1.2: The Modern Hydro Distillation Unit (rotary evaporator)

The Government wants to nurture the Malaysian herbal industry to be a leading international player. Therefore, the government had organized a lot of campaign and the latest one is "Celik Herba 2006 Campaign" and it was held on 19th to 20th September 2006 with FRIM collaboration. The local herbal industry is growing at an annual rate of between 15% and 20% and has a market value estimated at RM14 billion and will reach a size of RM24 billion by 2012[13].

Nowadays, in Malaysia, essential oil and oleoresin is gaining popularity as an herbal medication as it gave a lot of benefit to overcome the disease. It is because our people start to realize the important of healthy awareness. Hence, *Nigella Sativa* essential oil has a clear commercial value. So the identification of the constituents of *Nigella Sativa* has been carried out extensively over the last 10 years. Based on the knowledge on the constituent of our local *Nigella Sativa* and the potential commercial value essential oil as a whole, it is only appropriate if KUKTEM could further the research into producing and commercialize a new product from these invaluable herbs.

1.3 The Objective of the Research

To obtain essential oil from *Nigella Sativa* plant source using hydro distillation technique, competitive in terms of quantity and cost to essential oil produced by traditional methods, by investigating and understanding steam distillation extraction process.

1.4 The Scopes of the Research

To achieve the objective, scopes have been identified in this research. The scopes of this research are listed as below:-

1. To study the factor of influences of rate of extraction
    - Time
    - Surface area
2. To study the product analysis using HPLC

CHAPTER II

LITERATURE REVIEW

2.0 Overview of Nigella Sativa

Nowadays, the consumption of herbal based products are getting a wide spread acceptance among consumers because of the numerous beneficial therapeutic impacts they could give to our body and indirectly helps us sustaining a healthy condition. Although artificial and synthetic drugs are common in the market to combat a lot of chronic diseases, but these medications usually have negative effects to our body. Nowadays, people start to realize the differences between traditional and modern medication technique. Therefore, products from herbs, especially the Jintan Hitam (*Nigella Sativa)* are the right choice in treating certain kinds of ailments or diseases without introducing side effects to our body if consumed accordingly based on scientific findings and research. This is where our herbal products comes in which are confidently introduced to the public and backed by scientific research and findings from local and overseas scientist to prevent, reduce or to the extent in aiding the cure of certain types of chronic diseases or ailments.

The scientific term of Nigella Sativa have other synonyms such as Nigella damascena, Nigella ciliaris, Nigella arvensis and Nigella hispanica .Nigella Sativa is also known by its vernacular names such as Black Cumin (English), Cheveux de Vénus (France), Jinten hitam (Indonesia), Kalaunji (Punjabi), Habbet as-suda (Arabic), Jintan hitam (Malaysia), Hak jung chou (Chinese, Cantonese) and Thian dam (Thailand)[3]. This herb (Jintan Hitam known in Malaysia) is mentioned in the Quran, but today it is well known not only in Central and South Asia, but also in Western, its main application

area is Turkey, Lebanon and Iran. From Iran, nigella usage has spread to Northern India, particularly Punjab and Bengal

2.1 Characteristic of Nigella Sativa

Nigella Sativa is an annual flowering plant, native to southwest Asia. It grows to 20-30 cm tall, with finely divided, linear (but not thread-like) leaves. The flowers are delicate, and usually coloured pale blue and white, with 5-10 petals. The fruit is a large and inflated capsule composed of 3-7 united follicles, each containing numerous seeds. The seed is used as a spice [12]. Figure 2.0, 2.1 and 2.2 below show the flower, bud and the seeds of *Nigella Sativa*.

Figure 2.0: Nigella Sativa Flower

Nigella Sativa yield a seed capsule with five compartments each topped by a spike. The compartments open when dried to disperse the seeds. Nigella is native to western Asia where it grows both wild and cultivated. India, Egypt and the Middle East also cultivate it [12].

Figure 2.1: Nigella Sativa Unripe capsule

Nigella seeds are small, matte-black grains with a rough surface and an oily white interior. They are roughly triangulate, 1 1/2 - 3 mm (1/16 to 1/8 in) long. They are similar to onion seeds. The seeds have little bouquet, though when they are rubbed they give off an aroma reminiscent of oregano. It is also slightly bitter and peppery with a crunchy texture [12].

Figure 2.2: Nigella Seeds

2.2 Main constituents of Nigella Sativa

The seeds contain numerous esters of structurally unusual unsaturated fatty acids with terpene alcohols (7%); furthermore, traces of alkaloids are found which belong to two different types: isochinoline alkaloids are represented by nigellimin and nigellimin-*N*-oxide, and pyrazol alkaloids include nigellidin and nigellicin [14].

In the essential oil (avr. 0.5%, max. 1.5%), thymoquinone was identified as the main component (up to 50%) besides *p*-cymene (40%), α-pinene (up to 15%), dithymoquinone and thymohydroquinone. Other terpene derivatives were found only in trace amounts: Carvacrol, carvone, limonene, 4-terpineol, citronellol. Furthermore, the essential oil contains significant (10%) amounts of fatty acid ethyl esters. On storage,

thymoquinone yields dithymoquinonene and higher oligocondensation products (nigellone)[3].

The seeds also contain a fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50 – 60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%) which is characteristic for the genus. Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less. Commercial nigella oil ("Black Seed Oil", "Black Cumin Oil") may also contain parts of the essential oil, mostly thymoquinone, by which it acquires an aromatic flavour [3].

2.3 Uses and Benefit of Nigella Sativa

The seeds of the Nigella sativa plant are black in color and look something like sesame seeds. Both the seeds and oil from the seeds are used as a nutritional supplement. Black cumin seed is considered to have a number of beneficial properties when used as part of an overall holistic health program. Many studies show that, while black cumin seed is effective by itself, it is particularly potent when combined with other herbs in regimens used to treat specific ailments.

Black cumin seed (also referred to simply as "black seed") has been used as a nutritional supplement for centuries. Ancient traditions document the use of black cumin seed as an energy source, perhaps because of its rich nutritional value. The seeds are still believed to increase heat in the body, making metabolism more efficient. As a nutritional supplement in modern times, black cumin seed is used to treat respiratory conditions like bronchitis, asthma and emphysema. In addition, it is used to support stomach and

intestinal health as well as kidney and liver function. Black cumin seed is thought to have antihistamine-like properties that make it useful in treating congestion, and it is widely used as a general tonic to boost immune function and to help prevent cancer. Several skin conditions can be treated with black cumin seed, and it is also used to enhance circulation [5].

2.4 Overview of Separation Process

Separations are extremely important in Chemical manufacture. Separation processes are any set of operation that separate solutions of two or more components into two or more product that differ in composition. These may either removed a single components from a mixture or separate a solution into its almost pure components. This can be done by exploiting chemical and physical property differences between the substances through the used of a separating agent. There are three types of separation processes. 1st is gas –liquid separation, 2nd is liquid –liquid separation and 3rd is solid –liquid separation. In this research, solid-liquid separation processes will be conduct to separate the Nigella Sativa essential oil from the seeds [15].

2.5 Essential Oil Extraction Process

There are a lot of methods to extract the essential oils; the vast majority of true essential oils are produced by distillation. There are different processes used such as steam distillation, hydro distillation, cold pressing, vapor-cracking, turbo-extractor and many other solvent extractors. Hydro Distillation method will be use in this research because it’s very efficient and easy to conduct.

2.5.1 Hydro distillation

Hydro distillation is used in the manufacture and extraction of essential oils. The botanical material is immersed in the water then being boiled with the water. The hot water helps to release the aromatic molecules from the plant material since the hot water forces to break the pockets in which the oils are kept in the plant material. The molecules of these volatile oils then escape from the plant material and evaporate into the steam.

The temperature of the process needs to be carefully controlled - just enough to force the plant material to let go of the essential oil, yet not too hot as to burn the plant material or the essential oil. The steam which then contains the essential oil is passed through a cooling system to condense the steam, which form a liquid from which the essential oil and water is then separated.

During distillation, only very tiny molecules can evaporate, so they are the only ones, which leave the plant. These extremely small molecules make up an essential oil. Oils containing more of the smallest, and therefore most volatile of these tiny molecules, are termed ‘top notes’ in the perfumery world; those containing more of the heaviest and least volatile of the tiny molecules are called ‘base notes’. Those in between are known as middle notes[15].

2.5.2 Steam Distillation

Steam distillation is used in the manufacture and extraction of essential oils. The botanical material is placed in a still and steam is forced over the material. The hot steam helps to release the aromatic molecules from the plant material since the steam forces open the pockets in which the oils are kept in the plant material. The molecules of these volatile oils then escape from the plant material and evaporate into the steam.

The temperature of the steam needs to be carefully controlled - just enough to force the plant material to let go of the essential oil, yet not too hot as to burn the plant material or the essential oil. The steam which then contains the essential oil is passed through a cooling system to condense the steam, which form a liquid from which the essential oil and water is then separated.

During distillation, only very tiny molecules can evaporate, so they are the only ones, which leave the plant. These extremely small molecules make up an essential oil. Oils containing more of the smallest, and therefore most volatile of these tiny molecules, are termed ‘top notes’ in the perfumery world; those containing more of the heaviest and least volatile of the tiny molecules are called ‘base notes’. Those in between are known as middle notes [11].

2.6 *Nigella Sativa* Essential Oil Processing

2.6.1 Introduction to Rotary Evaporator

Rotary evaporators commonly found in organic laboratories. They are used to remove solvents from reaction mixtures and can accommodate large volumes of liquid. It is usually utilized to separate solvents such as n-hexane, acetone and ethanol from the essential oils produced in solvent extraction. Figure 2.3 below shows the technical specifications of rotary evaporator.

