ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO ENGINEERING

EXTRACTION, OPTIMIZATION AND CHARACTERIZATION OF OIL FROM ETHIOPIAN *JATROPHA SEED* KERNELS USING SOLVENT EXTRACTION METHOD

A Thesis Submitted to the School of Chemical and Bio Engineering in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in Chemical and Bio Engineering (Under Process Engineering Stream)

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ADDIS ABABA UNIVERSITY
ADDIS ABABA, ETHIOPIA
NOVEMBER, 2017

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This is to certify that the thesis prepared by Zinnabu Tassew entitled **"Extraction, Optimization, and Characterization of Oil from Ethiopian *Jatropha* Seed Kernels Using Solvent Extraction Method"** and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Chemical and Bio Engineering (Under Process Engineering Stream) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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DEDICATION

I dedicate my thesis work to my family and many friends. A special feeling of gratitude should go to my beloved younger brother, Haftu Tasew, whose words of encouragement and push for tenacity ring in my ears. My sister, Marzeneb Tasew, never left my side and is very special.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this thesis is my own work. I have followed all ethical principles of research in the preparation, data collection, data analysis and completion of this thesis. I affirm that I have cited and referenced all sources used in this document. Every serious effort has been made to avoid any plagiarism in the preparation of this thesis.

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ABSTRACT

In this study, the seed of Jatropha curcas was collected from Bati of Amhara Region and it was utilized for determination of proximate and mineral composition analysis of the seed, oil extraction, characterization, and optimization. The Jatropha curcas oil was extracted using normal hexane (62—78°C) by Soxhlet apparatus. The result showed that the seed kernels consisted of: 55.30% (w/w) oil, moisture (6.3621% w/w), total ash (5.25 % w/w), crude protein (21.59 % w/w), crude fat (46.57 % w/w), crude fiber (16.77 % w/w) and carbohydrate (3.4%w/w) as its proximate analysis; and Na (31.99 mg/100g), K (1159 mg/100g), Ca (8.65 ppm), Zn (0.308 ppm) and P (0.03g/100g) as the main mineral profile of the seed. After optimization using Design-Expert has been carried out, optimal conditions of temperature (70^{o}C), solvent to solid ratio (10:1), and particle size (0.66mm) have been obtained and an oil yield of 55.28% by wt. was found. After the optimum values have been obtained, an experiment has been conducted and an oil yield of 54.81% by wt. was found which is closely in agreement with the result obtained from the model and the experimental observation and hence validated the findings of the optimization. Physicochemical characterization of the optimized oil yield revealed that it has the following most important properties: moisture and volatile matter (15.4883% w/w), kinematic viscosity (0.014mm^2) at 40^{o}C, specific gravity (0.8567) at 20^{o}C, density (855.20kg/m^3) at 20^{o}C, pH (5.74), refractive index (1.4512) at 20^{o}C, acid value (13.0152 mg KOH g-1 oil), free fatty acid value (6.5076 % by wt.), saponification value (140.25 mg KOH g-1 oil), iodine value (104 g I2/100 g), and ash content (0.0811 % by wt.). From the FT-IR result, different functional components like alcohols, phenols, alkanes, easters, aldehydes were identified. The GC-MS analysis result of J. curcas oil showed that the presence of the following major fatty acids: oleic acid (44.61%), palmitic acid (22.55%), linolic acid (13.64%), stearic acid (8.64%), myristic acid (2.63%), 11-Hexadecenoic acid (1.85%) and other acids (6.08%).

Key words: Jatropha seed oil; extraction; physicochemical characterization; optimization; response surface methodology; yield

ACKNOWLEDGEMENTS

My greatest debt of gratitude is Dr. -Ing. Abubeker Yimam–from Addis Ababa University, Addis Ababa Institute of Technology, School of Chemical and Bio Engineering; for his invaluable backing and skillful contribution to this thesis and also his continuous guidance, encouragement and fine-tuning up to its accomplishment of this thesis work.

Especially, I am profoundly indebted to my employer organization—Addis Ababa University— who sponsored my full time study, and above all bearing the whole burden of covering the job which I would have to accomplish during my study time.

My especial indebtedness would go to all the staff of the School of Chemical and Bio Engineering and Mr. Chernet Denibu, a Ph.D. candidate in process engineering of the School of Chemical and Bio Engineering, for their invaluable cooperation.

I owe a special debt of gratitude to the Theme - SMALL SCALE SUSTAINABLE BIODIESEL PRODUCTION FROM JATROPHA Coordinator, Dr.-Ing Demiss Alemu, who bestowed me financial support in covering the whole cost of the research. This thesis work would not come to this end had it not been for the great help and support of the above Theme.

LIST OF ACRONYMS AND SYMBOLS

AAS	Atomic Absorption Spectroscopy
AD	Anaerobic Digestion
ASTM	American Society for Testing and Materials
EN	European Standard
EPA	US Environmental Protection Agency
FFA	Free Fatty Acids
FTIR	Fourier Transform Infra-Red Spectrometry
GC-MS	Gas Chromatography Mass Spectrometry
HPLC	High Performance Liquid Chromatography
ITD	Ion Trap Detector
K	Kelvin
KI	Potassium Iodide
KOH	Potassium Hydroxide
M	Molar (Molar concentration)
MAE	Microwave-Assisted Extraction
MPa	Mega Pascal
N	Normal
NESHAP	US National Emission Standards for Hazardous Air Pollutants
SFE	Supercritical Fluid Extraction
UAE	Ultrasound-Assisted Extraction
V_b	Titre for Blank
VOCs	Volatile Organic Compounds
w_o	Weight of Clean Dry Bottle
w_1	Weight of Oil Sample Before Drying
w_2	Weight of Oil Sample After Drying

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1. INTRODUCTION

1.1 Background of the Study

Jatropha curcas is a perennial poisonous shrub belonging to the Euphorbiaceae family. It is an uncultivated non-food wild-species, a perennial shrub, easy to adapt in marginal areas and resistant to a medium-long periods of dryness [1]. The high environmental adaptability allowed Jatropha to become widespread in arid, semi-arid, and tropical region in the world. Furthermore, its deep root system allows it to store carbon, maintain soil quality, and manage water and nutrients conservatively. The deep roots can prevent landslides and the shallow can defend soil erosion caused by wind or water. Moreover, scientists believe that toxins in the seed and plant make Jatropha non-edible; thus it will not compete with food [2].

Jatropha grows in various parts of Ethiopia, as a hedge around homesteads and farmlands, such as in Wolayita, Metekel, Southern Wollo, Northern and Eastern Shoa, Tigrai, Gamo Gofa zones and Gambella region. This suggests that Jatropha can be cultivated either as large-scale plantations on marginal areas, as small-scale hedges, or intercropped to assist rural livelihoods. Although Jatropha already exists in many places of Ethiopia; its economic importance is far from being realized due to absence of proper evaluation and promotion of the existing local Jatropha provenances for their oil content, oil yield and oil quality for biodiesel utilization.

Hardness, rapid growth, drought resistance and wide ranging usefulness are the main characteristics of the plant. The oil content of the Jatropha seeds is at least 30% by weight so it is considered as a plant with high oil content seeds. It has a yield per hectare of more than four times that of soybean and ten times that of corn. Among the different species of Jatropha, *Jatropha curcas l.* has a variety of uses and offers interesting scope of usage which could be of great economic significance. The oil of *Jatropha curcas l.*, simply known as Jatropha oil or crude Jatropha oil, is a non-edible oil which is used for producing soap, candles, engines lubricant and, more importantly, as an alternative to diesel. The excellent chemical properties, performance characteristics and ignition quality equivalent to diesel makes Jatropha oil a promising and sustainable alternative for diesel to overcome the source limitation problem.

Recently, most developing countries have experienced a significant increase in energy demand. In addition, some of the world's largest producers of oil have suffered from warfare and political and social instability. Diminishing fossil fuel resources, coupled with the steady increase in energy consumption, has spurred research interest in alternative and renewable energy sources. Biodiesel is among the most promising fossil fuel alternatives. Various renewable sources, including recycled oil, can be utilized as feed stocks.

There is an increasing interest in using vegetable oils as fuels or fuel sources. One of these vegetable oils is non-edible oil of Jatropha which has a tremendous potential to be used not only directly as fuel but also as a source for biodiesel production. Solid liquid extraction is the most common and efficient technique in producing oil for fuel and biodiesel production. Solid liquid extraction, mostly known as leaching in industry, involves separation of solute component from the solid phase. The methodis usually called extraction, and is carried out with organic solvents.

The separation (extraction) of oil contained in the seeds can be performed by mechanical pressing, with the use of organic solvents or supercritical fluids. Oil extraction by mechanical pressing is a method widely used by industries; however, the remaining solid fraction after pressing (cake) contains appreciable quantities of oil which is subsequently recovered by solvent extraction. For mechanical pressing, prior treatment of the seeds is required, including grinding, rolling, and heat treatment to increase process yield. The resulting oil usually has a high turbidity with high contents of water and metals when compared to solvent extracted oil. Thus, filtration, dehydration, degumming, and neutralization processes are necessary to use the oil in biodiesel production.

Extraction using solvents is generally conducted at a temperature near the boiling point of the solvent, which reduces oil viscosity and improves its solubility in the solvent, ensuring the efficiency of the process [3]. Extraction can be performed by immersing the seed particles in the solvent or by percolation of the solvent through a column of particles in a continuous process [4].

