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JUDUL: **EXTRACTION OF PHARMACOLOGICALLY ACTIVE THYMOQUINONE IN NIGELLA SATIVA L.**

SESI PENGAJIAN: **2006/2007**

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EXTRACTION OF PHARMACOLOGICALLY ACTIVE THYMOQUINONE IN NIGELLA SATIVA L.

NAZERI BIN NASARUDDIN

A report submitted in partial fulfillment of the requirements for the award of the degree of Bachelor of Technology (Chemical Engineering and Natural Resource)

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To my foundation of hope, happiness and love; My Mother.

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In the name of Allah; most gracious and most merciful.

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I cannot write every single name here, but your name is written all over my heart. Eternally!

Abstract

Black Seed Oil or Nigella Sativa L. is said to be the universal remedy. It has an amazing healing power and its greatness has been recorded in the Hadith stating that it could cure any diseases except death. Today black seed oil has been commercialized; research is carried out from time to time to improve its quality. This research purposely is to determine the best solvent to be used in extracting black seed oil, in terms of time and most importantly its yield. Rotary Evaporator employed with Ultra Sonic Bath is used in extraction process. Time and heat is chosen as the parameter to manipulate and several trials is carried out. Optimum condition will be recognized. After the extraction, oil is obtained then HPLC analysis is carried out to determine the existence of Thymoquinone which is the main compound in Black Seed Oil. Quantitatively, more Thymoquinone means better quality of Black Seed Oil.

Abstrak

Minyak Jintan Hitam atau Nigella Sativa L. adalah merupakan penyembuh universal. Ia mempunyai kuasa penyembuhan yang menakjubkan, bisa menyembuh pelbagai penyakit. Kehebatan kuasa penyembuhan Jintan Hitam ini ada di sebut di dalam Hadith yang menyatakan bahawa minyak jintan hitam mampu menyembuhkan segala macam penyakit kecuali satu penyakit iaitu kematian. Dewasa ini, pengeluaran minyak jintan hitam telah di komersialkan sekaligus banyak kajian-kajian telah di jalankan dari semasa ke semasa untuk meningkatkan mutu minyak jintan hitam. Kajian ini adalah bertujuan mengenal pasti pelarut manakah yang paling sesuai di gunakan untuk mengekstrak minyak jintan hitam. *Rotary Evaporator* di guna bersama *Ultrasonic Bath* untuk proses ekstrak. Masa dan haba telah di ambil kira sebagai parameter kajian untuk di manipulasi serta beberapa cubaan di lakukan bagi mengurangkan ralat. Keadaan optimum untuk ekstrak akan di ketahui. Setelah itu, analisa menerusi HPLC di jalankan untuk menguji kehadiran Thymoquinone yang merupakan komposisi utama di dalam minyak jintan hitam. Secara quantitatifnya, semakin tinggi kepekatan Thymoquinone semakin elok mutunya.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Black seed is considered to be one of the greatest healing herbs of all times. This herb has been used for millenniums to strengthen the immune system, cleanse the body, purify the blood, protect against irritants and support healthy longevity. Black Seed is also known as Black Cumin, Black Caraway Seed, Habba Sawda (the Black Seed) Habbatul Baraka (the Blessed Seed), and by its botanical name "Nigella Sativa.

Historically, the first essential oil encountered was the oil of rose. It was discovered by the Chinese prior to the Christian era. A layer of this oil was found on a pool that was filled with rose water. Essential oils contain DNA of the plant of herbs they are extracted from. Essential oils or sometimes called volatile oils are believed to be that small portion of the plant material, which imparts the characteristics odors and flavor most closely associated with the vegetative matter which they are obtained [1]. Most of the essential oils are used at about a level of 0.01-0.1 percent in the finished product. They are often slightly colored and have a specific gravity of about 1.

The advantages of essential oils are their flavor concentrations and their similarity to their corresponding sources. The majority of them are fairly stable and contain a few natural antioxidants. Although most are soluble in high strength alcohol (more than 90 percent), they have poor water solubility and most contain terpenes that contribute to their poor water solubility [8]. Some essential oil are adaptogenic. This implies that the essential oil increase resistance and resilience to stress, enabling the body to avoid reaching collapse. Adaptogenic essential oils aid the body in maintaining homeostasis throughout stressful periods.

While the Black Seed is highly effective by itself, ongoing studies with the combination of other herbs have produced remarkable results. Amazingly Black Seed's chemical composition is very rich and diverse. Aside from its primary ingredient, crystalline nigellone, Black Seed contains 15 amino acids, proteins, carbohydrates, both fixed oils (84% fatty acids, including linolenic, and oleic), and volatile oils, alkaloids, saponin, and crude fiber, as well as minerals such as calcium, iron, sodium and potassium. There are still many components in Black Seed that haven't been identified. But research is going on around the world

In general, there are many way to extract Black Seed (*Nigella Sativae*) to produce Black Seed oil. Black Seed oil is the value added product obtains from the dry seed using extraction method such as Supercritical Fluid, Supercritical CO_2, Solvent Extraction or Steam Distillation.

In this research, Rotary Evaporator is used to obtain Black Seed oil. To obtain a series of high quality extracts from black seed, the effect of heat is need to be study

1.2 Objectives

i. To extract the essential oils of Black Seed Oil (Nigella Sativa) using Rotary Evaporator.
ii. To study the existence of Thymoquinone in Black Seed Oil
iii. To compare commercial sample of Black Seed Oil with the one extracted using via solvent extractions.

1.3 Scope of Study

To accomplish the objectives, scopes have been decided in this research. The scopes of this research are to investigate the fastest solvent that need to use for solvent extraction, to investigate the heat effect at rotary evaporator. The experimental work for black seed oil is based on the fastest solvent that obtains from the lab scale experiment by using a rotary evaporator.

1.4 ` Problem Statement

Local Black Seed oil is on the verge of its establishment, hence it is crucial to ensure the quality is as good as the commercial Black Seed oil. It is vital to identify which is the most appropriate condition to extract Black Seed oil as the suitable parameter could lead to more yield of oil. Up till today the best way to extract black seed oil is still under research. Conventionally, black seed oil is extracted using cold pressed, however solvent extraction could be used as an alternative.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

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.

Nigella sativa seed is known variously as kalonji. In English it is called fennel flower, black caraway, nutmeg flower or Roman coriander [2]. Other names used, sometimes misleadingly, are onion seed and black sesame (both of which are similar-looking but unrelated). Frequently the seeds are referred to as black cumin, this is, however, also used for a different spice, Bunium persicum. It is also sometimes just referred to as nigella or black seed. An old English name gith is now used for the corncockle.

This potpourri of vernacular names for this plant reflects that its widespread use as a spice is relatively new in the English speaking world, and

largely associated with immigrants from areas where it is well known. Increasing use is likely to result in one of the names winning out, hopefully one which is unambiguous.

Nigella sativa has a pungent bitter taste and a faint smell of strawberries. It is used primarily in candies and liquors. The variety of naan bread called Peshawari naan is as a rule topped with kalonji seeds. In herbal medicine, Nigella sativa has hypertensive, carminative, and anthelminthic properties.

2.2 History of The Black Seed

For over two thousand years the black seed, a plant from the Ranunculaceae (buttercup) family, has been traditionally used by various cultures throughout the world as a natural remedy for several diseases and ailments and to improve health in general.

The ancient Egyptians knew and used the black seed and described it as a panacea (cure for problems and diseases). Tutankamun even had a bottle of the oil in his tomb.

The Romans also knew this seed and called it Greek Coriander and used it as a dietary supplement. In the first century, the Greek physician Dioscoredes recorded that the black seed were taken to treat headaches, nasal congestion, toothache and intestinal worms [4].

The black seed is also mentioned in the Bible in Isiah 28:25-27 as the ‘fitches’. Ibn Senna, known in the West as Avicenna, who wrote the great medical treatise 'The Canon of Medicine', referred to the black seed as the seed ‘that stimulates the body's energy and helps recovery from fatigue’.

2.3 Black Seed

Figure 2.1: Black Seed Flower (Kalonji)

An annual herbaceous plant, black cumin seed (botanical name is nigella sativa , or black seed for short, is believed to be indigenous to the Mediterranean region but has been cultivated into other parts of the world including the Arabian peninsula, northern Africa and parts of Asia [15].

The plant has no relation to the common kitchen herb, cumin. Tiny and hairy, being no more than 3mm in length, black seed originates from the common fennel flower plant (nigella sativa) of the buttercup (Ranunculaceae) family. Nigella sativa is sometimes mistakenly confused with the fennel herb plant (Foeniculum vulgare). The plant has finely divided foliage and pale bluish purple or white flowers. The flowers grow

terminally on its branches while the leaves grow opposite each other in pairs, on either side of the stem. Its lower leaves are small and petioled, and the upper leaves are long (6-10cm). the stalk of the plant reaches a height of twelve to eighteen inches as its fruit, the black seed, matures. Nigella sativa is bisexual and forms a fruit capsule which consists of many white triagonal seeds. Once the fruit capsule has matured, it opens up and the seeds contained within are exposed to the air, becoming black in color (black seeds). Nigella sativa and its black seed are known by other names, varying between places. some call it black caraway, habbat al barakah , and habbat sawda , others call it black cumin (kalounji), onion seeds or even coriander seeds. in English, the nigella sativa plant is commonly referred to as black cumin . Nevertheless, this is nigella sativa, which has been known and used from ancient times and is also known in Persian as Shonaiz.

2.4 Chemical Analysis of Black Seed Oil

Black Seed Oil contains several ingredients (in significant amounts) with potential value. The following chart reflects the composition of Black Seed Oil in terms of its active, nutrient components, and any other significant ingredients.

Essential Oil Composition (1.4%)	**Black Seed Oil**
Carvone	21.1%
Alfa-Pinene	7.4%
Sabinene	5.5%
Beta-Pinene	7.7%
P-cymene	46.8%
Others	11.5%

Fatty Acids	**Black Seed Oil.**
Myristic Acid (C14:0)	0.5%

Palmitic Acid (C16:0)	13.7%
Palmitoleic Acid (C16:1)	0.1%
Stearic Acid (C18:0)	2.6%
Oleic Acid (C18:1)	23.7%
Linoleic Acid (C18:2)(Omega-6)	57.9%
Linolenic Acid (18:3n-3) (Omega-3)	0.2%
Arachidic Acid (C20:0)	1.3%
Saturated & Unsaturated Fatty Acids	**Black Seed Oil**
Saturated Acid	18.1%
Monounsaturated Acids	23.8%
Polyunsaturated Acids	58.1%
Nutritional Value	**Black Seed Oil**
Protein	208 ug/g
Thiamin	15ug/g
Riboflavin	1 ug/g
Pyridoxine	5ug/g
Niacin	57 ug/g
Folacin	610 IU/g
Calcium	1.859 mg/g
Iron	105 ug/g
Copper	18 ug/g
Zinc	60 ug/g
Phosphorus	5.265 mg/g
Nutritional Composition	**Black Cumin Seed**
protein	21%
carbohydrates	35%
fats	35-38%

Table 2.1: Chemical Compositions in Black Seed Oil

Black Cumin (Nigella sativa) Seed is rich in nutritional values. Monosaccharides (single molecule sugars) in the form of glucose, rhamnose, xylose, and arabinose are

found in the black seed. The Black Cumin (Nigella sativa) Seed contains a non-starch polysaccharide component which is a useful source of dietary fiber.

