EXTRACTION AND CHARACTERIZATION OF *JATROPHA CURCAS LINNAUES* SEED OIL THROUGH SOXHLET METHOD

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SUPERVISOR’S DECLARATION

“I hereby declare that I have read this thesis and in my opinion this thesis has fulfilled the qualities and requirements for the award of Degree of Bachelor of Chemical Engineering”

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STUDENT'S DECLARATION

I declare that this thesis entitled "Extraction and Characterization of *Jatropha curcas Linnaeus* Seed Oil through Soxhlet Method" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

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Special thanks to my beloved father and mother for their constant encouragement
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LIST OF ABBREVIATIONS

AEOE	Aqueous Enzymatic Oil Extraction
FAME	Fatty Acid Methyl Ester
GC-MS	Gas Chromatography-Mass Spectrometer
HAP	Hazardous Air Pollutant
MACT	Maximum Achievable Control Technology
MAE	Microwave- Assisted Extraction
PTFE	Polytetrafluoroethylene
SFE	Supercritical Fluid Extraction
TAG	Triacylglyceride
UAE	Ultrasonic-Assisted Extraction
VOC	Volatile Organic Compound

LIST OF SYMBOLS

^0C	Degree Celcius
h	hour
mL	mili-liter
µL	micro-liter
g	Gram
%	Percentage
min	Minute
w/w	weight of oil/weight of plant materials

PENGESTRAKAN DAN PENCIRIAN MINYAK JARAK PAGAR DARI BIJI *JATROPHA CURCAS LINNAUES* MELALUI KAEDAH SOXHLET

ABSTRAK

Penurunan pengeluaran bahan api fosil berikutan dengan susutan sumber petroleum dan keprihatinan terhadap alam sekitar telah menyebabkan dunia beralih kepada tenaga boleh diperbaharui, biodiesel dan mengurangkan kebergantugan kepada bahan api fosil. Kini, minyak jarak pagar banyak digunakan sebagai bahan mentah untuk biodiesel disebabkan harganya yang murah berbanding bahan mentah lain seperti minyak soya dan minyak bunga matahari. Kajian ini bertujuan untuk menyiasat keberkesanan pengestrakan minyak jatropha melalui kaedah soxhlet. Pengestrakan minyak jarak pagar dari biji *Jatropha curcas Linnaeus* dijalankan bawah tiga faktor utama, pelarut pengestrakan, etil asetat dan etanol, masa pengestrakan 6 jam, 8 jam, 10 jam dan nisbah pelarut kepada sampel jatropha, 7.5:1, 10:1 dan 12.5:1. Pelarut pengestrakan yang sering digunakan dalam indutsri adalah n-heksana yang disenaraikan sebagai pencemar udara berbahaya. Oleh itu, pelarut alternatif yang lebih mesra alam dan memberi keputusan akhir yang hampir dengan n-heksana telah dikaji. Parameter pengestrakan terbaik didapati melalui kajian ini telah ditetapkan berdasarkan pengeluaran minyak jarak pagar yang paling maksima. Pelarut etil asetat, masa pengestrakan 8 jam dan nisbah yang paling tinggi telah ditetapkan sebagai parameter optimum kerana memberi minyak dengan hasil yang banyak iaitu 47.38% (kg/kg). Kemudian, sampel minyak pada masa pengestrakan yang berbeza telah dianalisis melalui GC-MS untuk menilai kualiti minyak dengan menentukan konstituen minyak. Antara konstituen utama yang telah dikesan dalam minyak yang diekstrak pada masa 8 jam dan pelarut etil asetat ialah asid oleik, asid linoleik, asid palmitik, asid stearik, oleoaldehyde, metil linolate, etil oleat, metil linolelaidate, metil stearat dan squalene, di mana asid oleik dan asid linoleik adalah komponen dominan bagi kebanyakan minyak yang diekstrak. Kaedah pengestrakan soxhlet adalah lebih berkesan daripada kaedah pengestrakan lain dari segi harga, kebolehgunaan, dan juga hasil minyak dari segi kualitatif dan kuantitatif. Selain itu, etil asetat telah dipilih sebagai pelarut alternatif yang sesuai dengan kriteria mesra alam dan keberkesanan dalam memberikan hasil setanding dengan n-heksana.

EXTRACTION AND CHARACTERIZATION OF *JATROPHA CURCAS LINNAEUS* SEED OIL THROUGH SOXHLET METHOD

ABSTRACT

Decrease in fossil fuel production due to depletion of petroleum resources and concern toward environmental have made the world to turn into renewable energy, biofuel and reduce its dependency on fossil fuel. Jatropha oil, less expensive feedstock as it is inedible oil has been used as biofuel raw material instead of edible oils which are more expensive because of its competitive food uses. This study was carried out to investigate the performance of soxhlet extraction in the extraction of jatropha oil from *Jatropha curcas Linnaeus* seed under three main factors which were extraction solvent, ethyl acetate and ethanol, extraction time, 6 hour, 8 hour and 10 hour and solvent to solid ratio, 7.5:1, 10:1 and 12.5:1. Current extraction solvent used for jatropha oil extraction in industry is n-hexane which is listed as hazardous air pollutant (HAP). Hence, an alternative solvent which is more environmental-friendly and yields comparable result with n-hexane are studied. The best extraction condition that has been determined for maximum jatropha oil production was under solvent ethyl acetate at 8 hour extraction time and highest solvent to solid ratio. This optimum condition was finalized based on its maximum yield of jatropha oil which was 47.38% (w/w). Then, the oil samples at different extraction time were analyzed to evaluate its quality by determining its chemical constituent through GC-MS. The main components detected in the jatropha oil with solvent ethyl acetate and 8 hour extraction time were oleic acid, linoleic acid, palmitic acid, stearic acid, oleoaldehyde, methyl linolate, ethyl oleate, methyl linolelaidate, methyl stearate and squalene where oleic acid and linoleic acid are the dominant component for most of the oil recovered through extraction. Soxhlet extraction method is much more efficient than other extraction method in term of cost, operability and also yield of oil, quantitatively and qualitatively. Besides, ethyl acetate has been chosen as suitable alternative environmentally friendly as it gives comparable result with n-hexane.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Jatropha curcas L. or its common name, physic nut, is from the family *Euphorbiaceae.* It is drought-resistant plant which grows well in marginal land of low to high rainfall areas and can be used as a commercial crop. Jatropha is easy to propagate and can easily survive with minimum care. It grows wild in many areas and even survives in infertile soil. The plant is easy to grow and produces seed for 50 years life span. The maximum oil content that has been reported for jatropha seeds has been close to 47%. However, the accepted average is 37 - 40%. Hence, it can be the best plant for future biodiesel production (Jongschaap, *et al.,* 2007). Besides using as a biodiesel feedstock, jatropha can also used for saponification of oil in process of making soap, cleaning, dye for clothes, medicinal purposes, and organic fertilizer and also as antidotes for snake bites. Jatropha oil can even be used to soften leather and lubricate machinery. Each part of jatropha plant, its leaves, flowers, fruits, and seeds has its own uses which make it a multi-beneficial plant (Gubitz, *et al.,* 1999, Felix and Clement, 2011).

Jatropha oil is a vegetable oil which is produced from *Jatropha curcas Linnaeus* (*Jatropha curcas L.)* seed. Due to the toxicity of jatropha seeds, the plant is cultivated for the sake of extracting the oil as it cannot be ingested by humans. Jatropha oil can be used as biodiesel feedstock where biodiesel means diesel fuel that is extracted from oil and fats of plants (Omotoso, *et al.,* 2011). Biodiesel is a renewable form of energy which is similar to conventional diesel fuel. Besides, this energy is environmental-friendly as jatropha oil has very low emission and it is also easily produced in rural areas.

Extraction of *Jatropha curcas L.* can be done through many methods such as ultrasonic-assisted extraction, microwave-assisted extraction, mechanical extraction, supercritical fluid extraction, aqueous enzymatic oil extraction and also soxhlet extraction (Felix & Clement, 2011). Soxhlet extraction is a method done to recover a component from either a solid or liquid (Ogbobe & Akano, 1991). The research involves extraction of *Jatropha curcas L.* seed through soxhlet method and characterization of the oil obtained through gas chromatography-mass spectrometry (GC-MS) method.

This research is mainly conducted to determine an environmental-friendly method of the extraction of *Jatropha curcas L.* seed. An optimum extraction condition which is also cost effective is required to increase the yield of the oil. The research is also to determine the constituents of the oil obtained followed by the applicability and commercialization of the oil.

1.2 Problem Statement

Recently, jatropha has been recognized as the major source of biodiesel production as it has the highest percentage of oil content which comes to an average of 37 to 40%. It has been a marked substitute in the fuel industries for automotive diesel of locomotives (Omotoso, *et al.*, 2011). According to the current ratio, the price of jatropha seeds is much lower than the oil price. But as the demand of jatropha oil as biofuel increases, the cost will increase too with consideration of jatropha plantation and the product price. Besides, the cost also depends on the quality of the oil extracted from the seed. The increasing demand of jatropha oil also has opened up wide opportunities for global marketing which leads to the requirement of competitive products which comes in advantage in term of quality, cost and production time (Openshaw, 2000). Hence, it is best to identify a best extraction technique, as to extract higher yield of oil with higher quality at lower cost.

Other than that, this research is also conducted to improve existing extraction process toward more environmental-friendly method. The existing process makes use of n-hexane, a solvent obtained from petrochemical sources which will be emitted during extraction and recovery process. Hexane is a volatile organic compound which can cause air pollution when it reacts with ozone in the atmosphere. But still, many existing processes are using n-hexane because it is lower in cost and gives good yield of oil recovery from the plant (Suzanna, *et al.*, 2003). Hence, it is necessary to come up with an alternative organic extraction solvent which gives approximately the same yield as n-hexane which is lower in cost and also environmental-friendly.

Many researches have been conducted before using jatropha and also soxhlet method, but still room for enhancement can be made. Existing extraction method can still be improved by optimizing extraction parameters such as extraction time, solvent to solid ratio and also type of solvent used. In this research, we are using ethyl acetate and ethanol as the extraction solvent replacing the current solvent hexane and study the effect of the solvent on the yield of the oil from jatropha seeds.

1.3 Objectives

The objectives of this research are

1) To study the performance of an environmental-friendly method of extraction of Jatropha curcas L. seed oil through Soxhlet method.

2) To identify the chemical composition of the oil extracted from Jatropha curcas L. seed oil through Soxhlet method using Gas Chromatography-Mass Spectrometer (GC-MS) method

1.4 Scope of Research

The scope of this research is to find the yield of jatropha oil extracted and also the efficiency of the soxhlet method. Besides that, the effect of various parameters such as extraction time, and solvent to solid ratio on the yield of jatropha oil will be studied. The extraction time used in this research will be 6 hour, 8 hour and 10 hour, where else the solvent to solid ratio will be 7.5:1, 10:1 and 12.5:1. Not only that, the effect of the type of extracting solvent such as ethyl acetate and ethanol on extraction of jatropha oil from its seed will be studied. Optimum operating condition will be obtained to maximize the yield of oil from extraction. Last but not least, the oil extracted from jatropha seed will be analyzed for its constituents using Gas Chromatography-Mass Spectrometry (GC-MS) method.

1.5 Significance of Proposed Study

This research will be helpful in determination of the performance of soxhlet method in the extraction of jatropha oil from *Jatropha curcas L.* seed based on the yield of the oil extracted. The result of this research would also signify the identification of the optimum extraction parameters for the production of jatropha oil from its seed to fulfill the high demand of jatropha oil as biofuel feedstock and also for other purposes such as medicinal use, soap making, and as natural hedge.

Besides, through this research an environmentally friendly method will be identified for the production of jatropha oil through the selection of suitable extracting solvent between ethanol and ethyl acetate. Soxhlet method has been chosen for this research as it is cheaper than other extraction method, hence reduces the production cost of the oil. This method also yields higher percentage of oil with good quality in comparison of other methods.

1.6 Conclusion

As a conclusion, Soxhlet extraction method and analysis of oil using GC-MS is one of the most reliable methods for jatropha oil extraction which is time, cost and energy saving. Despite its toxicity, jatropha oil has much benefit and has a higher prospect in the future to be commercialized in term of application of the product. Hence, an optimum extracting condition in term of time, solvent to solid ratio and extraction solvent will be identified to obtain higher yield of jatropha oil from its seed.

CHAPTER 2

LITERATURE REVIEW

2.1 An Introduction of *Jatropha curcas Linnaeus*

Jatropha is a genus of over 170 plants from the Euphorbiceaus family commonly found and utilized across most of the tropical and subtropical regions of the world. Among the different species of jatropha, *Jatropha curcas L.* has a wide range of uses and promises various significant benefits to human and industry. It has a yield per hectare of more than four times that of soybean and ten times of corn (Sepidar, *et al.,* 2009, Nobrega & Sinha, 2007). Taxonomy of *Jatropha curcas L.* is given in Table 2.1.

Table 2.1 Taxonomy of *Jatropha curcas Linnaeus*

Taxonomy	
Kingdom	*Plantae*
Division	*Embryophyta*
Class	*Spermatopsida*
Order	*Malpighiales*
Family	*Euphorbiaceae*
Genus	*Jatropha*
Species	*J. curcas*
Scientific name	*Jatropha curcas Linnaeus*
Common name	Physic nut, Barbados nut, Purging nut

(Source *:* Chemie, 1997)

Jatropha curcas L. is a very adaptable, perennial plant, which lives for more than two years and can be easily grown on hard soil. Jatropha plant is regarded as a shrub or small tree as its height generally ranges from 3-5 meter. It can grow in arid condition, on any kind of ground and does not require irrigation. Therefore, it can be easily cultivated in marginal land (Felix & Clement, 2011).

Growth of jatropha plant occurs during the rainy season. Flowering usually occurs during rainfall and seed will be produced at the end of the rainy season, usually in the first or second year of growth (Brittania & Lutaladio, 2010). The branches of the plant contain latex, a milky substance that hardens once out in the open air. The leaves are smooth and 10 to 15 cm in length and width. The seeds have thin shells in black colour and oblong shape. Picture of jatropha fruit and seed are given in Figure 2.1. The matured seeds are usually 2.5cm long and can be easily crushed to extract oil from them (Jongschaap, *et al.*, 2007).

Figure 2.1 Jatropha fruit and seeds

(Source : Brittania & Lutaladio, 2010)

Jatropha is believed to have been reach to countries in Africa and Asia by Portuguese seafarers from its centre of origin in Central America and Mexico via Cape Verde and Guinea Bissau. In Madagascar, Cape Verde and Benin, jatropha oil was used as mineral diesel substitute during the Second World War (Akbar, *et al.*, 2009, Agarwal, 2007).

The genus jatropha has 426 species and distributed throughout the world. *Jatropha curcas L.* was first described in 1973 by Carl Linnaeus, Swedish botanist. The name of this plant was derived from Greek where the first part of its name, ‘jatros’, meaning physician and the last part, ‘trophe’, meaning nutrition (Brittania & Lutaladio, 2010).

2.2 Jatropha Oil

Jatropha oil is a vegetable oil that is extracted from jatropha seeds of the *Jatropha curcas Linnaeus* (*Jatropha curcas L.)* plant. Jatropha oil is one of the most discussed vegetable oils that could be used as a feedstock for producing transesterified oil (biodiesel), mono alkyl esters of long chain fatty acids resulting from vegetable oil which confirm to specifications for using diesel engines. It is a clean and renewable source of fuel. The calorific value and cetane number of jatropha oil are equivalent to diesel and it can be substituted as biodiesel without any engine alteration and preheating of it (Karaj, *et al.*, 2008).

2.2.1 Chemical Properties of *Jatropha curcas Linnaeus* Oil

Chemical properties of oil are amongst the most important properties that determines the present condition of the oil. Free fatty acid (FFA) and peroxide values are valuable measures of oil quality and gives indication of the extent of deterioration of oil. The fatty acid component of jatropha oil usually contains oleic acid (41.5-48.8%), linoleic acid (34.6-44.4%), palmitic acid (10.5-13.0%) and stearic acid (2.3-2.8%) (Martinez, *et al.*, 2006). Higher iodine value of jatropha is caused by high content of unsaturated fatty acid such as oleic acid and linoleic acid (Gunstone, 2004, Akbar, *et al.*, 2009).

Jatropha oil is viscous oil, also known as heavy oil or thick oil. Viscosity is a measure of resistance of a fluid to deform under shear stress (Smalley, 2000). Due to its viscosity, jatropha oil can be made into soap, skin products, candles, perfumes and other personal care product (Sarquis & Woodward, 1999).

2.2.2 Application of *Jatropha curcas Linnaues* Oil

Jatropha oil has been found for using in many different fields for various applications around the world. There are some chemical compounds in the seed of jatropha which possesses poisonous and purgative elements and renders it from being edible to human. Therefore, jatropha oil has been commercialized in other field for non edible purposes. Some of the applications of jatropha oil which have been discussed by Felix & Clement (2011) and Parajuli (2009) have been listed below.

- Source of Biodiesel

  The extracted oil of jatropha is used as transesterified oil or biodiesel. Biodiesel is a clean and renewable energy replacing the diesel fuel. It has some advantages: it is non-flammable, non-explosive, biodegradable, and has significantly lower toxicity.

- Hedge

  Jatropha plant was commonly used as hedges or living fences to protects valuable plants from eaten by animals as it produces latex and is toxic. Besides, they also provide shade for other plants while the dropped leaves will decompose and become fertilizer.

- Fertilizer and insecticide

The residue from the oil extraction, pressed seed cake, is rich in nitrogen, phosphorous, potassium and more fertilizing nutrients. Besides this, they also have insecticidal properties which can reduce amount of nematodes in soil.

- Medicinal Uses

Tannins and latex extracted from the bark has anti microbial properties and astringent properties each. Extracts from *Jatropha curcas L.* have been shown to have anti-tumor activity, the leaves as a remedy for malaria and high fever, the seeds for treatment of constipation and the sap in accelerating wound healing procedure.

- Manufacturing

Jatropha oil gives a good foaming; white soap with positive effect on the skin is due to the glycerin content of the soap. It is also useful for manufacture of candles and cosmetic industry. The saponification of jatropha oil indicates the amount of sodium hydroxide necessary to make a solid soap. In China, it is used to make varnish while in England; it is used for wool spinning. The protein content of jatropha oil can be used as raw material for plastics and synthetic fibers.