Various solvents and solvent mixtures may be employed to extract the oil contained in the seeds. Normal-hexane (N-hexane) has been the most widely used because of its low boiling point (69°C) and low corrosive effect on the equipment [5]. Despite these advantages n-hexane is an air pollutant and its use in oil extraction plants can adversely affect the central nervous system of workers [2, 4, 5]. Furthermore, n-hexane is highly flammable and generates higher risks and harmful emissions, which makes the process expensive, especially for small capacity plants [6]. Even though there are disadvantages with regard to air pollution, if it is accurately controlled and recycled, then there are no risks. The other most important point with hexane is its advantages in giving the highest oil yields. For more about solvent selection, it is clearly in literature review.

Extraction of oil using solvents is the most effective method for oil recovery of almost 98%, especially with materials having low oil content. This method is not absolutely applicable to oil seeds with high oil content, like peanut and sunflower that require a prior step of pressing the seeds and then extracting oil from the cake produced using a solvent. When performed at low temperature, solvent extraction has another advantage over screw pressing because it gives better quality of produced oil [7].

Moreover, solvent extraction involves the use of chemicals referred to as the solvents in the extraction of oil from oil seeds. It is known for its high yielding of oil output, easiness and swiftness to carry out, and it is relatively cost effective. The use of this method requires a complete refining process to ensure that traces of the solvents are removed totally from the oil in order to avoid contamination. [8, 9]indicated that hexane, a petroleum-derived product had been extensively used as a solvent for oil extraction of some seeds because of its low vaporization temperature, high stability, low corrosiveness and low greasy residual effect [10]. Generally, the process of solvent extraction can be considered in three parts: first is the change of phase of the solute as it dissolves in the solvent, second is its diffusion through the solvent in the pores of the solid to the outside of the particle, and the third one is the transfer of the solute from the solution in contact with the particles to the main bulk [11].

If solvent extraction method is to be used to get oil from a plant seed, the values of the factors (input variables) affecting the extraction have to be carefully selected in order to get high yield of the oil, and the proper selection of these values can be achieved through optimization.

Optimization is defined as the process of finding the conditions that give minimum or maximum value of a function, where the function represents the effort required or the desired benefit. The use of statistical software like Design Expert is gaining high ground when finding the optimum conditions for a process such as the extraction of oil from Jatropha seed. Before optimizing, the software is normally employed to design the experiments that are required to be carried out for the optimization through the use of methods like response surface methodology (RSM). RSM is a widely used technology for rational experimental design optimization when the mechanistic information of the process is not available [12, 13, 14]. RSM initiates from Design of Experiments (DoE) to determine the values of the factors to be used for conducting experiments and collecting data. The data are then used to develop an empirical model that relates the process response to the factors. Subsequently, the model facilitates the search for better process response, which is validated through experiment(s). The above procedure is repeated until an optimal process is identified or the limit on experiemtal resources is reached [15, 16].

1.2 Statement of the Problem

The world is facing severe energy crisis attributed to global oil reserve depletion, increasing consumption rate, incessant oil price hike and above all environmental concerns (air pollution and emission of Greenhouse Gases) dictate that the world community must hunt for alternative and sustainable energy sources.

The increasing trend of land acquisition for biofuels has led to the widespread debate about food versus biofuel because of the perceived competition for land and water. To avoid the food versus fuel debate, the use of “marginal” land for biofuel feedstock production (Jatropha) has emerged as a dominant narrative. But both the availability and suitability of “marginal” land for commercial level Jatropha production is not well understood/examined, especially in Africa [17].

Since Ethiopia imports its huge amounts of fossil fuel from outside, then there should be a means to look for alternative renewable energy sources that are technically feasible, economically competitive and environmentally acceptable. Among alternative energy sources, biodiesel derived from Jatropha seeds, have been gaining increasing attention recently as a replacement for fossil fuels. The increasing fuel prices, concerns with climate change and future energy security have led to tremendous global interest in the use of liquid biofuels like biodiesel in the transport sector. One of the mitigation efforts considered in response to the crisis is finding substitute for petroleum oil. Since Jatropha oil is the primary ingredient required in the production of biofuels, the development of solvent extraction methods and the optimization of existing methods of extraction have become significant.

This study work is, therefore, quite important in finding the mitigation efforts for the environmental concerns and to substitute for petroleum oil products.

1.3 Scope of the Study

The thesis work generally covers proximate and mineral composition analysis of Jatropha seed kernels, Jatropha oil extraction, optimization of Jatropha oil extraction parameters, physicochemical characterization of the oil using standard procedures and test methods and the results were compared against to the standard specifications of ASTM and EN.

1.4 Objectives of the Study

1.4.1 General Objective

The general objective of this study work is extraction, optimization and characterization of crude Jatropha oil by using solvent extraction of Jatropha seed kernels obtained from Bati—Amhara Region.

1.4.2 Specific Objectives

The specific objectives include:-

- Proximate and mineral composition analysis of *Jatropha curcas l.* seeds
- Extraction of crude Jatropha oil by normal-hexane
- Obtain optimal extraction parameters (temperature, solvent to solid ratio and particle size) for maximum oil yield extraction
- Investigate the effects of temperature, particle size, solvent to solid ratio and their interaction effects on the oil yield
- Physicochemical characterization of the most important physicochemical properties of *Jatropha* oil

1.5 Significance of the Research

This study is an experimental study of solid-liquid extraction method, optimization and characterization of Jatropha oil. The study is believed to contribute a significant method of identification of high quality variety of Jatropha seeds which has given the highest oil yield.

Moreover, this study is important in giving a good direction in decreasing the greenhouse gas emissions due to the sustainable replacement of the petro based fuels by biofuels. It also improves the economic and social benefits of our country for the country is becoming a major destination for foreign direct investment in biofuels where it is important for the import substitute of petroleum based products. The study will also serve as a reference for future researchers on the subject of extraction of Ethiopian Jatropha seeds, the characterization and optimization of the oil for maximum oil yield.

2. LITERATURE REVIEW

2.1 Introduction

In this literature review, the discussion has centered the application of vegetable oils in industry, especially in the fuel field. The properties, characteristics and usage as fuel of Jatropha curcas l. which is the raw material used in this work were investigated. More importantly, different methods of oil extraction from Jatropha curcas, as well as their advantages and disadvantages, have also been explored.

2.2 Vegetable Oils

Vegetable oil consists of mostly triacylglycerols (90-98%), but it also contains minor components such as free fatty acids (FFA) (generally 1–5%), mono- and diacylglycerols, phospholipids, chlorophylls, carotenes, sterols, tocopherols, phenolic compounds, metals, sulfur compounds and traces of water[18]. Triglyceride is a lipid consisting of three molecules of fatty acids covalently bonded to one molecule of glycerol. Commonly found fatty acids in vegetable oils are palmitic, stearic, oleic, linoleic and linolenicacid.

Vegetable oils have a wide range of uses in many manufacturing industries. Beside extensive application in food industry, vegetable oils can be used for producing perfumes, soaps, lubricants, resins, emulsifiers and medicine. These oils can also be applied as insulators in electrical industry and as cooling agent in personal computers. Vegetable oils have excellent environmental credentials such as biodegradability, low toxicity, and renewability. Apart from various usages in industry, one of the most important applications of vegetable oils is to use them as fuel or fuel source which is aimed to overcome current fuel crisis of the world.

Triglycerides are the main constituents of vegetable oils and animal fats. Triglycerides have lower densities than water (they float on water), and at normal room temperatures may be solid or liquid. When solid, they are called "fats" or "butters" and when liquid they are called "oils". A triglyceride, also called triacylglycerol (TAG), is a chemical compound formed from one molecule of glycerol and three fatty acids.

Glycerol is a trihydric alcohol (containing three -OH hydroxyl groups) that can combine with up to three fatty acids to form monoglycerides, diglycerides, and triglycerides. Fatty acids may combine with any of the three hydroxyl groups to create a wide diversity of compounds. Monoglycerides, diglycerides, and triglycerides are classified as esters which are compounds created by the reaction between acids and alcohols that release water (H_2O) as a by-product.

2.2.1 Vegetable Oil as Fuel Alternative

Vegetable oils and their derivatives (especially methyl esters), commonly referred to as “biodiesel,” are prominent candidates as alternative diesel fuels. They have advanced from being purely experimental fuels to initial stages of commercialization. They are technically competitive with or offer technical advantages compared to conventional diesel fuel. Besides being a renewable and domestic resource, biodiesel reduces most emissions while engine performance and fuel economy are nearly identical compared to conventional fuels.

The main advantages of vegetable oils over petro-diesel are their ready availability, renewability, non-toxicity, and biodegradability. Meanwhile, the main disadvantages are the higher viscosity, lower volatility, and the reactivity of unsaturated hydrocarbon chains [19,20].All vegetable oils are extremely viscous with viscosities ranging from 9 to 17 times greater than petro-diesel fuel [21,22]. Trans-esterification is the common method used to reduce the viscosity of vegetable oils such that this product, termed biodiesel, can be used directly in conventional diesel engines [23]. The use of vegetable oil-derived fuels led to substantial reductions in emissions of carbon monoxide (CO), poly-aromatic hydrocarbons (PAH), smoke, and particulate matter (PM) [24, 25]. Furthermore, vegetable oil-derived fuels contribute to the reduction of greenhouse gas emissions in comparison to petro-diesel, since carbon dioxide (CO_2) emitted during combustion is recycled in the photosynthesis process in the plants [26].Reductions in net carbon dioxide emissions are estimated at 77–104 g/MJ of diesel displaced by biodiesel [27]. In addition, vegetable derived fuels have an output-to-input energy ratio higher than petro-diesel. Typical results show that the life-cycle output-to-input (fossil) energy ratio of soybean oil, soy-biodiesel and petro-diesel is around 6.2, 3.2, and 0.83, respectively [28].