It is rich in fatty acids, particularly the unsaturated and essential fatty acids (Linoleic and Linolenic acid). The EFAs, consisting of alpha-Linolenic acid (omega-3) and Linoleic acid (omega-6), are substances that cannot be manufactured in the body, and thus must be taken in as supplements or through high-EFA foods in order to sustain health.

Fifteen amino acids make up the protein content of the Black Cumin (Nigella sativa) Seed, including eight of the nine essential amino acids. Essential amino acids cannot be synthesized within our body in sufficient quantities and are thus required from our diet. Black seed contains Arginine which is essential for infant growth. Chemical analysis has further revealed that the Black Cumin (Nigella sativa) Seed contains carotene, which is converted by the liver into vitamin A.

The Black Cumin (Nigella sativa) Seed is also a source of calcium, iron, sodium, and potassium. Required only in small amounts by the body, these elements' main function is to act as essential cofactors in various enzyme functions.

2.5 Uses of Black Seed Oil

The common name "love in the mist" aptly describes the poetry of this exquisite plant. In the garden, one easily imagines etheric spirits flitting about amongst its evanescent bluish-white blossoms. Even the seedpods, which are so often used in dried flower arrangements, suggest an otherworldly sense of exotic enchantment. Is it possible

that such a delicately beautiful herb, with such potent medicinal properties would be so hardy as to easily reseed itself in our gardens year after year?

With an exalted position of use throughout the Middle East and to a somewhat lesser extent in India and other Eastern lands, the information about Nigella I owe to herbalist, plant-scientist extraordinaire, Jim Duke as presented in his book Medicinal Plants of the Bible. In it he describes Black Cumin as a Muslim Miracle Herb which, according to an Arab Proverb it is said that, 'in the black seed is the medicine for every disease except death.'

I have spoken with a Turkish colleague who reports that it the seeds are widely cultivated and traded in ton lots within his country throughout the Middle East, Northern Africa and India. The seeds are used both as a condiment in bread and cakes and various confections and like pepper or combined with pepper such as cayenne in sauces. The Ethiopians add along with other spices to flavor local alcoholic beverages. Still another use is to sprinkle them with woolen garments as a moth repellant.

The major uses I have employed it for are upper respiratory conditions, allergies, coughs, colds, bronchitis, fevers, flu, asthma and emphysema for which it is effective. Simply collect the abundance of seeds from the pods and grind them to a paste and mix with melted honey to a 'hahlava' (a Middle Eastern confection usually made with toasted sesame seeds and honey). Jim Duke confirms its folk use for these and a wide variety of other diseases and conditions including bilious ailments, calluses, cancer, colic, corns, eruptions, headache, jaundice, myrmecia, orchitis, puerperal fever, sclerosis, skin, snakebite, stomachache, swellings, tumors of the abdomen and eyes, and warts. In Algeria, the roasted seeds are combined with butter for cough and honey and taken for colic.

For upper respiratory conditions, at least a few of its constituents have shown an antihistamine-like action, which explains is positive effects for upper respiratory diseases including asthma, bronchitis, and cough. The oils of the seed increase milk flow which explains its folk use as a galactagogue. In large quantities, however, the seeds have also been used to abortion.

It is unusual for a hot spicy herb to have a positive effect on liver diseases as it is used by the Lebanese. Of course, one of its most obvious uses is for diarrhea and dysentery, combined with astringents. Externally the seeds can be ground to a powder, mixed with a little flour as a binder and applied directly to abscesses, on the forehead for headache, nasal ulcers, orchitis, and rheumatism. The seeds also are a rich source of sterols, especially beta-sitosterol, which is known to have anticarcinogenic activity. This substantiates its folk use for indurations and/or tumors of the abdomen, eyes and liver.

In India, Nigella seeds are combined with various purgatives to allay gripping and colic and also help kill and expel parasites. Middle Eastern Unani medicine affirms its abortifacient properties and also use it as a diuretic to relieve ascites, for coughs, eye-sores, hydrophobia, jaundice, paralysis, piles and tertian fever

2.6 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. Separation process is used for three primary functions which are purifications, concentration and fractionation.

In purification, the principal is to remove undesired components in a feed mixture from the desired species. For example is the purification of acid gases such as sulfur dioxide must be removed from power plant combustion gas effluents before discharge into atmosphere.

In concentration, the principal is to obtain higher proportion of desired components that are initially dilute in a feed steam. For example is concentration of metals present in an electroplating process by removal water. This separation allows one to recycle the metals back to the electroplating process rather than discharge to environment.

In fractionation, a feed stream of two or more components is segregated into product streams of different components, typically relatively pure streams of each component.

Separation also divided into two classes which is equilibrium based and rate-based processes. These classes are designated using thermodynamic equilibrium relationships between phases and the rate of transfer of a species from one phase to another respectively.

Equilibrium processes are those in which cascades of individual units called stages are operated with two system typically flowing countercurrents to each other. Examples of equilibrium based processes are including extraction and solid extraction or leaching. Extraction is the removal of species from a liquid in which it dissolved by means to another liquid for which it has a higher affinity. Leaching is the removal of a species from a solid phase by means of liquid for which it has stronger affinity.

Rate-based processes are limited by the rate of mass transfer of individual components from one phase to another under influence of physical stimuli. One mass transfer based process is gas absorption a process by which a vapor is removed from its mixture with an inert gas by means of a liquid in which the vapor is soluble.

2.7 Essential Oil Extraction Processes

There are a few conventional and modern methods of extracting essential oils. It can be extracted by steam distillation, water distillation, hydrodistillation, supercritical fluid extraction, vapo-cracking, turbo-extractor and microwave extraction.

2.8 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

Table 2.2: 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 (R. Toreki, 2005).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 (R. Toreki, 2005). Figure 2.4 and 2.5 below shows the whole part of rotary evaporator.

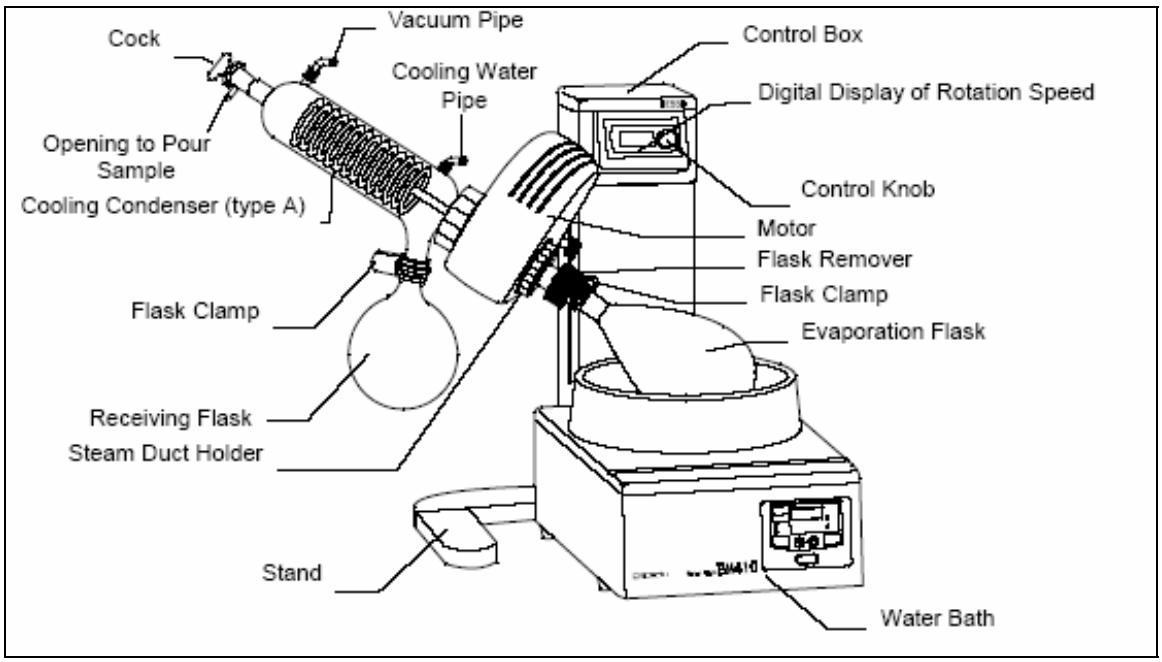


Figure 2.2: Rotary evaporator components

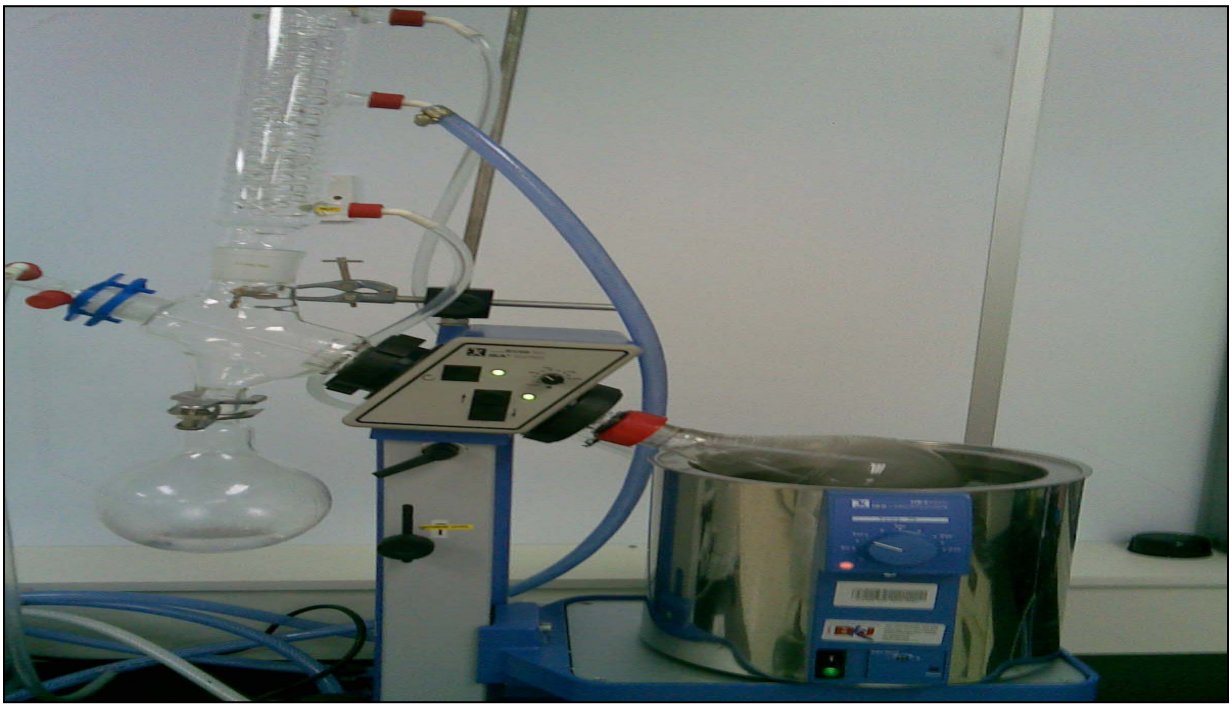


Figure 2.3: Rotary evaporator

2.9 Solvent Extraction

In 1997, Kreuter, M.H. [10], *et.al* invented and patended a process for the preparation of a stable, homogeneous extract of plants. They used a 400 kg dried herbs sample mixed with 1600 kg ethanol and extraction was done at a temperature between 60-70^{0}C. solvent removal of the patented process was done under evaporation, reduced pressure and elevated temperature.