2.3 Extraction of Jatropha Oil from *Jatropha curcas Linnaues* seed

The extraction process can be classified based on combination of phases (solid, liquid, gas, supercritical fluid). For solid – liquid, this extraction is useful for the isolation and purification of naturally occurring sources while liquid – liquid is a more common method depending on solubility properties of components.

Various solvent are used for extraction such as organic solvents and inorganic solvents where, organic solvents are less dense than water while inorganic solvents are denser than water. Commonly used organic solvents are diethyl ether, toluene, hexane, ethyl acetate, ethanol, and inorganic solvents are dichloromethane, chloroform and carbon tetrachloride.

The most oil content of jatropha is in the seed of the plant where it has about 40% of oil. The oil can be extracted using various methods as discussed below.

2.3.1 Mechanical Extraction

Mechanical pressing is the oldest and simplest method for oil extraction. No chemical is used for oil extraction. Continuous screw-presses replaced the conventional hydraulic presser equipment (Bargale, 1997). Mechanical extraction of the oil is accomplished by exerting sufficient force on confined seed. Under this condition, pressure is high enough to rupture the cells and force oil from the seed to “escape”. Extraction is accomplished by compressing the material in a container that has small perforations, either round or slotted, that allow the liquid component to leave (Antony, *et al.,* 2011).

2.3.2 Ultrasonic-Assisted Extraction (UAE)

Oil extraction method based on ultrasonic waves. Ultrasound is used for the extraction of intra-cellular compounds through disintegration of cell structures which is also known as lysis of the cell (Allinger, 1975). The process of extracting oil through this method is known as cavitations. Cavitations occur when vapour bubbles of a liquid form in an area where pressure of the liquid is lower than its vapour pressure (Paula, & Roberto, 2011).

2.3.3 Microwave-Assisted Extraction (MAE)

MAE is an extraction technique which utilizes microwave energy to heat the solvent and the sample to increase the mass transfer rate of the solutes from the sample matrix into the solvent. Closed vessels are used to contain the sample and solvent, and the solvent are heated directly through the vessel where the temperature would be consistent (Manish, *et al.,* 2009).

2.3.4 Supercritical Fluid Extraction (SFE)

The basic principle of SFE is the feed material is contacted with a supercritical fluid and when the soluble materials are dissolved; the supercritical fluid containing the dissolved materials is removed from the feed material. The extracted component is separated from supercritical fluid by means of temperature or pressure change. Supercritical fluid which will be used is carbon dioxide without using organic solvent as carbon dioxide is a non-flammable and harmless medium. The oil saturated with CO_2 can be released by lowering the pressure or reducing the temperature (Eggers & Sievers, 1989).

2.3.5 Aqueous Enzymatic Oil Extraction (AEOE)

Enzymes are used to facilitate oil release in the aqueous process of oil extraction instead of solvent. Finely ground seed is dispersed in water and the dispersion is separated into solid, oil and water in the presence of enzyme (Rosenthal, *et al.*, 1996). The use of hydrolytic enzymes in this extraction process breaks the structure of the cell wall and makes the seed structure more permeable (Sajid, 2009).

2.3.6 Soxhlet Extraction

Soxhlet extraction is required when the desired compound has limited solubility in a solvent, and the impurity is insoluble in that solvent. It is the most economical, efficient and widely used process for high oil content seeds and medium oil content seeds (Sajid, 2009).

Extraction of jatropha oil through soxhlet method needs to consider few parameters such as selection of solvent and extraction conditions. Jatropha is a non-polar compound and according to the rule, “like dissolves like”; non-polar solvents yield the best results when extracting jatropha oil. Besides that, the temperature, pH and residence time could have an effect on the yield and selectivity of the extraction process (Madhavan, 2011).

Soxhlet method allows for near 100% active material recovery and user friendliness. It has several advantages; it gives higher yield and less turbid oil than mechanical extraction. Mechanical extraction has low oil recovery, where it leaves about one-third of the oil in the seed cake. Hence, heat pre-treatment is applied to improve the oil extractability. It also operates on higher cost as there is need for higher pressure plant and is labour intensive (Eggers, *et al.,* 1985). Higher pressure applied to the seed will damage the seed protein where else extreme heat generation will cause the darkening of the oil due to burning and loss of protein amino acids (Bargale, 1997).

Compared to other modern method of oil extraction, conventional soxhlet method is used to extract oil from *Jatropha curcas L.* as it allows more complete extraction at low operating cost. Extraction using ultrasonic-assisted extraction, microwave-assisted extraction and supercritical fluid extraction, the oil obtained has very high purity; however the operating and investment cost is very high as all the methods have high energy consumption (Amin, *et al.,* 2010).

Aqueous enzymatic oil extraction is safer and environmental-friendly compared to soxhlet extraction, but, it has lower oil yields and it produces significant amount of aqueous effluent. Furthermore, use of enzyme in this method increases the cost as enzymes are expensive than organic solvent. The extracted oil through aqueous enzymatic method also has higher risk of microbial contamination which discourages the oil’s commercial value (Sajid, 2009). Different studies of extraction method have been listed in the table following.

Table 2.2 Different studies for extraction of plant seeds using various extraction methods.

NO	EXTRACTION METHOD	SUBJECT	TYPE OF SOLVENT	PARAMETERS STUDIED	FINDINGS	REFERENCES
1	Mechanical extraction	- Soybean - Sunflower seed	-	Pretreatment method = enzymatic hydrolysis = extrusion cooking	Extrusion cooking increases the oil yield than enzymatic pretreatment	Bargale (1997)
2	Mechanical extraction = Oil presses = Oil expellers = Traditional methods	*Jatropha curcas L.*	-	Type of mechanical extraction and its method - Biodiesel production optimization = temperature = time = reactant ratio = catalyst concentration	Optimized parameters = 60 ^{0}C reaction temperature = 60 minutes reaction time = 6:1 methanol to oil = 0.92% NaOH catalyst	Antony, et al. (2011)
3	- High Pressure Mechanical Extraction - Soxhlet extraction	Rapeseed	-Soxhlet extraction = n-hexane	Energy consumption	- High pressure mechanical extraction = Mechanical energy – >200 kWh/t = Heating – >90E-4 kJ/t = Cooling – >60E-4 kJ/t	Eggers, *et al.* (1985)

Table 2.2 Continued

					- Soxhlet extraction = Mechanical energy – 75 kWh/t = Heating – 75E-4 kJ/t = Cooling – 42E-4 kJ/t	
4	- Aqueous Enzymatic Oil Extraction (AEOE) - Soxhlet extraction	- Vegetable oilseeds = Canola = Sunflower = Sesame = Cotton = Hemp = *Moringa oleifera* = *Moringa concanensis*	- Soxhlet extraction = n-hexane	Quality evaluation and comparison of AEOE method and conventional method	- Higher level of free fatty acid content and colour in AEOE than conventional method -Iodine and saponification value comparable - Higher oil yield for conventional method than AEOE	Sajid (2009)
5	- Aqueous Enzymatic Oil Extraction (AEOE) -Soxhlet extraction	Edible oils = Coconut = Palm = Peanut = Peanut = Rapeseed = Sunflower = Lupin seed	- Soxhlet extraction = n-hexane	Advantages and disadvantages of both extraction methods	AEOE are safer extraction method as it does not use solvent for extraction whereas soxhlet extraction uses n-hexane which is not environmentally friendly and not cost effective	Rosenthal, *et al* (1996)

Table 2.2 Continued

6	- Solvent-liquid extraction - Mechanical extraction	*Jatropha curcas L.*	Aqueous HCL, H_2SO_4, and H_3PO_4 with n–hexane	- Extraction time - Extraction temperature - Oil yield	Aqueous acidic hexane solvent gives better result, = shorter extraction time, 3 hr = lower temperature, 60 ^{0}C = higher oil yield, 23.5% than conventional hexane solvent and mechanical pressing	Amin, *et al.* (2010)
7	- Supercritical carbon dioxide extraction - Soxhlet extraction	Rapeseed	- Soxhlet extraction = n-hexane	- Pretreatment method - Extraction time - Solvent requirement	- Pretreatment method = Flaked press cake - Extraction at 75MPa gives shorter extraction time and less water is co-extracted with oil	Manish, *et al.* (2009)
8	Soxhlet extraction	*Jatropha curcas L.*	Hexane	Analysis of physico-chemical properties of oil	Oil content = 63.16%	Akbar, *et al.* (2009)

Table 2.2 Continued

9	- Aqueous Enzymatic Oil Extraction (AEOE) - Soxhlet extraction	*Jatropha curcas L.*	- Hexane - Petroleum ether - Isopropanol	Analysis of physico-chemical properties of oil - FFA content	- AEOE (21.4%) - Soxhlet (78%) = Hexane, 20-78% = Petroleum ether, 10-53% = Isopropanol, 15-59%	Shivani, *et al.* (2011)
10	Soxhlet extraction	*Jatropha curcas L*	- Hexane - Petroleum ether	- Extraction temperature = from room temperature to boiling point of solvent - Extraction time = 6 – 9 hour - Solvent to solid ratio = 4:1 to 7:1 - Particle size = below 0.5mm = 0.5mm – 0.75mm	- Temperature (near boiling temperature) = Hexane ,68^{0}C = Petroleum ether, 68^{0}C - Time = 8hr - Solvent to solid ratio =6:1 - Particle size = 0.5 –0.75mm - Solvent = Hexane , 47.3% = Petroleum ether, 46%	Sayyar, *et al.* (2009)

Table 2.2 Continued

11	Soxhlet extraction	*Jatropha gossipifolia*	Petroleum ether	Analysis of physico-chemical properties of oil - FFA content	Oil content = 35.44%	Ogbobe & Akano (1991)
12	Soxhlet extraction	*Jatropha curcas L.*	Hexane	Extraction temperature = 40, 50, 60, 70, 80 ^{0}C	At 40 ^{0}C = 33.35% 50 ^{0}C = 38.88% 60 ^{0}C = 44.43% 70 ^{0}C = 43.83% 80 ^{0}C = 41.50%	Felix & Clement (2011)
13	- Soxhlet method = Conventional Soxhlet extraction = Ultrasound assisted-soxhlet extraction (UAE) - Batchwise extraction = control = ultrasound cleaning bath = ultrasound probe	Rapeseed	Hexane	- Particle size = ≤ 234, ≤ 600 and ≤ 850 μm - Moisture content = dried seed and non-dried seed - Extraction time	- Particle size (μm) ≤ 234 = 26.0% ≤ 600 = 31.3% ≤ 850 = 36.52% - Moisture dried = 38.16% - Non-dried = 38.68% - Extraction time UAE = 1.5hr Soxhlet = 4hr - Oil recovery = Soxhlet100%	Ibiari, *et al.* (2010)

Table 2.2 Continued

					= UAE lower than soxhlet	
14	Soxhlet extraction	*Jatropha curcas L.*	Hexane	-Average weight of the seeds = divided into 4 groups T1=0.2-0.35g T2=0.36–0.5g T3=0.51– 0.7g T4=0.71–0.9g	Oil content T1 = 7.19% T2 = 18.66% T3 = 33.01% T4 = 38.94%	Karaj, *et al. (*2008)
15	Soxhlet extraction	*Jatropha curcas L.*	Hexane	- Origin of seeds = Malaysia = Thailand = Borneo = India = Indonesia = Cape Verde = South Africa = Philiphines = Vietnam	- Oil content Malaysia = 40% Thailand = 41.2% Borneo = 45.4% India = 45.5% Indonesia = 45.2% Cape Verde = 44.2% South Africa = 42.2% Philiphines = 44.3% Vietnam = 48.4%	Islam, *et al* . (2011)
16	Soxhlet extraction	*Jatropha curcas L.*	Petroleum ether	Fatty acid composition of oil extracted	Oil content = 46.27%	Joshi,*et al*. (2011)

The most outstanding advantages of using soxhlet method is the sample is repeatedly brought into contact with the fresh portions of the solvent, which accounts for its near 100% recovery (Luque & Garcia, 1998). No filtration is required in this method and the sample throughput also can be increased by simultaneous extraction in parallel, since this method is inexpensive (Ibiari, *et al.*, 2010). Hence, by using a soxhlet apparatus to perform the extractions, the amount of solvent will be significantly reduced, and consistency improved.

2.4 Soxhlet Apparatus

Soxhlet apparatus mainly consist of condenser, porous container and distilling pot as in the Figure 2.2. In soxhlet method, the sample is placed in a thimble-holder, and during operation gradually filled with condensate fresh solvent from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solute of the thimble-holder and unloads it back into distillation flask; carrying the extracted analytes into the bulk liquid. This operation is repeated until complete extraction is achieved. The condenser functions to cool the solvent vapour and cause it to condense where else the porous container functions to hold the solid sample and allow for the condensed solvent to saturate and pass through, thereby extracting active materials. Not only that, the distilling pot functions to hold the solvent pool and serve as a reservoir for the concentrated material (Luque & Garcia, 1998).

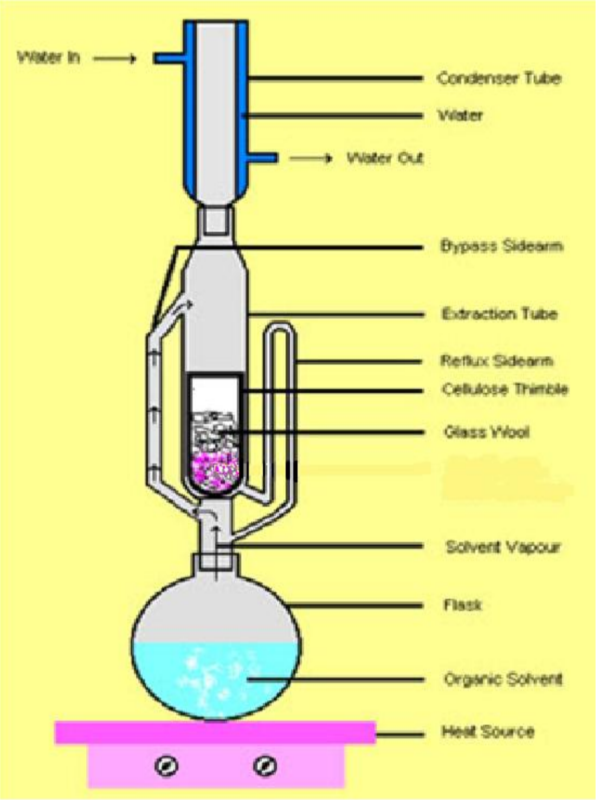


Figure 2.2 Soxhlet Apparatus

2.5 Analysis

In order to examine the quality of the jatropha oil, a fine analysis on its composition need to be carried out. This is because; the types and amount of composition of particular components can reveal the characteristics of the oil. There are a number of analysis methods available in industry to carry out such analysis.

2.5.1 Gas Chromatography-Mass Spectrometry (GC-MS)

Gas Chromatography-Mass Spectrometry (GC-MS) is an instrumental technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS), by which complex mixtures of chemicals may be separated, identified and quantified. Schematic diagram of GC-MS is as shown in Figure 2.3. In order for a compound to be analyzed by GC-MS it must be sufficiently volatile and thermally stable.

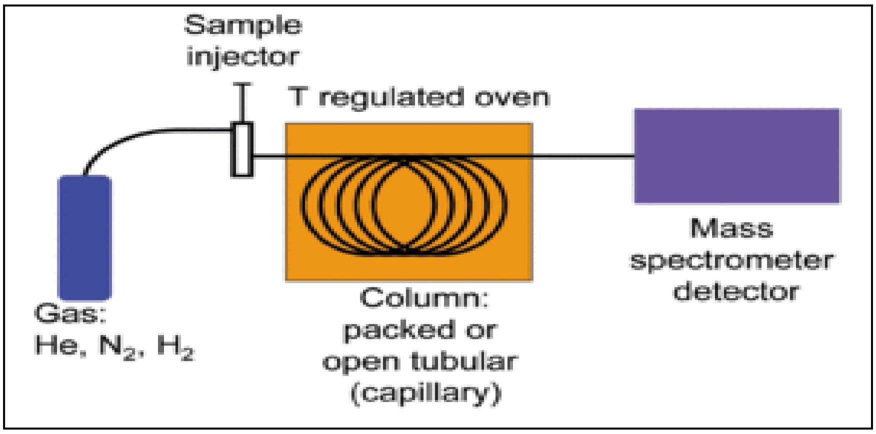


Figure 2.3 Schematic Diagram of GC-MS

(Source : Murray, 2006)

2.5.2 Principle of Process of GC-MS

The GC-MS functions where GC component will separate chemical mixture while MS component act as an identifier of components at a molecular level. The GC works on the principle that a mixture will separate, as the sample travels the length of the column, into individual substances when heated which is based on its volatility. The heated gases are carried through a column with an inert gas (such as helium).

The molecules take different amounts of time which called as retention time to come out of or elute from the gas chromatography.

As the separated substances emerge from the column opening, they flow into the MS and it will identify compounds by the mass of the analyte molecule. The MS does this by breaking each molecule into ionized fragments. A “library” of known mass spectra, covering several thousand compounds, is stored on a computer. The data from a MS is sent to a computer and plotted on a graph called a mass spectrum (Oregon State University, 2011).

2.6 Conclusion

Jatropha plant is a suitable plant for oil extraction as it has maximum oil content in it which is about 40% compared to other plant seeds. The conventional soxhlet extraction method is the most applicable to the extraction in accordance to good selection of solvent, and other parameters such as temperature, pH and solubility. On the other hand, the chemical composition of the oil can be determined through GC-MS.

CHAPTER 3

METHODOLOGY

3.1 Materials

The materials which were used in this study are jatropha seeds *(Jatropha curcas Linnaeus*) collected from the Jatropha Plantation Industrial Bionas Sdn. Bhd, extraction solvent, ethyl acetate and ethanol and n-hexane for GC-MS analysis.

3.2 Overall Methodology

The experimental work started with the pretreatment of the raw material, jatropha seeds. This pretreatment includes the drying and grinding process followed by extraction of oil from *Jatropha curcas L*. by soxhlet method. In this part, the effect of extraction was analyzed based on different parameters which were extraction time, solvent to solid ratio and type of solvents. Finally, analysis on the samples(oil extracted from the raw material) was carried out using Gas Chromatography-Mass Spectrometry (GC-MS).

3.3 Pretreatment Method

Jatropha curcas L. was selected according to their condition where only seeds in good condition were used further while the damaged ones were discarded. The selected jatropha seeds were cracked and the shell was removed. De-shelling the seeds to remove the shell from the kernels, oil bearing portion of the seed is essential as to eliminate the parts that have little or no nutritional value.