Rotary Evaporator	Water Bath
Speed range:20-190 rpm	Temperature range: ambient to 95°C
Vacuum: <1 mmHg	Capacity: 3.5 Liters
Lift distance: 150 mm	Heater power: 1300W
Dimension: (w x d x h) – 385 x 335 x 470 - 610mm, excluding glassware	Dimension: (w x d x h) – 260 x 280 x 200mm

Figure 2.3: Technical specifications of a rotary evaporator

Rotary evaporator has several parts. The main parts of a rotary evaporator include a water bath, a speed motor, a condenser and a vacuum supply. A typical rotary evaporator has a water bath that can be heated in either a metal container or crystallization dish to keep the solvent from freezing during the evaporation process. Water or silicon oil is used as the heating medium. Besides that, the evaporator normally uses a variable speed sparkles induction motor that spins at 0- 220 rpm and provides

high constant torque [12]. This enables the flask containing solution to rotate continuously according to the speed set as well as enhances the evaporation of solvent. Vacuum is used to evaporate the solvent while the condenser condenses the vapor trapped to liquid that is later collected for easy reuse or disposal. Rotary evaporator cannot be used for air and water-sensitive materials unless special precautions are taken.

A vacuum is usually applied to the setup and this shows that the boiling points of the solvents are going to be significantly lower than at ambient pressure. Since the flask is rotated during the evaporation process, the surface area is larger which increases the evaporation rate. These two factors combined make it a very useful tool in synthetic chemistry to remove solvents. Apart from that, the need for lower temperatures also avoids overheating of the target compounds [12]. Figure 2.3 and 2.4 below shows the whole part of rotary evaporator.

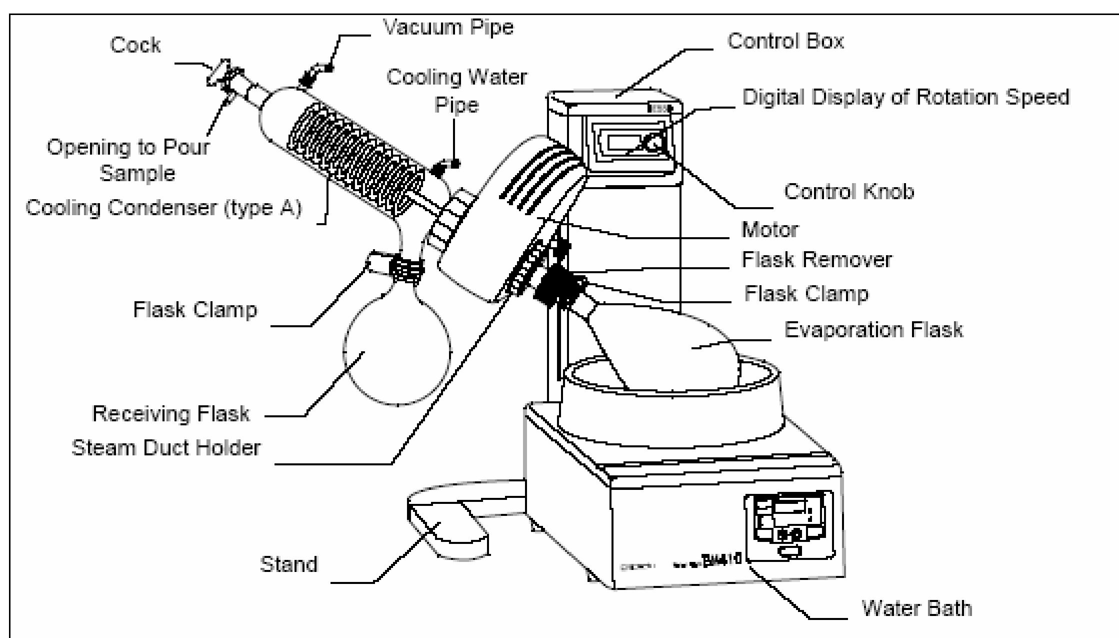

Figure 2.3: Rotary evaporator components

Figure 2.4: Rotary evaporator

2.7 *Nigella Sativa*: The Analysis

The most important part in research is analysis of the plant extracts. This is very important to make sure the product is in a good quality, and to make sure the extraction process is efficient to get the high quality product.

\

2.7.1 Separation and Purification Techniques

2.7.1.1 Gas Chromatography

Gas chromatography - specifically gas-liquid chromatography - involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

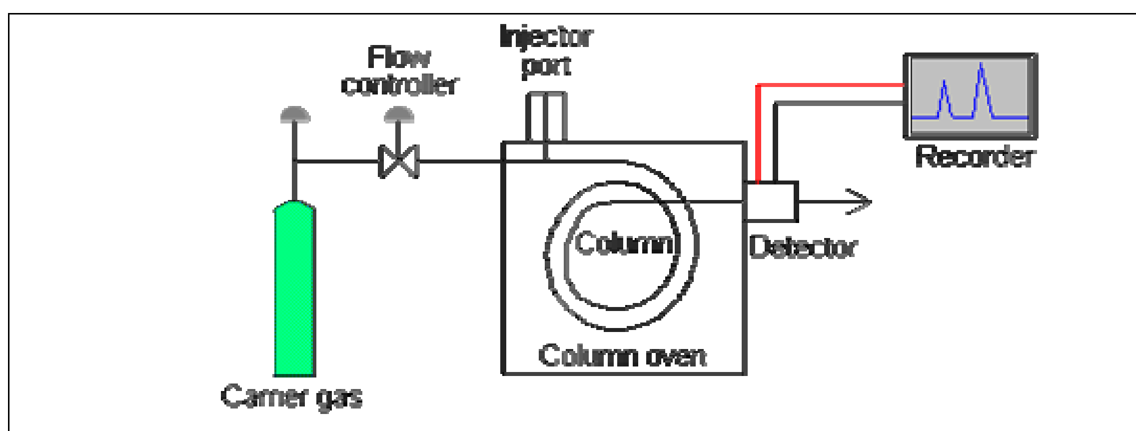

Figure 2.5: Schematic Diagram of a Gas Chromatography

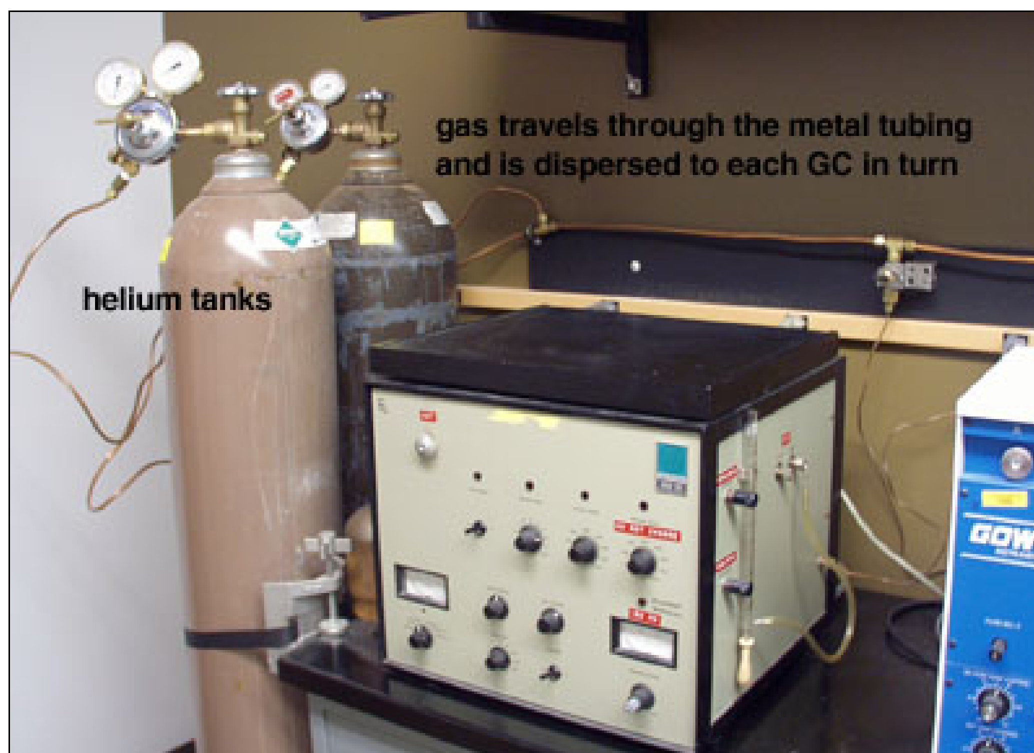

Figure 2.6: Gas Chromatography

2.7.1.1.1 Carrier gas

The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependant upon the type of detector which is used. The carrier gas system also contains a molecular sieve to remove water and other impurities [6].

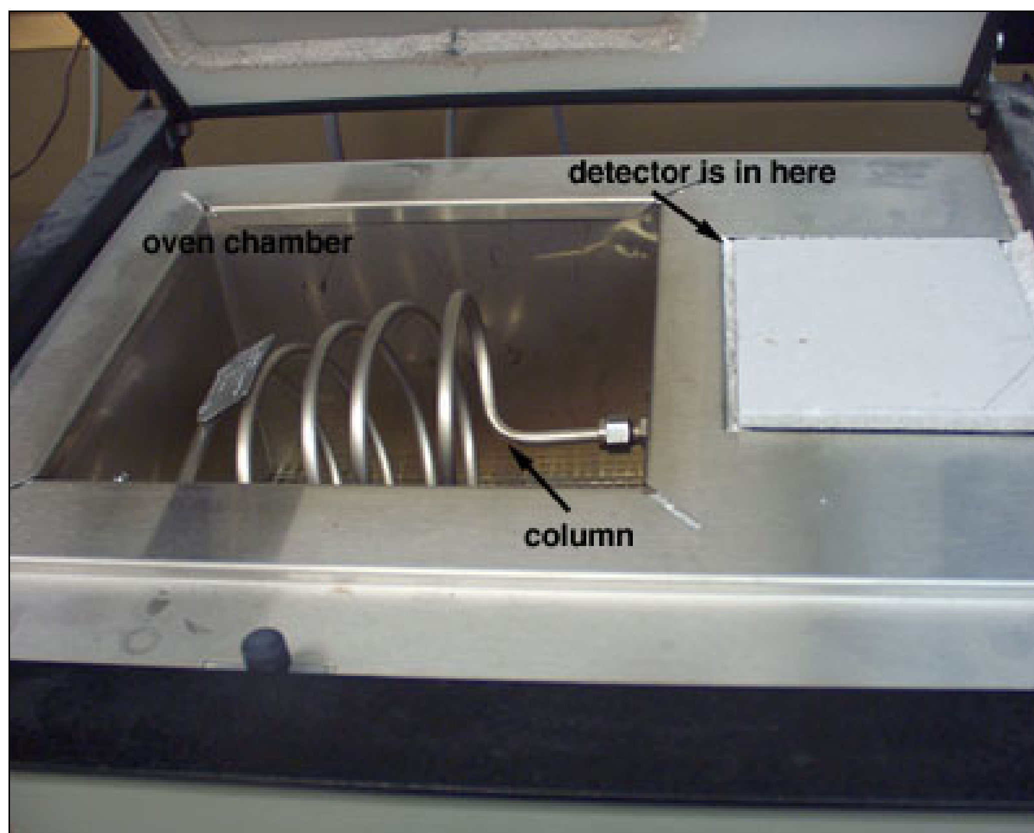

Figure 2.7: Inside of Gas Chromatography

2.7.1.1.2 Sample injection port

For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a "plug" of vapor - slow injection of large samples causes band broadening and loss of resolution. The most common injection method is where a micro syringe is used to inject sample through a rubber septum into a flash vaporizer port at the head of the column. The temperature of the sample port is usually about 50 C higher than the boiling point of the least volatile component of the sample. For packed columns, sample size ranges from tenths of a micro liter up to 20 micro

liters. Capillary columns, on the other hand, need much less sample, typically around 10^{-3} L. For capillary GC, split/split less injection is used.