Purseglove, J.W. *et.al*, stated that the solvents most widely used commercially for obtaining ginger oleoresin from dried ginger are acetone, ethanol and chlorinated

hydrocarbons. Their statement was further confirmed by Govindarajan in 1982 [15]. The yield and quality of the oleoresin can be variable depent on the source material, solvent and preparative methods used. a study was conducted on Malaysian ginger oleoresin extraction using petroleum ether as solvent and the yield was comparatively low, which was 2.8% [16]. With acetone, yields from Australian grown ginger have been found to vary between 5 and 11% on a dried weight basis. On the same ginger, oleoresin yields of up to 20% have been obtain using ethanol as extractant [10].

Extraction may be improved by using dried material. But when drying is used some decomposition can occur, and aromatic materials from natural sources differ widely in their physical and chemical properties, particularly in their volatility and solubility, so the choice of the solvent may determine the success of the extraction [10]. The solvent is removed from the solution by fractional distillation. The residue is termed the "concrete" and consists of volatile matter and oleoresins as well. Industrial practice is to treat the concrete with alcohol to obtain a selective removal of the volatile constituents.

Oleoresins are extracted by a process of solvent extraction, followed by removal of the solvent to extremely low levels typically less than 25-30 parts per million. In 1998, Balladin, *et.al* [17] used pilot plant extraction to extract oleoresin from ginger. The solvent used followed its designated route to the vertically oriented water cooled 20 liters leaching vessel. The process continued for 10 hours until the majority of the oleoresin was extracted, determined by the transparent appearance of the extracting solvent.

The quality of an oleoresin is typically evaluated on the basis of presence of the active ingredients in desired levels which is the "bite" giving resin portion containing a combination of alkaloids, gums, pigments, etc. The aroma giving volatile/essential oil component is also considered to be the most important part in oleoresin quality indication. In 1998, Spiro, M. *et.al* [18] conducted a kinetic study using solvent mixtures stirred at 450 r.m.p. with a glass paddle stirrer so as not to damage the particles. The

extraction of oleoresin from the comminuted dry ginger using solvent extraction is influenced by factors below [16] :

I. Particle size

To increase the rate of solvent extraction, it is desirable that the range of particle size to be small. This is due to the greater interfacial areas between the solid and liquid and therefore the higher is the rate of transfer of material.

II. Choice of solvent

The liquid chosen should be good selective solvent , less hazardous for mass production, low viscosity and economical. The organic solvents more frequently used are :

- Aliphatic hydrocarbons: propane, butane, hexane
- Alcohols: methanol, ethanol, 2-propanol
- Hydrocarbons with a carbonyl group: acetone, methyl acetate
- Halogen derivation: dichloro methane, dichloroethane, freons

III. The fluid agitation

Agitation of the solvent is important because it is increase the eddy diffusion and therefore increase the transfer of material from the surface of the particles to the bulk of the solution

IV. The temperature

The solubility of the material which is being extracted will increase with temperature to give a higher rate of extraction.

2.10 Heat Effect

Heat effect is important because it is relative to the simple chemical-manufacturing process. It also associates with the reactions. Sensible heat effect are characterize by temperature changes, the heat effect of chemical reaction, phase transition and the formation and separation of solutions are determined from experimental measurement made at constant temperature.

2.11.1 Standard Heat of Reaction

Heat effect is not only for physical processes, but in chemical reaction it accompanied either by the transfer of heat or by temperature changes during the course of reaction. These effects are manifestation of the differences in molecular structure, in energy of the product and reactants. Each reaction carried out in a particular way accompanied by a particular heat effect.

Tabulation of heat effect for all possible reaction can be calculate with the heat effect for reaction carried out in diverse ways from data for reactions carried out in a standard ways. The heat is associate with a specific chemical reaction depends on the temperature of both reactant and product.

A consistent basis for treatment of reaction heat effect result when the product of reaction and the reactants are all at same temperature. Heat effect can be calculated using equation;

$$Q = \Delta H$$

Where, Q = heat absorbed

ΔH = enthalpy change of reaction

2.11.2 Standard Heat of Formation

Standard heat of formation is a reaction which forms a single compound from its constituent elements. Formation reaction is understood to result in the formation of 1 mole of the compound and therefore heat of formation is based on 1 mole of the compound formed.

The standard heat of formation of compound at standard temperature is representing by the symbol ΔH^{o}_{f298}. The f value shows that it is a heat of formation and 298 is the approximate absolute temperature in Kelvin (^{o}K). Formation equation and standard heat of formation may always be combined to produce any desired equation and its accompanying standard heat of reaction.

2.11.3 Latent Heat of Pure Substances

Latent heat occurs when a pure substance is liquefied from the solid state or vaporized from the liquid at constant pressure there is no change in temperature. The process required the transfer of a finite amount of heat to the substance. The latent heat processes coexistence of two phases. According to the phase rule, a two phase system consisting of a single species in invariant and intensive state is determined by the specification of just one intensive property

Latent heat is phase change is a function of temperature only and related to other system properties by an exact thermodynamic equation;

$$\Delta H = T \Delta V (dP_{sat} / dT)$$

Where (dP_{sat} / dT) is the slope of vapor pressure versus temperature curve. ΔV is the difference between molar volumes of saturated vapor and saturated liquid. ΔH is the latent heat of vaporization. ΔH also can be calculated from vapor-pressure and volumetric data. Approximate methods are used for estimates of the heat effect accompanying a phase change.

2.11 Heat Transferred

2.11.4 Introduction

Heat is defined as the form of energy that is transferred between two systems (or a system and its surroundings) by virtue of a temperature differences. In daily life, heat frequently refers to the sensible and latent forms of internal energy as thermal energy and it is different from heat transfer. Heat flow, heat addition, heat rejection, heat absorption, heat removal, heat gain, heat loss, heat storage, resistant heating, heat of reaction, specific heat, process heat and heat source are not consistent with the strict thermodynamic meaning of the term heat. So in thermodynamics, the term of heat simply means heat transfer.

Heat transfer Q during the process between two state is denoted by Q_{12} or just Q and for per unit mass;

$$q = Q/m \text{ (kJ / kg)}$$

When Q varies with time, the amount of heat transfer during a process is determined by integrating Q over the time interval of the process which is;

$$Q = \int Q \, dt \text{ (kJ) from } t_1 \text{ to } t_2$$

Heat is transferred by three mechanisms which are conduction, convection and radiation. Heat of conduction is the transfer of energy from the more energetic particle of a substance to these adjacent less energetic ones as a result of interaction between particles. Heat of radiation is the transferred of energy due to the emission of electromagnetic waves. Heat of convection is the transferred energy between a solid surface and the adjacent fluid that is in motion and it involves the combined effect of conduction and fluid motion.

In the vernacular of the time, heat transferred is indeed a relevant subject not to mention an inherently fascinating part of the engineering sciences. Heat transferred phenomena play an important role in many industrial and environmental problems. Heat transferred processes effect the performance of propulsion system such as the internal combustion.

2.11.5 Heat of Conduction

At mention of word "conduction" it conjure up concepts of atomic and molecular activity for it is processes at these levels that sustain this mode of heat transfer. Conduction is most easily understood by considering heat flow in homogeneous isotropic solids in these and there is no convection and the effect of radiation is negligible unless the solid is translucent to electromagnetic waves.

Conduction may be viewed as the transfer of energy from the more energetic to less energetic particles of substance due to interactions between particles. According to

Fourier Law, the heat flux is proportional to the temperature gradient and opposite to it sign. Fourier law equation;

$$q_x /A = -k (dT / dx)$$

Heat conduction Q/ Time = (Thermal conductivity) x (Area) x (Thot - Tcold)/

Where q is the rate of heat flow (W), A is a surface area (m^2), T is temperature in Kelvin (K), k is the thermal conductivity (W / m.K) and x is the distant (m).

At steady state heat transfer;

$$q /A = [k / (x_2-x_1)] \times (T_1-T_2)$$

2.11.6 Thermal Conductivity

The proportionality constant k is a physical property of the substance. Fourier laws state that k is independent of the temperature gradient but not necessarily of temperature itself. 'k' is function of temperature but not strong one. For small ranges of temperature, k may be considered constant. For large temperature range, k can usually be approximated by an equation;

$$k = a + bT$$

Where a and b are empirical constant.

For most liquid, k is lower than solids. Gases have the smallest k and solid have low k value. In heat transfer analysis, the ratio of the thermal conductivity to the heat capacity is an important property termed the thermal diffusivity α, which has units of m_2/s.

$$\alpha = k / \rho Cp$$

It measures the ability of material to conduct thermal relative to it ability to store thermal energy.

2.11.7 Total Heat Transferred

The total heat Q_T transferred to the solid time t_T through a unit area of a surface is often of interest. The heat required to raise the temperature of a unit mass of solid from T_a to T_b is Cp (T_b-T_a). The slab thickness 2S and density ρ.

Total A = 1 / s ρ

Q_T = s ρ Cp (T_b-T_a)

For a long cylinder;

$Q_T / A = \underline{r\ m\ \rho\ Cp\ (T_b\text{-}T_a)}$

2.11.8 Heat of Convection

Heat of convection is the transferred energy between a solid surface and the adjacent fluid that is in motion and it involves the combined effect of conduction and fluid motion. There are two main classification of convective heat transfer. First is free or natural convection and the second one is forced convection.