Then, the seeds were dried at high temperature of 100 – 105 ^{0}C for 35 minutes to remove the moisture content of the seeds. Drying the seed reduces the moisture content of oil seeds to below 10 percent. The dried seeds should be stored in place with adequate ventilation or aeration to ensure low moisture levels are maintained and microbial development is avoided without loss of oil content. The sun-drying of oil seeds with high moisture content such as jatropha, is slow and inefficient. Therefore, the seeds were heat-treated using oven, which liquefies oil in the plant cells and facilitates its release during extraction. Besides, oven drying of the seeds are much faster and saves time of operation (Food and Agriculture Organization [FAO], 1995).

Next, the seeds were grinded with a grinder to increase the exposed surface area of jatropha for extraction and sieved using sieve plate. Grinding the seed not only can increase the surface area of the seed for extraction but can also break the cell walls and free the oil entrapped within the seeds. Smaller particle sizes of the samples give larger contact surface areas and are less resistance to penetrate the solvent into the samples and oil diffusion, which results in higher oil yield. More oil will be transferred from inside the smaller particles to the surrounding solution compared to the larger ones (Ebewele, *et al*., 2010)

3.4 Extraction Method

Extraction of oil from these raw materials was conducted by soxhlet method at three different parameters to optimize the extraction operating conditions for achieving maximum oil yield. 20g of grinded meals were placed inside a thimble and placed in the soxhlet extractor while the extraction solvent, ethyl acetate or ethanol is placed in a round bottom flask. The extraction time was fixed at 6, 8 and 10 hour and at three different solvent to solid ratio, 7.5:1, 10:1, and 12.5:1. The extraction temperature was fixed at boiling point of the solvent, 77 ^{0}C for ethanol and 78 ^{0}C for ethyl acetate. These parameters were varied one at a time to identify the optimum conditions for each type of solvent. Overall conditions of the extraction were summarized in Table 3.1.

Following that, the solvent was separated from the oil using rotary vacuum evaporator at boiling point of solvent and was collected in the receiving flask. The oil remained in the sample flask was weighed after the process was completed. The volume of the oil was measured by using measuring cylinder. The extracted oil was stored in freezer at 4 ^{0}C for further physicochemical analysis. Complete soxhlet extractor is shown in Figure 3.1.

Table 3.1 Overall Condition of Soxhlet Extraction

Parameter	Description
Temperature ^{0}C	Depends on the solvents boiling point
Extraction time, hour	6, 8 and 10
Solvent used	Ethyl acetate and ethanol
Solvent to Solid Ratio	7.5:1, 10:1, 12.5:1

Figure 3.1 Soxhlet extractor

3.5 Analysis of Extracted Oil Using Gas Chromatography–Mass Spectrometry (GC-MS)

The analysis of oil samples was carried out by using Gas Chromatography Mass Spectrometry (GC-MS) Agilent 6890 gas chromatography instrument couple to an Agilent 5973 mass spectrometer and an Agilent Chem. The column type is HP 5ms with dimension 30 x 0.250 x 0.25. Split less injection was performed with a purge time of 1.0 min. The carrier gas was helium at a flow rate of 1mL min^{-1}. The column temperature was maintained at 50 ^{0}C for 3 min, then programmed at 5 ^{0}C min^{-1} to 80 ^{0}C and then at 10 ^{0}C min^{-1} to 340 ^{0}C. The inlet and detector temperatures were 250 and 340 ^{0}C, respectively and the solvent delay was 4 min. Extracted oil constituents was identified by comparing retention times of the chromatogram peaks with those of reference compound run under identical conditions. Analytical results were obtained using the reference compounds based on the area ratio between target components and the reference compound.

First, for prepare the sample for GC-MS analysis, the extracted jatropha oil from *Jatropha curcas Linnaeus* seed were filtered using syringes and 0.45µm PTFE filters. Then the filtered samples were diluted with n-hexane to a concentration of 2% by adding 20µL of pure essential oil to 980µL of n-hexane to prepare 1mL samples. All the diluted samples were then put on the vortex mixer for 2 minutes to make the samples in homogeneous form. Then, 1.5mL of the diluted samples was inserted into GC-MS vial for further analysis.

3.6 Calculation of Yield of Extracts

The amount of yield obtained from the extraction need to be calculated to evaluate the performance of soxhlet in jatropha oil extraction. The following equation can be applied:

$$Yield\ of\ jatropha\ oil\ (w/w\ \%) = \frac{amount\ of\ oil\ (g) obtained}{amount\ of\ raw\ material\ (g)\ used}\ x\ \ 100\%$$

3.7 Methodology Flow Chart

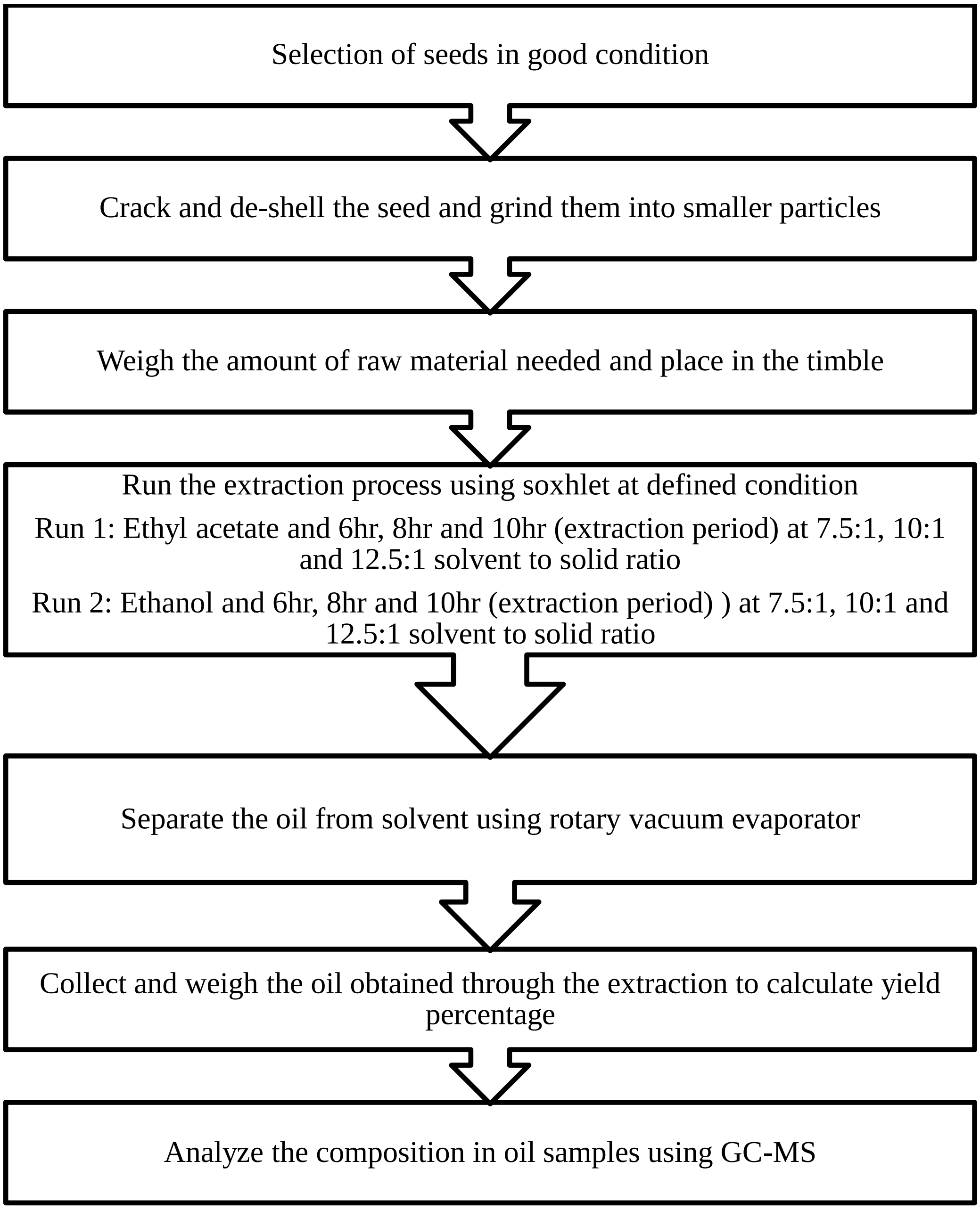


Figure 3.2 Summary of methodology

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

This research was carried to study the efficiency of soxhlet extraction method on the extraction of jatropha oil from *Jatropha curcas Linnaeus* seed. Therefore, the experiment was run at different parameters condition in order to identify the most optimize condition to obtain a maximum yield of oil.

Parameters used for optimization of extraction condition were type of solvent (ethyl acetate and ethanol), extraction time (6 hour, 8 hour and 10 hour), and solvent to solid ratio (7.5:1, 10:1 and 12.5:1) while the amount of sample were kept constant at 20g. Besides, the quality of the jatropha oil obtained through soxhlet method has also been analyzed using the GC-MS by identifying its chemical constituents.

4.2 Effect of Reaction Time on Oil Extraction

The extraction time is an important parameter for soxhlet extraction. It helps in deciding the optimum residence time required in extractor. Figure 4.1 and 4.2 shows the yield of jatropha oil extracted from *Jatropha curcas Linnaues* seed at different extraction time and different solvent to solid ratio for solvent ethyl acetate and ethanol respectively.

Table 4.1 Oil yield (%) for solvent ethyl acetate at different solvent to solid ratio and extraction time

Time (h)	Solvent-solid ratio	7.5:1	10:1	12.5:1
6	Oil yield (%)	24.84	32.66	34.04
8		33.12	42.32	45.08
10		36.34	45.54	47.38

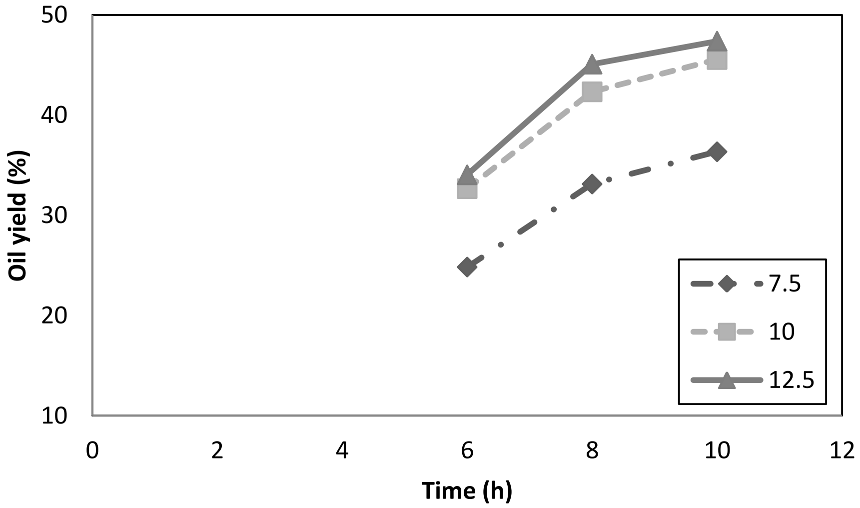


Figure 4.1 Percentage of extracted oil yield by extraction time at different solvent to solid ratio for solvent ethyl acetate

Table 4.2 Oil yield (%) for solvent ethanol at different solvent to solid ratio and extraction time

Time (h)	Solvent-solid ratio	7.5:1	10:1	12.5:1
6	Oil yield (%)	7.50	9.07	8.90
8		9.47	11.84	12.43
10		10.06	11.83	12.72

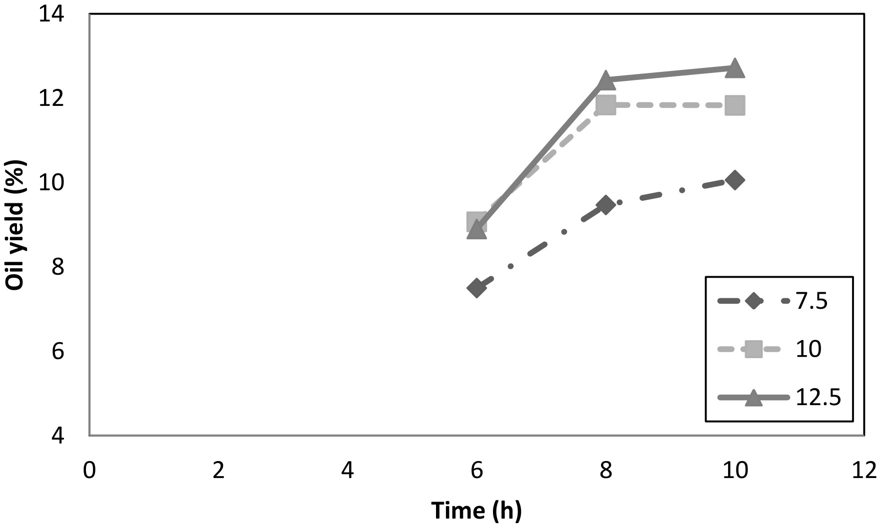


Figure 4.2 Percentage of extracted oil yield by extraction time at different solvent to solid ratio for solvent ethanol

From the graph, the amount of yield does not change significantly after 8 hour. Most of the oil is extracted at 8 hour, although maximum extracted oil is achieved after 10 hour with 36.34%, 45.54% and 47.38% for ethyl acetate and 10.06%, 11.83% and 12.72% for ethanol at solvent to solid ratio, 7.5:1, 10:1 and 12.5:1 respectively. Extraction time is an important parameter as there must be enough time for the dissolving and flow processes to occur (Anderson, 2011). The extraction rate is fast at the beginning of the extraction but gets slow gradually. The reason is that when the meal is exposed to the fresh solvent, the free oil on the surface of seeds is solubilised and oil gets extracted quickly which shows fast increase in the extraction rate (Saxena, *et al.*, 2011). As the time of extraction increases, the rate of extraction of the oil decreases because the oil content in the sample also decreases with time.

4.3 Effect of Solvent to Solid Ratio on Oil Extraction

The effect of different solvent to solid ratio on yield of *Jatropha curcas Linnaues* seed at different extraction time and different type of solvent are presented in Figure 4.3 and Figure 4.4. The amount of solvent, ethyl acetate and ethanol used is varied based on the solvent to solid ratio.

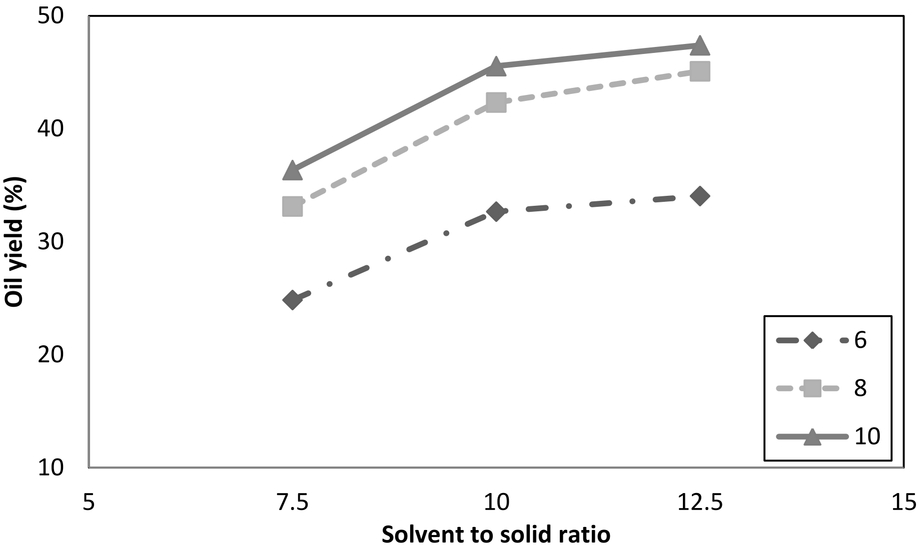


Figure 4.3 Percentage of extracted oil yield by extraction time at different solvent to solid ratio for solvent ethyl acetate

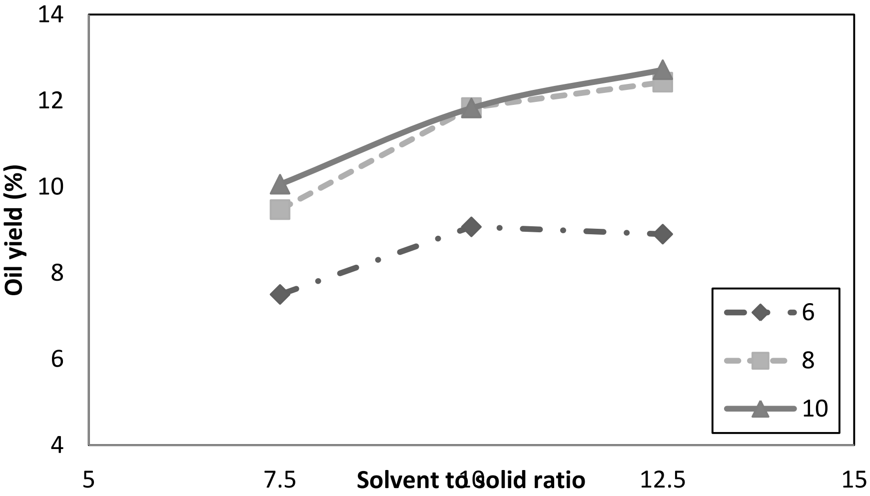


Figure 4.4 Percentage of extracted oil yield by extraction time at different solvent to solid ratio for solvent ethanol

Both the solvent had illustrated similar output pattern on the variability of solvent to solid ratio where the highest yield obtained at ratio 12.5:1 followed by 10:1 and 7.5:1. When ethanol is used as extraction solvent, the maximum extraction at 6 hour, 8 hour and 10 hour was found to be 8.90%, 12.43%, 12.72%, respectively while in ethyl acetate at 6 hour, 8 hour and 10 hour, it was 34.04%, 45.08% and 47.38% respectively.

At lower solvent to solid ratio, the oil extraction may be restricted due to lower solubility limit of oil in solvent. The amount of oil yield increases rapidly from 7.5:1 to 10:1, but the yield increased only very slowly or does not show any changes from 10:1 to 12.5:1, because the oil in the meal had been almost completely extracted (Saxena, *et al.,* 2011).

4.4 Effect of Extraction Solvent on Oil Extraction

Figure 4.5 shows the effect of type of solvent, ethyl acetate and ethanol on extraction of jatropha oil at 12.5:1 solvent to solid ratio and different extraction time.