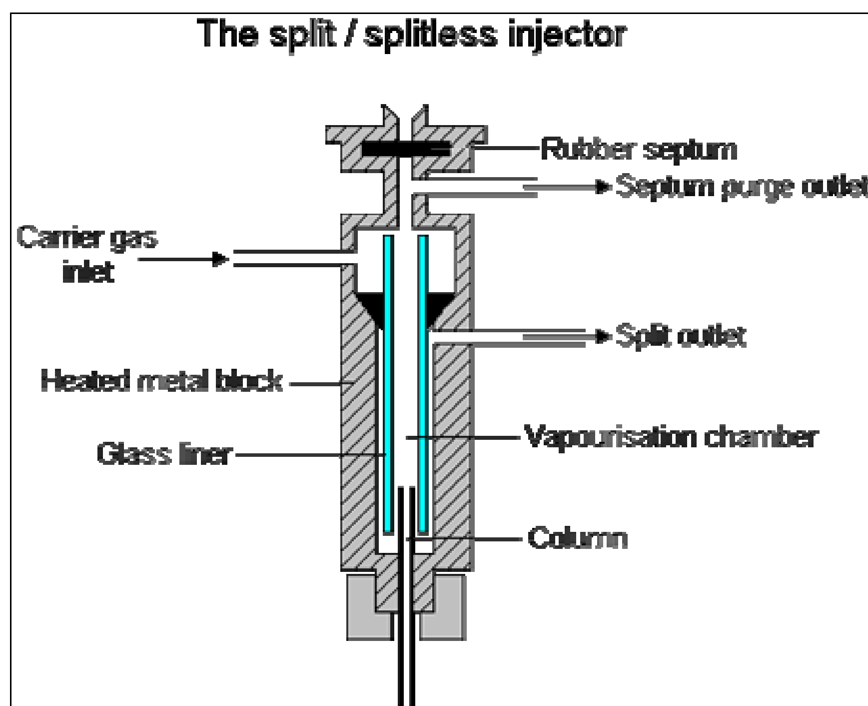

Figure 2.8: The Split Injector

The injector can be used in one of two modes; split or split less. The injector contains a heated chamber containing a glass liner into which the sample is injected through the septum. The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode). The sample vaporizes to form a mixture of carrier gas, vaporized solvent and vaporized solutes. A proportion of this mixture passes onto the column, but most exits through the split outlet. The septum purge outlet prevents septum bleed components from entering the column.

2.7.1.1.3 Columns

There are two general types of column, *packed* and *capillary* (also known as *open tubular*). Packed columns contain a finely divided, inert, solid support material (commonly based on *diatomaceous earth*) coated with liquid stationary phase. Most packed columns are 1.5 - 10m in length and have an internal diameter of 2 - 4mm.

Capillary columns have an internal diameter of a few tenths of a millimeter. They can be one of two types; *wall-coated open tubular* (WCOT) or *support-coated open tubular* (SCOT). Wall-coated columns consist of a capillary tube whose walls are coated with liquid stationary phase. In support-coated columns, the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.

In 1979, a new type of WCOT column was devised - the *Fused Silica Open Tubular* (FSOT) column.

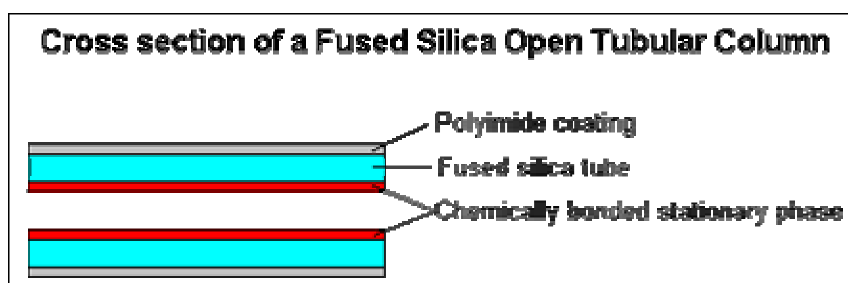

Figure 2.9: Cross Section of a Fused Silica Open Tubular Column

These have much thinner walls than the glass capillary columns, and are given strength by the polyimide coating. These columns are flexible and can be wound into coils. They have the advantages of physical strength, flexibility and low reactivity.

2.7.1.1.4 Column temperature

For precise work, column temperature must be controlled to within tenths of a degree. The optimum column temperature is dependant upon the boiling point of the sample. As a rule of thumb, a temperature slightly above the average boiling point of the sample results in an elution time of 2 - 30 minutes. Minimal temperatures give good resolution, but increase elution times. If a sample has a wide boiling range, then temperature programming can be useful. The column temperature is increased (either continuously or in steps) as separation proceeds.

2.7.1.1.5 Detectors

There are many detectors which can be used in gas chromatography. Different detectors will give different types of selectivity. A *non-selective* detector responds to all compounds except the carrier gas, a *selective detector* responds to a range of compounds with a common physical or chemical property and a *specific detector* responds to a single chemical compound. Detectors can also be grouped into *concentration dependant detectors* and *mass flow dependant detectors*. The signal from a concentration dependant detector is related to the concentration of solute in the detector, and does not usually destroy the sample Dilution of with make-up gas will lower the detectors response. Mass flow dependant detectors usually destroy the sample, and the signal is related to the rate

at which solute molecules enter the detector. The response of a mass flow dependant detector is unaffected by make-up gas. Have a look at this tabular summary of common GC detectors [6].

Detector	Type	Support gases	Selectivity	Detectability	Dynamic range
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic cpds.	100 pg	10^7
Thermal conductivity (TCD)	Concentration	Reference	Universal	1 ng	10^7
Electron capture (ECD)	Concentration	Make-up	Halides, nitrates, nitriles, peroxides, anhydrides, organometallics	50 fg	10^5
Nitrogen-phosphorus	Mass flow	Hydrogen and air	Nitrogen, phosphorus	10 pg	10^6
Flame photometric (FPD)	Mass flow	Hydrogen and air possibly oxygen	Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium	100 pg	10^3
Photo-ionization	Concentration	Make-up	Aliphatics, aromatics, ketones,	2 pg	10^7

(PID)			esters, aldehydes, amines, heterocyclics, organosulphurs, some organometallics		
Hall electrolytic conductivity	Mass flow	Hydrogen, oxygen	Halide, nitrogen, nitrosamine, sulphur		

Figure 2.10: Tabular Summary of Common GC Detectors

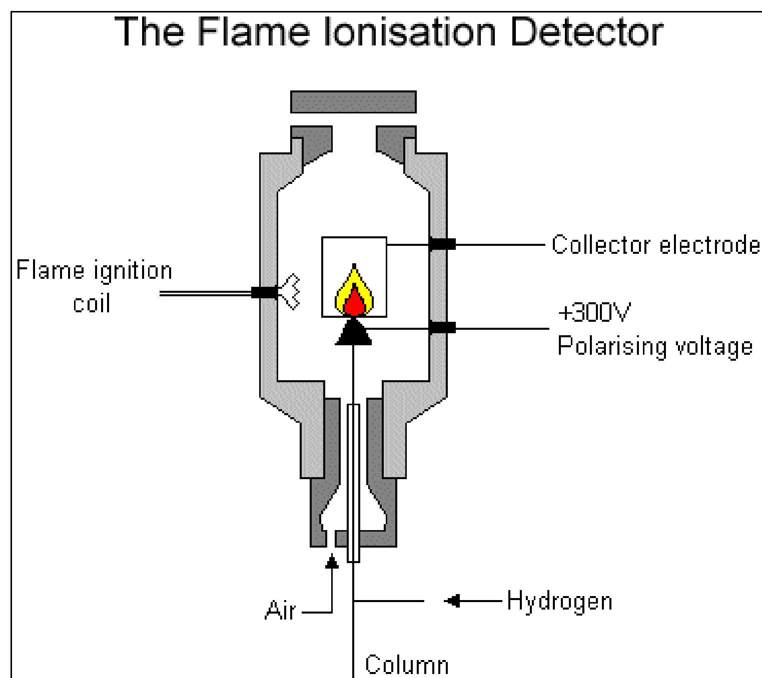

Figure 2.11: The Flame Ionisation Detector

The effluent from the column is mixed with hydrogen and air, and ignited. Organic compounds burning in the flame produce ions and electrons which can conduct electricity through the flame. A large electrical potential is applied at the burner tip, and a collector electrode is located above the flame. The current resulting from the pyrolysis of any organic compounds is measured. FIDs are mass sensitive rather than concentration sensitive; this gives the advantage that changes in mobile phase flow rate do not affect the detector's response. The FID is a useful general detector for the analysis of organic compounds; it has high sensitivity, a large linear response range, and low noise. It is also robust and easy to use, but unfortunately, it destroys the sample [6].