Natural convection is where the motion of the fluid result from the density changes in heat transfer. Forced convection is forced to flow by pressure difference, a pump, a fan and etc. To correlate the data for heat transfer coefficient, dimensionless numbers such as the Reynolds and Prandlt number are used.

Prandlt number is the ratio of the shear component of diffusivity for momentum μ/p to the diffusivity for heat k/ρCp

$$N_{Pr} = (\mu/p) / (k/\rho Cp) = (Cp\ \mu) / k$$

The dimensionless Nusselt number N_{Nu} is used to relate data for the heat transfer coefficient h to thermal conductivity k of the fluid and characteristic dimension D.

$$N_{Nu} = (h\ D) / k$$

CHAPTER 3

METHODOLOGY

3.1 Methodology Overview

The overall methodology involved all the steps in achieving Black Seed oil. The whole study is divided into three major section:

i.) Sample preparation of dried black seed
ii.) Black seed oil extraction
iii.) Analysis via HPLC

3.2 Sample Preparation of Dried Black Seed

Black Seed was purchased from local herbs shop then it was dried using sun dried to remove the moisture from black seed. Prior to drying, sample is cleaned thoroughly to remove other things, for instance worms, so that extraction could be carried out accurately. The dried black seed at this stage had reached its constants weight. The constant weight is the residual moisture in the black seed.

The percent of moisture lost (ML) of black seed was determine by the following formula:

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

After identifying the ideal moisture loss of the ginger suitable for the extraction process, the sample is put in a basin before mix it with ethanol for solvent extraction process.

3.3 Black Seed oil Extraction

3.3.1 Experiment 1: The Extraction Time for Ethanol

The black seed sample is first extracted with the ethanol for 24 hours at atmospheric pressure and elevated temperature. After that the sample is placed in Ultrasonic Bath for 30 minutes to break down the cell wall hence oil diffusion will occur easily. Then solvent is removed through distillation under vacuum. This is done using rotary evaporator. Extraction of sample is done batch wisely using rotary evaporator. After the experiment is finished the justification is made . The procedure and operating condition of the experiment is summarized in figure 3.0.

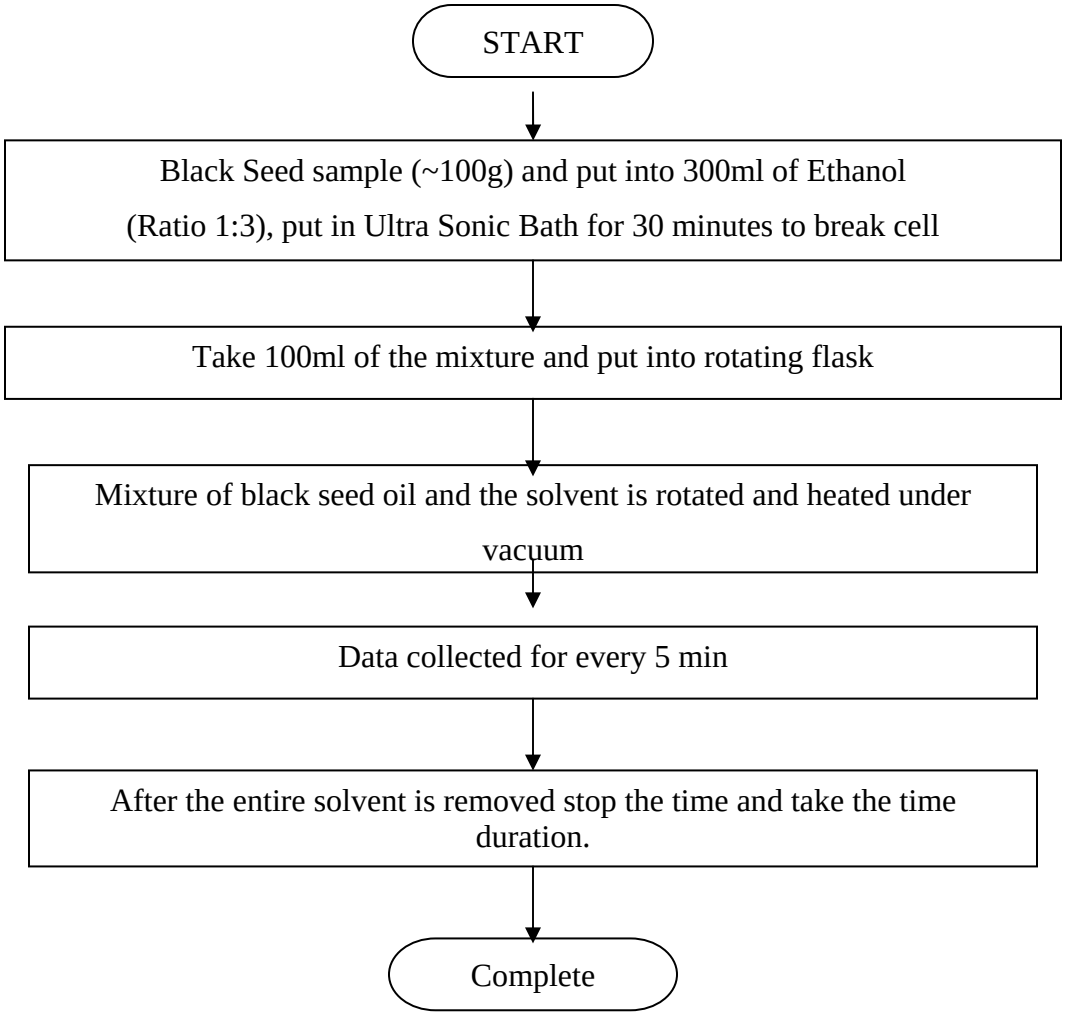


Figure 3.0: Summary of the fastest extraction time experiment for ethanol

3.3.2 Experiment 2: Extraction of Black Seed Oil with Various Temperatures

The extraction of black seed oil using ethanol as solvent. The black seed sample is first extracted with the solvent for 24 hours at atmospheric pressure and elevated temperature. After that sample is placed in ultra sonic bath for approximately 30 minutes to promote diffusion of the oil before solvent removal through distillation under vacuum. This is done using rotary evaporator. Extraction of sample is done batch wisely using

rotary evaporator. The procedure and operating condition of the experiment is summarized in figure 3.1.

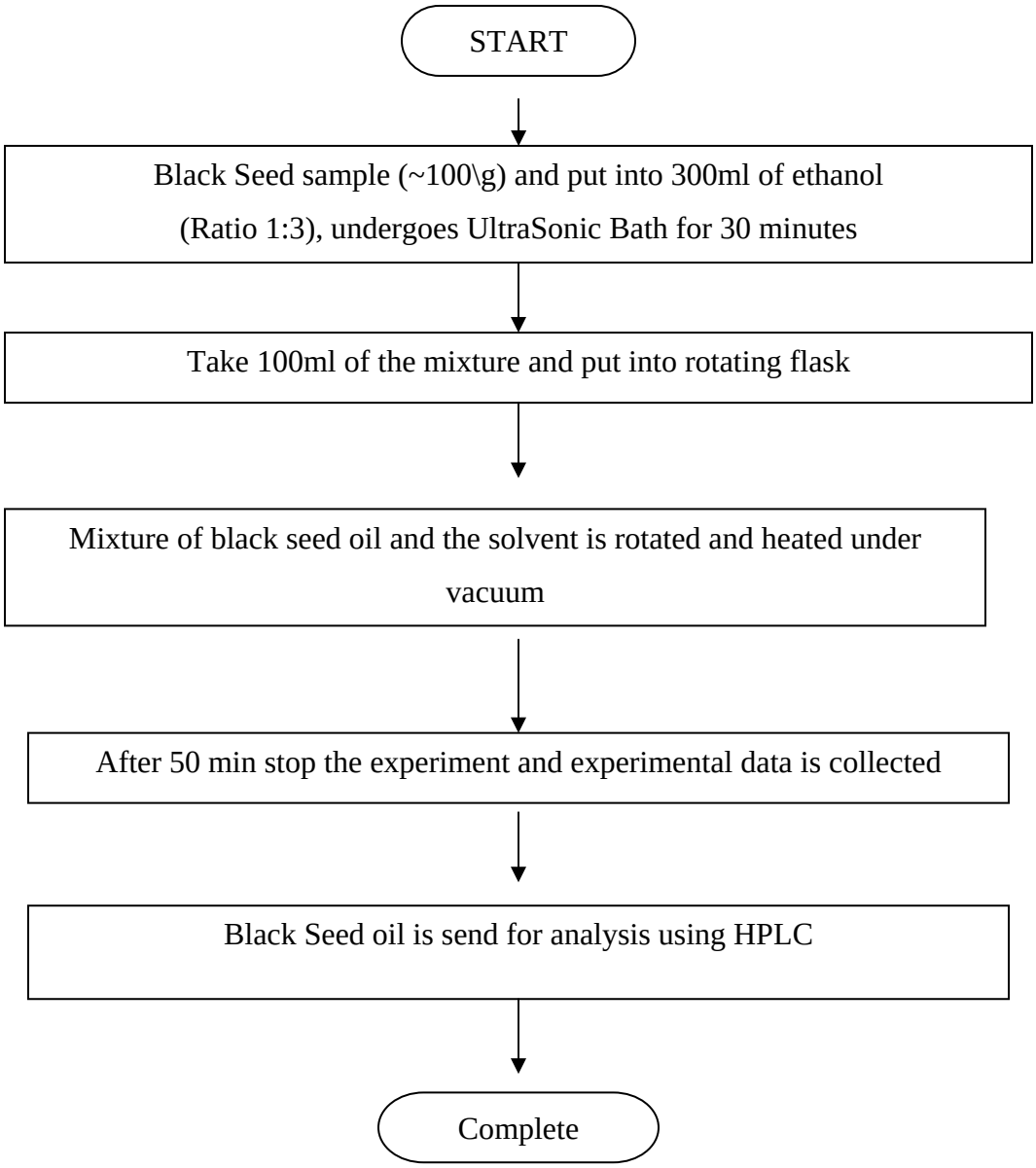


Figure 3.1: Extraction of black seed oil with Ethanol

3.4 Chemicals and Materials

Oil analysis: seed samples (20 g) from each sowing date were homogenized in 100 mL deionized water, together with 3 g of NaCl to avoid foaming, and the oil was distilled for 3 h by simultaneous distillation and extraction (SDE) in a LikensNickerson apparatus, using 50 mL of diethyl ether as the organic solvent. The extract was dried over anhydrous Na_2SO_4; the solvent was then evaporated under vacuum in a rotary evaporator. The oil content was determined gravimetrically and expressed on a dry weight basis.