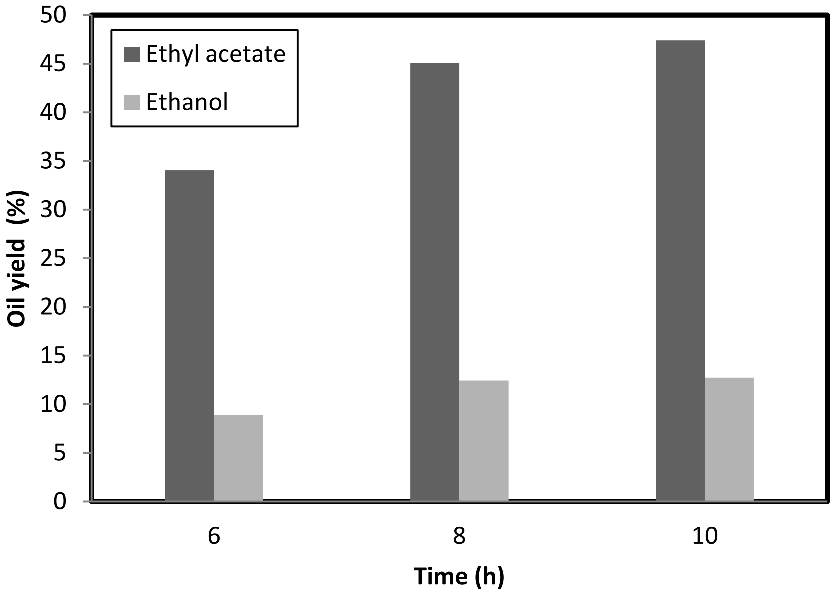
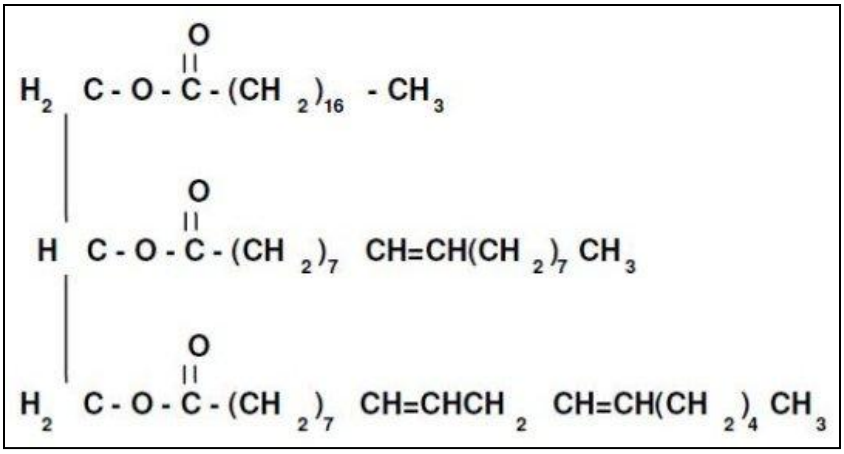


Figure 4.5 Percentage of jatropha oil yield for different extracting solvent at solvent to solid ratio 12.5:1

Extraction of jatropha oil at 6 hour, 8 hour, and 10 hour using ethyl acetate as solvent gives higher yield, which are 34.04%, 45.08% and 47.38%, respectively, rather than ethanol which gives yield as much as 8.90%, 12.43% and 12.72%, respectively.

Jatropha oil is a non-polar compound, which is shown in the structure below.



$$\begin{array}{l} H_2C-O-\overset{O}{\overset{\|}{C}}-(CH_2)_{16}-CH_3 \\ \quad | \\ HC-O-\overset{O}{\overset{\|}{C}}-(CH_2)_7\ CH{=}CH(CH_2)_7\ CH_3 \\ \quad | \\ H_2C-O-\overset{O}{\overset{\|}{C}}-(CH_2)_7\ CH{=}CHCH_2\ CH{=}CH(CH_2)_4\ CH_3 \end{array}$$

Figure 4.6 Structure of jatropha oil

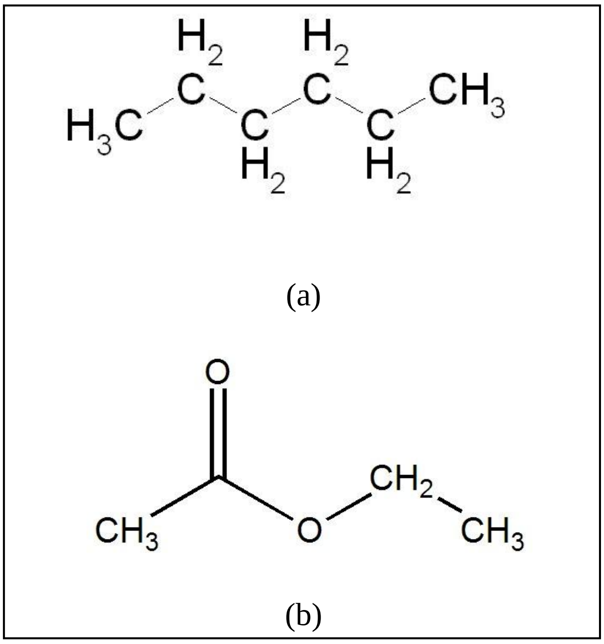
According to the theory, “like dissolves like”, non-polar solvents yield the best results when extracting non-polar oils like jatropha oil. Currently, hexane, a fossil fuel and a non-polar compound, is the most conventional solvent commercially used for jatropha oil extraction which gives highest oil yield.

Hexane is a mixture of 5 to 6 carbon paraffins and is classified as volatile organic compound (VOC) as it can undergo photo oxidation in the air to form ozone. Larger plants operating with hexane are required to have an operating permit and pay fee for emission of the VOC. Besides that, the plants are also required to apply maximum achievable control technology (MACT) to minimize the hexane loss

(Ferreira-Dias, *et al.*, 2003). The control technology includes condenser, water separator, reboiler, mineral oil scrubber, counter current de-solventizer and toaster. Hence, use of n-hexane as an extraction solvent will increase the cost of operation of the extraction even though the price of n-hexane is lower. Long-tem exposure of n-hexane would pose a particular health hazard as it may cause peripheral nerve damage. It is a flammable, harmful, dangerous to the environment and toxic to reproduction. Hence, n-hexane is classified as substance with some 'danger of serious damage to health by prolonged exposure' (AEA Energy & Environment, 2009).

Therefore, an alternative solvent to replace hexane in terms of its operability and also cost were tested. The ethyl acetate extracted oils were comparable in quantity with oils extracted from hexane. Hexane extracts almost completely oil from jatropha seed which contains around 48% of oil content. In this experiment, ethyl acetate gives the highest result of oil extracted, which is 47.38% at 12.5:1 ratio and 10 hour extraction time.

Comparable results of hexane and ethyl acetate are due to the similar ends of the chemical structure of hexane and ethyl acetate, as shown in the Figure 4.7.



(a)

(b)

Figure 4.7 Structure of (a) hexane and (b) ethyl acetate

(Source : Kahama, 2010)

These compounds begin and end with the sequence CH_3, making them as non-polar compounds. The non-polar end of ethyl acetate is what makes the results of jatropha oil extraction comparable to hexane as a solvent.

However, ethanol is not a good alternative to hexane, as it gives very low yield of oil compared to ethyl acetate. The oil extracted by ethyl acetate and ethanol gives difference from 69.81% to maximum 74.02%. Ethanol has a polar and non-polar end, which is shown in the Figure 4.8.

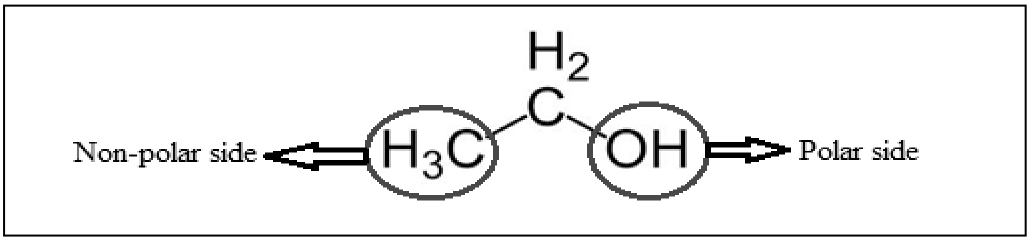


Figure 4.8 Structure of ethanol

(Source : Kahama, 2010)

Lower oil extraction of ethanol may be the result of the low solubility of the solvent with jatropha oil. The non-polar end of ethanol extracts jatropha oil, but the polar end enables it to extract other organic compounds, resulting in highly impure oil. This explains the impurities present in the oil extracted by ethanol (Kahama, 2010).

4.5 Identification and Quantification of Extracted Jatropha Oil

In the analysis of GC-MS on jatropha oil (*Jatropha curcas Linnaues*), there were total of 27 components identified with solvent ethyl acetate and 40 components identified with solvent ethanol. The major fatty acids in jatropha oil were oleic acid, linoleic acid, plamitic acid and stearic acid. Oleic acid would be in the highest percentage followed by linoleic acid, where jatropha oil can also be classified as oleic –linoleic oil (Akbar, *et al*, 2009).

The main components detected in the jatropha oil with solvent ethyl acetate were oleic acid, linoleic acid, palmitic acid, stearic acid, oleoaldehyde, methyl linolate, ethyl oleate, methyl linolelaidate, methyl stearate and squalene. The main components detected in the jatropha oil with solvent ethanol were the same as the one extracted with ethyl acetate except for some additional components which are methyl palmitate, 7-octadecenoic acid methyl ester, 13-octadecenal, (Z)-, 9-eicosyne and methyl elaidate. The dominant component in the jatropha oil in term of quantity and quality is oleic acid which has the highest percentage of area and also quality of 99.

4.5.1 Effect of Extraction Time and Extraction Solvent (Ethyl Acetate) on the Chemical Constituents of Jatropha (*Jatropha curcas Linnaeus*) Oil

Table 4.3 Major chemical constituents of jatropha (*Jatropha curcas Linneaus*) oil at different extraction time and ethyl acetate as extraction solvent

Constituents	Relative Peak Area, % at Different Extraction Time		
	6 hour	8 hour	10 hour
Squalene	3.3	-	15.44
Palmitic acid	-	13.44	4.22
Stearic acid	-	10.01	-
Oleic acid	20.13	68.93	29.82
Linoleic acid	15.7	2.06	9.12
Methyl stearate	4.86	-	-
Ethyl oleate	7.07	-	-
Methyl linolelaidate	-	-	7.37
Olealdehyde	-	-	14.74
Methyl linolate	9.5	-	-

The main chemical constituents identified from the jatropha oil of *Jatropha curcas Linnaues* at different extraction time, 6 hour, 8 hour and 10 hour and extraction solvent ethyl acetate are presented in Table 4.3. Each extraction time gives different main constituent in different amount. The GC-MS chromatogram for these major constituents and other constituents were included in the appendix.

The oil sample at 6 hour gives six main components which were squalene (3.3%), oleic acid (20.13%), linoleic acid (15.7%), methyl stearate (4.86%), ethyl oleate (7.07%) and methyl linolate (9.5%). The amount of each constituent within the jatropha oil in descending order: oleic acid > linoleic acid > methyl linolate > ethyl oleate > methyl stearate > squalene.

The four major components identified in the jatropha oil at 8 hour were oleic acid (68.93%), palmitic acid (13.44%), linoleic acid (2.06%) and stearic acid (10.01%). List of these constituent in the descending order: oleic acid > palmitic acid > stearic acid > linoleic acid. The amount of linoleic acid is lower in the oil may be due to degradation of the oil during the removal of solvent from the oil or during the storage period which is caused by the exposure to the air and moisture.

Where else, the major components in 10 hour of jatropha oil were squalene (15.44%), palmitic acid (4.22%), oleic acid (29.82%), linoleic acid (9.12%), methyl linolelaidate (7.37%), and olealdehyde (14.74%). The amount of each constituent in the order of descending: oleic acid > squalene > olealdehyde > linoleic acid > methyl linolelaidate > palmitic acid.

It can be seen from the table that oleic acid is the key component at all extraction time with the highest amount in 8 hour extraction, 68.93%. Two components that identified as major components in all jatropha oil were oleic acid and linoleic acid.

4.5.2 Effect of Extraction Time and Extraction Solvent (Ethanol) on the Chemical Constituents of Jatropha (*Jatropha curcas Linnaeus*) Oil

Table 4.4 Major chemical constituents of jatropha (*Jatropha curcas Linneaus*) oil at different extraction time and ethanol as extraction solvent

Constituents	Relative Peak Area, % at Different Extraction Time		
	6 hour	8 hour	10 hour
Palmitic acid	12.45	13.53	8.65
Stearic acid	4.27	5.24	-
Oleic acid	23.51	20.31	29.3
Linoleic acid	38.97	43.63	7.09
Methyl linolate	2.12	-	-
Methyl palmitate	-	-	4.33
7-Octadecenoic acid, methyl ester	-	-	10.9
13-Octadecenal, (Z)-	-	-	6.2
9-Eicosyne	-	-	14.49
Methyl elaidate	-	3.99	-

The main chemical constituents identified from the jatropha oil of *Jatropha curcas Linnaues* at different extraction time, 6 hour, 8 hour and 10 hour and extraction solvent ethanol are presented in Table 4.4. Each extraction time gives different main constituent in different amount. The GC-MS chromatogram for these major constituents and other constituents were included in the appendix.

The oil sample at 6 hour gives five main components which were palmitic acid c, stearic acid (4.27%), oleic acid (23.51%), linoleic acid (38.97%), and methyl linolate (2.12%). List of the constituent in the descending order: linoleic acid > oleic acid > palmitic acid > stearic acid > methyl linolate.

The major components identified in the jatropha oil at 8 hour were oleic acid (20.31%), palmitic acid (13.53%), linoleic acid (43.63%), stearic acid (5.24%) and methyl elaidate (3.99%). List of these constituent in the descending order: linoleic acid > oleic acid > palmitic acid > stearic acid > methyl elaidate. Where else, the major components in 10 hour of jatropha oil were palmitic acid (8.65%), oleic acid (29.3%), linoleic acid (7.09%), methyl palmitate (4.33%), and 7-octadecenoic acid methyl ester (10.9%), 13-octadecenal, (z)- (6.2%) and 9-eicosyne (14.49). The amount of each constituent in the order of descending: oleic acid > 9- eicosyne > 7-octadecenoic acid methyl ester > palmitic acid > linoleic acid > 13-octadecenal, (z)- > methyl palmitate.

Jatropha oil is composed of various triacylglycerols (TAGs), molecules that consist of three fatty acid chains which can be converted into biodiesel. The fatty acid of the oil has direct impact on ignition quality, heat of combustion and oxidative stability. Due to its high viscosity, jatropha oil is not suitable for direct use as engine fuel as it will result in operational problems, such as carbon deposits, oil ring sticking and thickening of lubricating oil (Qu, *et al.,* 2012).

The conversion of TAGs from jatropha oil into fatty acid methyl esters (FAME) is necessary to decrease their viscosity to levels comparable to fossil fuel in order to minimize problems due to incomplete combustion. The quality and performance of the biodiesel depends on the chemical composition of the fatty acids present in the oil before conversion. An ideal biodiesel composition should have more monounsaturated fatty acids and less polyunsaturated acids (Bahadur, *et al.*, n.d.)

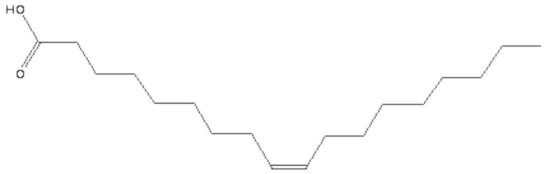
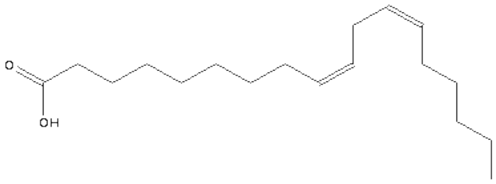
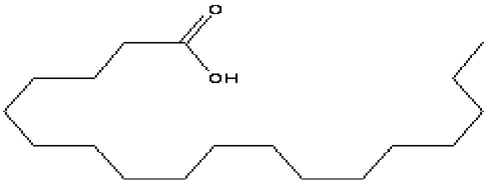
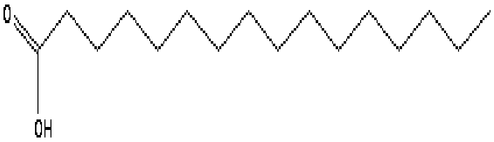
Jatropha oil extracted with ethyl acetate has higher percentage of oleic acid than linoleic acid, where oleic acid is a monounsaturated acid and linoleic acid is polyunsaturated acid. In oil extracted with ethanol, percentage of linoleic acid is higher than oleic acid which indicates lower quality of biodiesel when the oil is converted into FAME.

Besides, when jatropha oil is converted into FAME, oleate esters are more stable than linoleate esters. The oxidation of polyunsaturated esters of linoleate results in the initial accumulation of hydroperoxides, which eventually polymerizes forming insoluble sediments that may cause all the operational problems listed above. In addition to it, engines operating on high ratio of polyunsaturated biodiesel produce higher NO_2 emission than fossil fuel. Hence, jatropha oil extracted with ethyl acetate which has higher oleic acid content than linoleic acid gives oil with good quality than jatropha oil extracted with ethanol (Bahadur, *et al.*, n.d.).

Furthermore, the higher numbers of components in jatropha oil extracted with solvent ethanol are caused by its polar end which is capable of extracting other organic compounds, resulting in highly impure oil (Kahama, 2010).

The chemical equation and structure of the major constituents in the jatropha oil of *Jatropha curcas Linnaues* are shown in the table 4.5(NIST, 2011).

Table 4.5 Chemical Formula and Structure of Major Constituents of Jatropha (*Jatropha curcas Linnaues) Oil*

No	Constituents	Formula	Structure
1	Oleic acid	$C_{18}H_{34}O_2$	
2	Linoleic acid	$C_{18}H_{32}O_2$	
3	Stearic acid	$C_{18}H_{36}O_2$	
4	Palmitic acid	$C_{16}H_{32}O_2$	

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This research was carried out to investigate the performance of soxhlet extraction method in the extraction of jatropha oil from *Jatropha curcas Linnaeus* seed by calculating their yield. Three main operating parameters affecting the soxhlet method on extraction of jatropha oil were optimized based on the maximum yield extracted from the plant seed. The optimum condition for the lab scale soxhlet extraction method was obtained under solvent ethyl acetate and 8 hour of extraction time at highest solvent to solid ratio with highest oil yield (w/w) of 47.38% for *Jatropha curcas Linnaues*. Extraction of jatropha oil from *Jatropha curcas Linnaues* using soxhlet method yields the maximum oil content of the seed, around 48-50% of oil content, which shows the high efficiency of the soxhlet extraction method (Sajid, 2009 & Shivani, *et al.*, 2011).