Table 1 Fuel properties of common vegetable oils used as biodiesel feedstock

Type of Oil	CN	LHV MJ/Jg	Density (kg/L)	Kin. Visc. mm^2/s	FP ºC	CP ºC	PP ºC	Ref.
Diesel fuel #2	45.8	45.2	0.847	2.39	78	-19	-23	[30]
Edible Oil								
Palm Oil	49	40.14	0.9139	40.33	275	23	12	[31,32]
Rapeseed	37.6	39.709	0.9115	37.3	246	-3.9	31.7	[29,33]
Sunflower	37.1	39.575	0.9161	34.4	274	7.2	-15	[29,33]
Soybean	37.9	39.623	0.9138	33.1	254	-3.9	-12.2	[29,33]
Non-edible Oil								
Jatropha	38	39.071	0.917	35.98	229	9	4	[34,35]

Kinematic viscosity was measured at 38ºC, CN - cetane number, LHV - lower heating value, FP-flash point, CP-cloud point, PP-pour point

Several problems, however, have impaired the widespread use of biodiesel. They are related to the economics and properties of biodiesel. For example, neat vegetable oils reported to cause engine deposits. Attempting to solve these problems by using methyl esters causes operational problems at low temperatures. Furthermore, problems related to combustion and emissions remain to be solved. The problems associated with the use of biodiesel are thus very complex and no satisfactory solution has yet been achieved despite the efforts of many researchers around the world.

2.3. Growing Conditions and Harvesting

The seed yields are highly dependent on environmental conditions (temperature, altitude, rainfall, sunlight, and soil conditions), genetics, plant age and plant management (use of pesticide and fertilizer, irrigation, plant density, etc) [36, 37, 38, 39]. At low input condition, the dry Jatropha seed yield is in the range of 2-3 tons/ha/yr. In more favorable conditions, dry Jatropha seeds yield up to 5 tons/ha/yr have been achieved.

The harvesting time is one of the critical steps in seeds production. Oil content has been related to fruit maturity. The Jatropha seed can be classified into four maturity stages: early maturity (green fruit), physiological maturity (yellow fruit), over maturity (brown fruit), and senescence (black-dry fruit). The best fruit maturity stage for seed oil content was found in yellow and brown fruit [40]. It is recommended that seed should be harvested at low moisture content [41]. But if the seeds are harvested at high moisture tent, seeds should be dried immediately.

2.3.1 Advantages of Jatropha

Though there are a number of crops that are available for the production of bio-fuel, the question that arises in the mind of the cultivators is that why Jatropha should be chosen? Researchers say that the answer to this question is very simple. They say that cultivating and processing of Jatropha has many advantages than any other plant.

Following are the advantages of the Jatropha plant:

- Low cost seeds
- High oil content
- Drought resistant
- Small development period
- Grow on good and despoiled soil
- Grow in low and high rainfall areas
- Does not require any special maintenance
- The plant can be harvested for about 50 years
- Can be harvested in non-rainy season
- Has the ability to grow well on poor and infertility soil, in marginal areas and can withstand any type of climate
- Multi products are developed using a single Jatropha plant. The products include bio-diesel, soap, mosquito repellent, and organic fertilizer [42].

2.4 Seed Characteristics and Storage Behavior of Jatropha curcas L.

The storage behavior of Jatropha curcas seeds is one of the focuses of current researches because of the potential of this crop as a source of biodiesel. After harvest, the seedbegins to deteriorate. Seed deterioration (also seed aging) is best described as the loss of seed quality in time. Seed deterioration is a process that is inexorable, inevitable, and irreversible and varies among species, varieties within species seed lots within a variety and seeds within a seed lot.

The two most important environmental factors that influence the life span of seeds are relative humidity and temperature. The effects of relative humidity (and its subsequent effect on seed moisture) and the temperature of storage environment are highly interdependent. Therefore, for to slow down the deterioration process, optimum storage conditions must be given to seeds.

2.5 Characteristics and Components of Jatropha Seed

Dry *Jatropha curcas* fruit contains about 30-40% hccull and 60-70% seed. The fruits are 2.5 cm long, ovoid, and have 2–3 seeds **[43].** The seed contains about 30-40% shell and 60-70% kernel with the weight of the seeds is about 0.45-0.86 g [44].The oil content in Jatropha seed is reported to be in the range of 29 to 37% by weight of the seed or from 44 to 62% weight of the kernel. The kernels are also rich in crude protein (22-35%) [45].The oil contains 63.9-91.6% unsaturated fatty acids (Table 2.2) with an iodine value of 92-112 which classifies it as a semi-drying oil (partially hardens when the oil is exposed to air) [46, 47].

A representative figure of *Jatropha curcas* tree can be seen in Figure 1.

Figure 1 Mature Jatropha curcas plant (left); Mature Jatropha curcas fruit ready for harvest (right)

Table 2 Fatty acid composition of Jatropha oil [48]

No	Fatty Acid Compoition	Content (%) in Range	Average Content in (%)
1	Myristic Acid	0-0.1	0.05
2	Palmitate Acid	14.1-15.3	14.7
3	Palmitoleic Acid	0-1.3	0.65
4	Stearic Acid	3.7-9.8	6.75
5	Oleic Acid	34.3-45.8	40
6	Linoleic Acid	29.0-44.2	36.6
7	Linolenic Acid	0-0.3	0.15
8	Arachidic Acid	0-0.3	0.15
9	Bahenic Acid	0-0.2	0.1
10	Total Saturated	17.8-25.6	21.7
11	Total Unsaturated	63.3-91.6	77.45

The relationship between Jatropha capsules, seeds, oil and seed cake are shown in the following figure

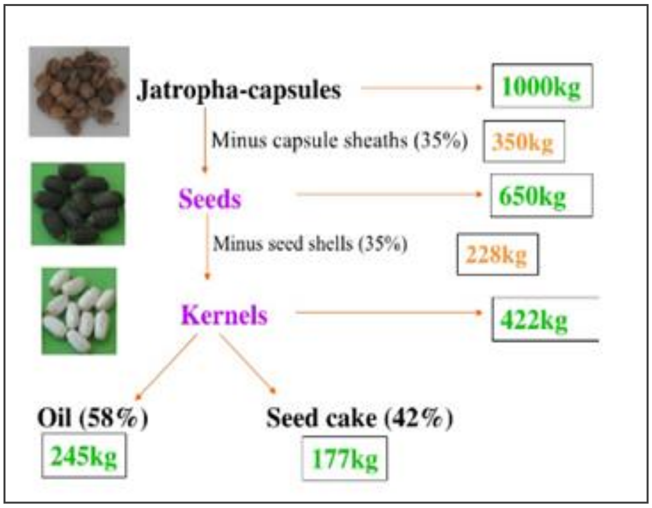

Figure 2 Components of a Jatropha seed

2.6 Oil Content of Ethiopian Jatropha Populations

The oil content values for different study sites of the Ethiopia are shown in Table 3 where solvent extraction method was used. There is more oil content in the samples collected from SNNPR and lowest from Oromia region. The existing Ethiopian Jatropha provenances demonstrated an overall oil content of 32.19±3.48. Jatropha provenances having oil content ranging between 30-35% are considered good for oil production. Considering the existing Ethiopian Jatropha provenances growing in different testing sites, 15.79% of the provenances demonstrated oil content value of > 35%, 71.05% between 30 and 35% and the remaining 13.16% demonstrated oil content value of < 30%. This indicates that Ethiopian Jatropha provenances can be exploited for the production of oil for different purposes from their existing growing conditions. Likewise, the observed variation confirms the potential of the local population of *Jatropha* to produce a high-yielding genetic material through selection.

Table 3 Average oil content (%) values of Ethiopian Jatropha populations [49]

No̲	**Region**	**Oil Content (%) Range**	**Average Oil Content (%)**
1	SNNPR	31.18-35.91	34.34
2	Oromia	21.23-33.26	29.88
3	Tigray	30.18-33.16	31.89
4	Amhara	21.89-34.99	30.25
5	Benishangul Gumuze	31.03-35.27	33.09

1. Important Terminologies Used for Physicochemical Properties of Fuels

A. Viscosity

It is the resistance to flow of a fluid under the influence of gravity. The kinematic viscosity is equal to the dynamic viscosity divided by density. The kinematic viscosity is a basic design specification for the fuel injectors used in diesel engines; moreover the high a viscosity implies the injectors fail to perform properly.

B. Density

It is defined as mass per unit volume of a substance at a specific temperature. The higher density of biofuel indicates presence of large mass in specific a volume, which is a heavy biofuel.

C. Flash point

It is the lowest temperature at which application of the test flame causes the vapor and air mixture above the sample to ignite. Flash point specifies the temperature to which a fuel needs to be heated for the vapor and air above the fuel could be ignited.

D. Ash content

It is the residue remaining after a fuel sample has been burned. For biodiesel, this test is an indicator of the quantity of residual materials in the fuel that came from the catalyst used in the transesterification reaction and raw material used.

E. Acid number

It is an indication of the presence of free fatty acids in biodiesel. The free fatty acids can lead to corrosion and may be an indication of water in the fuel during manufacturing.

F. Iodine value

It indicates the level of saturation of the molecules which according to ASTM, the iodine number helps to indicate the oxidation stability of the biodiesel. The higher iodine number represents the lower oxidation stability.