The oil was analyzed by means oi a Hewlett Packard 5890 gas Chromatograph under the following conditions: BPX-5 fused silica capillary column (30 m × 0.25 mm, 0.25 μm film thickness); carrier gas, helium; injector, 240°C; detector, 280°C; temperature program: from 80°C (2 min) to 150°C (10 min) at 3°C/min, then to 240°C at 10°C/min. GC/MS analysis was carried out by means of a 3300 Variai) gas Chromatograph coupled with a Finuigan MAT ITD-40 mass spectrometer.

HPLC grade methanol and 2-propanol (Agilent Series) were used. Milipore filtered water was obtained by passing distilled water through a Mili-Q system (Milipore Corp., Milford, MA). Thymoquinone, thymol and dipehyl sulfone were purchased. Dithymoquinone was prepared from thymoquinone. The commercial Nigella Sativa oil was purchased. The local Nigella Sativa was purchased from Hamid Bros.

3.5 HPLC Analysis of Black Seed Oil

3.5.1 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.

3.5.2 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.

3.5.3 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.

3.5.4 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.

3.5.5 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.

3.5.6 Calibration Curves

Calibration curves of peak are ratios obtained by co-injecting different quantities of each analyte with a constant amount of diphenyl sulfon, This is 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 r2 > 0.99. The limit of detection for each of the analytes was carried out based on previous studies by Omar A. Ghosheh et. Al.

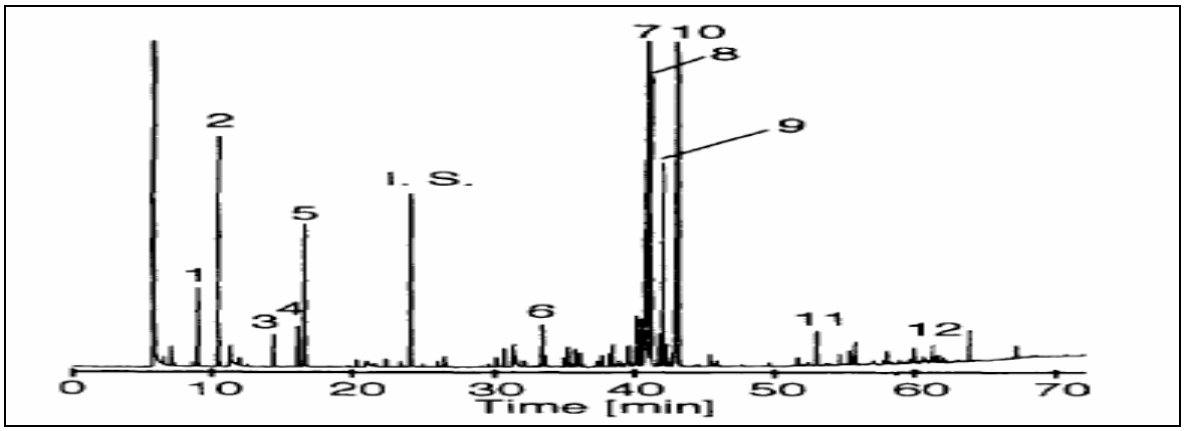


Figure 3.2: High Liquid Perfomance chromatogram of Black Seed Oil extraction (expected)

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

Upon completion the experiment part of this research,, the desired results were accomplished, producing some crucial data to be analyzed such as the fastest extraction time for solvent and also identification of the best operational temperature for black seed oil production . Figure 4.0 and 4.1 below shows the black seed oil after the experiment has been done.

Figure 4.1: Black Seed oil by using Ethanol as solvent

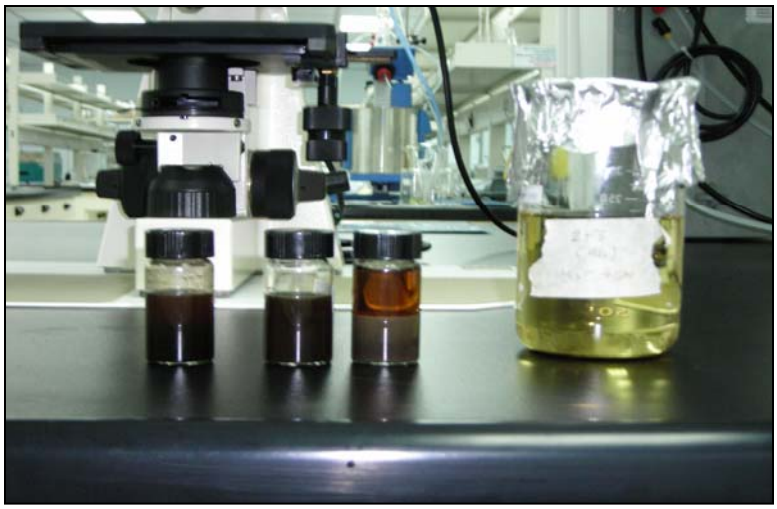


Figure 4.2: Black Seed oil by using water extraction

4.2 Experiment 1: Comparison on The Extraction Time for Difference Solvent

After the sample has been prepared, experiment has been done to find the fastest extraction time for each solvent to produce black seed oil. The extraction using rotary evaporator was done in 5 trials. Table 4.0 and Table 4.1 below show the result:

Table 4.1: Solvent removal using rotary evaporator

Sample: black seed	1	2	3
Weight(g)	100	100	100
Solvent	ethanol	ethanol	ethanol
Ratio solvent/sample	3:1	3:1	3:1
Duration of mixing(hr)	24	24	24
Vacuum Pressure(bar)	1.00	1.00	1.00
Temperature(^{0}C)	65	65	65
Extraction time(min)	50	52	48
Yield(ml)	28	30	20

Table 4.2: Solvent removal using rotary evaporator

Sample: black seed	1	2	3
Weight(g)	100	100	100
Solvent	water	water	water
Ratio solvent/sample	3:1	3:1	3:1
Duration of mixing(hr)	24	24	24
Vacuum Pressure(bar)	1.00	1.00	1.00
Temperature(^{0}C)	70	70	70
Extraction time(min)	120	120	150
Yield(ml)	20	18.6	22.4

The table above describes the difference in black seed oil yield and the difference of solvent extraction time for difference solvent. The result shows that ethanol extraction

time is faster than water extraction time. This is due to the fact that n-hexane is of the alkane group where its bond is susceptible to be diminished with a lesser amount of energy as appose to ethanol of alcohol group, bearing an atomic chain that requires a great deal of energy to be broken down.

Both Figure 4.3 and Figure 4.4.shows the extraction time for black seed oil. From the graph it is obvious that the fastest extraction time for ethanol is 48min while for water is 120min or 2hours. So ethanol was chosen for further experiment. It is because the time for experiment can be reduced.

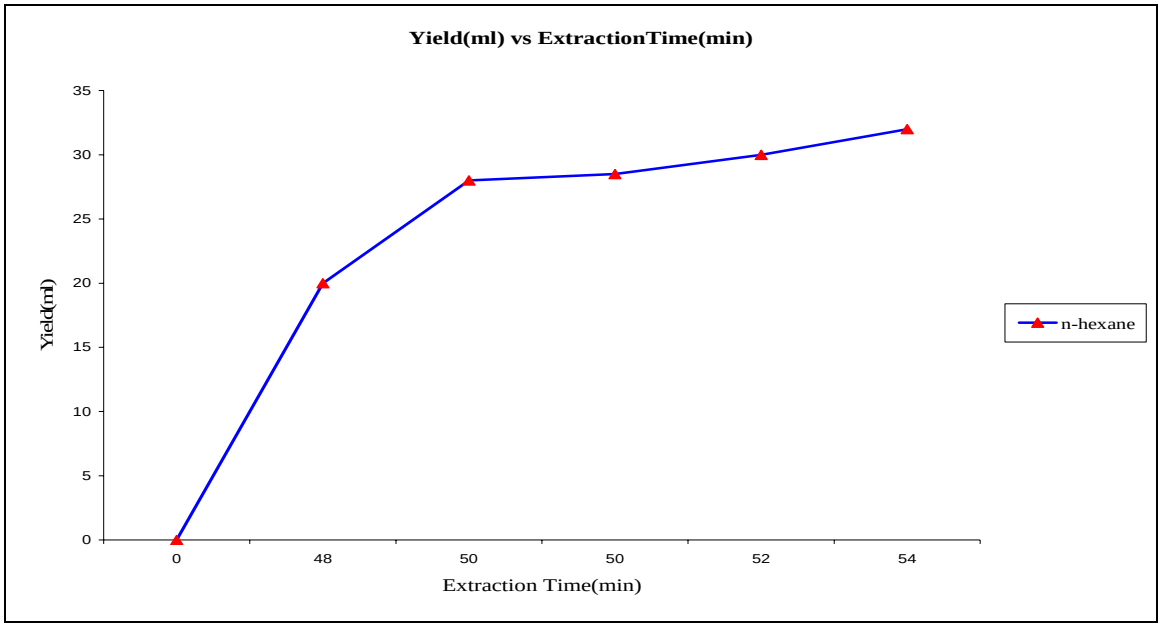


Figure 4.3: Yield of black seed oil (ml) versus ethanol extraction time (min)

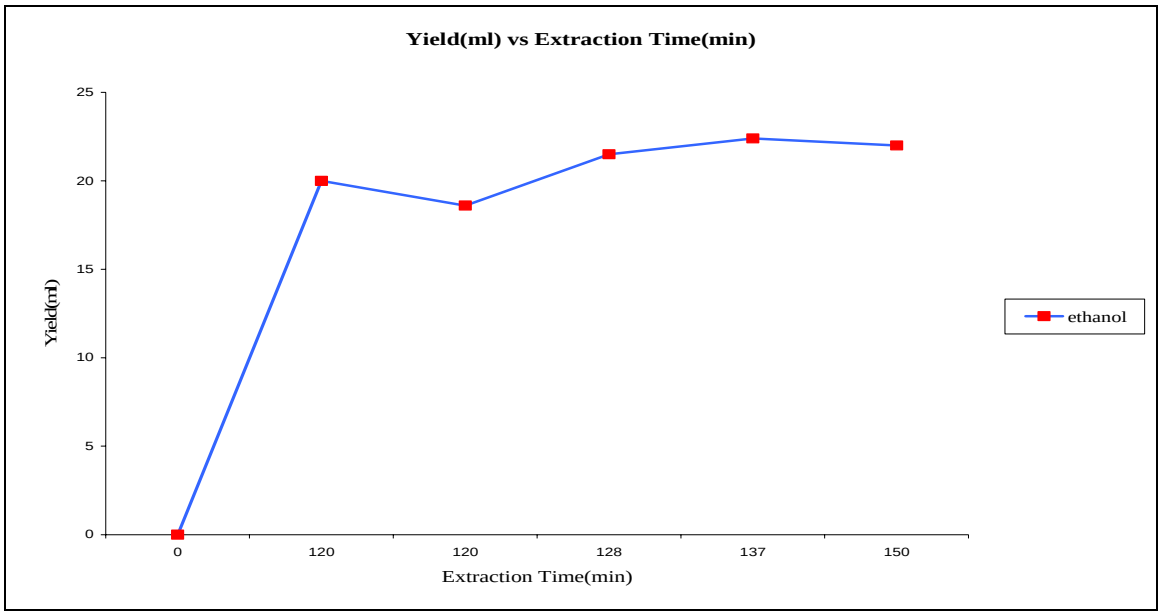


Figure 4.4: Yield of black seed oil (ml) versus water extraction time (min)