The major constituents found in the oil of *Jatropha curcas Linnaeus* extracted with ethyl acetate were oleic acid, linoleic acid, palmitic acid, stearic acid, oleoaldehyde, methyl linolate, ethyl oleate, methyl linolelaidate, methyl stearate and squalene. Wherelse, the main components in the jatropha oil extracted with ethanol were oleic acid, linoleic acid, palmitic acid, stearic acid, methyl linolate, methyl palmitate, 7-octadecenoic acid methyl ester, 13-octadecenal, (Z)-, 9-eicosyne and methyl elaidate. The dominant component in the jatropha oil of *Jatropha curcas Linnaeus* which were detected in all samples of oil (6 hour to 10 hour) were oleic acid and linoleic acid. These two components are very important for biodiesel characteristics of the oil (Bahadur, *et al.*, n.d.).

Higher monounsaturated acid, oleic acid content compared to polyunsaturated acid, linoleic acid in jatropha oil extracted with ethyl acetate indicates it is a better extraction solvent than ethanol for *Jatropha curcas Linnaeus*. Besides that, presence of a lot of other impurities in the jatropha oil extracted from ethanol is due to the presence of polar end in ethanol structure which makes it less suitable as an extraction solvent.

The quality of the oil extracted with ethyl acetate are also comparable with oil extracted with hexane as extraction solvent, where the major constituents are oleic acid, palmitic acid, stearic acid and linoleic acid (Akbar, *et al.*, 2009). Ethanol is not suitable as an extraction solvent as it does not yield oil with essential constituents for biodiesel production, major use of jatropha oil. Hence, it can be concluded that soxhlet extraction method with ethyl acetate as extraction solvent at 8 hour extraction time and higher solvent to solid ratio offers advantage in term of quantity and quality of oil.

5.2 Recommendation

There are three recommendations to improve the yield of jatropha oil, where the first is to grind jatropha seed just before the extraction and transfer the grinded sample directly into cellulose thimble from the grinder to prevent loss of oil content in the seed during the transfer of the sample from one surface to another. Besides, the grinded jatropha should not be kept for too long as the storage period of the sample also may affect the oil yield from the extraction. Furthermore, speed of the grinder should be maintained at medium, around 8000 rpm and the each grinding time should be around 10 seconds and not longer than that. This is to prevent release of oil from the seed through grinding (mechanical extraction) and to enable easier sieving of the seed powder. The grinded sample should be sieved using sieve shaker in the future as to maintain a constant sample size as the sample size also affects the extraction oil yield.

The temperature of the rotary evaporator during the solvent removal step should be kept lower than the boiling point of the solvent as rotary evaporator are operated at vacuum, high pressure. Increasing the boiling point will deteriorate the quality of the oil as some of the constituents in the oil will get evaporated at higher temperature. Besides that, higher temperature also would cause burning of the oil which will also affect the oils constituents and also change the colour of the oil. and not rise very high than that to avoid thermal degradation of the oil and also to avoid burning of the oil. Through this study we can see that highest oil yield is obtained at highest solvent-to-solid ratio, and effect of increasing the solvent-to-solid ratio should be studied further.

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APPENDIX A

GC-MS ANALYSIS RESULT

(a) Ethyl Acetate: 6 Hour

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Instrument : GCMSD
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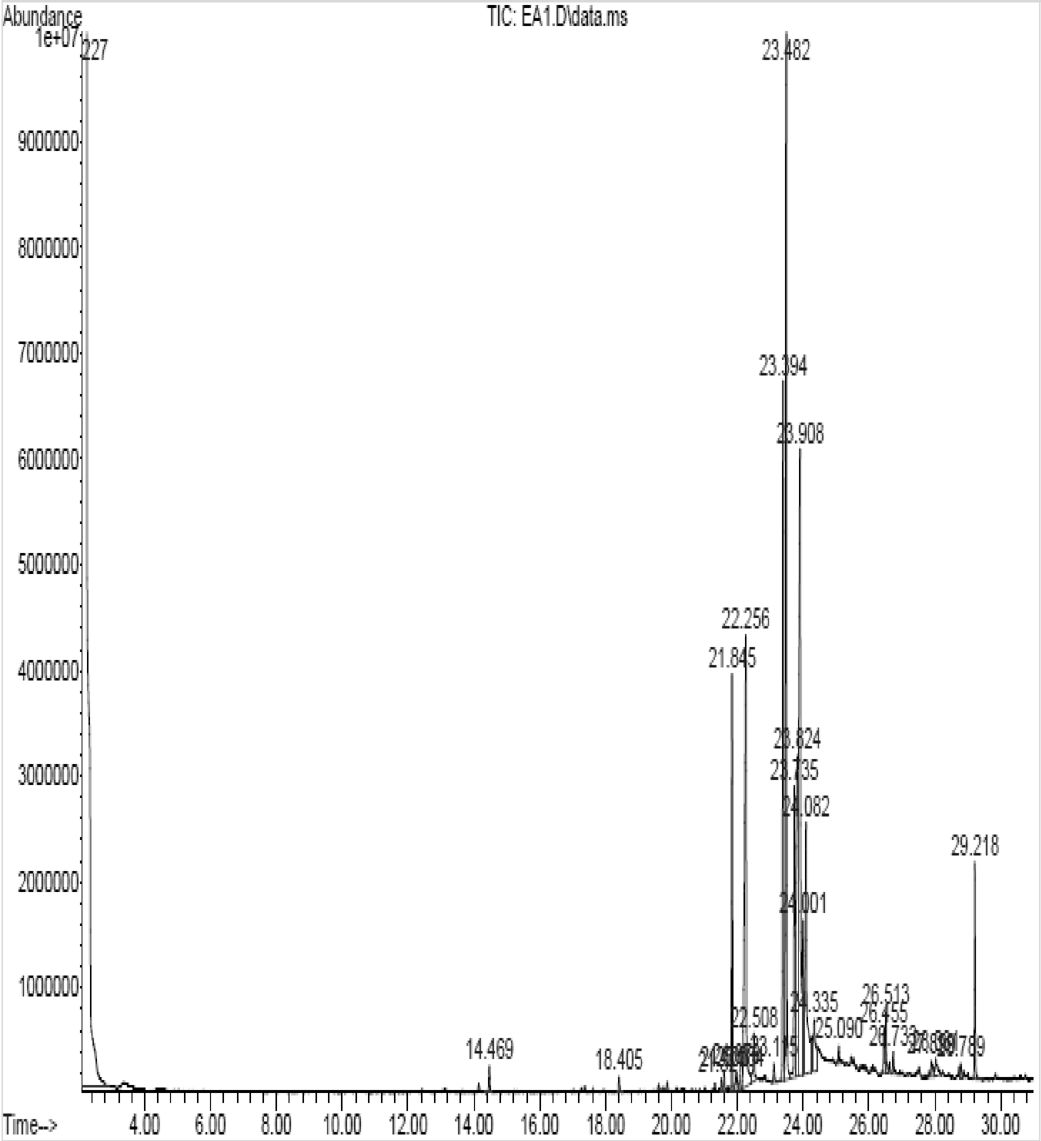
Misc Info :
Vial Number: 1



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Library Search Report
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Acq On : 11 Dec 2012 15:19
Operator : fiza
Sample : EA1
Misc :
ALS Vial : 1 Sample Multiplier: 1
Search Libraries: C:\Database\NIST05a.L Minimum Quality: 0
Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e
Pk#      RT      Area%   Library/ID                          Ref#     CAS#          Qual
_______________________________________________________________________________________

1        2.226   76.95   C:\Database\NIST05a.L
                         Hexane                              1791     000110-54-3   58
                         Hexane                              1792     000110-54-3   43
                         Hexane                              1790     000110-54-3   43

2        14.467  0.09    C:\Database\NIST05a.L
                         2,4-Decadienal, (E,E)-              24070    025152-84-5   90
                         2,4-Decadienal, (E,E)-              24069    025152-84-5   87
                         2,4-Nonadienal                      16909    006750-03-4   78

3        18.409  0.06    C:\Database\NIST05a.L
                         Benzophenone                        44597    000119-61-9   97
                         Benzophenone                        44598    000119-61-9   96
                         Benzophenone                        44596    000119-61-9   94

4        21.528  0.05    C:\Database\NIST05a.L
                         7,9-Di-tert-butyl-1-oxaspiro
                         (4,5)d eca-6,9-diene-2,8-dione      109509   082304-66-3   93
                         7,9-Di-tert-butyl-1-oxaspiro(4,5)
                         deca-6,9-diene-2,8-dione            109508   082304-66-3   92
                         2,5-Cyclohexadien-1-one,
                         2,6-bis(   ,1-dimethylethyl)-4-ethylidene-180001 006738-27-8   47

5        21.603 0.06     C:\Database\NIST05a.L
                         9-Hexadecenoic acid,methyl ester,Z)- 104152 001120-25-8    98
                         7-Hexadecenoic acid,methyl ester,(Z)-104151 056875-67-3    95
                         9-Octadecenoic acid (Z)-,methyl ester122321 000112-62-9    93

6         21.849 1.20    C:\Database\NIST05a.L
                         Pentadecanoic acid, 14-methyl-,     105662   005129-60-2   97
                         methyl ester
                         Hexadecanoic acid, methyl ester     105639   000112-39-0   97
                         Hexadecanoic acid, methyl ester     105644   000112-39-0   96

7        21.977 0.12     C:\Database\NIST05a.L
                         9-Hexadecenoic acid                 94742    002091-29-4   99
                         Hexadecenoic acid, Z-11-            94748    002416-20-8   98
                         Hexadecenoic acid, Z-11-            94745    002416-20-8   70

8        22.084 0.07      C:\Database\NIST05a.L
                         Eicosane, 7-hexyl-                  159841   055333-99-8   90
                         Hexacosane                          159836   000630-01-3   86
                         Pentacosane                         153747   000629-99-2   86

9        22.255  2.95    C:\Database\NIST05a.L
                         n-Hexadecanoic acid                 96235    000057-10-3   99
                         n-Hexadecanoic acid                 96234    000057-10-3   98
                         n-Hexadecanoic acid                 96233    000057-10-3   95
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Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
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			Hexadecanoic acid, ethyl ester	114844	000628-97-7	97
			Hexadecanoic acid, ethyl ester	114847	000628-97-7	91
			Decanoic acid, ethyl ester	57085	000110-38-3	81
11	23.120	0.07	C:\Database\NIST05a.L			
			13-Hexyloxacyclotridec-10-en-2-one	112000	127062-51-5	70
			Z,E-7,11-Hexadecadien-1-yl acetate	111998	051607-94-4	47
			Z,Z-11,13-Hexadecadien-1-ol acetat	112003	1000130-88-3	46
12	23.398	2.19	C:\Database\NIST05a.L			
			9,12-Octadecadienoic acid (Z,Z)-, methyl ester	121105	000112-63-0	99
			8,11-Octadecadienoic acid, methyl ester	121095	056599-58-7	99
			9,12-Octadecadienoic acid, methyl Ester	121093	002462-85-3	99
13	23.483	3.62	C:\Database\NIST05a.L			
			9-Octadecenoic acid, methyl ester	122299	002462-84-2	99
			9-Octadecenoic acid (Z)-, methyl ester	122323	000112-62-9	99
			11-Octadecenoic acid, methyl ester, (Z)-	122331	001937-63-9	99
14	23.739	1.12	C:\Database\NIST05a.L			
			Octadecanoic acid, methyl ester	123709	000112-61-8	98
			Heptadecanoic acid, 16-methyl-, methyl ester	123732	005129-61-3	98
			Octadecanoic acid, methyl ester	123700	000112-61-8	96
15	23.825	2.18	C:\Database\NIST05a.L			
			9,17-Octadecadienal, (Z)-	101505	056554-35-9	93
			9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	93
			2-Chloroethyl linoleate	148820	025525-76-2	83
16	23.910	4.64	C:\Database\NIST05a.L			
			Oleic Acid	113354	000112-80-1	98
			9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	155406	000111-03-5	90
			Oleic Acid	113353	000112-80-1	89
17	24.006	0.75	C:\Database\NIST05a.L			
			Linoleic acid ethyl ester	129812	000544-35-4	99
			9,12-Octadecadienoic acid, ethyl ester	129833	007619-08-1	99
			Linoleic acid ethyl ester	129811	000544-35-4	99
18	24.081	1.63	C:\Database\NIST05a.L			
			Ethyl Oleate	131051	000111-62-6	70
			Ethyl Oleate	131050	000111-62-6	68
			9-Octadecenoic acid, ethyl ester	131087	006512-99-8	55
19	24.338	0.36	C:\Database\NIST05a.L			
			11-Dodecen-1-ol trifluoroacetate	111638	128792-46-1	60
			Heptadecanoic acid, 15-methyl-, ethyl ester	132360	057274-46-1	50
			Octadecanoic acid, ethyl ester 1	32321	000111-61-5	46

Pk#	RT Area%	Library/ID	Ref#	CAS#	Qual
20	25.085 0.09	C:\Database\NIST05a.L			
		9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	91
		9,12-Octadecadienoic acid (Z,Z)-	111992	000060-33-3	90
		6-Octadecenoic acid, (Z)-	113359	000593-39-5	83
21	26.453 0.15	C:\Database\NIST05a.L			
		9,12-Octadecadienoic acid, methyl ester, (E,E)-	121109	002566-97-4	70
		9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	64
		9,12-Octadecadienal	101501	026537-70-2	62
22	26.517 0.24	C:\Database\NIST05a.L			
		Cyclopropaneoctanal, 2-octyl-	112082	056196-06-6	90
		11,13-Dimethyl-12-tetradecen-1-ol acetate	113394	1000130-81-0	87
		2-Methyl-Z,Z-3,13-octadecadienol	112083	1000130-90-5	86
23	26.730 0.09	C:\Database\NIST05a.L			
		Benzene, 1-isocyano-2-methyl-	8348	010468-64-1	46
		Benzonitrile,4-butyl-	29161	020651-73-4	43
		Benzyl nitrile	8331	000140-29-4	43
24	27.895 0.09	C:\Database\NIST05a.L			
		2-Methyl-Z,Z-3,13-octadecadienol	112083	1000130-90-5	72
		8-Dodecenol	45954	042513-42-8	42
		Oleic Acid	113353	000112-80-1	41
25	28.034 0.18	C:\Database\NIST05a.L			
		9,17-Octadecadienal, (Z)-	101505	056554-35-9	91
		9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	90
		Z,E-3,13-Octadecadien-1-ol	102833	1000131-10-4	56
26	28.792 0.06	C:\Database\NIST05a.L			
		9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	155406	000111-03-5	97
		2,3-Dihydroxypropyl elaidate	155383	002716-53-2	94
		cis-7,cis-11-Hexadecadien-1-yl acetate	112005	052207-99-5	72
27	29.219 0.76	C:\Database\NIST05a.L			
		2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	173573	000111-02-4	96
		Squalene	173556	007683-64-9	91
		2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	173572	000111-02-4	91

(b) Ethyl acetate: 8 hour

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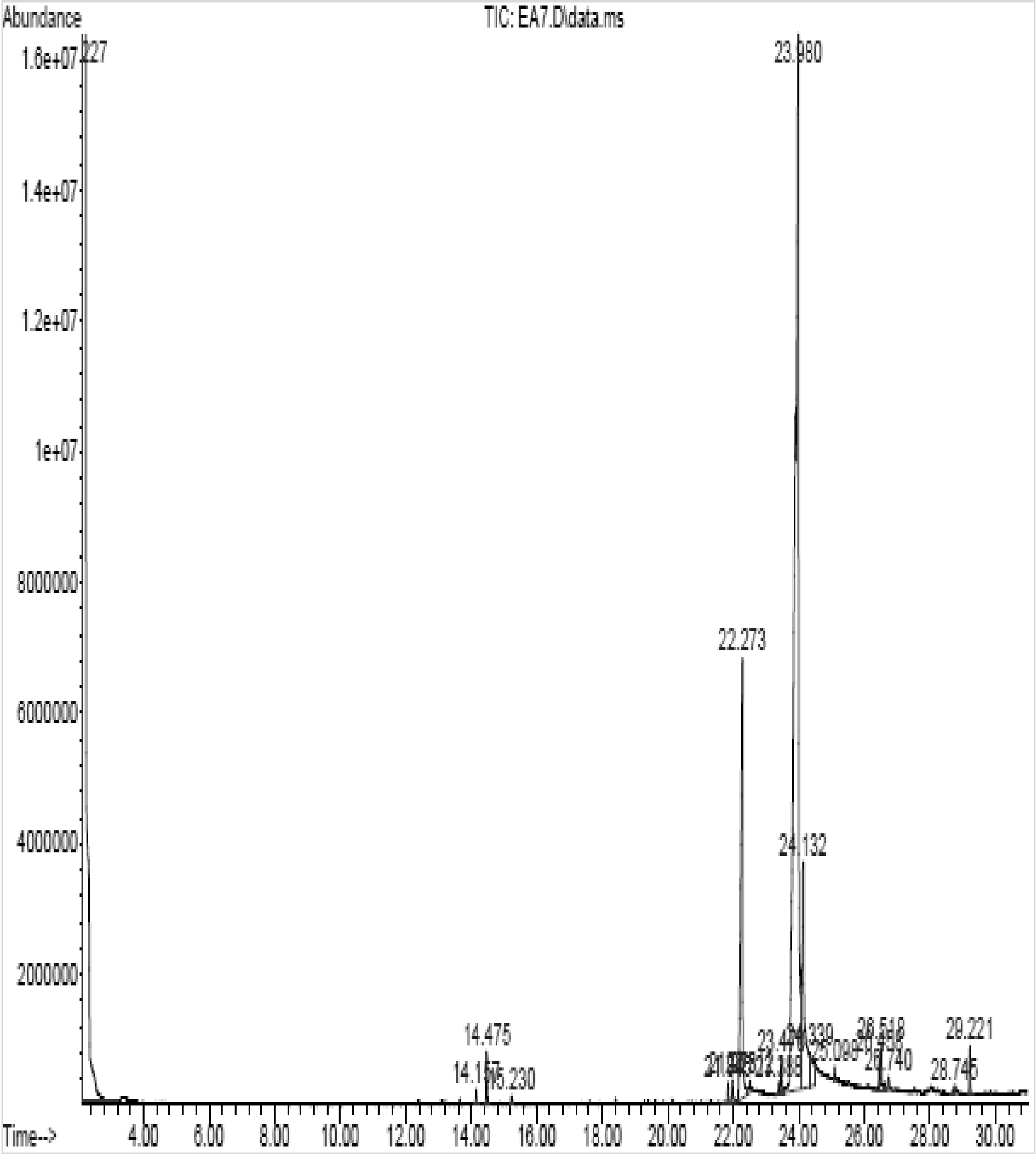
Misc Info :
Vial Number: 7