2.7.1.1.6 Factors Which Affect GC separations

Efficient separation of compounds in GC is dependent on the compounds traveling through the column at different rates. The rate at which a compound travels through a particular GC system depends on the factors listed below:

- **Volatility of compound**: Low boiling (volatile) components will travel faster through the column than will high boiling components
- **Polarity of compounds**: Polar compounds will move more slowly, especially if the column is polar.
- **Column temperature**: Raising the column temperature speeds up all the compounds in a mixture.
- C**olumn packing polarity**: Usually, all compounds will move slower on polar columns, but polar compounds will show a larger effect.
- **Flow rate of the gas** through the column: Speeding up the carrier gas flow increases the speed with which all compounds move through the column.
- **Length of the column**: The longer the column, the longer it will take all compounds to elute. Longer columns are employed to obtain better separation.

Generally the number one factor to consider in separation of compounds on the GCs in the teaching labs is the **boiling points of the different components** . Differences in polarity of the compounds is only important if you are separating a mixture of compounds which have widely different polarities. Column temperature, the polarity of the column, flow rate, and length of a column are constant in GC runs in the Organic Chemistry Teaching Labs. For each planned GC experiment, these factors have been optimized to separate your compounds and the instrument set up by the staff.

2.7.1.2 High Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by injecting a plug of the sample mixture onto the column. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase [7]. HPLC is a separation technique where solutes migrate through a column containing a micro particulate stationary phase at rates dependent on their distribution ratios. Solutes are transported through the column by a pressurized flow of liquid mobile phase, and are detected as they are eluted. It consists of 5 major components:

- solvent delivery system
- sample injection valve
- column
- detection and recording system
- microcomputer with control and data-processing

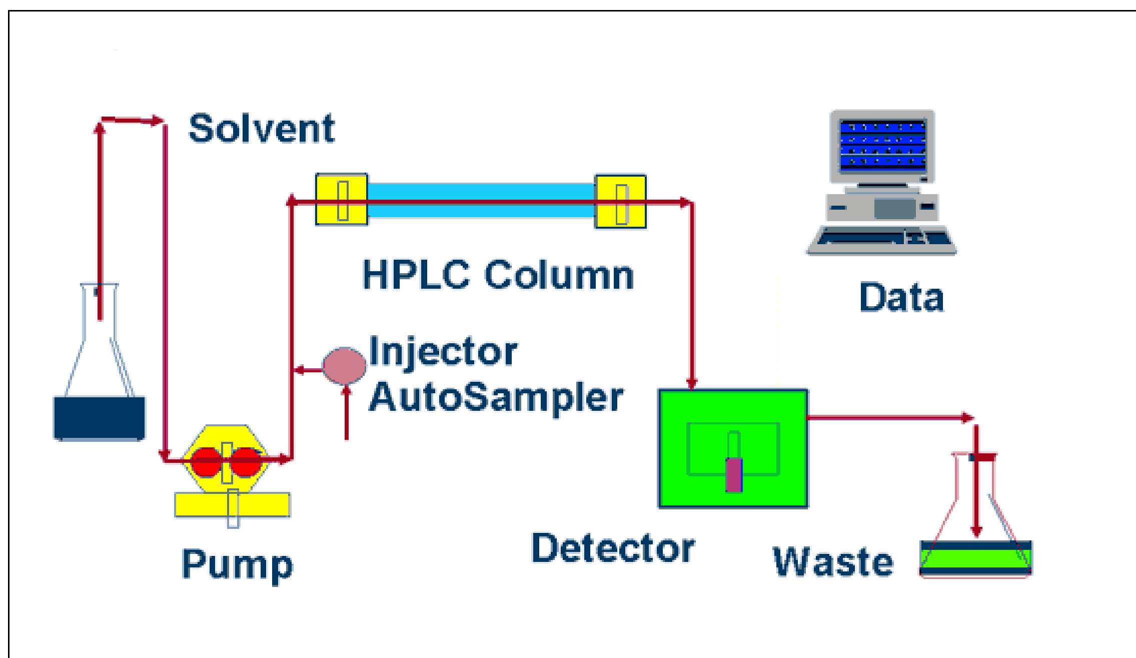

Figure 2.12: Schematic Diagram for HPLC

Figure 2.13: High Performances Liquid Chromatography (HPLC)

CHAPTHER III

METHODOLOGY

3.0 Introduction

Base on study, the best available technique in extracting the *Nigella Sativa* essential oil is by using hydro-distillation unit. The study stated that this method can protect the oils so extracted to a certain degree since the surrounding water acts as a barrier to prevent it from overheating. Condensed material, the water and essential oil extracted then can be easily separated using the oil decanted because of its density differentiation. Based on the literature, there are three methods that have been developed before doing an extraction. They are also known as pre-treatment process, which are drying, grinding and soaking, this all is conclude in sample preparation of dried *Nigella Sativa Seeds*.

3.1 The Overall Methodology

The overall methodology involved all the steps in achieving Nigella Sativa essential oil. The whole study is divided into three major sections:

i.) Sample preparation of dried Nigella Sativa Seeds.
ii.) Nigella Sativa oil extraction

iii.) Analysis

3.2 Sample Preparation of Dried Nigella Sativa Seeds

3.2.1 Drying Process

1st step of sample preparation is dried The Nigella Sativa using tray dryer, type Guntt Hamburg CE130 in our lab to remove the moisture from the seeds. The dried seeds at this stage had reached its constants weight. The constant weight is the residual moisture in the Nigella Sativa seeds.

The percent of moisture lost (ML) of seeds determine by the following formula:

$$ML\ (\%) = \frac{\text{Current weight of sample (g)} - \text{Initial weight of sample (g)}}{\text{Initial weight (g)}} \times 100\%$$

Figure 3.2 Tray dryer types Guntt Hamburg CE130

3.2.2 Grinding Process

After that, the seeds of *Nigella Sativa* are ground until the size become 0.01mm. In leaching process, the rate of extraction is increase when the area of contact between the steam or hot water and solid is high. So, the higher surface area of Nigella Sativa grinded seeds, more Nigella Sativa essential oil can be extracted. Below is the grinder that is use in grinding process.

Figure 3.1 Grinder types Disk Mill FFC23

Nigella Sativa is then packed in seal bag so it can be weight and labeled for easier use in the experiment.

3.3 Extraction Process

The apparatus for the experiment is set up (rotary evaporator). Then the raw materials are gathered. Then the Nigella Sativa seeds are grind until fine using the grinder. Then, the grinded seeds immerse with water in the rotary flask. Then rotary flask immerse in the hot water bath contain glycerol or thermal oil for the heat transfer. The experiment is then run. This causes a vapor mixture of water and essential oil. Then, this is vapor condenses in the condenser creating back water and essential oil. The essential oil will float above water and thus it is collected. The oil is then sent for high performance liquid chromatography (HPLC) sampling.

Figure 3.3: Rotary Evaporator

3.4 Data Collecting

Through all of the manipulated parameters, yield of the essential oil is collected in every parameter that been studied. The yield of essential oil is calculated using equation below:

$$\text{Oil Yield (\%)} = \frac{\text{Weight of Oil Extracted (g)}}{\text{Dry Weight Sample (g)}} \times 100$$

The product collected is labeled so that the analysis can be done. Graph of essential oil yield (%) versus sample collected also can be plotted to show at what condition in term of time of extraction and soaking different method that give high yield.

3.5 Summary of methodology

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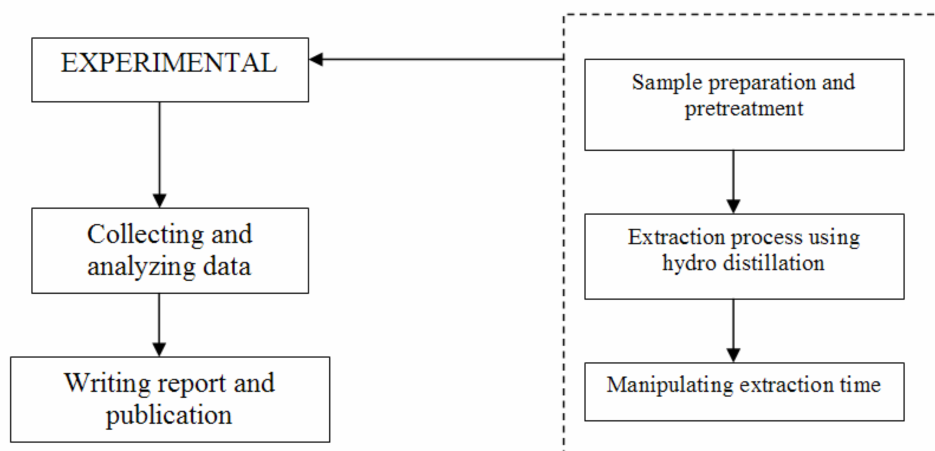