4.3 Experiment 2: Extraction of Black Seed Oil with Various Temperatures

After the suitable solvent has been identified, experiment is continued with difference temperatures to find the optimum temperature that produce q maximum yield of black seed oil. The extraction using rotary evaporator was carried out in 3 trials. Table 4.3 below show the result:

Table 4.3: Solvent removal using rotary evaporator with various temperatures

Sample: black seed	1	2	3
Weight(g)	100	100	100
Solvent	ethanol	ethanol	ethanol
Ratio solvent/sample	3:1	3:1	3:1
Duration of mixing(hr)	24	24	24
Vacuum Pressure (bar)	1.00	1.00	1.00
Temperature(^{0}C)	50	50	50
Extraction time(hr)	1.00	1.00	1.00
Yield(ml)	**43.0**	**45.0**	**43.0**
Temperature(^{0}C)	60	60	60
Extraction time(hr)	1.00	1.00	1.00
Yield(ml)	**39.0**	**35.0**	**35.0**
Temperature(^{0}C)	70	70	70
Extraction time(hr)	1.00	1.00	1.00
Yield(ml)	**24.0**	**23.5**	**24.0**
Temperature(^{0}C)	80	80	80
Extraction time(hr)	1.00	1.00	1.00
Yield(ml)	**4.0**	**3.5**	**4.5**
Temperature(^{0}C)	90	90	90

Temperature is one of the parameters that need a detailed analysis in determining the optimal condition for black seed oil. Excess heat will eventually decompose or transform the active ingredient such as quinone in black seed oil. Figures 4.4 below shows that higher black seed oil volume obtained when feed temperature were increased.

In this experiment, difference temperature has been used to determine the optimum temperature that produce optimum yield of black seed oil.

After the experiment, the data shows that, at 50^0C to 70^0C, black seed oil volume collected was decreased, meaning that further than these temperature will cause overheated black seed oil. Furthermore it will not be feasible to continue heating as feed temperature can be optimized between these two temperatures

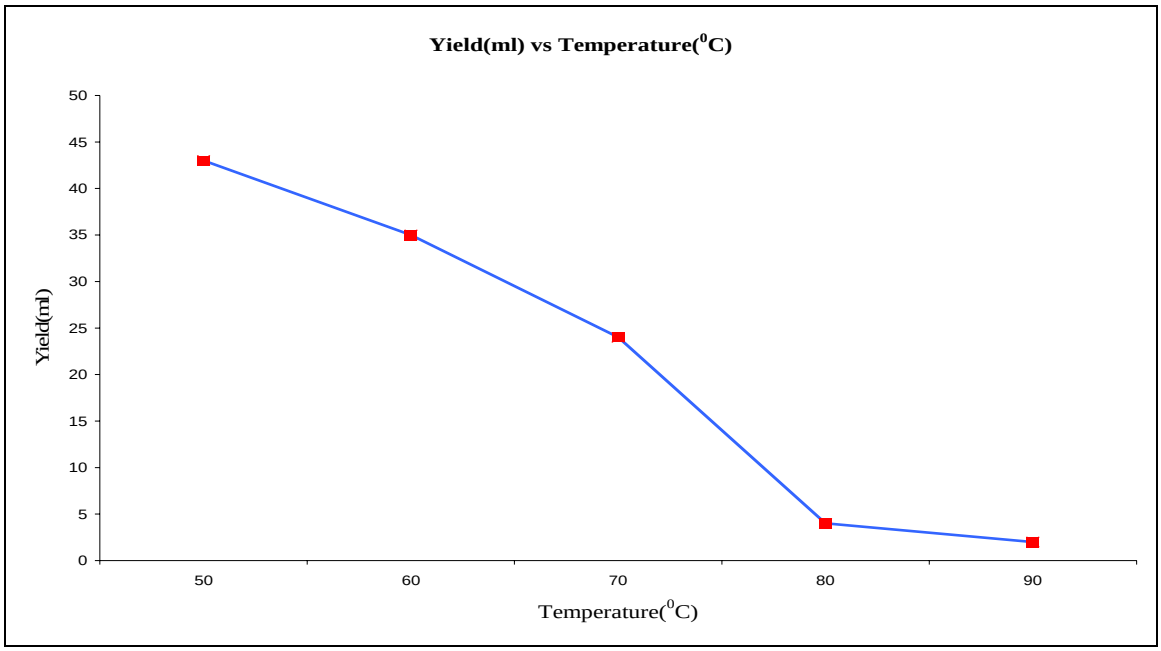


Figure 4.5: Yield (ml) versus Temperature (^{0}C)

For each experiment, prior entering rotary evaporator, each sample underwent a total of 30 minutes in the Ultrasonic Bath to promote diffusivity. Ultrasonic bath break the cell wall allowing the essential oil to slowly diffuse. Via this method, more yield of oild could be obtained.

Essential oil yield was 0.39±0.21% and 0.39±0.08% of seed diy weight for N. sativa. The higher variability of N. sativa was due to the effect of sowing date. The oil composition was very different between the two species. In fact, more than 90% of N. sativa oil was represented by monoterpenes.

Nigella Sativa oil was characterized by a high p-cymene content, often recognized as a typical component of the oil of this species, whereas the abundance of thymol and the low amount of thymoquinone are in contrast to what has been reported in the literature (3,4). Thymoquinone has been found recently in N. sativa oil (4), whereas other sources (8) report a 10:1 thymoquinone/thymol ratio as minor constituents in commercial fatty oil of N. sativa.

Most of the literature refers to seeds obtained in hot dry areas of north Africa and western Asia, where temperature and water availability during seed ripening are quite different from those during these trials, which corresponded to a late-spring, higher latitude climate. Thymoquinone is easily obtained by oxidation of thymol (9). It can therefore be supposed that the ratio between the two components is determined by the different environmental factors occurring during their biosynthesis.

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:

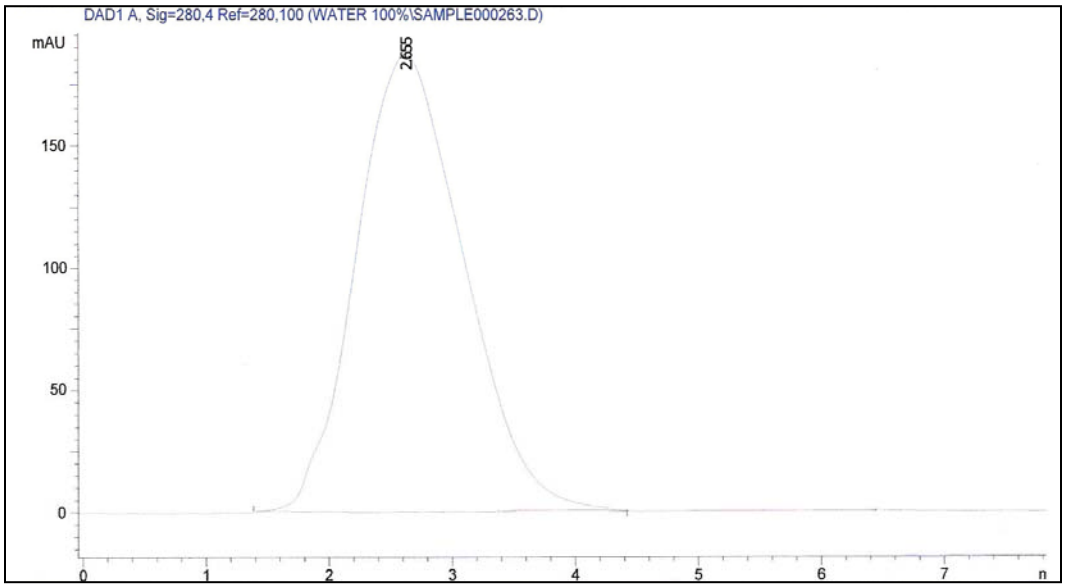


Figure 4.6: Standard curve for Thymoquinone

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. Theoretically, 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 or minimized, 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 RECOMMENDATION

5.1 Conclusion

Black Seed Oil could be extracted using solvent extraction and ethanol appears to be the best solvent to do so. Thymoquinone is the major constituent in Black Seed Oil. Temperature and time of extraction play a major part in determining the highest yield of black seed oil. This research; is very crucial in determining the vital compounds that verifies and distinguished Black Seed Oil Samples from around the globe. Expectantly, through this research our local product can and will be improved. Black Seed Oil varies based on origins as it is cultivated differently. The most active compound would absolutely be Thymoquinone as it offers numerous heath benefits. Analysis of Black Seed Oil could be carried out using HPLC.

5.2 Recommendation

It is highly recommended that this research is continued with other solvents and other method so that the best extraction method to extract Black Seed oil could be pin-point. In terms of analysis, it is a good idea to run the sample using LC-MS as the equipment tends to generate a better reading and result since it is entirely based on molecular weight. Molecular weight would not change regardless of the pH and retention time. For future works, more compounds of Black Seed oil should be analyzed since this oil consists of numerous compounds that confer health benefits.

Meticulous attention should be paid especially during preparation of sample prior analyzing using HPLC as HPLC is a very sensitive equipment. This is essential in ensuring a better reading and a better life span for HPLC itself.

REFRENCES

1. T.G. Tutin, Nigella L. In: Flora Europaea. Edits., T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters and D.A. Webb, pp 209-210, University Press, Cambridge (1972).
2. Saeed, M. Afzal Rizvi and L. Ahmed, Cultivation of medicinal herbs at Madinat al-Hikmah. Hamdard Med., 39(2), 23-26 (1996).
3. M. Riaz, M. Syed and P.M. Chaudhary, Chemistry of the medicinal plants of the genus Nigella. Hamdard Med., 39(2), 40-45 (1996).
4. F.S. Mozzafari, M. Ghorbanli, A. Babai and M. Farzami Scpehr, The effect of water stress on the seed oil of Nigella sativa L. J. Essent. oil Res., 12,36-38(2000).
5. A.A. Siddiqui and P.K.R. Sharma, Clinical importance of Nigella sativa L.-a review. Hamdard Med., 39(4), 38-42 (1996).
6. T. Shibamoto, Retention indices in essential oil analysis. In: Capillary gas chromatography in essential oil analysis. Edits., P. Sandra and C. Bicchi, pp 259-274, Huethig Verlag, Heidelberg (1987).
7. R.P. Adams, Identification of essential oil components by gas chromatography/massspectrometry. Allured Publ. Corp., Carol Stream, IL (1995).