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Library Search Report
Data Path : D:\Data\psm 2_2012\shalini _mani\
Data File : EA7.D
Acq On : 11 Dec 2012 18:58
Operator : fiza
Sample : EA7
ALS Vial : 7 Sample Multiplier: 1
Search Libraries: C:\Database\NIST05a.L Minimum Quality: 0
Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e
Pk#     RT      Area%           Library/ID                      Ref#     CAS#           Qual
_____________________________________________________________________________________

1       2.226 67.94     C:\Database\NIST05a.L
                        Hexane                                  1790     000110-54-3    50
                        Hexane                                  1791     000110-54-3    43
                        Hexane                                  1792     000110-54-3    43

2       14.157 0.08     C:\Database\NIST05a.L
                        2,4-Decadienal, (E,E)-                  24069    025152-84-5    90
                        2,4-Decadienal, (E,E)-                  24067    025152-84-5    87
                        2,4-Decadienal                          24041    002363-88-4    87

3       14.478 0.24     C:\Database\NIST05a.L
                        2,4-Decadienal, (E,E)-                  24070    025152-84-5    91
                        2,4-Decadienal                          24041    002363-88-4    91
                        2,4-Decadienal, (E,E)-                  24069    025152-84-5    91

4       15.226 0.03     C:\Database\NIST05a.L
                        2-Tridecenal, (E)-                      54431    007069-41-2    91
                        2-Undecenal                             34832    002463-77-6    91
                        2-Dodecenal, (E)-                       44525    020407-84-5    83

5       21.848 0.08     C:\Database\NIST05a.L
                        Pentadecanoic acid, 14-
                        methyl-, methyl ester                   105661   005129-60-2    96
                        Pentadecanoic acid, 14-
                        methyl-, methyl ester                   105662   005129-60-2    95
                        Hexadecanoic acid, methyl
                        ester
                                                                105644   000112-39-0    95
6       21.977 0.19     C:\Database\NIST05a.L
                        Z-7-Hexadecenoic acid                   94744    1000130-90-8   97
                        Hexadecenoic acid, Z-11-                94748     002416-20-8   95
                        9-Hexadecenoic acid                     94742    002091-29-4    90

7       22.276 4.31     C:\Database\NIST05a.L
                        n-Hexadecanoic acid                     96235    000057-10-3    98
                        n-Hexadecanoic acid                     96234    000057-10-3    97
                        Tridecanoic acid                        67137    000638-53-9    74

8       22.511 0.10     C:\Database\NIST05a.L
                        Octadecanoic acid                       114818   000057-11-4    86
                        Octadecanoic acid                       114822   000057-11-4    86
                        Hexadecanoic acid, ethyl ester1         14847    000628-97-7    83

9       23.387 0.06     C:\Database\NIST05a.L
                        9,12-Octadecadienoic acid,              121093   002462-85-3    99
                        methyl ester
                        8,11-Octadecadienoic acid,              121092   056599-58-7    99
                        methyl ester
                        9,12-Octadecadienoic acid (Z,Z)-        111993   000060-33-3    99
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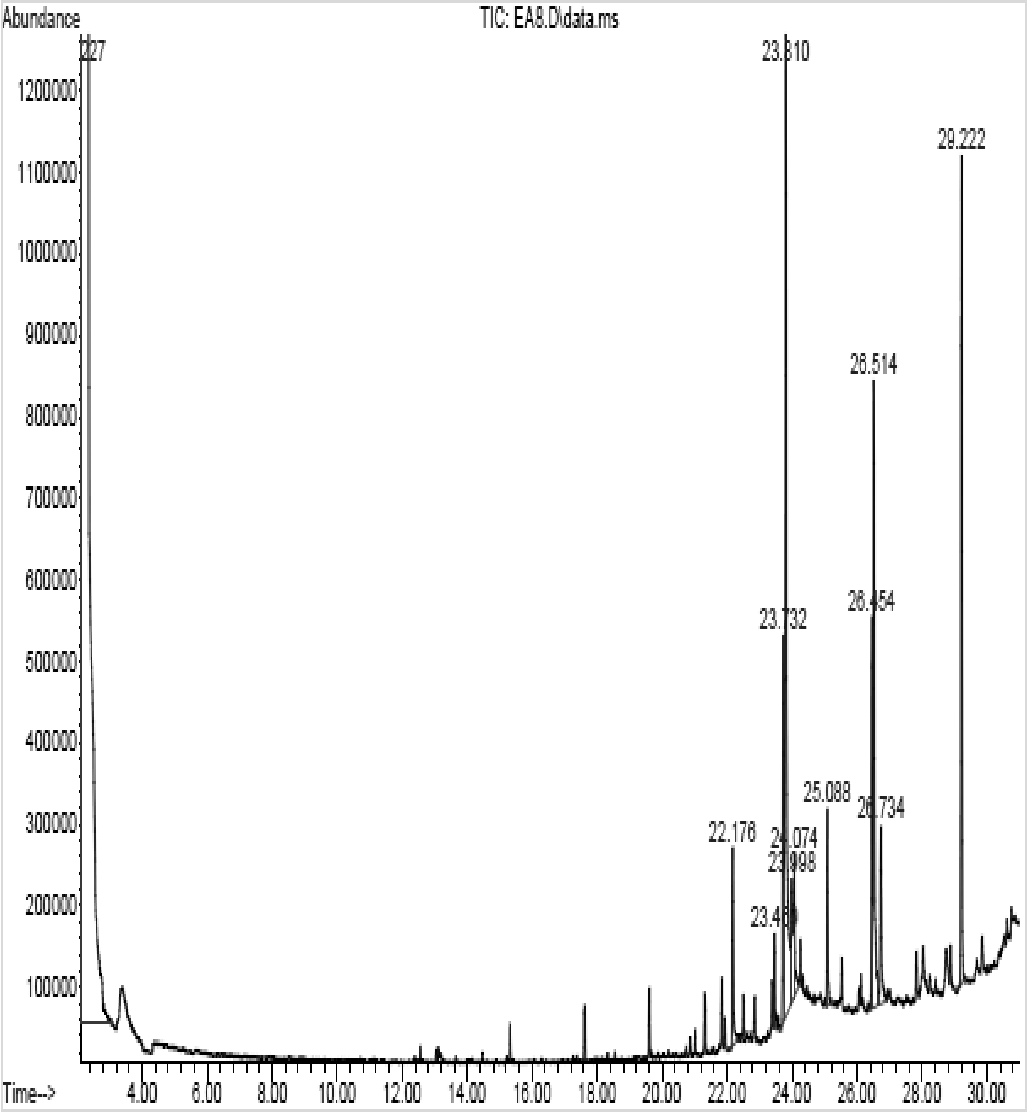
Pk#	RT Area%	Library/ID	Ref#	CAS#	Qual
10	23.472 0.17	C:\Database\NIST05a.L			
		9-Octadecenoic acid (Z)-, methyl ester	122323	000112-62-9	99
		7-Octadecenoic acid, methyl ester	122298	057396-98-2	99
		11-Octadecenoic acid, methyl ester	122316	052380-33-3	99
11	23.985 22.10	C:\Database\NIST05a.L			
		Oleic Acid	113354	000112-80-1	95
		Oleic Acid	113353	000112-80-1	90
		Octadec-9-enoic acid	113356	1000190-13-7	81
12	24.134 3.21	C:\Database\NIST05a.L			
		Octadecanoic acid	114822	000057-11-4	96
		Octadecanoic acid	114821	000057-11-4	93
		Octadecanoic acid	114818	000057-11-4	93
13	24.337 0.66	C:\Database\NIST05a.L			
		9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	95
		Oleic Acid	113353	000112-80-1	95
		9-Octadecenoic acid, (E)-	113363	000112-79-8	94
14	25.096 0.08	C:\Database\NIST05a.L			
		9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	93
		9,12-Octadecadienoic acid (Z,Z)-	111992	000060-33-3	91
		9,17-Octadecadienal, (Z)-	101505	056554-35-9	90
15	26.463 0.14	C:\Database\NIST05a.L			
		13-Tetradece-11-yn-1-ol	629461	000131-00-4	89
		Isopropyl linoleate	138084	022882-95-7	87
		1,2-15,16-Diepoxyhexadecane 9	47621	000192-65-0	83
16	26.516 0.24	C:\Database\NIST05a.L			
		Cyclopropaneoctanal, 2-octyl-	112082	056196-06-6	95
		9-Octadecenal, (Z)-	102821	002423-10-1	91
		12-Methyl-E,E-2,13-octadecadien-1- ol	112087	1000130-90-4	90
17	26.741 0.09	C:\Database\NIST05a.L			
		2,3-Dihydroxypropyl elaidate	155383	002716-53-2	87
		6-Octadecenoic acid, (Z)-	113359	000593-39-5	86
		Oleic Acid	113355	000112-80-1	78
18	28.749 0.07	C:\Database\NIST05a.L			
		Oleic Acid	113353	000112-80-1	43
		2,3-Dihydroxypropyl elaidate	155383	002716-53-2	35
		1,3,12-Nonadecatriene	100226	1000131-11-1	35
19	29.219 0.22	C:\Database\NIST05a.L			
		Squalene	173555	007683-64-9	99
		2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	173571	000111-02-4	98
		2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	173573	000111-02-4	95

(c) Ethyl acetate: 10 hour

File :D:\Data\psm 2_2012\shalini _mani\EA8.D
Operator : fiza
Acquired : 11 Dec 2012 19:34 using AcqMethod FAME CJO.M
Instrument : GCMSD
Sample Name: EA8
Misc Info :
Vial Number: 8



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Library Search Report
Data Path : D:\Data\psm 2_2012\shalini _mani\
Data File : EA8.D
Acq On : 11 Dec 2012 19:34
Operator : fiza
Sample : EA8
ALS Vial : 8 Sample Multiplier: 1
Search Libraries: C:\Database\NIST05a.L Minimum Quality: 0
Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e
Pk#     RT Area%              Library/ID               Ref#     CAS#            Qual
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1       2.226 97.15     C:\Database\NIST05a.L
                        Hexane                    1791     000110-54-3       43
                        Hexane                    1792     000110-54-3       43
                        Hydroperoxide, hexyl               8555     004312-76-9     39

2       22.180 0.12     C:\Database\NIST05a.L
                        n-Hexadecanoic acid                96235    000057-10-3     95
                        n-Hexadecanoic acid                96234    000057-10-3     94
                        n-Hexadecanoic acid                96233    000057-10-3     90

3       23.472 0.04     C:\Database\NIST05a.L
                        9-Octadecenoic acid (Z)-,  122323 000112-62-9        99
                        methyl ester
                        11-Octadecenoic acid,              122316 052380-33-3       99
                        methyl ester
                        10-Octadecenoic acid,              122312 013481-95-3       99
                        methyl ester

4       23.729 0.26      C:\Database\NIST05a.L
                        9,17-Octadecadienal, (Z)-  101505   056554-35-9      94
                        9,12-Octadecadienoic acid,         121093 002462-85-3       91
                        methyl ester
                        9,12-Octadecadienoic acid (Z,Z)- 111993 000060-33-3 90

5       23.814 0.85     C:\Database\NIST05a.L
                        Oleic Acid                         113354 000112-80-1       95
                        Oleic Acid                         113353 000112-80-1       86
                        Octadec-9-enoic acid               113356 1000190-13-7      83

6       23.996 0.13     C:\Database\NIST05a.L
                        9,12-Octadecadienoic acid (Z,Z)- 111993 000060-33-3 99
                        9,12-Octadecadienoic acid,         129833 007619-08-1       95
                        ethyl ester
                        Linoleic acid ethyl ester  129812 000544-35-4        95

7       24.071 0.11     C:\Database\NIST05a.L
                        Oleic Acid                         113354 000112-80-1       97
                        Oleic Acid                         113353 000112-80-1       91
                        13-Octadecenal, (Z)-               102822 058594-45-9       86

8       25.085 0.11     C:\Database\NIST05a.L
                        Myristoyl chloride                 89240    000112-64-1     46
                        Undecanedioic acid                 68187    001852-04-6      38
                        1H-Tetrazole-1-ethanol, 5-amino- 12372 015284-29-4 35

9       26.453 0.21     C:\Database\NIST05a.L
                        9,12-Octadecadienoic acid,         121109 002566-97-4       91
                        methyl ester, (E,E)-
                        4-Tetradecyne                      52899    060212-33-1     89

                        9,17-Octadecadienal, (Z)-  101505 056554-35-9        87

10      26.517 0.42     C:\Database\NIST05a.L
                        9-Octadecenal, (Z)-                102821 002423-10-1       96
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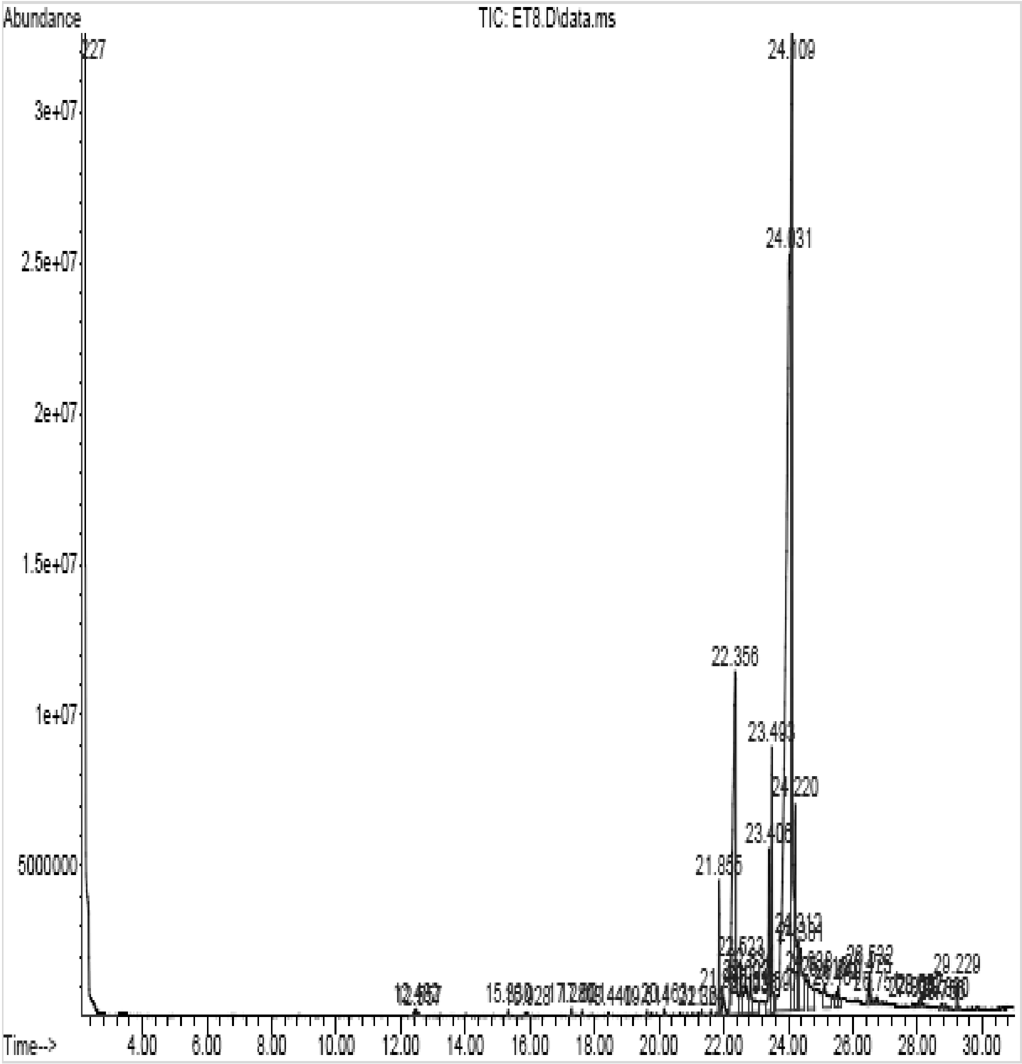
Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			9,17-Octadecadienal, (Z)-	101505	056554-35-9	92
			Cyclopropaneoctanal, 2-octyl-	112082	056196-06-6	64
11	26.730	0.15	C:\Database\NIST05a.L			
			1-Cyano-4-(5-hexenyl)benzene	46770	142779-16-6	35
			Benzonitrile, 4-methyl-	8342	000104-85-8	30
			Benzonitrile, 4-methyl-	8344	000104-85-8	30
12	29.219	0.44	C:\Database\NIST05a.L			
			Squalene	173555	007683-64-9	99
			2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	173571	000111-02-4	99
			Squalene 1	73554	007683-64-9	98

(d) Ethanol: 6 hour

File :D:\Data\psm 2_2012\shalini _mani\ET8.D
Operator : fiza
Acquired : 12 Dec 2012 00:25 using AcqMethod FAME CJO.M
Instrument : GCMSD
Sample Name: ET8
Misc Info :
Vial Number: 16



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Library Search Report
Data Path : D:\Data\psm 2_2012\shalini _mani\
Data File : ET8.D
Acq On : 12 Dec 2012 00:25
Operator : fiza
Sample : ET8
ALS Vial : 16 Sample Multiplier: 1
Search Libraries: C:\Database\NIST05a.L Minimum Quality: 0
Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e
Pk#    RT Area%              Library/ID                    Ref#    CAS#          Qual
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1      2.226 41.85   C:\Database\NIST05a.L
                     Hexane                                1791    000110-54-3   62
                     Hexane                                1792    000110-54-3   50
                     Hexane                                1790    000110-54-3   50

2      12.480 0.12   C:\Database\NIST05a.L
                     Octanoic Acid                         20065   000124-07-2   94
                     Octanoic Acid                         20066   000124-07-2   91
                     Octanoic Acid                         20063   000124-07-2   86