G. Caloric value

It is the standard heat of reaction at constant pressure where the fuel burns completely with oxygen and higher caloric value fuel gives higher heat output.

H. Cloud point

It is the temperature at which wax crystals first begin to be formed. Below the cloud temperature, filters will start to become blocked and potentially starve the engine of the fuel.

I. Water content

It is the amount of water content in the biodiesel determines the caloric value and the shelf life of the fuel. Biodiesel with higher water content has lower oxidation stability and the greater probability of oxidation products will be formed during long storage period.

J. Cetane number

It is the measure of fuel's ignition and combustion quality. The higher cetane number biodiesel has lower ignition time delay, which means it, ignites immediately. Fuels with lower cetane numbers will cause hard starting, rough operation, noise and increased smoke opacity in engines.

Table 4 Standards for crude Jatropha oil

No	**Property**	**Unit**	**ASTM D 6751-02**	**DIN EN 14214**
1	Density at 15°C	kg/m^3	875-900	860-900
2	Kinematic viscosity at 40°C	mm^2/s	1.9-6.0	3.5-5.0
3	Flash point	°C	>130	>120
4	Pour point	°C	-	-
5	Water content	%	<0.03	<0.05
6	Ash content	%	<0.02	<0.02
7	Carbon residue	%	-	<0.3
8	Sulphur content	%	0.05	-
9	Acid value	mg KOH/g	<0.8	<0.5
10	Iodine value	-	-	-
11	Saponification value	-	-	-
12	Calorific value	MJ/kg	-	-
13	Cetane number	-	-	-

2.7Extraction Methods of Jatropha Oil

2.7.1 Introduction

It is often considered that a more effective extraction technique would yield greater quantities of oil. This is partly inaccurate, since an effective extraction method would only yield the optimum quantity and not more than that. The optimum oil content in Jatropha plants varies between species and genetic variants.

The process of extraction consists of five operations: extraction, evaporation, solvent recovery, desolventizing-toasting, and drying. Figure 3 presents an overview of the Jatropha seed oil extraction process.

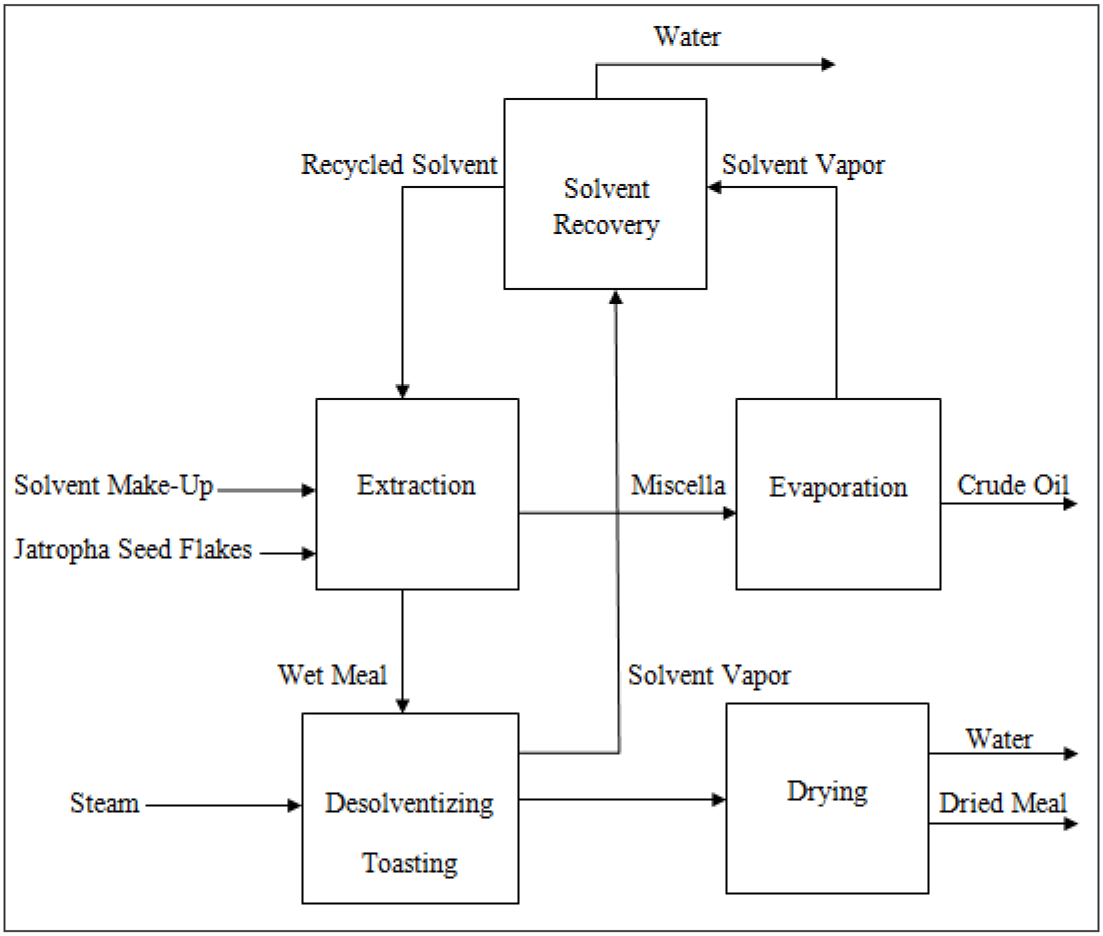

Figure 3 Process overview of Jatropha seed oil extraction

Most vegetable oils are obtained from beans or seeds, which generally furnish two valuable commodities-oil and a protein-rich meal.

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 solvents 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.

2.7.2 Different Extraction Technologies of Oils

1. Mechanical Oil 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. 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. The mechanical extraction method has several advantages compared to the other methods, such as simple equipment and low investment, low operating cost, and the oil does not undergo solvent separation process, etc. some of the most important mechanical extraction methods include the following:

- **Oil Presses:** Oil presses have been used for the purpose of oil extraction as simple mechanical devices - either powered or manually driven. Among the different oil presses that are used for Jatropha oil extraction, the most commonly used presses include the Bielenberg ram press. The Bielenberg ram press involves the traditional press method to extract oil and prepares oil cakes as well as soaps. It is a simple device that yields around 3 liters of oil per 12 kg of seed input.
- **Oil Expellers:** Different kinds of oil expellers are used for the purpose of Jatropha oil extraction. The most commonly used ones are the Sayari oil expeller (also called the Sundhara oil expeller) and the Komet Expeller. The Komet expeller is a single-screw oil expeller that is often used for extracting Jatropha oil from the seeds and also for the preparation of oil cakes.

2. Aqueous Enzymatic Extraction

Conventional oil extraction method such as solvent extraction raises an environmental concern due to the release of volatile organic compound (VOCs) into the atmosphere. This circumstance triggers interest in aqueous extraction which is perceived as a more environment-friendly and cleaner alternative. Nevertheless, aqueous extraction has lower oil yield and needs longer duration for extraction. Enzymatic aqueous extraction has been introduced to enhance oil release in the 1970s. Nowadays, enzymatic assisted aqueous extraction on *Jatropha curcas* oil is carried out at various conditions to study its effect on oil yield and quality. Both singular and combinatory enzyme systems comprising of alkaline protease and cellulase are engaged at various experimental variables which includes the extraction temperature, duration, particle size, pH, enzyme concentration and ratio of *Jatropha curcas* seed to water (by mass to volume). The step-wise addition of enzymes studies are done to investigate the sequential enzymatic mechanism. Highest oil yield, 31% (based on total oil content) is obtained at the optimum condition for both alcalase and celluclast-assisted extraction. The incorporation of centrifugation and cooling processes has further enhanced oil separation and recovery in the post-extraction stage. In addition, the effect of ultrasonication prior to the enzymatic assisted extraction is also investigated using polarizing microscope.

3. Solvent Oil Extraction (Chemical Extraction)

Solvent extraction is the process in which the oil is removed from a solid by means of a liquid solvent; it is also known as leaching [50]. Chemical extraction using n-hexane method results in the highest oil yield which makes it the most commonly used solvent [51, 52].

Although the cost of oil extraction technique by mechanical screw press is low it is ineffective due to relatively lower oil yields. On the contrary, the chemical oil extraction method was found to be very effective because of high oil yield and for its consistent performance.

Solvent extraction with hexane is the standard practice in today`s modern processing facilities. There are three major steps in solvent extraction: seed preparation, oil extraction, and desolventizing of the oil and meal. Conventional seed preparation includes drying, cleaning, cracking, optional dehulling or decortications, conditioning, and flaking of the seeds. The option of expanding after flaking is used to improve oil extraction, percolation, and solvent drainage, and is accompanied by a doubling of the throughput.

Solvent extraction in vegetable oil production has been recognized by the US Environmental Protection Agency (EPA) as a major hazardous air pollutant and 'National Emission Standards for Hazardous Air Pollutants' (NESHAP) for oil extraction were established (Federal Register 2001).

It has been observed that there are many factors affecting the rate of solvent extraction such as particle size, the type of solvent used, temperature, solvent to solid ration, and time of extraction [53, 54].