8. O.A. Ghosheh, A.E. Abdulghani and P.A. Crooks, High performance liquid Chromatographie analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (Nigella sativa L.). J. Pharm. Biomed. Anal., 19, 757-762 (1999).
9. R.R.L. Martins, M.G.P.M.S. Neves, AJ.D. Silvestre, A.M.S. Suva and J.A.S. Cavaleiro, Oxidation of monoterpenes with hydrogen peroxide catalysed by Mn(III) porphirin complexes. J. Molec. Catal. A: Chem., 137, 41 -47 (1999).
10. F. Tillequin, C. Leconte and M. Paris, Carbures sesquiterpeniques des graines de Nigella damascena. Planta Med., 30, 59-61 (1976).
11. T.G. Tutin, Nigella L. In: Flora Europaea. Edits., T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters and D.A. Webb, pp 209-210, University Press, Cambridge (1972)
12. A. Saeed, M. Afzal Rizvi and L. Ahmed, Cultivation of medicinal herbs at Madinat al-Hikmah. Hamdard Med., 39(2), 23-26 (1996).
13. M. Riaz, M. Syed and P.M. Chaudhary, Chemistry of the medicinal plants of the genus Nigella. Hamdard Med., 39(2), 40-45 (1996)
14. F.S. Mozzafari, M. Ghorbanli, A. Babai and M. Farzami Scpehr, The effect of water stress on the seed oil of Nigella sativa L. J. Essent. oil Res., 12,36-38(2000).
15. A.A. Siddiqui and P.K.R. Sharma, Clinical importance of Nigella sativa L.-a review. Hamdard Med., 39(4), 38-42 (1996).

16. T. Shibamoto, Retention indices in essential oil analysis. In: Capillary gas chromatography in essential oil analysis. Edits., P. Sandra and C. Bicchi, pp 259-274, Huethig Verlag, Heidelberg (1987).
17. R.P. Adams, Identification of essential oil components by gas chromatography/massspectrometry. Allured Publ. Corp., Carol Stream, IL (1995).

APPENDIX

Material Safety Data Sheet
Thymoquinone, 99%

ACC# 97941

Section 1 - Chemical Product and Company Identification

MSDS Name: Thymoquinone, 99%
Catalog Numbers: AC305070000, AC305070010
Synonyms: 2-Isopropyl-5-methyl-1,4-benzoquinone
Company Identification:

Acros Organics N.V.
One Reagent Lane
Fair Lawn, NJ 07410

For information in North America, call: 800-ACROS-01
For emergencies in the US, call CHEMTREC: 800-424-9300

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
490-79-9	2,5-Dihydroxybenzoic acid, 99%		207-718-5

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: gold solid.
Caution! May cause eye and skin irritation. May cause respiratory and digestive tract irritation. The toxicological properties of this material have not been fully investigated.
Target Organs: None known.

Potential Health Effects
Eye: May cause eye irritation.
Skin: May cause skin irritation.
Ingestion: May cause irritation of the digestive tract. The toxicological properties of this substance have not been fully investigated.
Inhalation: May cause respiratory tract irritation. The toxicological properties of this substance have not been fully investigated.
Chronic: None

Section 4 - First Aid Measures

Eyes: Flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid.
Skin: Get medical aid if irritation develops or persists. Flush skin with plenty of soap and water.
Ingestion: If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid.
Inhalation: Remove from exposure and move to fresh air immediately. Get medical aid if cough or other symptoms appear.
Notes to Physician: Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information: As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear.
Extinguishing Media: Use water spray, dry chemical, carbon dioxide, or chemical foam.
Flash Point: 103 deg C (217.40 deg F)
Autoignition Temperature: Not available.
Explosion Limits, Lower: Not available.
Upper: Not available.
NFPA Rating: Not published.

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks: Sweep up or absorb material, then place into a suitable clean, dry, closed container for disposal. Avoid generating dusty conditions.

Section 7 - Handling and Storage

Handling: Wash thoroughly after handling. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Keep container tightly closed. Avoid ingestion and inhalation.
Storage: Store in a tightly closed container. Store in a cool, dry, well-ventilated area away from incompatible substances.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Use adequate ventilation to keep airborne concentrations low.
Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
2,5-Dihydroxybenzoic acid, 99%	none listed	none listed	none listed

OSHA Vacated PELs: 2,5-Dihydroxybenzoic acid, 99%: No OSHA Vacated PELs are listed for this chemical.
Personal Protective Equipment
Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.
Skin: Wear appropriate protective gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to prevent skin exposure.
Respirators: Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

Section 9 - Physical and Chemical Properties

Physical State: Solid
Appearance: gold
Odor: Not available.
pH: Not available.
Vapor Pressure: Not available.
Vapor Density: Not available.
Evaporation Rate: Not available.
Viscosity: Not available.
Boiling Point: 232 deg C

Freezing/Melting Point:45.5 deg C
Decomposition Temperature: Not available.
Solubility: Not available.
Specific Gravity/Density: Not available.
Molecular Formula:C10H12O2
Molecular Weight:164.20

Section 10 - Stability and Reactivity

Chemical Stability: Stability unknown.
Conditions to Avoid: Incompatible materials.
Incompatibilities with Other Materials: Strong oxidizing agents.
Hazardous Decomposition Products: Carbon monoxide, carbon dioxide.
Hazardous Polymerization: Has not been reported

Section 11 - Toxicological Information

RTECS#:
CAS# 490-79-9: LY3850000
LD50/LC50:
CAS# 490-79-9:
Oral, mouse: LD50 = 4500 mg/kg;
.

Carcinogenicity:
CAS# 490-79-9: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

Epidemiology: No data available.
Teratogenicity: No data available.
Reproductive Effects: No data available.
Mutagenicity: Mutation:see RTECS
Neurotoxicity: No data available.
Other Studies:

Section 12 - Ecological Information

No information available.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.
RCRA P-Series: None listed.
RCRA U-Series: None listed.

Section 14 - Transport Information

	US DOT	Canada TDG
Shipping Name:	DOT regulated - small quantity provisions apply (see 49CFR173.4)	No information available.
Hazard Class:		

UN Number:
Packing Group:

Section 15 - Regulatory Information

US FEDERAL

TSCA
CAS# 490-79-9 is listed on the TSCA inventory.
Health & Safety Reporting List
None of the chemicals are on the Health & Safety Reporting List.
Chemical Test Rules
None of the chemicals in this product are under a Chemical Test Rule.
Section 12b
None of the chemicals are listed under TSCA Section 12b.
TSCA Significant New Use Rule
None of the chemicals in this material have a SNUR under TSCA.
CERCLA Hazardous Substances and corresponding RQs
None of the chemicals in this material have an RQ.
SARA Section 302 Extremely Hazardous Substances
None of the chemicals in this product have a TPQ.
SARA Codes
CAS # 490-79-9: immediate.
Section 313 No chemicals are reportable under Section 313.
Clean Air Act:
This material does not contain any hazardous air pollutants.
This material does not contain any Class 1 Ozone depletors.
This material does not contain any Class 2 Ozone depletors.
Clean Water Act:
None of the chemicals in this product are listed as Hazardous Substances under the CWA.
None of the chemicals in this product are listed as Priority Pollutants under the CWA.
None of the chemicals in this product are listed as Toxic Pollutants under the CWA.
OSHA:
None of the chemicals in this product are considered highly hazardous by OSHA.
STATE
CAS# 490-79-9 is not present on state lists from CA, PA, MN, MA, FL, or NJ.

California Prop 65

California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations
European Labeling in Accordance with EC Directives
Hazard Symbols:
Not available.
Risk Phrases:

Safety Phrases:
S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S 37/39 Wear suitable gloves and eye/face protection.

WGK (Water Danger/Protection)
CAS# 490-79-9: No information available.
Canada - DSL/NDSL
CAS# 490-79-9 is listed on Canada's DSL List.
Canada - WHMIS
WHMIS: Not available.
This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all of the information required by those regulations.

Canadian Ingredient Disclosure List

Section 16 - Additional Information

MSDS Creation Date: 9/02/1997
Revision #3 Date: 10/03/2005

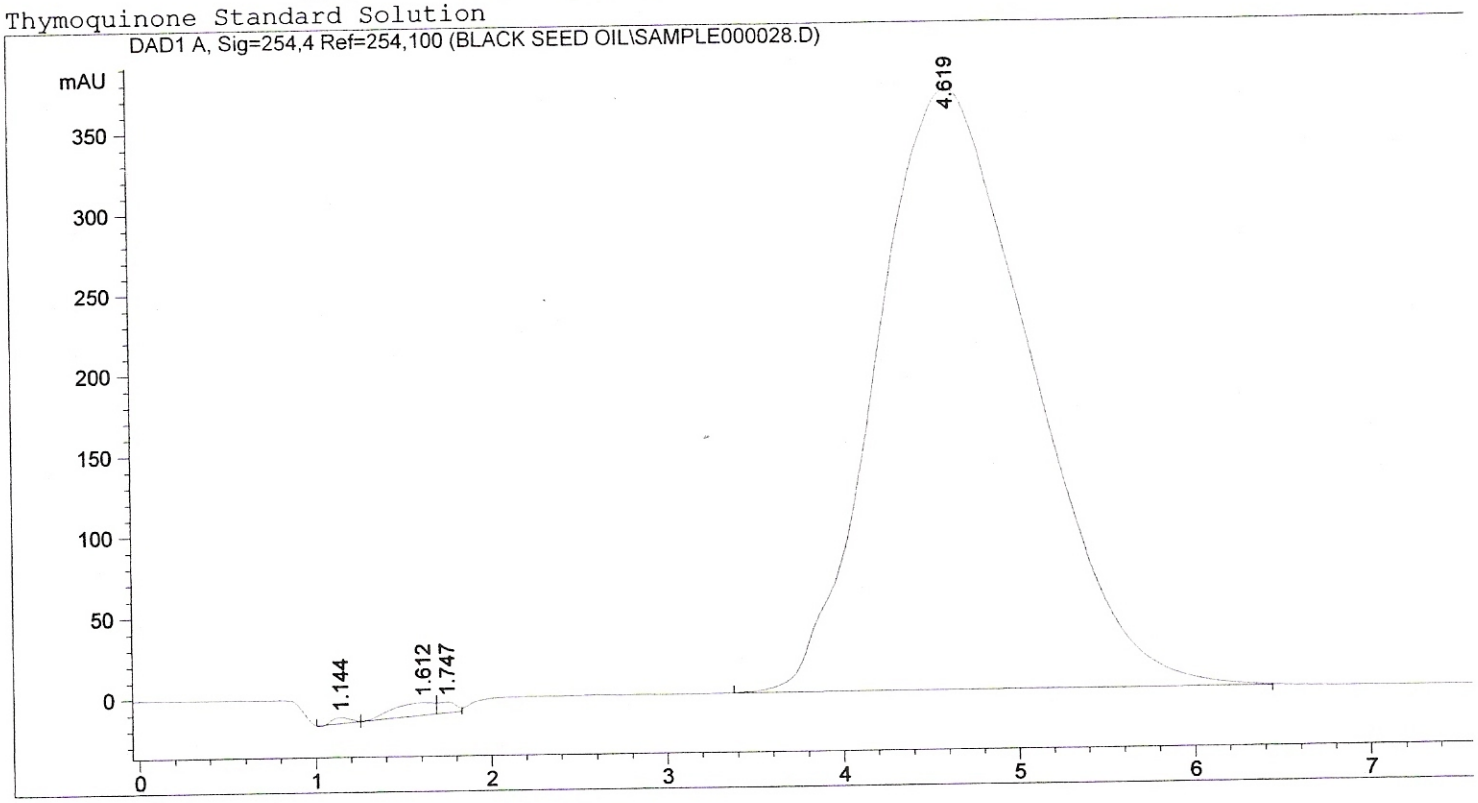
The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall Fisher be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Fisher has been advised of the possibility of such damages.