3      12.555 0.03    C:\Database\NIST05a.L
                     Cyclopentasiloxane, decamethyl-       161017  000541-02-6   90
                     Cyclopentasiloxane, decamethyl-       61015   000541-02-6   87
                     Cyclopentasiloxane, decamethyl-       161016  000541-02-6   83

4      15.332 0.03   C:\Database\NIST05a.L
                     Cyclohexasiloxane, dodecamethyl- 179152  000540-97-6   91
                     Cyclohexasiloxane, dodecamethyl- 179153  000540-97-6   90
                     Cyclohexasiloxane, dodecamethyl- 179151  000540-97-6   38

5      15.931 0.07   C:\Database\NIST05a.L
                     3-Tetradecene, (Z)-                   54518   041446-67-7   97
                     5-Tetradecene, (E)-                   54528   041446-66-6   95
                     5-Tetradecene, (E)-                   54532   041446-66-6   95

6      17.277 0.05   C:\Database\NIST05a.L
                     Phenol, 2,4-bis(1,1-dimethylethyl)    61438   000096-76-4   96
                     Phenol, 2,5-bis(1,1-dimethylethyl)    61441   005875-45-6   94
                     Phenol, 2,4-bis(1,1-dimethylethyl)    61449   000096-76-4   94

7      17.618 0.03   C:\Database\NIST05a.L
                     Cycloheptasiloxane, tetradecamethy 185541 000107-50-6    93
                     Pentasiloxane, dodecamethyl-          166194  000141-63-9   43
                     4-(Anisylideneamino)-cinnamic acid 112579 025959-50-6   27
8      18.441 0.02   C:\Database\NIST05a.L
                     1-Hexadecene                          74521    000629-73-2  95
                     1-Hexadecene                          74519    000629-73-2  92
                     9-Eicosene, (E)-                      112106  074685-29-3   91

9      19.616 0.03   C:\Database\NIST05a.L
                     Cyclooctasiloxane, hexadecamethyl- 188231 000556-68-3   68
                     Cyclooctasiloxane, hexadecamethyl- 188230 000556-68-3   64
                     Benzeneacetic acid, .alpha.,3,4-tr    182236  037148-65-5   50
                     is[(trimethylsilyl)oxy]-, trimethylsilyl ester

10     20.161 0.05   C:\Database\NIST05a.L
                     Tetradecanoic acid                    77275   000544-63-8   95
                     Tetradecanoic acid                    77273   000544-63-8   94
                     Tetradecanoic acid                    77276   000544-63-8   93
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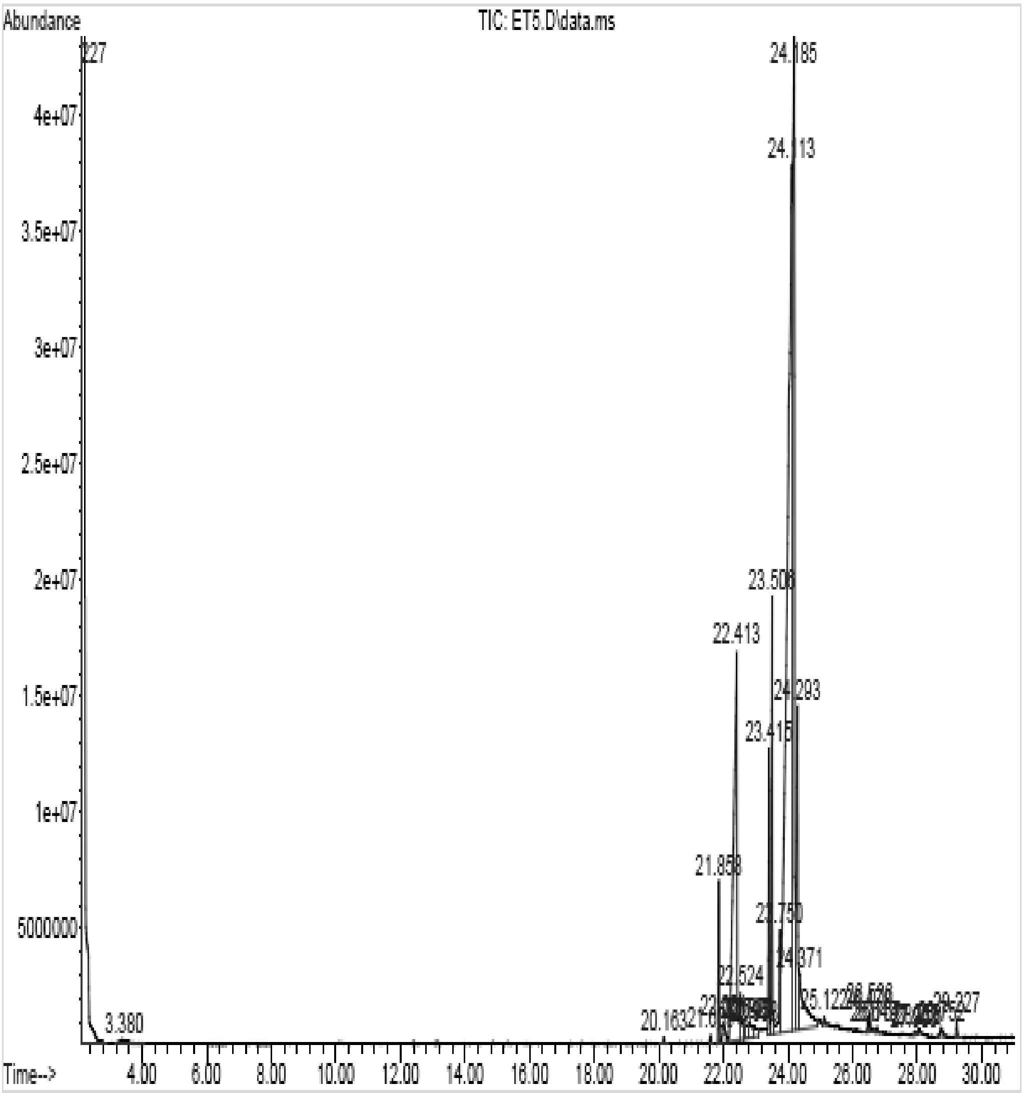
Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
11	21.325	0.03	C:\Database\NIST05a.			
			Cyclononasiloxane, octadecamethyl-	189575	000556-71-8	62
			1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	179156	038147-00-1	59
			Cyclononasiloxane, octadecamethyl-	189576	000556-71-8	55
12	21.613	0.04	C:\Database\NIST05a.L			
			9-Hexadecenoic acid, methyl ester, (Z)-	104152	001120-25-8	99
			7-Hexadecenoic acid, methyl ester, (Z)-	104151	056875-67-3	86
			9-Octadecenoic acid (Z)-, methyl eSter	122321	000112-62-9	76
13	21.859	0.71	C:\Database\NIST05a.L			
			Hexadecanoic acid, methyl ester	105639	000112-39-0	99
			Pentadecanoic acid, 14-methyl-, methyl ester	105662	005129-60-2	97
			Hexadecanoic acid, methyl ester	105644	000112-39-0	96
14	21.998	0.35	C:\Database\NIST05a.L			
			Octadec-9-enoic acid 1	13356	1000190-13-7	95
			9-Hexadecenoic acid	94742	002091-29-4	94
			Hexadecenoic acid, Z-11-	94748	002416-20-8	93
15	22.361	7.23	C:\Database\NIST05a.L			
			n-Hexadecanoic acid	96234	000057-10-3	98
			n-Hexadecanoic acid	96235	000057-10-3	97
			n-Hexadecanoic acid	96233	000057-10-3	95
16	22.521	0.48	C:\Database\NIST05a.L			
			Hexadecanoic acid, ethyl ester	114844	000628-97-7	96
			Hexadecanoic acid, ethyl ester	114847	000628-97-7	93
			Octadecanoic acid 1	14818	000057-11-4	91
17	22.692	0.90	C:\Database\NIST05a.L			
			n-Hexadecanoic acid	96235	000057-10-3	97
			n-Hexadecanoic acid	96233	000057-10-3	96
			n-Hexadecanoic acid 9	6234	000057-10-3	93
18	22.831	0.43	C:\Database\NIST05a.L			
			Isopropyl Palmitate	123689	000142-91-6	64
			n-Hexadecanoic acid	96234	000057-10-3	50
			n-Hexadecanoic acid	96235	000057-10-3	46
19	22.938	0.49	C:\Database\NIST05a.L			
			n-Hexadecanoic acid	96235	000057-10-3	60
			n-Hexadecanoic acid	96234	000057-10-3	60
			Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-	112117	056009-20-2	55
20	23.408	1.23	C:\Database\NIST05a.L			
			9,12-Octadecadienoic acid (Z,Z)-, methyl ester	121105	000112-63-0	99
			8,11-Octadecadienoic acid, methyl ester	121095	056599-58-7	99
			9,12-Octadecadienoic acid (Z,Z)-, 1 methyl ester	21107	000112-63-0	99

Pk#	RT Area%	Library/ID	Ref#	CAS#	Qual
21	23.493 1.77	C:\Database\NIST05a.L			
		11-Octadecenoic acid, methyl ester	122331	001937-63-9	99
		9-Octadecenoic acid, methyl ester,	122328	001937-62-8	99
		11-Octadecenoic acid, methyl ester	122316	052380-33-3	99
22	23.600 0.13	C:\Database\NIST05a.L			
		Oleic Acid	113353	000112-80-1	95
		Cyclopropaneoctanal, 2-octyl-	112082	056196-06-6	90
		Octadecanoic acid	114821	000057-11-4	80
23	24.028 22.62	C:\Database\NIST05a.L			
		9,12-Octadecadienoic acid (Z,Z)-	11992	000060-33-3	94
		E-2-Octadecadecen-1-ol	104257	1000131-10-2	90
		9,17-Octadecadienal, (Z)-	101505	056554-35-9	90
24	24.113 13.65	C:\Database\NIST05a.L			
		6-Octadecenoic acid, (Z)-	113359	000593-39-5	91
		Oleic Acid	113353	000112-80-1	90
		15-Tetracosenoic acid, methyl ester, (Z)-	165280	002733-88-2	83
25	24.220 2.48	C:\Database\NIST05a.L			
		Octadecanoic acid	114822	000057-11-4	98
		Octadecanoic acid	114820	000057-11-4	91
		Octadecanoic acid	114821	000057-11-4	90
26	24.316 0.60	C:\Database\NIST05a.L			
		9,17-Octadecadienal, (Z)-	101505	056554-35-9	98
		9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	97
		11,14-Eicosadienoic acid, methyl ester	138089	002463-02-7	93
27	24.380 1.49	C:\Database\NIST05a.L			
		Elaidic acid, isopropyl ester	139170	022147-34-8	95
		Oleic Acid	113354	000112-80-1	91
		13-Octadecenal, (Z)-	102822	058594-45-9	87
28	24.636 1.01	C:\Database\NIST05a.L			
		9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	95
		9,17-Octadecadienal, (Z)-	101505	056554-35-9	91
		Octadecanoic acid	114821	000057-11-4	86
29	25.117 0.64	C:\Database\NIST05a.L			
		9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	94
		9,12-Octadecadienoic acid (Z,Z)-	111992	000060-33-3	93
		6-Octadecenoic acid, (Z)-	113359	000593-39-5	89
30	25.480 0.18	C:\Database\NIST05a.L			
		9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	98
		9,17-Octadecadienal, (Z)-	101505	056554-35-9	97
		Oleic Acid	113353	000112-80-1	95
31	25.544 0.27	C:\Database\NIST05a.L			
		9-Octadecenamide, (Z)-	112657	000301-02-0	60
		9-Octadecenamide, (Z)-	112656	000301-02-0	60
		2-Ethoxyethyl acetate	13818	000111-15-9	43

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
32	26.474	0.17	C:\Database\NIST05a.L			
			9,12-Octadecadien-1-ol, (Z,Z)-	102838	000506-43-4	96
			9,12-Octadecadienoic acid, ethyl ester	129833	007619-08-1	90
			Oxirane, tetradecyl-	85503	007320-37-8	90
33	26.538	0.25	C:\Database\NIST05a.L			
			9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	95
			9-Octadecenal, (Z)-	102821	002423-10-1 9	42
			-Methyl-Z,Z-3,13-octadecadienol	112083	1000130-90-5	93
34	26.751	0.10	C:\Database\NIST05a.L			
			1-Eicosene	112102	003452-07-1	92
			2,3-Dihydroxypropyl elaidate	155383	002716-53-2	92
			9,17-Octadecadienal, (Z)-	101505	056554-35-9	91
35	27.841	0.03	C:\Database\NIST05a.L			
			Cyclononasiloxane, octadecamethyl-	189575	000556-71-8	47
			Cycloheptasiloxane, tetradecamethy	185541	000107-50-6	38
			Cyclooctasiloxane, hexadecamethyl-	188231	000556-68-3	27
36	28.055	0.05	C:\Database\NIST05a.L			
			9,17-Octadecadienal, (Z)-	101505	056554-35-9	96
			9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	96
			9,12-Octadecadienal	101501	026537-70-2	91
37	28.087	0.10	C:\Database\NIST05a.L			
			9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	155406	000111-03-5	97
			9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	155412	003443-84-3	93
			Oleic Acid	113353	000112-80-1	81
38	28.760	0.06	C:\Database\NIST05a.L			
			17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-vinylhexadecahydrocyclopenta[a]phenanthren-3-ol	174427	1000210-86-9	50
			Tetradecanoic acid, dodecyl ester	170269	002040-64-4	46
			.gamma.-Sitosterol	174403	000083-47-6	42
39	28.877	0.04	C:\Database\NIST05a.L			
			Cyclononasiloxane, octadecamethyl-	189575	000556-71-8	43
			Cyclononasiloxane, octadecamethyl-	189576	000556-71-8	33
			Cyclodecasiloxane, eicosamethyl-	190220	018772-36-6	32
40	29.230	0.18	C:\Database\NIST05a.L			
			2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	173571	000111-02-4	98
			2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	173573	000111-02-4	94
			2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	173572	000111-02-4	91

(e) Ethanol: 8 hour

File :D:\Data\psm 2_2012\shalini _mani\ET5.D
Operator : fiza
Acquired : 11 Dec 2012 22:36 using AcqMethod FAME CJO.M
Instrument : GCMSD
Sample Name: ET5
Misc Info :
Vial Number: 13



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Library Search Report
Data Path : D:\Data\psm 2_2012\shalini _mani\
Data File : EA5.D
Acq On : 11 Dec 2012 17:45
Operator : fiza
Sample : EA5
ALS Vial : 5 Sample Multiplier: 1
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                        Hexane                                1792     000110-54-3     43
                        Hexane                                1791     000110-54-3     43
                        Hydroperoxide, hexyl                  8555     004312-76-9     39

2       13.121 0.04     C:\Database\NIST05a.L
                        1,2,3-Propanetriol, monoacetate       14546    026446-35-5     72
                        2-Propanone, 1-hydroxy-               800      000116-09-6      9
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3       18.409 0.05      C:\Database\NIST05a.L
                        Benzophenone                          44597    000119-61-9     96
                        Benzophenone                          44598    000119-61-9     96
                        Benzophenone                          44596    000119-61-9     95

4       21.838 0.05     C:\Database\NIST05a.L
                        Hexadecanoic acid, methyl ester       105639   00112-39-0      96
                        Pentadecanoic acid, 14-methyl-, me    105661   005129-60-2     95
                        thyl ester
                        Hexadecanoic acid, methyl ester       105646   000112-39-0     95

5        21.977 0.21    C:\Database\NIST05a.L
                        Z-7-Hexadecenoic acid                 94744    1000130-90-8    97
                        Hexadecenoic acid, Z-11-              94748    002416-20-8     95
                        Hexadecenoic acid, Z-11-              94745    002416-20-8     94

6       22.286 5.38     C:\Database\NIST05a.L
                        n-Hexadecanoic acid                   96235    000057-10-3     98
                        n-Hexadecanoic acid                   96234    000057-10-3     98
                        Tetradecanoic acid                    77276    000544-63-8     93

7       22.511 0.14     C:\Database\NIST05a.L
                        Hexadecanoic acid, ethyl ester        114847   000628-97-7     93
                        Hexadecanoic acid, ethyl ester        114844   000628-97-7     81
                        Hexadecanoic acid, ethyl ester        114846   000628-97-7     70

8       23.387 0.04     C:\Database\NIST05a.L
                        9,12-Octadecadienoic acid, methyl     121093   002462-85-3     99
                        ester
                        9,12-Octadecadienoic acid (Z,Z)-      111993   000060-33-3     99
                        10,13-Octadecadienoic acid, methyl    121100   056554-62-2     98
                        Ester