The solvent has to be selected in such a way that it would be a good selective solvent and its viscosity would be sufficiently low to circulate freely. Sayyar et al. [55] extracted J. curcas oil by n-hexane and petroleum ether and found that the extraction yield with n-hexane to be about 1.3% more than that of petroleum ether (47.3% and 46.0% wt, respectively) under similar conditions. The authors recognized n-hexane as a more preferable solvent for extraction of Jatropha oil as compared to petroleum ether. In the extraction of olive oil using organic solvents like hexane, ethanol, petroleum ether, isopropyl alcohol and carbon tetrachloride by a Soxhlet extractor, Banat et al. [56]did also obtain the highest oil yield (12.7%) by n-hexane. However, it has been observed that this method consumes much more time compared to other techniques. The solvent extraction is only economical attractive at a large-scale of production (more than 50 ton biodiesel per day) as reported [57]. In addition, n-hexane solvent extraction has a negative environmental impact because of the wastewater generation, higher specific energy consumption and higher emissions of volatile organic compounds and human health impacts [58]. According to Achten et al. [59] and Mahanta and Shrivastava [60], there are three other types of solvent extraction technique: hot water extraction, soxhlet extraction and ultrasonication technique that could be use instead of hexane solvent extraction. Jahirul et al. [61] reported that in oil extraction from beauty leaf a seed (Calophylluminophyllum) by mechanical method (using the screw press) and chemical extraction (using hexane as a solvent), each method has advantages and disadvantages. The advantages and disadvantages of oil extraction by mechanical extraction and chemical extraction from beauty leaf seeds is presented in Table 5.

A. Extraction Temperature

In most cases, the solubility of the material which is being extracted will increase with temperature to give a higher rate of extraction. Further, the diffusion coefficient will be expected to increase with rise in temperature and this will also improve the rate of extraction. In some cases, the upper limit of temperature is determined by secondary considerations, such as, the necessity to avoid enzyme action during the extraction of sugar.

B. Particle Size

Particle size influences the extraction rate in a number of ways. The smaller the size, the greater is the interfacial area between the solid and liquid, and therefore the higher is the rate of transfer of material and the smaller is the distance the solute must diffuse within the solid as already indicated.

On the other hand, the surface may not be so effectively used with a very fine material if circulation of the liquid is impeded, and separation of the particles from the liquid and drainage of the solid residue are made more difficult. It is generally desirable that the range of particle size should be small so that each particle requires approximately the same time for extraction and, in particular, the production of a large amount of fine material should be avoided as this may wedge in the interstices of the larger particles and impede the flow of the solvent.

C. Solvent Selection

This is one of the most important factors to get a better yield of oil extraction. The solvent should possess the following properties:

- It should completely and quickly dissolve all the materials being extracted.
- It should be able to extract a considerable amount of oil and it should have high triglyceride selectivity
- It should possess a sufficiently low boiling point to permit its being easily removed without restoring to higher temperature; yet the boiling point should not be too low, as this would involve considerable solvent loss by evaporation in the warm climate.
- It must not dissolve in water
- It must have a low viscosity so that it will circulate freely
- It must be chemically inert, that is, it should not react chemically with the extract, nor should it readily decompose;
- It should have a uniform boiling point when evaporated and does not leave any residue.
- The cost of the solvent should be very low, non- flammable.

There are different solvents that can be used for the extraction of oil from jatropha seed. The following are the most commonly used organic solvents: diethyl ether, toluene, hexane, ethyl acetate, ethanol, isopropyl alcohol and inorganic solvents are dichloromethane, chloroform and carbon tetrachloride.

Separation or purification is one of the most important factors in solvent selection. If the solvent extracts other organic compounds, then the resulting oil will be impure. The environmental concerns that will be incurred by the solvent are also another very important factor that should be considered during the selection of solvent. Hexane gives the highest oil yield although it is a fossil fuel and a major air pollutant. Therefore, since hexane fulfills the above criterion for solvents then it is selected.

D. Extraction Time

The rate of extraction is fast at the beginning because the free oil on the surface of the seeds will be solubilised and the oil will be extracted quickly, which will cause a rapid increase in the extraction rate. Moreover, the concentration of the oil in the solvent is low during the initial period of extraction. Hence, the high-concentration gradient between the solute in the solvent and in the solid phase accelerated the mass transfer of the oil from the meal to the liquid phase. As the time of the extraction process increased, the rate of diffusion will be decreased as the concentration of the oil increased in the solvent. Eventually, the extraction process is said to have reached its saturation capacity when the highest oil yield is obtained and remained unchanged.

4. Accelerated Solvent Extraction (ASE)

Accelerated solvent extraction (ASE) is also referred to as pressurized solvent extraction (PSE) is another modern oil extraction technique which uses organic and/or aqueous solvents at elevated temperatures and pressures [62].It has been observed that high temperature accelerates the extraction rate, while elevated pressure prevents boiling at temperatures above the normal boiling point of the solvent. Khattab and Zeitoun[63] have extracted oil of flaxseed by different methods by supercritical fluid extraction (SFE), accelerated solvent extraction (ASE) and conventional solvent extraction (SE) and found the highest oil yield (42.40%) by SE using n-hexane which did not differ significantly from the one obtained by accelerated solvent extraction ASE in terms of oil quantity (41.90%) and their physicochemical properties and fatty acid profiles. The supercritical fluid extraction (SFE), however, showed significantly lower oil yield (36.49%) in this particular oil extraction from flaxseed. Sarip et al.[64] have also extracted crude palm oil from palm mesocarp by using hot compressed water extraction method and obtained 70 ± 0.5% of the oil with averaged free fatty acid of 0.81 ± 0.08%. Moreover, it was also reported that ASE has been used for the extraction of different materials including wheat germ [65]. In ASE, time as well as solvent consumption is significantly reduced compared to the other solvent extraction techniques [66, 67].

Table 5 Advantages and Disadvantages of three Main Oil Extraction Methods [68, 69, 70]

№	**Methods**	**Advantages**	**Disadvantages**
1	Oil Press	Virgin oil more sought after	Time and labor intensive
		No potential for solvent contamination	Relatively low oil yields
		Relatively inexpensive after initial capital costs	Operators require experience to achieve best results
		Minor consumable costs	High dependence on kernel moisture content
		Whole seeds or kernels can be processed	Relatively dirty process
		No environmental problem	Filtration or degumming process of oil is required
			Low and inconsistent oil production
			High oil loss
2	n-Hexane	Repeatable and reproducible results and process	Less sought after than virgin oil
		High oil yields	High potential for solvent contamination
		Relatively simple and quick	Safety issues and environmental concerns
		Suitable for bulk oil extraction	Very costly if the hexane cannot be recovered
		No especial equipment required with hexane can be recovered and reused, reducing cost significantly	Only kernel can be processed
3	ASE	Automatic technique	Very high capital investment
		Condition can be optimized	High preparation required

	More efficient Clean process	Special equipment and skill required Potential for solvent contamination
	Relatively less solvent consumption with less time and labor intensive with high oil yields	Only kernel can be processed

5. 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. The process of extracting oil through this method is known as cavitations. Cavitations occur when vapor bubbles of a liquid form in an area where pressure of the liquid is lower than its vapor pressure.

6. Steam Distillation

In steam distillation, steam is introduced into a macerated solid sample. The target analytes are co-distilled out of the sample container with the steam, and are collected after condensation of the steam to liquid water. In the circulating method, the condensate is extracted by liquid-liquid extraction using a non-polar solvent. Steam distillation is used for compounds with a relatively high vapor pressure which are not soluble in cold water. Steam distillation is an operation which has both extraction and separation / fractionation functions. Because it can separate target compounds from non-volatile compounds and sulfur contained in sediments, purification after extraction is easy.

7. Microwave-Assisted Extraction (MAE)

The fundamentals of the microwave extraction (MAE) process are different from those of conventional methods (solid–liquid or simply extraction) because the extraction occurs as the result of changes in the cell structure caused by electromagnetic waves.

In MAE, the process acceleration and high extraction yield may be the result of a synergistic combination of two transport phenomena: heat and mass gradients working in the same direction [71]. On the other hand, in conventional extractions the mass transfer occurs from inside to the outside, although the heat transfer occurs from the outside to the inside of the substrate (Figure 4). In addition, although in conventional extraction the heat is transferred from the heating medium to the interior of the sample, in MAE the heat is dissipated volumetrically inside the irradiated medium.

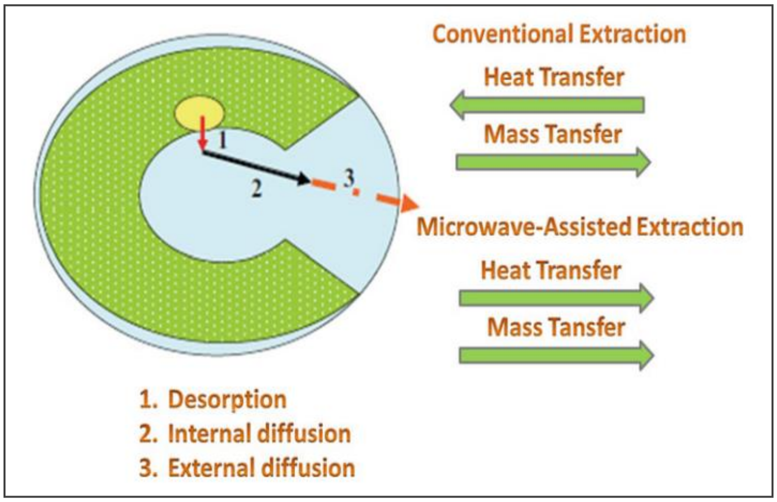

Figure 4 Basic heat and mass transfer mechanisms in microwave and conventional extraction of natural products. (Adapted from Périno-Issartier et al.[72]

During the extraction process, the rate of recovery of the extract is not a linear function of time: the concentration of solute inside the solid varies, leading to a non-stationary or unsteady condition. A series of phenomenological steps must occur during the period of interaction between the solid-containing particle and the solvent effectuating the separation, including:

- Penetration of the solvent into the solid matrix;
- Solubilization and/or breakdown of components;
- Transport of the solute out of the solid matrix;
- Migration of the extracted solute from the external surface of the solid into the bulk solution;
- Movement of the extract with respect to the solid; and
- Separation and discharge of the extract and solid [73]

Therefore, the solvent penetrates into the solid matrix by diffusion (effective), and the solute is dissolved until reaching a concentration limited by the characteristics of the solid. The solution containing the solute diffuses to the surface by effective diffusion. Finally, by natural or forced convection, the solution is transferred from the surface to the bulk solution (Figure 5).