Figure 3.4: Flow chart of research methodology

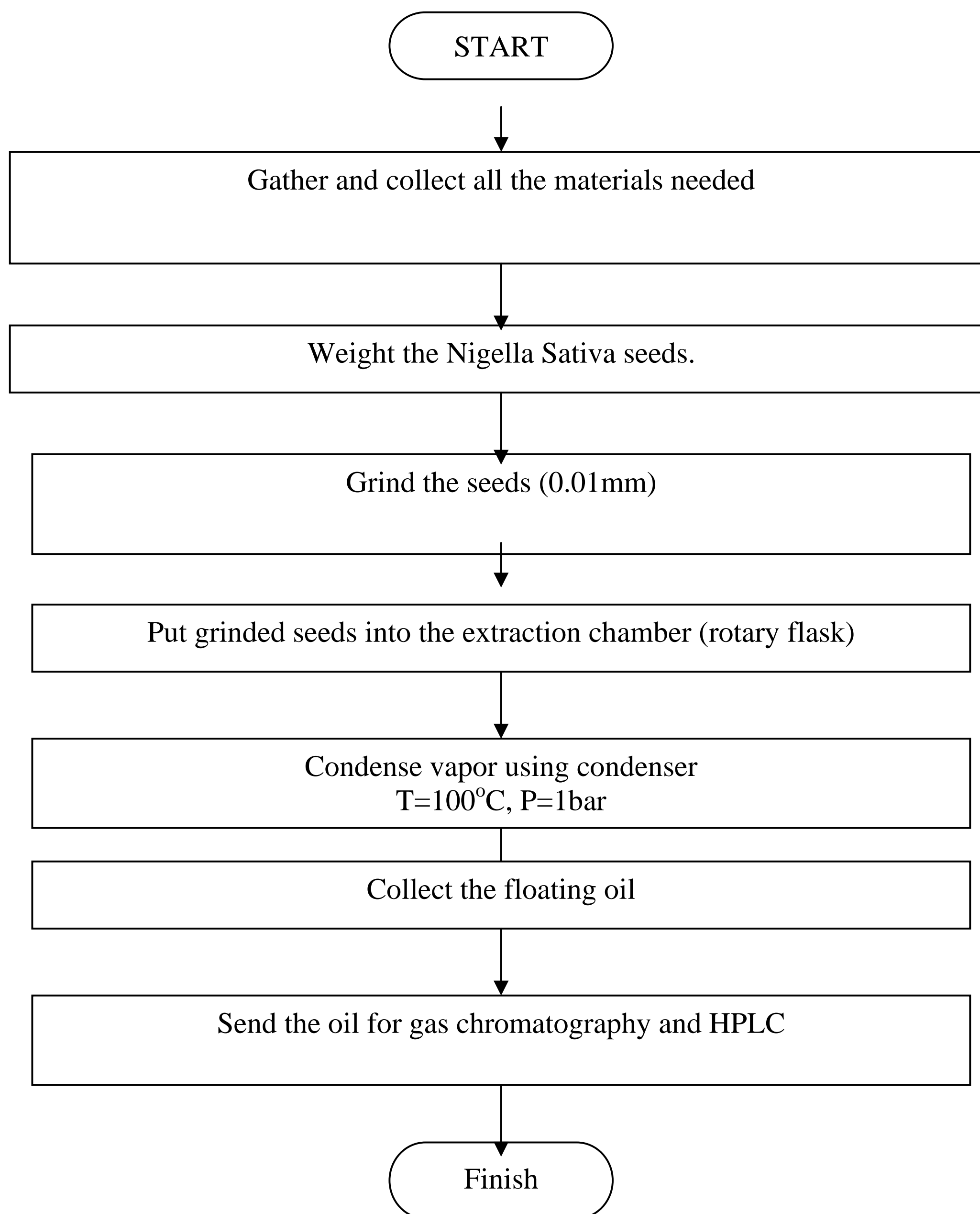

Figure 3.5: Flow Diagram for Nigella Sativa Oil Extraction Process

3.6 HPLC Analysis of Black Seed Oil

3.6.1 Apparatus

An HPLC system (Agilent Series) composed of a model 5700 solvent delivery system and a model 7125 sample injector connected in series to a model 480 lambda variable wavelength UV detector was utilized. The output signals were monitored on a model 3392A reporting integrator. Extractions were conducted using C18 PrepSep solid phase extraction columns. GC is also used to analyze the chemical compositions.

3.6.2 Column

A C18 reversed-phase Micro-Bondapak analytical column connected to a C18 reversed-phase guard column was used in all HPLC studies. As for GC, 30 m x 0.25 mm with Internal Diameter of 0.25 mm is used in all GC studies with helium as the carrier gas.

3.6.3 Chromatographic Conditions

The isocratic mobile phase utilized was composed of water: methanol:2-propanol and was filtered through a 0.45 micrometer Millipore filter and daerated before use. Analyses was performed at room temperature. UV monitoring of the eluted solutes was carried out at 254 nm for Thymoquinone. A flow rate of 2.0 ml min^{-1} was used. Prior to analysis, precautions were taken to assure stability of the analysis samples, which are light and heat sensitive, since quinines of this type undergo facile formation of radicals when when exposed to light. Thus, immediately after preparation, vials containing seed oil extract were refrigerated and covered by aluminum foil, to protect from light. Under these conditions the extracts were stable for at least 2 months.

3.6.4 Calibration curves

Calibration curves of peak area ratios obtained by co-injecting different quantities of each analyte with a constant amount of diphenyl sulfone, the calibration standard. At least three analyses were utilized for each point on the calibration curve, and each calibration curve had at least four points; all curves had $r^2 > 0.99$. The limit of detection for each of the analytes was carried out based on the study by Omar A. Ghosheh et. Al.

3.6.5 Purification procedure

N. Sativa seed oil was purified by passing the oil through a C18 PrepSep solid phase extraction column (preeluted with methanol) prior to HPLC analysis; 20 microliter samples of seed oil followed by 800 microliter of methanol were passed through the column to afford an eluate free from greasy and fatty materials. The calibration standard, DPS, was added to the PrepSep eluate, and 20microliter was injected onto the HPLC

column. The recovery from the extraction procedure was carried out for different amounts of each pure analyte. The recovery was determined by eluting 20microliter of a methanolic solution of each pure authentic analyte through the solid phase extraction column with 800 microliter of methanol eluent. To an accurate volume of the resulting eluate was added an accurate amount of DPS as the calibration standard and 20 microliter of this solution was injected onto the HPLC analytical system. The results were then compared to the values obtained using a similar procedure in which the solid phase extraction was omitted. Recovery was found to be > 95% for all analytes over the mass range used.

CHAPTHER IV

RESULT AND DISCUSSION

4.0 Introduction

After completing 5 month of research period, the scheduled experiments were accomplished, producing some important data to be analyzed, such as quantity of oil that obtain from the grinded (0.01 mm) and not grinded *Nigella Sativa Seeds* and also identification of the optimum time to obtain maximum quantity of oil. Figure 4.0 below show the *Nigella Sativa* essential oil after the experiment has been done

Figure 4.0: *Nigella Sativa* oil (grinded seeds)

4.1 Experiment 1 : Comparison on The Extraction Time for Grinded Seeds

After the sample has been prepared, experiment has been done to find the optimum time to obtain maximum quantity of oil. The extraction using rotary evaporator was done in 6 trials. Table 4.0 below show the result:

Time (h)	Raw Material Quantity (g)	Essential oil Quantity (ml)	Yields
5	200g + 40ml water	3.9	2.028
6	200g + 40ml water	9.6	4.992
7	200g + 40ml water	9.2	4.784
8	200g + 40ml water	10.2	5.304
9	200g + 40ml water	10.6	5.512
10	200g + 40ml water	10.6	5.512

Table 4.0: Grinded seeds (0.01mm) using rotary evaporator

The yields of seeds determine by the following formula:

$$\text{Yields} = \frac{\text{Essential oil quantity (ml) x Density of oil at 100°C (g/ml)}}{\text{Raw Material weight (g)}}$$

The table above describes the difference in quantity of the oil produce due to the time of the extraction. The result shows that, 9 hours of extraction time is the optimum time to get the maximum quantity of the oil. After that, the quantity of the oil that obtain is seem to be constant. The table also will produce yield graph base on 1.04 g/ml density of the oil at 100 °C and 1atm. Therefore figure 4.1 and 4.2 below show the yield graph of the *Nigella Sativa* oil extraction versus time.

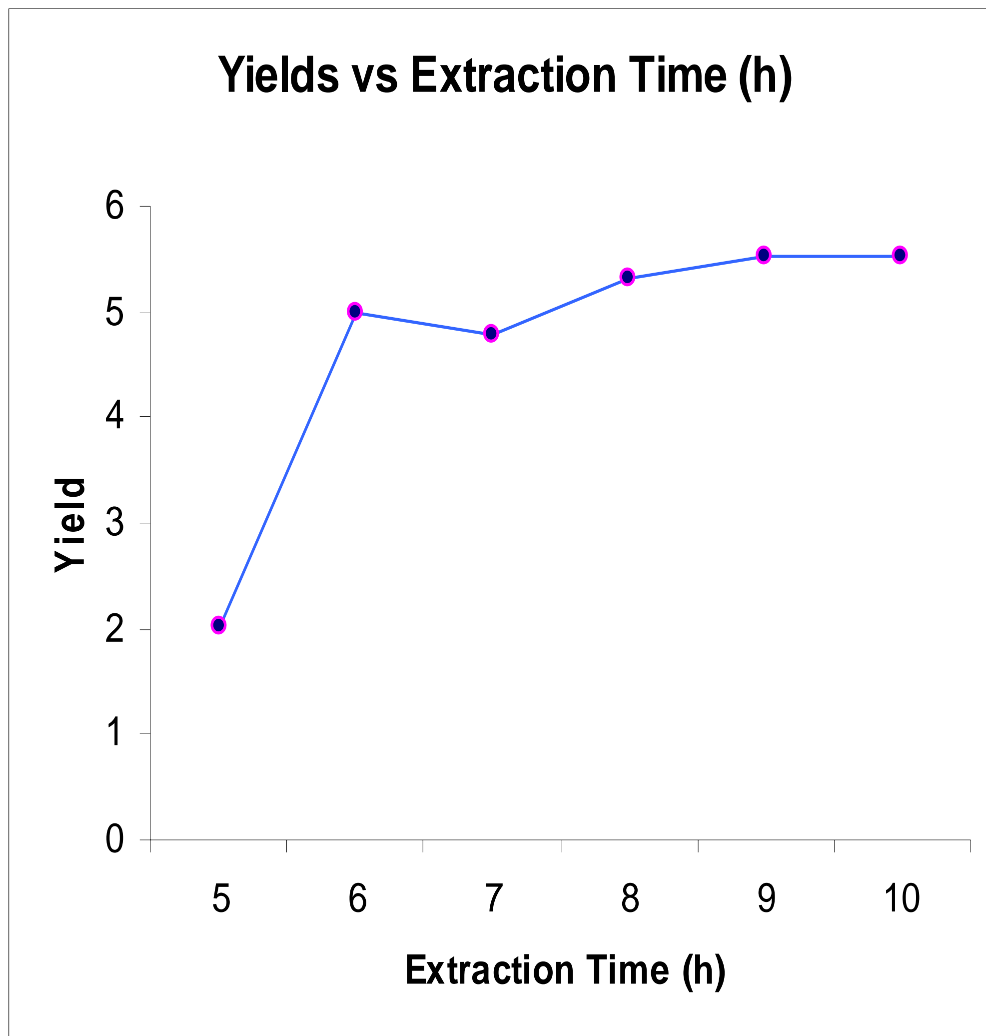

Figure 4.1: Yields of *Nigella Sativa* oil (grinded sample) versus Extraction time (hour)

4.2 Experiment 2 : Comparison on The Extraction Time for Non Grinded Seeds

After the sample has been prepared, experiment has been done to find the optimum time to obtain maximum quantity of oil for non grinded seeds. The extraction using rotary evaporator was done in 6 trials. For the last trial, it will take 10 hours as extraction time to find whether it still have essential oil or not. Table 4.1 and Figure 4.2 below show the result:

Time (h)	Raw Material Quantity (g)	Essential oil Quantity (ml)	Yields
5	200g + 40ml water	2.1	1.092
6	200g + 40ml water	5.8	3.016
7	200g + 40ml water	5.5	2.86
8	200g + 40ml water	6.3	3.276
9	200g + 40ml water	6.8	3.536
10	200g + 40ml water	6.8	3.536

Table 4.1: Non Grinded seeds (0.01mm) Using rotary evaporator

The table above describes the quantity of Nigella Sativa essential oil produce depend on time of the extraction for non grinded sample. The result shows that, 9 hours is the optimum time to get the maximum quantity of the oil. After that, the quantity of the oil that obtain is seem to be constant. The table also will produce yield graph of the Nigella Sativa essential oil at temperature 100 °C and pressure 1 atm.