9       23.472 0.09     C:\Database\NIST05a.L
                        9-Octadecenoic acid (Z)-, methyl e    122323   000112-62-9     99
                        ster
                        8-Octadecenoic acid, methyl ester,    122325   026528-50-7     98
                        (E)-
                        11-Octadecenoic acid, methyl ester    122316   052380-33-3     97
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		Cyclopropaneoctanal, 2-octyl-	112082	056196-06-6	64
		Cyclohexane, 1-(1,5-dimethylhexyl) -4-(4-methylpentyl)-	112117	056009-20-2	55
11	23.899 10.05	C:\Database\NIST05a.L			
		9,17-Octadecadienal, (Z)-	101505	056554-35-9	96
		E-1,9-Tetradecadiene	52923	1000245-70-7	94
		9,12-Octadecadienoic acid (Z,Z)-	111992	000060-33-3	94
12	23.985 11.07	C:\Database\NIST05a.L			
		Oleic Acid 113354 000112-80-1 96			
		9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	155406	000111-03-5	87
		Oleic Acid	113353	000112-80-1	81
13	24.092 0.76	C:\Database\NIST05a.L			
		9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	90
		13-Octadecenal, (Z)-	102822	058594-45-9	87
		9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	155406	000111-03-5	70
14	24.134 1.81	C:\Database\NIST05a.L			
		Octadecanoic acid	114822	000057-11-4	96
		Octadecanoic acid	114821	000057-11-4	94
		Octadecanoic acid	114818	000057-11-4	90
15	25.096 0.06	C:\Database\NIST05a.L			
		9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	95
		9,12-Octadecadienoic acid (Z,Z)-	111992	000060-33-3	92
		9,17-Octadecadienal, (Z)-	101505	056554-35-9	86
16	26.452 0.13	C:\Database\NIST05a.L			
		6-Tetradecyne	52900	003730-08-3	90
		1,2-15,16-Diepoxyhexadecane	94762	1000192-65-0	86
		9,17-Octadecadienal, (Z)-	101505	056554-35-9	86
17	26.517 0.20	C:\Database\NIST05a.L			
		9,17-Octadecadienal, (Z)-	101505	056554-35-9	94
		13-Octadecenal, (Z)-	102822	058594-45-9	92
		Cyclopropaneoctanal, 2-octyl-	112082	056196-06-6	92
18	26.741 0.08	C:\Database\NIST05a.L			
		2,3-Dihydroxypropyl elaidate	155383	002716-53-2	94
		cis-11-Hexadecenal	83994	053939-28-9	81
		cis-9-Hexadecenal	83993	056219-04-6	80
19	28.738 0.12	C:\Database\NIST05a.L			
		.beta.-Sitosterol	174399	000083-46-5	93
		.beta.-Sitosterol	174400	000083-46-5	49
		.gamma.-Sitosterol	174403	000083-47-6	49
20	29.219 0.37	C:\Database\NIST05a.L			
		Squalene	173555	007683-64-9	99
		Squalene	173554	007683-64-9	98
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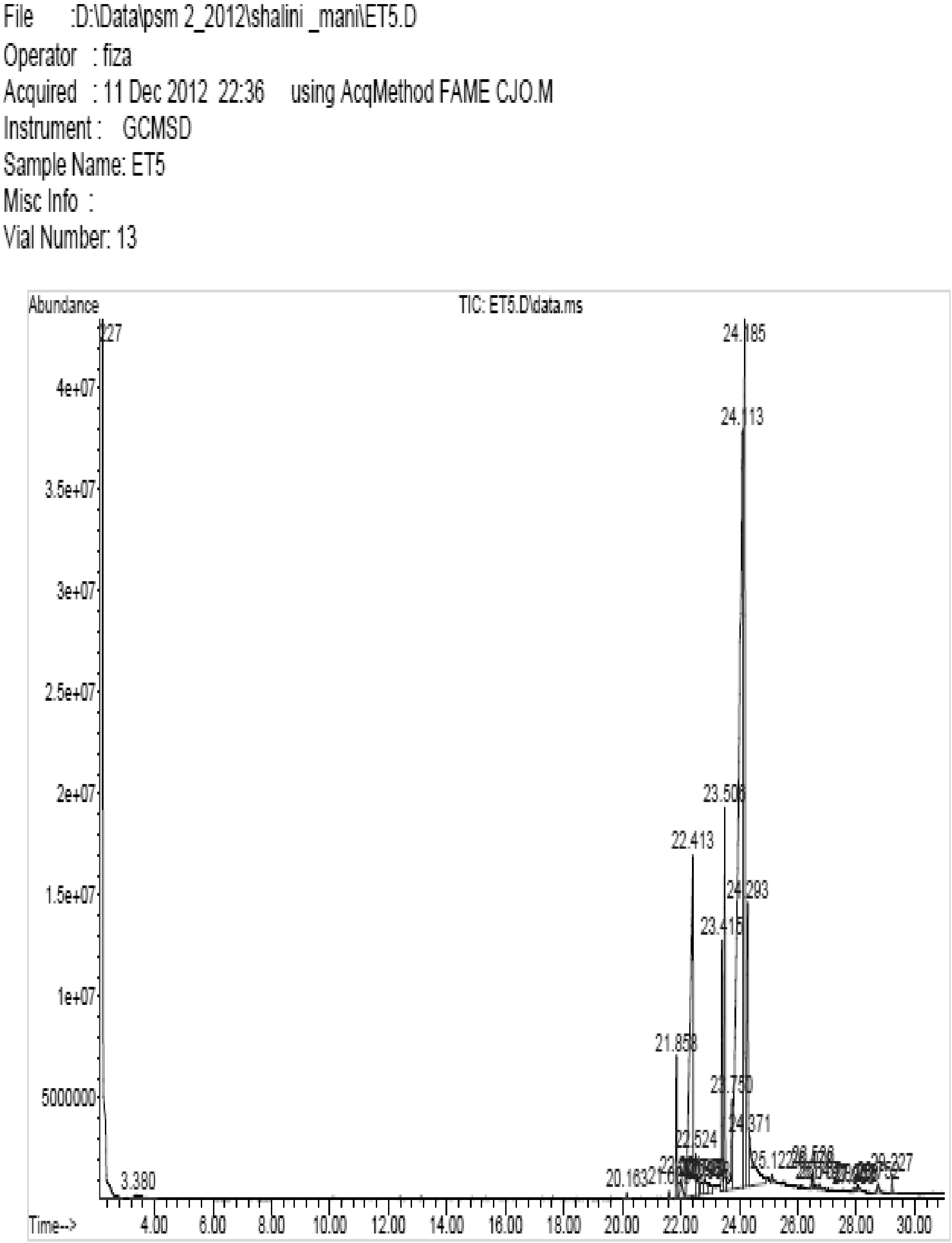
(f) Ethanol: 10 hour



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Acquired : 11 Dec 2012 22:36 using AcqMethod FAME CJO.M
Instrument : GCMSD
Sample Name: ET5
Misc Info :
Vial Number: 13

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Sample : ET5
ALS Vial : 13 Sample Multiplier: 1
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Qual
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                       3-Buten-1-ol, 3-methyl-             1754     000763-32-6     40

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                       Octane                              7420      000111-65-9    87
                       Octane                              7419     000111-65-9     86
                       Octane                               7421    000111-65-9     76

3       20.161  0.04   C:\Database\NIST05a.L
                       Tetradecanoic acid                  77276    000544-63-8     99
                       Tetradecanoic acid                  77275    000544-63-8     96
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4       21.614   0.05  C:\Database\NIST05a.L
                       9-Hexadecenoic acid, methyl ester,  104152    001120-25-8     99
                       (Z)-
                       7-Hexadecenoic acid, methyl ester,  104151   056875-67-3     95
                       (Z)-
                       9-Hexadecenoic acid, methyl ester,  104156   001120-25-8     95
                       (Z)-

5       21.859  0.95   C:\Database\NIST05a.L
                       Pentadecanoic acid, 14-methyl-, me  105662   005129-60-2      97
                       thyl ester
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                       Z-7-Hexadecenoic acid               94744    1000130-90-8    98
                       Octadec-9-enoic acid                113356    1000190-13-7   98
                       Hexadecenoic acid, Z-11-            94748    002416-20-8     97

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                       n-Hexadecanoic acid                 96234    000057-10-3     98
                       n-Hexadecanoic acid                 96235    000057-10-3     97
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8       22.522 0.56    C:\Database\NIST05a.
                       Hexadecanoic acid, ethyl ester      114844   000628-97-7     95
                       Hexadecanoic acid, ethyl ester      114847   000628-97-7      91
                       Hexadecanoic acid, ethyl ester      114846   000628-97-7     64

9       22.639  0.22   C:\Database\NIST05a.L
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                       n-Hexadecanoic acid                 96235    000057-10-3     91
                       Tridecanoic acid                    67136    000638-53-9     83

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			Oleic Acid	113353	000112-80-1	83
			n-Hexadecanoic acid	96235	000057-10-3	83
12	22.960	0.35	C:\Database\NIST05a.L			
			n-Hexadecanoic acid	96235	000057-10-3	95
			n-Hexadecanoic acid	96234	000057-10-3	90
			Oleic Acid	113353	000112-80-1	90
13	23.419	1.83	C:\Database\NIST05a.L			
			9,12-Octadecadienoic acid, methyl ester	121093	002462-85-3	99
			10,13-Octadecadienoic acid, methyl ester	121100	056554-62-2	99
			9,12-Octadecadienoic acid, methyl ester, (E,E)-	121112	002566-97-4	99
14	23.504	2.70	C:\Database\NIST05a.L			
			9-Octadecenoic acid, methyl ester, (E)-	122326	001937-62-8	99
			8-Octadecenoic acid, methyl ester	122297	002345-29-1	99
			9-Octadecenoic acid (Z)-, methyl ester	122323	000112-62-9	99
15	23.750	1.00	C:\Database\NIST05a.L			
			Octadecanoic acid, methyl ester	123709	000112-61-8	99
			Octadecanoic acid, methyl ester	123707	000112-61-8	98
			Heptadecanoic acid, 16-methyl-, methyl ester	123732	005129-61-3	97
16	24.113	29.54	C:\Database\NIST05a.L			
			9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	95
			1,9-Tetradecadiene	529151	12929-06-3	94
			9,17-Octadecadienal, (Z)-	101505	056554-35-9	94
17	24.188	13.75	C:\Database\NIST05a.L			
			Oleic Acid	113353	000112-80-1	94
			9-Octadecenoic acid, (E)-	113363	000112-79-8	76
			Z-7-Pentadecenol	76035	1000130-76-6	72
18	24.295	3.55	C:\Database\NIST05a.L			
			Octadecanoic acid	114822	000057-11-4	99
			Octadecanoic acid	114821	000057-11-4	95
			Octadecanoic acid	114820	000057-11-4	94
19	24.370	2.02	C:\Database\NIST05a.L			
			9,12-Octadecadienoic acid (Z,Z)-	111992	000060-33-3	70
			Cyclopropaneoctanal, 2-octyl-	112082	056196-06-6	70
			1-Eicosene	112103	003452-07-1	68
20	25.117	0.08	C:\Database\NIST05a.L			
			9,12-Octadecadienoic acid (Z,Z)-	111992	000060-33-3	93
			cis-9-Hexadecenal	83993	056219-04-6	89
			Cyclopentadecanone, 2-hydroxy-	85349	004727-18-8	87
21	26.474	0.12	C:\Database\NIST05a.L			
			10,13-Octadecadienoic acid, methyl ester	121100	056554-62-2	90
			4-Tetradecyne	52899	060212-33-1	83
			9,12-Octadecadienal	101501	026537-70-2	78
22	26.538	0.16	C:\Database\NIST05a.L			
			9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	95

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			12-Methyl-E,E-2,13-octadecadien-1-ol	112087	1000130-90-4	91
			9-Octadecenal, (Z)-	102821	002423-10-1	91
23	26.645	0.03	C:\Database\NIST05a.L			
			11-Dodecen-1-ol trifluoroacetate	111638	128792-46-1	45
			Oleic Acid	113355	000112-80-1	45
			Cyclopropaneoctanal, 2-octyl-	112082	056196-06-6	42
24	26.762	0.06	C:\Database\NIST05a.L			
			Oleic Acid	113355	000112-80-1	95
			9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	92
			Oleic Acid	113353	000112-80-1	91
25	27.905	0.01	C:\Database\NIST05a.L			
			9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	96
			9,17-Octadecadienal, (Z)-	101505	056554-35-9	95
			9,12-Octadecadienal	101501	026537-70-2	76
26	27.948	0.02	C:\Database\NIST05a.L			
			9-Oxabicyclo[6.1.0]nonane, cis-	11083	004925-71-7	45
			9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	155412	003443-84-3	41
			Difluoro(methylamino)phosphine sulFide	13585	1000306-16-8	27
27	28.055	0.05	C:\Database\NIST05a.L			
			9,17-Octadecadienal, (Z)-	101505	056554-35-9	98
			9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	95
			9,12-Octadecadienal	101501	026537-70-2	93
28	28.749	0.14	C:\Database\NIST05a.L			
			.gamma.-Sitosterol	174402	000083-47-6	96
			Stigmasterol, 22,23-dihydro-	174408	1000214-20-7	92
			4-Oxatricyclo[20.8.0.0(7,16)]triaconta-1(20),7(16)-diene	174424	1000155-84-7	86
29	29.230	0.13	C:\Database\NIST05a.L			
			2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	173571	000111-02-4	98
			2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	173573	000111-02-4	94
			Squalene	173556	007683-64-9	90

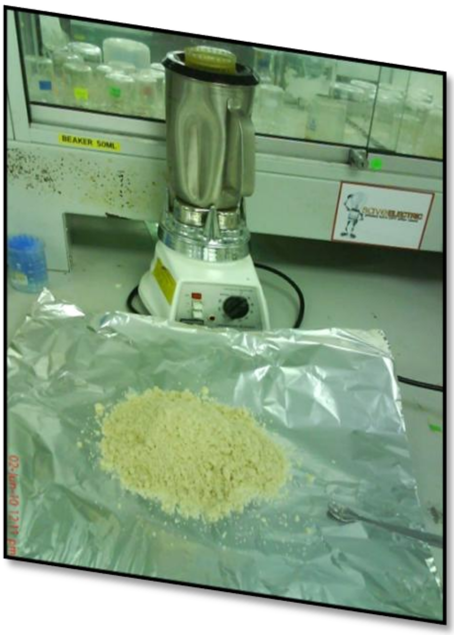
APPENDIX B

PICTURES OF METHODOLOGY

Jatropha curcas Linnaues seeds

Jatropha curcas kernels

Drying jatropha curcas kernels to remove moisture



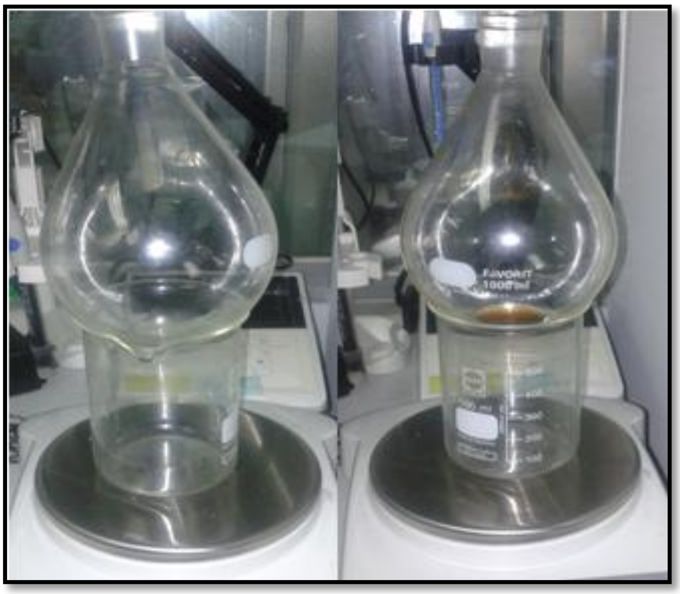
Grind seeds using blender

Grind seeds using grinder

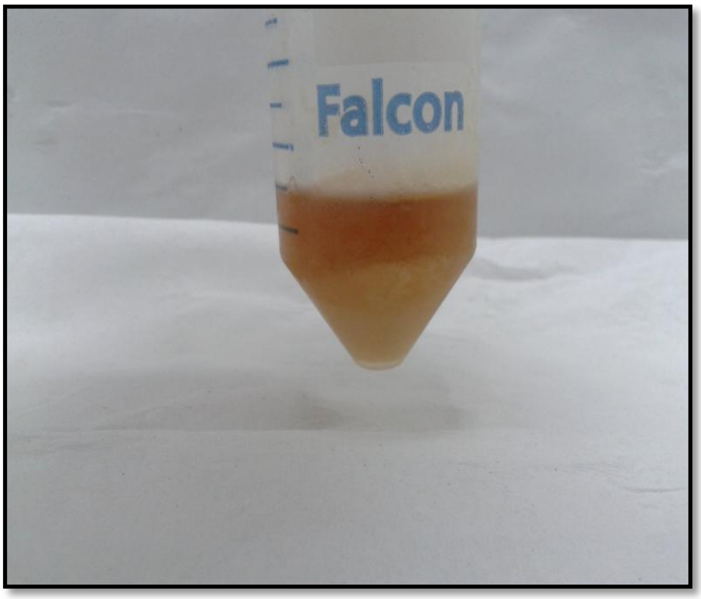
Weigh 20g grinded jatropha curcas kernels

Soxhlet extraction

Separation of solvent using rotary evaporator

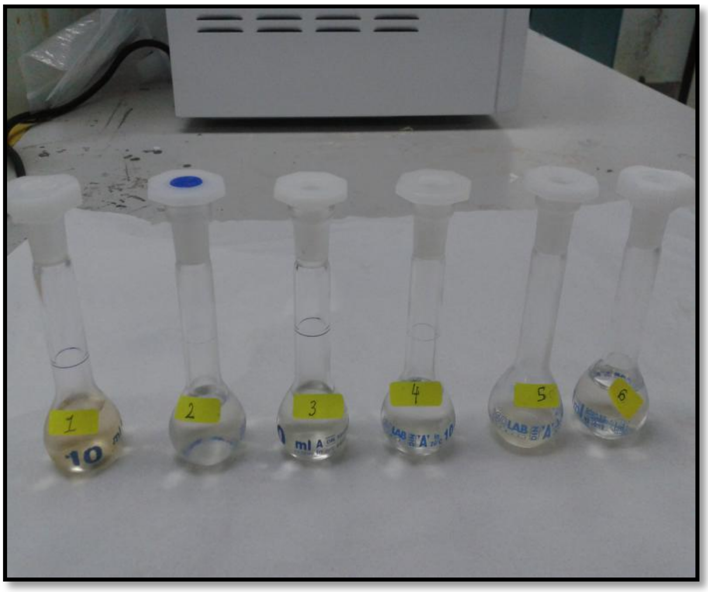


Measure weight of oil extracted

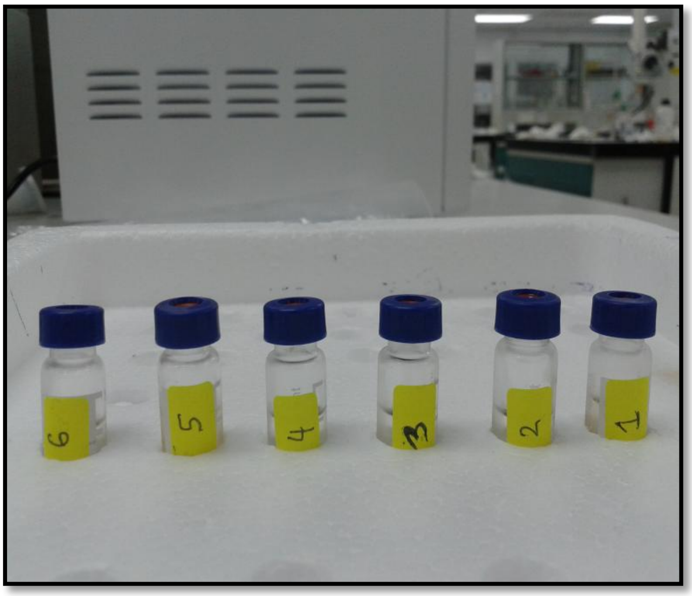


Impurities in oil extracted

Filter impurities out of the oil



Dilution of oil for GC-MS analysis



Sample prepared for GC-MS analysis

Run GC-MS analysis