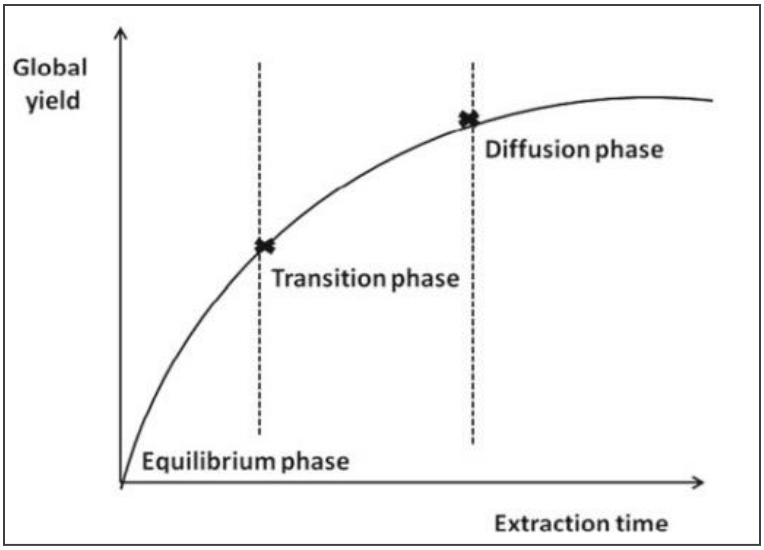

Figure 5 Schematic representation of yield versus time in extraction processes. (Adapted from Raynie [74]

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.

8. Supercritical Fluid Extraction (SFE)

Gases such as CO_2 and N_2O become fluid when temperature and pressure reach a critical point called the supercritical phase. In supercritical fluid extraction (SFE), this fluid is used to extract target substances in solid samples. This is a relatively new method which has recently begun to be applied extensively. Instruments connecting the SFE apparatus and on line GC have been developed in which the series of steps from extraction to analysis have been automated.

The sample is placed in an extraction chamber through which the supercritical fluid is forced, the target substances are extracted from the samples and trapped in vials by small amounts of methanol or ODS resin. The amount of organic solvent used is much smaller than Soxhlet extraction.

The fluids used for SFE include CO_2, N_2O, SF_6, methanol, and water. The fluid used is chosen to meet the analytical purpose. The most commonly used is CO_2. However, supercritical CO_2 is very non-polar, so modifiers are added to improve extraction efficiency for polar compounds. Modifiers for CO_2 include methanol, dichloromethane, acetonitrile, water etc. Modifiers are mixed with the

CO_2 using a pump, or spiked directly into the samples [75].Supercritical carbon dioxide extraction (SCE) has been extensively used in the extraction of natural products. The advantages of using SCE are that the high quality of oil does not need to be refined and the high oil recovery is achieved without using any organic solvent. However, intensive energy consumption and technical challenge for continuous operation restricted it to be used only in small amounts and for high value products. 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.

Table 6 Summary of the Different Methods for Jatropha Oil Extraction

№	**Extraction Method**	**Ref.**	**Temperature (^{o}c)**	**Pressure (bar)**	**pH**	**Time (Hr)**	**Yield (%)**
1	n-hexane oil extraction	(Gubitz et al,1999)	-		-	8	95-99
2	Aqueous oil extraction with 10 min ultrasonic pretreatment	(Shah et al., 2005)	50		9	6	67
3	Aqueous enzymatic oil extraction with 5 min ultrasonic pretreatment	(Shah et al., 2005)	50		9	6	74
4	Three-phase partitioning	(Shah et al., 2005)	25		9	2	97
5	Super critical carbon dioxide extraction	Min et al 2010	55	400	-	-	92

3. METHODOLOGY

3.1Materials and Equipments

3.1.1 Chemicals and Materials Used

The different chemicals and materials that have been used during the experimental analysis of the extraction Jatropha seed are listed as follows where most of the reagents are analytical and some are HPLC grade chemicals: sodium hydroxide (99%), normal hexane (99%), potassium hydroxide (85%), saturated sodium carbonate, acetone, phenolphthalein, potassium iodide, distilled water, and an Ethiopian Jatropha curcus seed.

3.1.2 Equipments Used

The instruments and apparatuses used throughout the experiment are listed in the Table below.

Table 7 List of laboratory apparatuses

№	Instrument/ Apparatus	Manufacturer and Model	Use
1	Rotary vacuum evaporator	Ingos(RVO 400)	Efficient and gentle removal of solventsfrom samples by evaporation
2	Jaw crusher	Retsch KG (BB1/A)	Used to reduce the size, or change the form
3	Centrifugal miller	Retsch KG (SK1)	Size reduction of crushed seed in to microns
4	Vibro viscometer	A & D Company Ltd (SV10)	Viscosity measurement
5	Water distiller	Beijing ZhogxingingWeiye Instrument Co. (DZ 5L)	Water distillers (steam distillers)
6	Digital refractometer	Bellingham Stanley (RFM960)	Refractive index measurement
7	FT-IR	Perkin Elmer	Used for the analysis of functional groups
8	Gas chromatography	GC HP 7820 series	Used for the analysis of the different components of a sample like fatty acids
9	Mass spectroscopy	HP 5977 series	Ionization of chemical species and sorts the ions based on their mass-to-charge ratio
10	Nebertherm furnace	Nebertherm(P 310)	Ash content determination
11	Density meter	Anton Paar GmbH	Determination of density

		(DMA 4100M)	
12	Chiller	Lauda (RE 307)	Removes heat from a liquid-cooling
13	Soxhlet extractor	Witeg (NS 60/46)	Used for extraction of oil from sample
14	Magnetic stirrer	Witeg (MSH-20D)	Device that employs a rotating magnetic field to cause a stir bar for stirring a sample
15	Oven	Intercontinental equipements (DAS42000)	Thermally insulated chamber used for the heating
16	Heated circulating bath	Grant (TXF 200)	Devices used for heating by offering precise temperature control
17	Digital weighing balance	Explorer Pro (EP214C)	Weight measuring devices
18	PH meter	Jenway (3505 pH meter)	Measurement of pH

3.2Experimental Methods

3.2.1 Raw Material Preparation

A. Preparation of Jatropha Seed

Jatropha seed has been collected from Bati of Amhara Region. After the seeds were collected, the foreign materials like dust and stone have been removed manually and the seed become pure. This has made the size reduction equipments safe.

Figure 6 Jatrophacurcas seed obtained from Bati—Amhara Region, Ethiopia

Figure 7 The shell (left); mixture of shell and kernel (middle); kernel of Jatropha curcas l. (right) after cracking and separation

B. Determination of Moisture Content of the Seed

Three representative samples of seed were carefully selected, weighed and dried in an oven at 105^0C and the weight was measured every two hours. The procedure has been repeated until a constant weight will be obtained and the percentage moisture content of kernel has been determined.

$$\%\,\mathrm{MoistureContent} = \frac{\mathrm{w1} - \mathrm{w2}}{\mathrm{w1}} * 100 \qquad (3.1)$$

Where: w_1 = original weight of the sample before drying; w_2 = weight of the sample after drying.

C. Size Reduction and Sieve Analysis

After the moisture is removed by placing the sample in an oven at 105^0C for 30 minutes, the dried Jatropha seed was first fed to a jaw crusher and the seeds have been crushed and hence the size has been reduced in to smaller pieces. This smaller size of the seed has been fed in to a centrifugal miller with a size of 2 mm for further size reduction. After the sample has been fed to a vibrating shaker for 5 minutes with amplitude of 10mm, the ranges of particle sizes has been separated. Literature reveled that to have a higher yield of oil, particle sizes should be less than 5mm and higher than 0.2mm.Particle size ranges, which are in the range used in this literature, of 0.66mm—.85 mm, 0.85—1.4 mm, and between 1.4—1.59mm, have been used for this study.