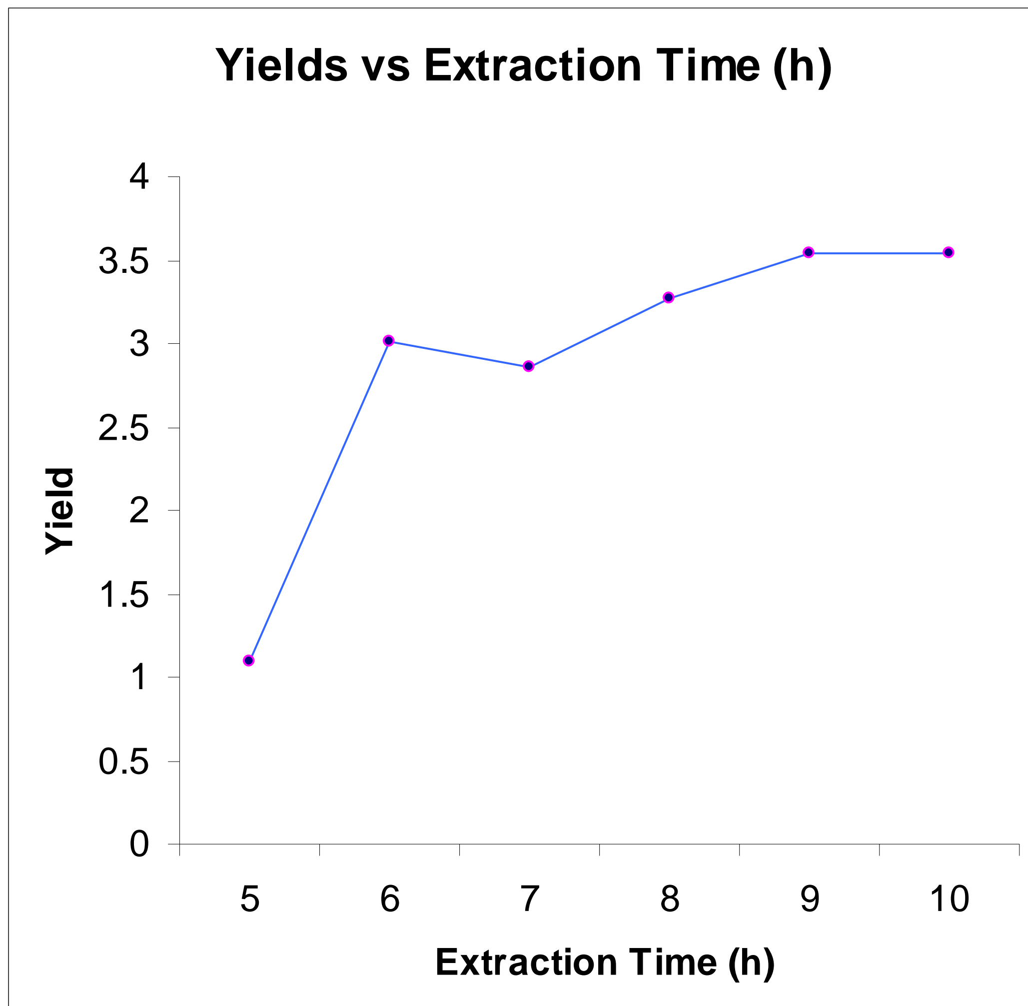

Figure 4.2: Yields of *Nigella Sativa* oil (non grinded sample) versus Extraction time (hour)

4.3 Comparison between Grinded Sample (0.01mm) and Non Grinded Sample

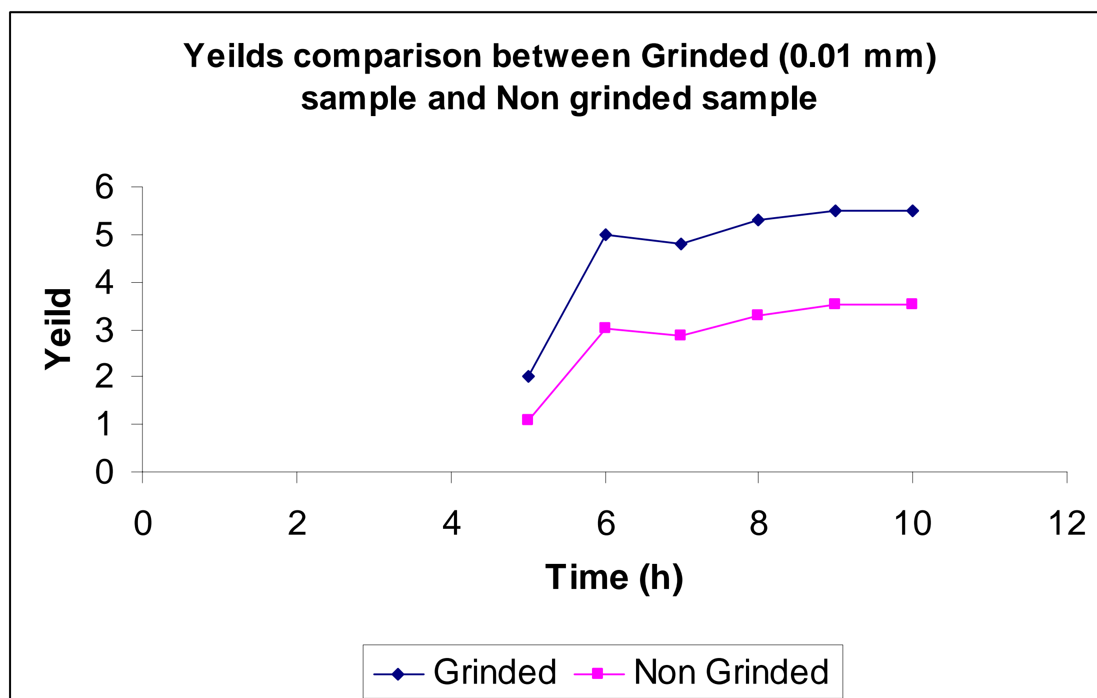

Figure 4.3: Yields comparison between Grinded and Non Grinded Sample

The time and quantity of raw material for experiment 2 will be the same as in the experiment 1. From the graph, we can see that there are different between Grinded Sample (0.01mm) and Non Grinded Sample. We can conclude that Grinded sample particle size have high yield and can produce more essential oil rather than non grinded sample. It is because the factor of particle size gives a major effect in the production of Nigella Sativa essential oil. When the particle is small, the surface area that expose to the extraction process is more compare to the large particle, so that it can breaks up the bags of the seeds and release the essential oil. As result, it will produce more essential oil rather than non grinded sample.

4.4 Analysis results of Black Seed Oil

Black Seed Oil should contain a major amount of Thymoquinone. After the extraction process, HPLC analysis of the black seed oil is carried out and the results is as followed:

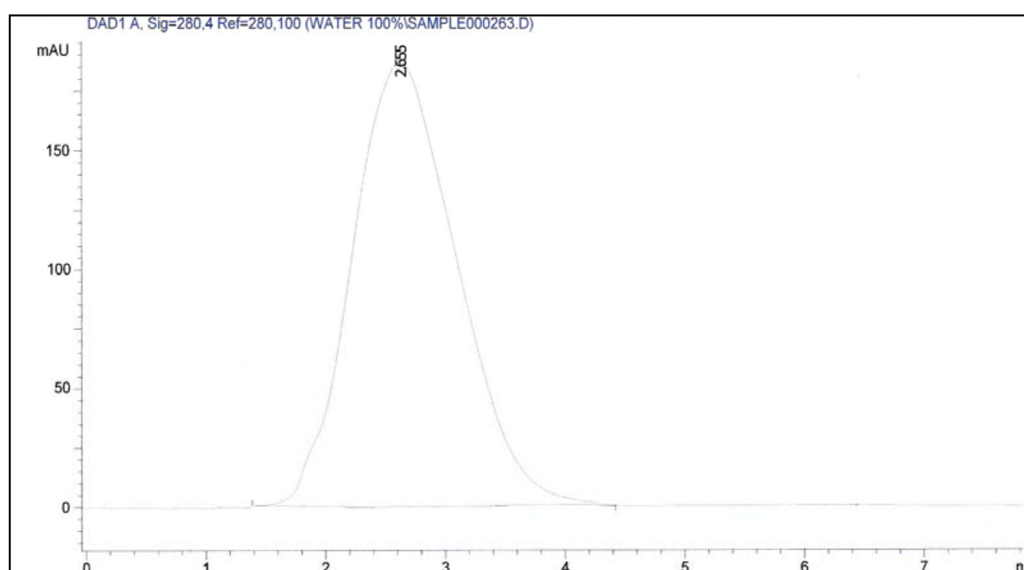

Figure 4.6: Standard curve for Thymoquinone

Above is the standard Thymoquinone curve. As seen above Thymoquinone seems to appear at around 2.7 minutes. Obtained, is sharp graph free of impurities for the first run of the standard. Unfortunately, for the second run due to several inevitable kinks, a sharp graph could not be obtained. The graph is full with impurities that affect its reading. Supposively there should only be one peak, however due to several factors proper graph failed to be generated. HPLC in used, is probably not in its best condition due too many usage but no washing. Hence, the column in use contained numerous impurities. Moreover, lack of experts when it comes to handling HPLC is a major drawback. At times the HPLC appear to be overpressure. Even so, still the peak is there

only the impurities is also there making the graph less standard. In terms of samples, all 4 samples analyzed showed the same retention time. Obviously, the existence of Thymoquinone could be recorded qualitatively but not quantitatively. With regards to the shifting of sample peak than the standard peak is due to the pH differences that need to be consider prior injecting into the HPLC. The pH should more or less be the same to ensure same retention time.