Data File C:\Chem32\1\DATA\BLACK SEED OIL\SAMPLE000028.D Standard
Sample Name: Thymoquinone

T
hymoquinone

```
==========================================================================
Injection Date  : 10/14/2006 11:41:39 AM
Sample Name     : Thymoquinone                    Location : Vial 1
Acq. Operator   : Nigella Sativa
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




```
==========================================================================
                        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 Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.144 BV    0.1167   24.11486    3.57386   0.1060
   2   1.612 VV    0.2778  134.66527    7.78679   0.5918
   3   1.747 VV    0.1077   48.31226    6.57759   0.2123
   4   4.619 BB    0.9092 2.25476e4  371.98486  99.0899

Totals :                   2.27547e4  389.92311

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

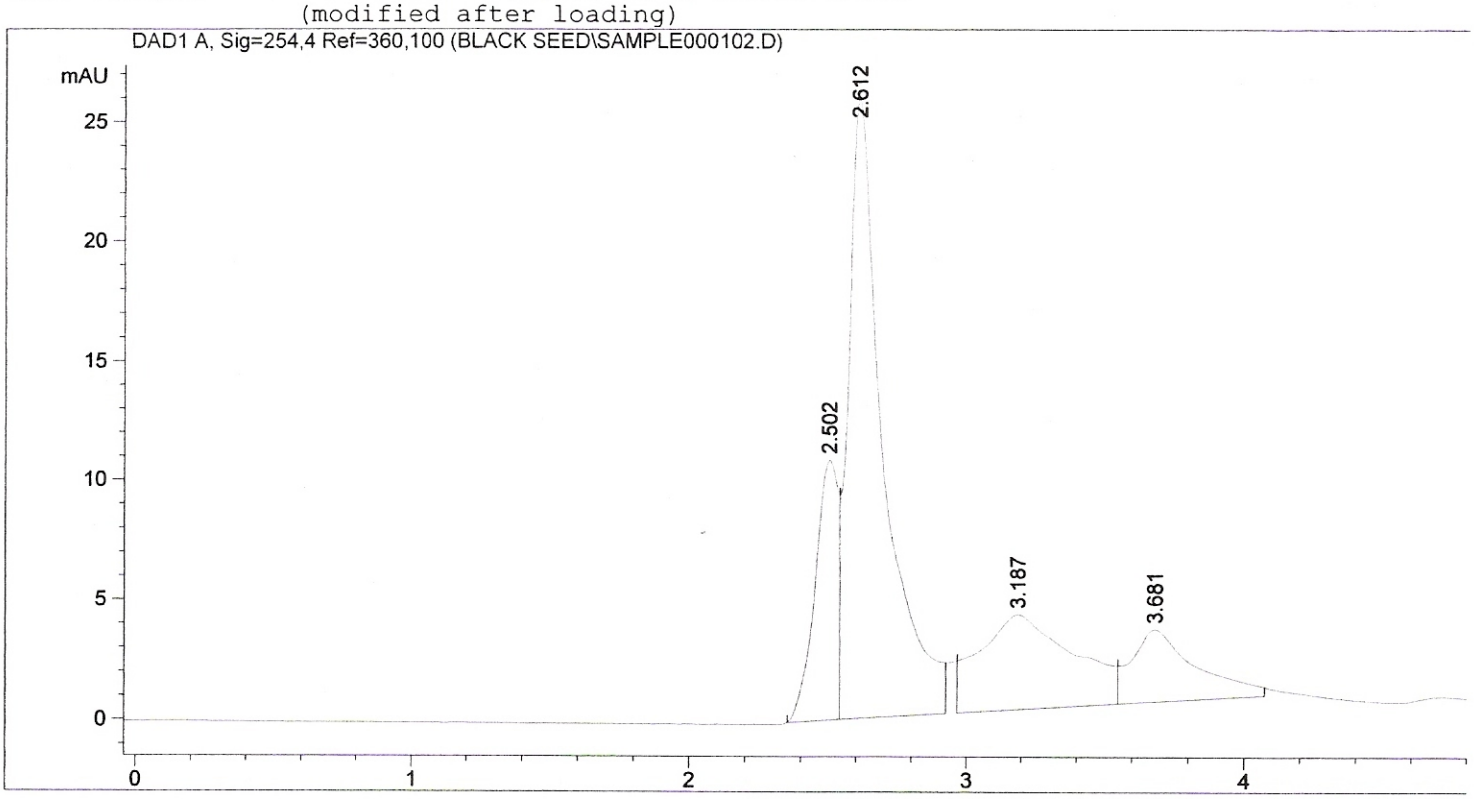
Data File C:\Chem32\1\DATA\BLACK SEED\SAMPLE000102.D
Sample Name: thymoquinone

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)



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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=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	

==========

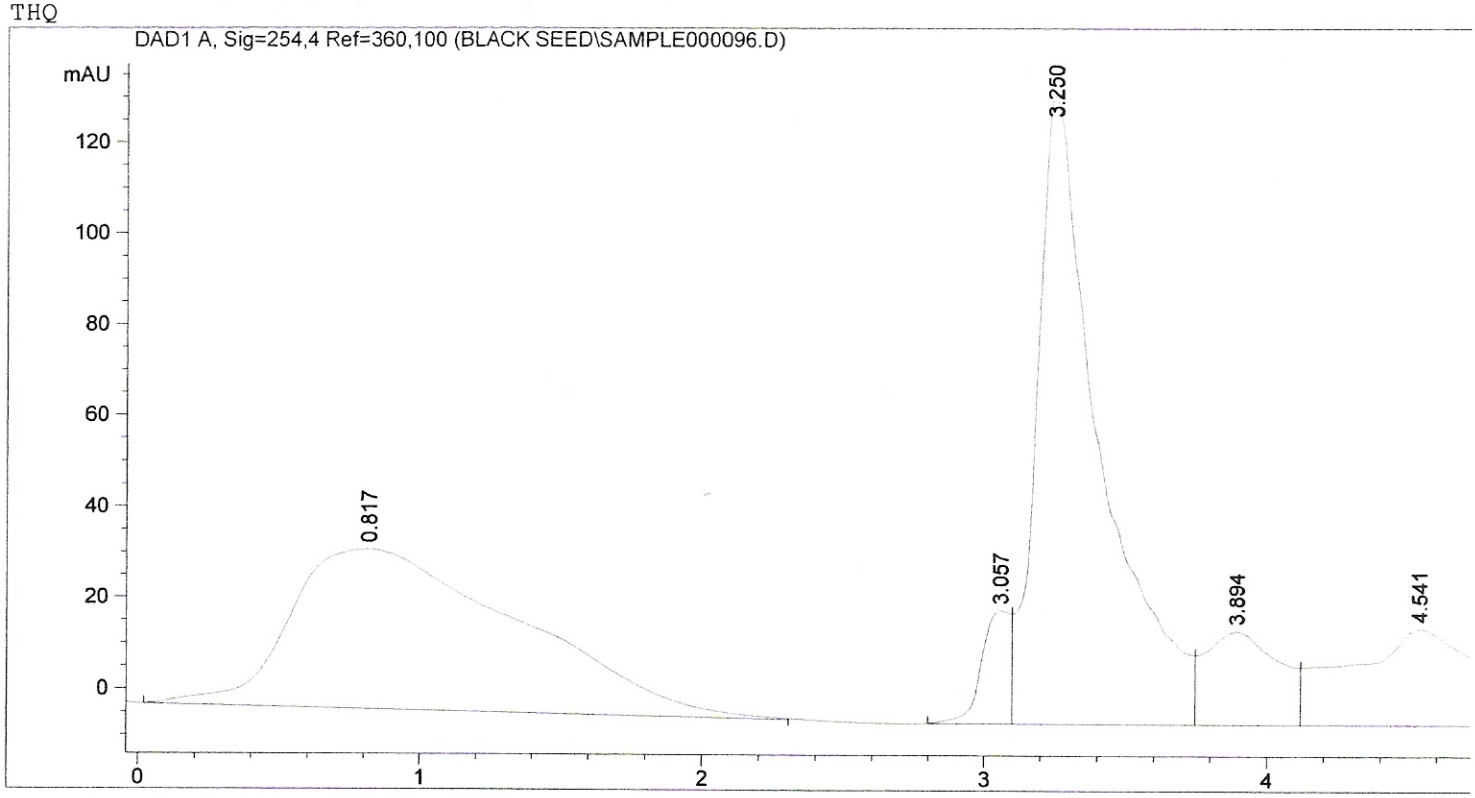
*** End of Report ***

Data File C:\Chem32\1\DATA\BLACK SEED\SAMPLE000096.D
Sample Name: thymoquinone

Thymoquinone

```
================================================================================
Injection Date  : 10/15/2006 10:21:47 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 10:19:45 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 Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.817 BB    0.7993 1976.39539   34.97383  36.4846
   2   3.057 BV    0.1088  175.41833   24.72391   3.2383
   3   3.250 VV    0.2179 2169.94775  137.64276  40.0576
   4   3.894 VV    0.2520  370.57870   20.38948   6.8410
   5   4.541 VBA   0.4482  724.72290   21.02242  13.3785

Totals :                  5417.06308  238.75240

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