3.2.2 Proximate and Mineral Composition Analysis of the Seed

The proximate and mineral composition of *Jatropha curcas l.* seed were investigated as follows:

1. Proximate Composition Analysis of the Seed

a) Moisture Content

The moisture contents of Jatropha curcas seeds were estimated by drying the samples in an Air Forced Draft Oven (Model: DO-1-30/02, PCSIR, Pakistan) at 105±5°C till constant weight will be obtained (AACC, 2000; Method No. 44-15A).

b) Total Ash

Ash content has been estimated by direct incineration of sample; igniting it in a Muffle Furnace (MF-1/02, PCSIR, Pakistan) at 550°C till grayish white residue (AACC, 2000; Method No. 08-01).

c) Crude Protein

The crude protein content was determined by using Kjeldhal Apparatus (Model: D-40599, Behr Labor Technik, Gmbh-Germany) as described in AACC (2000) Method No. 46-30.

d) Crude Fat

Crude fat content was determined using hexane as a solvent in Soxtec System (Model: H-2 1045 Extraction Unit, Hoganas, Sweden) according to the procedure given in AACC (2000) Method No. 30-25.

e) Crude Fiber

Crude fiber was estimated in fat free samples by treating with 1.25% H_2SO_4; left over material will be subjected to further treatment with 1.25% NaOH solutions. Crude fibers of the samples have been determined through Labconco Fibertech (Labconco Corporation Kansas, USA) as per procedure in AACC (2000) Method No. 32-10.

f) Carbohydrate (CBH)

The carbohydrate content was calculated according to the following expression:

CBH % = 100 – (weight in grams [Moisture contents + Crude protein + Crude fat + Crude fiber + Ash] in 100 g of sample)

2. Mineral Composition Analysis of the Seed

The mineral profile of Jatropha curcas seed was analyzed by AOAC (2016). The concentrations of the heavy metals: Calcium (Method 968.08), Zinc (Method 991.11), and Phosphorous (AOAC 995.11:2016) has been determined by Atomic Absorption Spectrophotometer (Varian AA240, Australia), while sodiumand potassium by flame Photometerhave been measured through Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge).

3.2.3Oil Extraction Using Soxhlet Apparatus

In the solvent extraction process, Soxhlet extraction using normal hexane as solvent has been used.

A. Extraction with volatile solvents

The principle of extraction with volatile solvents is simple. Fresh feed is charged in to specially constructed extractors and extracted systematically at a specified temperature. The solvent penetrates the feed material and dissolves it. Although solvent extraction is used extensively, some people do not believe that it should be used for aromatherapy oils since a residue of solvent could be present in the finished product. Some reports site a solvent residue of 6—20% still present in the finished extraction, but this was normally the case when benzene was the standard solvent used. With hexane as the solvent material, the solvent residue goes down to about 10 ppm (parts per million) and this is extremely low concentration of solvent in the resultant product.

B. Experimental procedures of solvent extraction using hexane

A sample of Jatropha seed is first packed with filter paper and placed on the extraction Soxhlet. The packed filter paper is a sample holder used for solvent extraction systems and it is suited for powdered samples and evaporable solvents are easily penetrated inside. The solvent hexane has been chosen for the extraction process. The selection of the appropriate solvents was briefly discussed in section Page 17.

After the solvent has been added, the temperature on the water bath was turned on. When it is heating, the solvent was evaporated into a vapor, and it was then cooled down into a liquid in a condenser and passed into the sample collection tube. This continues several hours until the lipid was removed from the sample. After the mixture of the extracted oil and the solvent were collected in a flask, the oil has been separated from the solvent using conventional distillation. Fifty grams in powder form of the sample Jatropha seed with particle sizes of 0.66—0.85mm, 0.85—1.4mm and between 1.4—1.59mm was fed to the Soxhlet extractor with 450, 500 and 550 ml of the solvent. The process of solvent extraction was shown in Figure 8 below.

Figure 8 Extraction of Jatropha oil using solvent extraction method

C. Determination of Percentage of Oil Extracted

The percentage oil yield (% OY by wt.) was calculated by the following relation.

$$\text{Percentage Oil Yield } (\%\text{ by wt.}) = \frac{\text{Mass of Oil}}{\text{Mass of Sample}} * 100 \qquad (3.2)$$

3.3 Optimization of the Process Parameters

The most important factors affecting the yield and quality of extraction of the seed are: extraction temperature, type of solvent, solvent to solid ratio, particle size of the meal and extraction time. For this thesis work, the effect of three main factors which are temperature, solvent to solid ratio, and particle size were investigated to optimize the extraction operating conditions for achieving maximum oil yield. 50g of grinded powder was extracted with n-hexane. The parameters temperature (62—78ºC), solvent to solid ratio (9:1—11.7:1) and particle size (0.66mm to 0.85mm, 0.85—1.4 mm and 1.4—1.59mm) on Jatropha curcas l. seeds were used for the extraction. At the end of the extraction, the solvent was separated from the oil using rotary evaporator and was collected in the receiving flask. The oil which will be remained in the sample flask has been weighed after the process is completed. The percentage of extracted oil was then calculated by dividing the amount of oil obtained by the amount of the seeds multiplied by 100.

Table 8 Ranges of applicability of extraction parameters

No	Parameter	Range
1	Extraction temperature	62—78^{o}C
2	Particle size	0.66—1.59 mm
3	Solvent to solid ratio (v/w)	9—11.7
4	Extraction time	6 hours
5	Type of solvent	Normal hexane

3.4 Design of Experiments using Central Composite Experimental Design

In this study, the effect of three factors on Jatropha oil extraction including temperature, solvent to solid ratio, and particle size were selected as factors. As a response, percentage oil yield was chosen; a total number of 20 experiments were employed for the response surface modeling. The Response Surface Methodology (RSM) based on Central Composite Design (CCD) was used to evaluate and optimize the effect of the factors on the response function.

The interaction effects, optimal parameters, and the coefficients of the second-order fitting equation were obtained by using Design Expert software. The significance of the independent variables and their interactions were tested by means of analysis of variance (ANOVA) with a 95% confidence level. The model suitability was tested using the ANOVA test.

The results were assessed with various descriptive statistics such as the p-value, F-value, and the degree of freedom (df); the determination coefficient (R^2) of each coefficient which was determined by Fisher's F-test and values of probability >F. As shown in Table 16, a small probability value ($p < 0.001$) indicates that the model was highly significant and could be used to predict the response function accurately. Goodness-of-fit for the model was also evaluated by coefficients of determination R^2 (correlation coefficient) and adjusted coefficients of determination R. If the value the correlation coefficient R^2 is large, then it will indicate a high reliability of the model in predicting of getting the best operating parameters for the oil extraction. Finally, regression models for the model were established for the dependent variables to fit the experimental data for the response function.

The factors and levels that were selected for the experiment were:

- Temperature (^{o}C): 62—78
- Solvent to solid ratio (ml/g): 9:1—11.7:1
- Particle size (mm): 0.66mm—0.85mm, 0.85—1.4mm and 1.4—1.59mm

3.5 Characterization of Crude Jatropha Oil

3.5.1Determination of Physical Properties

A. Determination of Moisture and Volatile Matter of Oil

Procedure: Five gm of oil was weighted and put in a dish and then dried in an oven at 105^{0}C for 1 hour. The dish was removed from the oven and cooled in a dissector and weighed. The process was repeated until a constant weight was observed and the moisture and volatile matter of the oil was determined.

$$\%\ \text{Moisture Content} = \left(\frac{\text{w1} - \text{w2}}{\text{w1}}\right) * 100 \qquad (3.3)$$

B. Determination of Specific Gravity

The density and specific gravity of the oil was determined using digital density meter (Model: DMA 4100M) at 19.97^{o}C.

C. Determination of Kinematic Viscosity

The kinematic viscosity of the oil was calculated after the dynamic /absolute viscosity of the oil was measured using vibro viscometer.

Procedure: First fill a clean sample cup with the oil heated to 40^{o}C. The sample depth should be between the 35 and 45 ml. then lower the stage all the way down, if this has not been done already. Slide the sample cup under the stage clips. Check that protector bar is in down (lowered) position.

The stage was adjusted until the oil level is even with the narrowest part of the paddles. Then finally make the “ON: OFF” button on the display unit. Wait for the unit to enter Standby mode. After the “START” was pressed, then the paddles began to vibrate, and after about 15 seconds, the measured viscosity value was displayed.

The kinematic viscosity was calculated using the following equation:

$$v = \frac{\mu}{\rho} \tag{3.4}$$

Where:

v=kinematic viscosity (m^2/s)

μ=absolute or dynamic viscosity (N s/m^2)

ρ=density (kg/m^3)

D. Determination of Refractive Index

The refractive index of seed sources of Jatropha curcas oils was determined by a standard instrument employing the principle of the critical angle using diffused daylight as suggested in IS: 326-1968. The experiments were conducted at 20°C and reported as a number corrected to four decimal places.

Procedure: Two drops of the extracted oil was put into the lens of the refractometer. Water at 30ºC was circulated round the lens to keep its temperature uniform. Through the eyepiece of the refractometer, the dark portion viewed was adjusted to be in line with the intersection of the cross. At no parallax error, the pointer on the scale pointed to the refractive index which was read against the internal monochromatic source of light in the equipment. This was repeated 3 times and the mean value noted and recorded as the refractive index.

E. Determination of pH

The pH is the degree of the acidity of the oil. The pH meter's electrode was lowered into a buffer solution, with the temperature then adjusted to 20°C using the temperature regulator. The instrument was then calibrated at a buffer of pH 7. The electrode was then removed from the buffer and rinsed with distilled water. Next, it was then dipped into the test tube containing 2 gm of oil and the pH on the screen of pH meter was recorded (A.O.A.C official method of analysis 960.19, 2000).

3.5.2 Determination of Chemical Properties

1. Determination of Acid Value and Free Fatty Acid

Standard alcoholic potassium hydroxide solution (0.1 N) was prepared by dissolving KOH (pellet) with 95%ethanol. The solution was filtered and stored in brown bottle for five days. A phenolphthalein 10 g per liter of 95% v/v ethanol was used us an indicator. Furthermore, a mixture of 1 to 1 ratio (v/v) 95% ethanol and diethyl ether was prepared by mixing 500 ml diethyl ether and 500 ml of ethanol. 25 ml of diethyl ether was mixed with 25 ml of hot ethanol and 1 ml of phenolphthalein solution (1%). Next, the mixture was carefully neutralized with 0.1N KOH. About 5g of the melted oil was dissolved in the mixed neutral solvent and titrated with aqueous 0.1N ethanolic KOH, shaking constantly until a pink colour which persisted for 15 seconds was obtained. The volume of 0.1 N ethanolic KOH (V) for the titration was noted [76].