Purification procedure has been overlooked therefore the results obtained digress slightly from journals. However, the result is still viable since purposely this research is to determine the existence of Thymoquinone. Successfully, Thymoquinone could be detected only the amount or the concentration failed to be determined. Isolation should also be carried out to promote better reading of the HPLC, however due to time constraint this step has been skipped.

Should the entire factor be eliminated, the outcome of the HPLC analysis would generate a sharp peak with no impurities. Even so, what’s important is that Thymoquinone is proved to be the major compound in black seed oil since all samples generate the Thymoquinone peak.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

This study on Extraction of Nigella Sativa Essential Oils Using Hydro-Distillation had been successfully done. The purpose of this study is to evaluate the correlation between the times of extraction versus the production of the essential oil. From this research, several conclusions can be made which are:

1) Generally the yield of Nigella Sativa essential oil is proportional to the time of extraction until certain time it will be constant. When the time of extraction increase, the yield of oil also increased. This statement apply to all both of processes, extraction for grinded and non grinded sample. The only things that different for both processes are the surface area that expose to the extraction process

Time (h)	Grinded Yield %	Non Grinded Yield %
1	2.028	1.092
2	4.992	3.016
3	4.784	2.86
4	5.304	3.276
5	5.512	3.536
6	5.512	3.536

Table 5.0 Yield of extraction processes for both experiment

2) The result indicates that yield of extraction *Nigella Sativa* is based on the oil density at 100°C and 1atm which is 1.04 g/ml. The yield is using those formula;

$$\text{Yields} = \frac{\text{Essential oil quantity (ml) x Density of oil at 100°C (g/ml)}}{\text{Raw Material weight (g)}}$$

5.2 RECOMMENDATIONS

Nigella Sativa oil extraction by using hydro-distillation study is an important research that must be continued due to the value and demand of the oil nowadays. Thus by doing this research can improve the newly method discovered in order to extract the essential oil. More importantly it is not only can improve the research study but also can helped local entrepreneur who involve in this oil extraction. From study that has been performing during this research, there are several important recommendations that should be done in future study to improve the yield and quality the oil extracted, so the recommendation base on 2 paths which are extraction process and analysis process.

For the extraction process;

1) For this time, one of the experiment is running using the steam distillation unit that just arrive, the equipment should be upgrade because during the experiment the are a lot of errors occur such as leakage and some of the oil compounds vaporize trough the air because of some hole at this equipment .
2) Try to use some high technology equipment such as supercritical CO_2 extraction that been claim efficiently extract oil from plant material.

3) Try to use some other extraction equipment such as microwave extraction or ultrasonic extraction to compare which result is better in term of quantity and quality.
4) Using scale up equipment to get more essential oil in the same time when using lab scale, and then compare the quality of the oil.
5) Student should have fund to access some of the journal at science direct.

For the analysis path;

1. Use specific column for each type of sample not the same column for every sample. This will cause impurities.
2. Maintenance should been done after each time of experiments to avoid impurities for the next sample that need to analyze.
3. Some of the analyze equipment cannot be use because of nobody knows how to operate it, such as Liquid Chromatography Mass Spectrometer (LCMS).This is very important because that equipment could give better result in analysis.

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APPENDIX

Grinded Sample 5 ppm

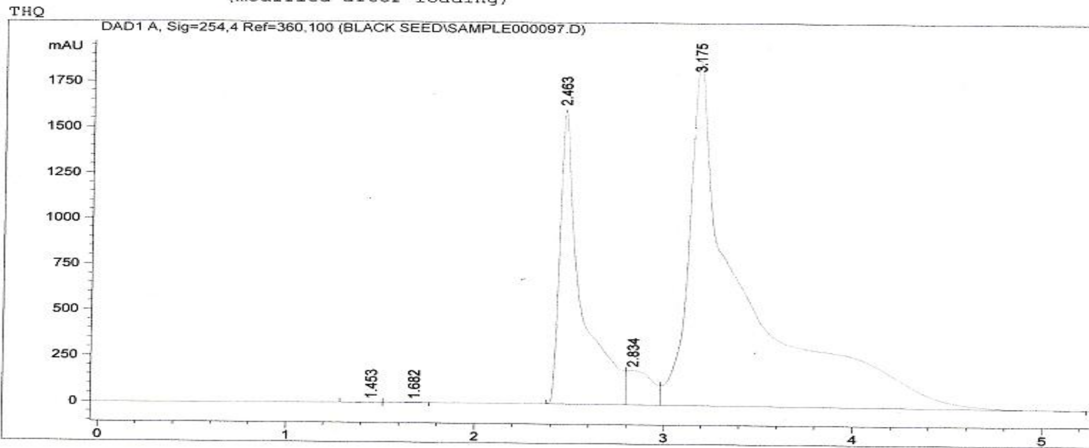
Data File C:\Chem32\1\DATA\BLACK SEED\SAMPLE000097.D
Sample Name: thymoquinone

Steam distillation

Thymoquinone

Injection Date	: 10/15/2006 10:52:11 AM		
Sample Name	: thymoquinone	Location :	Vial 1
Acq. Operator	: Jintan Hitam		
Acq. Instrument	: Instrument 1	Inj Volume :	5 µl
Method	: C:\CHEM32\1\METHODS\THYMOQUINONE.M		
Last changed	: 10/15/2006 10:50:24 AM by Jintan Hitam (modified after loading)		

THQ



Area Percent Report

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

Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.453	BV	0.1181	10.28543	1.22344	0.0194
2	1.682	VV	0.1516	12.71890	1.12793	0.0239
3	2.463	BV	0.1082	1.26985e4	1607.26746	23.8898
4	2.834	VV	0.1463	1770.02319	187.14290	3.3300
5	3.175	VB	0.2639	3.86628e4	1880.33020	72.7369
Totals :				5.31543e4	3677.09192	

*** End of Report ***

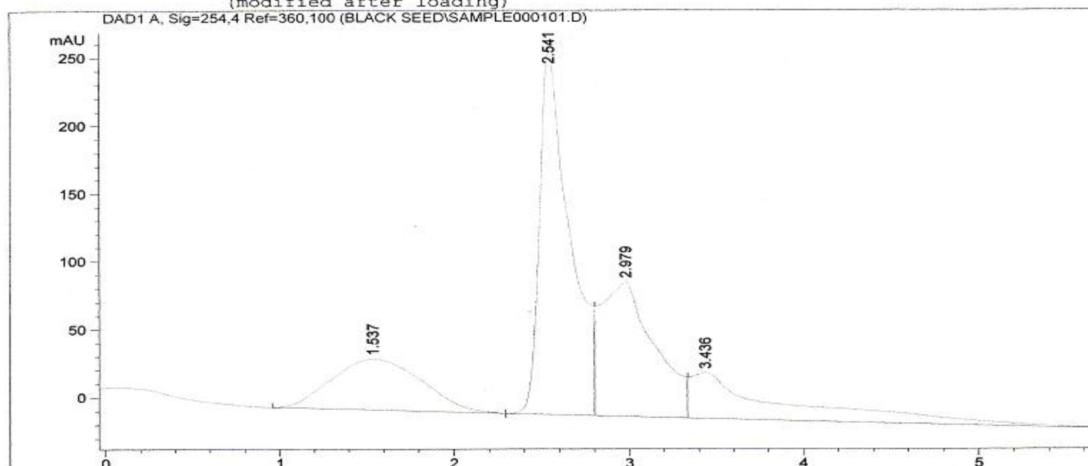
Grinded Sample 10 ppm

Data File C:\Chem32\1\DATA\BLACK SEED\SAMPLE000101.D
Sample Name: thymoquinone

Thymoquinone

n-hexane

```
Injection Date  : 10/15/2006 11:22:41 AM
Sample Name     : thymoquinone                  Location : Vial 1
Acq. Operator   : Jintan Hitam
Acq. Instrument : Instrument 1               Inj Volume : 5 µl
Method          : C:\CHEM32\1\METHODS\THYMOQUINONE.M
Last changed    : 10/15/2006 11:11:19 AM by Jintan Hitam
                  (modified after loading)
```



Area Percent Report

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

Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.537	BV	0.5572	1336.58289	37.37224	16.8609
2	2.541	VV	0.1654	3194.03638	267.26392	40.2926
3	2.979	VV	0.2716	2118.17017	98.99396	26.7206
4	3.436	VB	0.4817	1278.31299	33.61108	16.1259
Totals :				7927.10242	437.24119	

*** End of Report ***

Instrument 1 10/15/2006 11:29:07 AM Jintan Hitam   Page 1 of

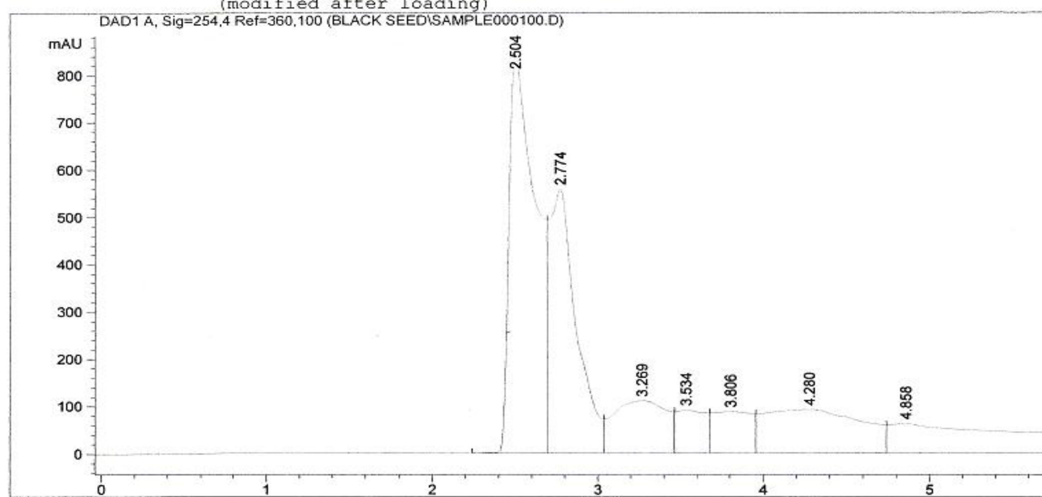
Grinded Sample 25 ppm

Data File C:\Chem32\1\DATA\BLACK SEED\SAMPLE000100.D
Sample Name: thymoquinone

Ethanol

. Thymoquinone

Injection Date : 10/15/2006 11:13:42 AM
Sample Name : thymoquinone Location : Vial 1
Acq. Operator : Jintan Hitam
Acq. Instrument : Instrument 1 Inj Volume : 5 µl
Method : C:\CHEM32\1\METHODS\THYMOQUINONE.M
Last changed : 10/15/2006 11:11:19 AM by Jintan Hitam
(modified after loading)



Area Percent Report

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

Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.504	BV	0.1542	9362.79688	839.11340	33.2568
2	2.774	VV	0.1516	6281.34229	556.74011	22.3114
3	3.269	VV	0.3375	2462.80103	109.86483	8.7479
4	3.534	VV	0.1632	1110.63123	90.48615	3.9450
5	3.806	VV	0.2228	1410.31873	87.14243	5.0095
6	4.280	VV	0.5360	3759.51489	91.75092	13.3539
7	4.858	VBA	0.7508	3765.61523	61.80253	13.3755
Totals :				2.81530e4	1836.90038	

*** End of Report ***

Instrument 1 10/15/2006 11:20:14 AM Jintan Hitam Page 1 of

Non Grinded Sample 5 ppm

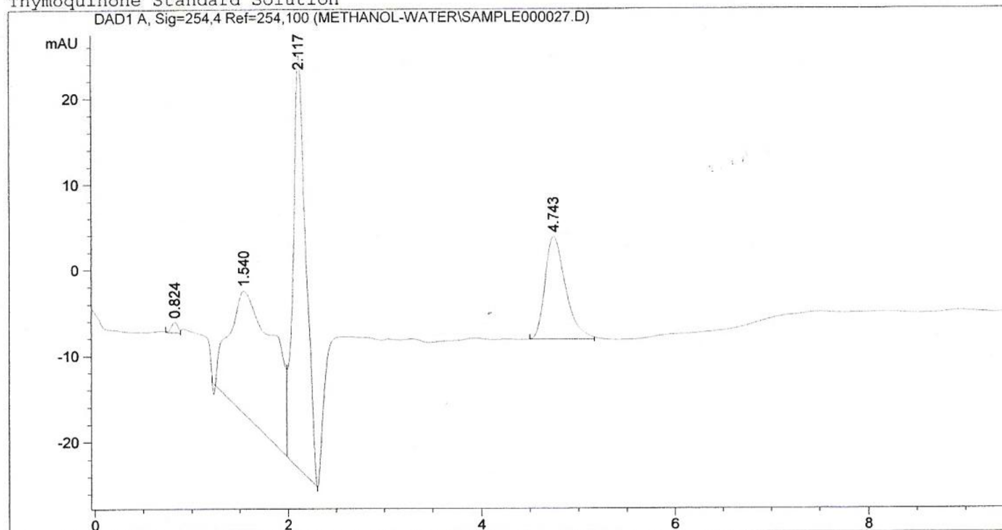
)ata File C:\Chem32\1\DATA\METHANOL-WATER\SAMPLE000027.D
3ample Name: thymoquinone

thymoquinone

===

Injection Date : 10/14/2006 11:27:50 AM
Sample Name : thymoquinone Location : Vial 1
Acq. Operator : nazeri
Acq. Instrument : Instrument 1 Inj Volume : 10 µl
Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 10/14/2006 11:26:50 AM by nazeri
(modified after loading)

Thymoquinone Standard Solution



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Area Percent Report

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

Signal 1: DAD1 A, Sig=254,4 Ref=254,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.824	BV	0.0753	5.72876	1.20767	0.5203
2	1.540	BV	0.4467	476.52591	14.22976	43.2782
3	2.117	VV	0.1315	443.26974	48.10057	40.2578
4	4.743	BB	0.2256	175.55246	11.93855	15.9437

Totals : 1101.07687 75.47656

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*** End of Report ***

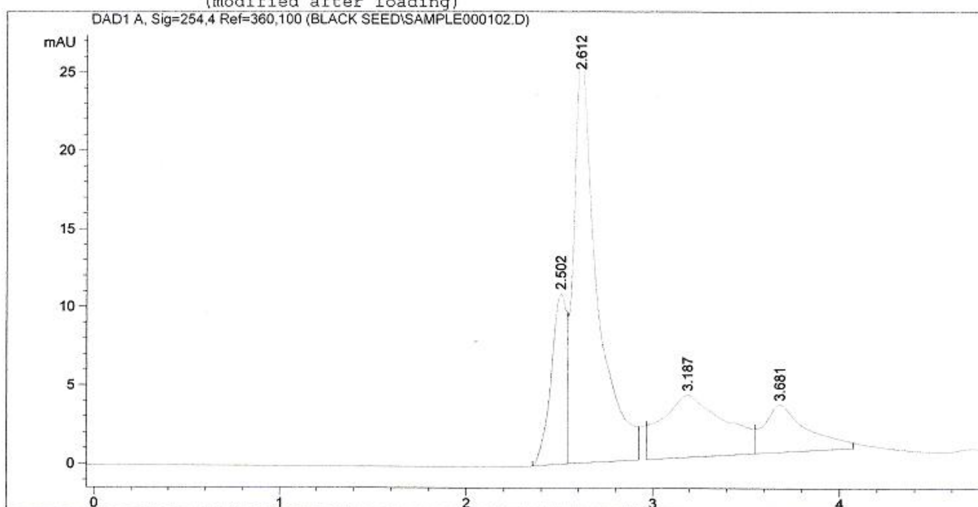
Instrument 1 10/14/2006 11:38:09 AM nazeri Page 1 of

Non Grinded 10 ppm

Data File C:\Chem32\1\DATA\BLACK SEED\SAMPLE000102.D
Sample Name: thymoquinone   water

Thymoquinone

Injection Date : 10/15/2006 11:33:45 AM
Sample Name : thymoquinone   Location : Vial 1
Acq. Operator : Jintan Hitam
Acq. Instrument : Instrument 1   Inj Volume : 5 µl
Method : C:\CHEM32\1\METHODS\THYMOQUINONE.M
Last changed : 10/15/2006 11:11:19 AM by Jintan Hitam
(modified after loading)



Area Percent Report

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

Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.502	BV	0.0851	62.57877	10.87925	14.4100
2	2.612	VB	0.1249	229.34004	26.00777	52.8101
3	3.187	BV	0.3078	93.79553	3.96833	21.5983
4	3.681	VB	0.2195	48.55902	3.02094	11.1817

Totals : 434.27337 43.87629

*** End of Report ***

Instrument 1 10/15/2006 11:39:16 AM Jintan Hitam   Page 1 of

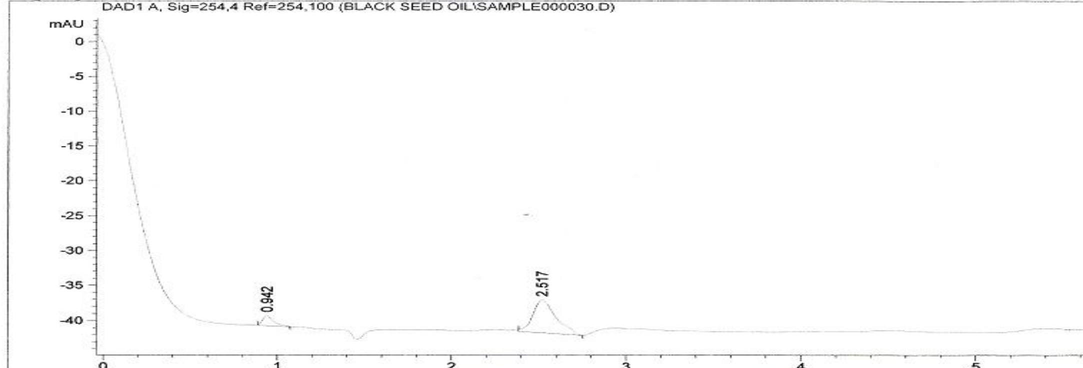
Non Grinded 25 ppm

Data File C:\Chem32\1\DATA\BLACK SEED OIL\SAMPLE000030.D
Sample Name: Thymoquinone

Thymoquinone

Injection Date	: 10/14/2006 12:08:08 PM		
Sample Name	: Thymoquinone	Location :	Vial 1
Acq. Operator	: Nigella Sativa		
Acq. Instrument	: Instrument 1	Inj Volume :	5 µl
Method	: C:\Chem32\1\METHODS\DEF_LC.M		
Last changed	: 10/14/2006 12:07:01 PM by Nigella Sativa (modified after loading)		

Thymoquinone Standard Solution



Area Percent Report

Sorted By	:	Signal
Multiplier	:	1.0000
Dilution	:	1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=254,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.942	BB	0.0639	6.34105	1.48385	12.8422
2	2.517	BV	0.1348	43.03568	4.70313	87.1578
Totals :				49.37673	6.18698	

*** End of Report ***

Instrument 1 10/14/2006 12:14:27 PM Nigella Sativa   Page 1 of