The total acidity (acid number) in mgKOH/g oil was calculated using equation 3.5 (a). The free fatty acid, FFA, of the oil was calculated from the acid value using equation 3.5 (b).

$$\mathrm{AcidNumber} = \frac{V * N * 56.1}{M} \qquad (3.5\ (a))$$

Where:-

V - Volume expressed in milliliter of 0.1N solution of ethanolic KOH
M - Mass in gram of test portion
N - Concentration of ethanolic KOH in mol/L
56.1- Potassium hydroxide molar mass, g/mol

The FFA value was calculated from the acid value using the relation:

$$\text{Acid Value} = \text{Free fatty acid} * 2 \qquad (3.5\ (b))$$

Therefore,

$$\text{Free fatty acids} = \frac{\text{Acid value}}{2} \qquad (3.6)$$

2. Determination of Saponifcation Value

Potassium hydroxide solution of 0.5M, in 95 % (v/v ethanol) was prepared. 35 g of KOH pellet was dissolved in 20 ml water, and the solution was mixed with 1000 ml of 95% ethanol. The solution was allowed to stand for 12 hours at room temperature and a clear supernatant solution decanted off. The filtered solution was then kept in the dark for storage. Hydrochloric acid of 0.5M was standardized and a 2% phenolphthalein as indicator was prepared. 2 g of the oil sample was introduced into a 250 ml conical flask connected to a reflux condenser and 25 ml of alcoholic KOH solution was added into it. The flask was heated at 78^0C (boiling temperature of ethanol) for 1hr, swirling intermittently using magnetic stirrer until complete saponification. The flask was removed from the heat mantel, and then 5 drops of phenolphthalein indicator was added, and the hot soap solution obtained was slowly titrated against the 0.5N HCl. The end point was reached when pinkish coloration changed to colorless. Blank determination was also conducted along with that of sample, using the same reagents minus sample (and volume the saponification value (SV) (expressed as the number of milligram of KOH required to saponify 1g fat) was calculated using equation 3.7 [77].

$$SaponificationNumber = \frac{(Vb - Va) \ * N * 56.1}{W} \tag{3.7}$$

Where:-

W - Weight of oil taken in gram
N - Normality of HCl solution
Va - Volume of HCl solution used in the test in milliliter
Vb- Volume of HCl solution used in black in milliliter

3. Determination of Iodine Values

The iodine value of vegetable oils and FAME can be determined using the empirical formula suggested by researchers for determination of higher heating value by using equations 3.7 to 3.9. After rearrangement, the iodine value was calculated from equation 3.10. Equation 3.7 was developed for the calculation of the Higher Heating Value (HHV) of vegetable oils and FAME from their viscosity (ν). Relationships derived from physical properties of vegetable oil and biodiesel fuels [78].The equations between viscosity and higher heating values are:

For vegetable oils,

$$\mathrm{HHV} = 0.0317\mathrm{v} + 38.053 \tag{3.8}$$

For FAME,

$$\mathrm{HHV} = 0.4625\mathrm{v} + 39.450 \tag{3.9}$$

$$\mathrm{HHV} = 49.43 - [0.041\ (\mathrm{SN}) + 0.015\ (\mathrm{IV})] \tag{3.10}$$

$$\mathrm{IV} = \frac{-\mathrm{HHV} + 49.43 - 0.041\ (\mathrm{SN})}{0.015} \tag{3.11}$$

Where: SN – Saponification Numer

4. Determination of Ash Content

The ash content of Jatropha oil was measured as per the standard ASTM D482. An electric muffle furnace (Nebertherm P 310 Model) was used in the experiment. In order to measure the ash content, sample was taken in a silica dish. The dish was first weighed empty and then with the oil sample. The sample weight was obtained from the difference between the initial and final weight of the dish. The sample was then placed in the muffle furnace and heated at 775 ± 25^0C for two hours. The dish was then cooled to room temperature in a desiccator. Thereafter, the dish was weighed to the nearest 0.01 mg using an electronic balance. The ash content was obtained using the equation given below:

$$\mathrm{As} = \frac{\mathrm{Wa}}{\mathrm{Ws}} * 100 \tag{3.12}$$

Where As=Ash content, percent

W_a=Weight of ash, g

W_s=Weight of sample,g

3.5.3 Fourier Transform Infrared Analysis

Fourier transform infrared spectrometry (FTIR) was used to evaluate the possible functional groups present in Jatropha oil. It is an easy way to identify the presence of functional groups in the sample and its structure based on the energies associated with the molecular vibration when irradiated. The response of the functional groups are characterized by observing the transmission of infrared radiations and comparing it with known standards in order to identify the type and the nature of functional groups present in the samples.

The major component of the FTIR system are; the radiation source, the interferometer, the slit, beam splitter and the detector. The radiation source generates a radiation in form of light which was directed to the sample through the interferometer. The interferometer separates the source radiation into its different wavelength, and the slit selects the collection of wavelength that passes through the sample at any given time. The beam splitter separates the incident beam into two; half of the incident beam goes to the fuel sample and the other half to the reference standard. The sample absorbs light according to its chemical properties. A detector collects the radiation that passes through the fuel sample and compares its energy to that going through the reference, and then put the electric signer which is normally sent directly to a recorder linked back to the interferometer so as to allow the interpretation of energy as a function of frequency or wavelength translated into a finger print which appear in a computer monitor attached directly to the detector, and printed out as hard copy. The functional groups are identified by comparing the peaks generated by the fuel sample with that of the reference standard.

The FTIR analyses for the Jatropha oil were conducted using Perkin Elmer spectrum 65 FTIR Spectrometer in the average wave number region from 4000 to 400cm^{-1}(in the mid-infrared range).This spectrometer is available in Chemistry Department of Addis Ababa University. The Jatropha crude oil was mixed with KBr particles to make it suitable to infrared analysis. The mixture was then pressed to a small thickness, slightly below 1mm, required for FTIR analysis. The functional groups were determined with the help of IR correlation charts. The IR spectra were reported in % absorbance.

3.5.4 GC-MS Analysis of Jatropha Oil

A. Conditions GC-MS Analysis

The component identification was achieved by the GC-MS analysis using HP 7820 series GC equipped with mass selective detector (MSD), HP 5977 series (German) in Addis Ababa University, Department of Chemistry. Column detector and MSD DB-5ms (60m x 250um, 1.4um thickness), Helium was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 1 μl was employed, injector temperature 250°C; Ion-source temperature of 280°C. The oven temperature was programmed from 50 °C (isothermal for 4min.), with an increase of 3^0C/min, to 280 ^{0}C and held for 10min at 3^0C/min, isothermal at 280 °C and Mass scan range from 29—800amu.

B. Identification of Components

Separation of a mixture into its components depends on the solubility differences of the sample vapor in a liquid (the stationary phase). The stationary phase is coated in a thin layer on solid particles (solid support) of large surface area and then packed uniformly into a column. A constant flow of the carrier gas passes through the column and transports solute molecules in the gas phase. The column is wound to fit inside an oven for precise temperature control.

A sample of the analyte is introduced by syringe injection into the heated injector tube, where it is vaporized and mixed with carrier gas. As the sample vapor is carried through the column by the carrier gas, the analyte partitions between the gas and liquid phases according to the analyte components' solubility in the liquid at the column operating temperature. This equilibrium partitioning continues as the sample is moved through the column by the carrier gas. The rate at which the sample travels through the column is determined by the sample solubility in the stationary phase, the carrier gas flow rate, and the temperature. Each component travels at a characteristic rate, and if the column has sufficient length and resolving power, the sample will be completely separated by the time it reaches the detector.

The detector located at the column exit is the ITD mass spectrometer. It records the total number of ions entering the mass analyzer from the column. The chromatogram produced is called the total ion chromatogram. Each point in the chromatogram is a mass spectrum. Each component is identified by comparing its "retention time", the length of time that it remains in the column, to that of a standard. The retention time of a vapor depends on the column temperature limits and ramp rate, the column length, type of stationary phase, and carrier gas velocity. If these variables are kept constant, the retention time of a component maybe tentatively identified by comparison to the retention time of a known standard run under identical operating conditions. If the response of the detector is linear, the area under a peak accurately represents the quantity of the component present. If it is not, calibration for detector response to the types of components expected to be in the analyte yields a set of response factors which convert the reported area percentages to quantitative weight percentages.

The MS instrument produces the output by drawing an array of peaks on a chart, the "mass spectrum." Each peak represents a value for a fragment mass. A peak's height increases with the number of fragments detected with one particular mass. As in the case of the GC detectors, a peak may differ in height with the sensitivity of the detector used.

4. RESULTS AND DISCUSSIONS

4.1 Characterization of Jatropha Seed

4.1.1 Moisture Content of the Seed

Table 9 Moisture Content of Jatropha curcas l. Seed

№	Time	Weight of Sample (g)
1	Initial weight	9.6823
2	After 2 hours	9.1992
3	After 4 hours	9.1321
4	After 6 hours	9.1095
5	After 8 hours	9.0663
6	After 10 hours	9.0663
	Final Moisture Content	**6.3621% by wt.**

The moisture content of the seed, 6.3621% by wt. which is in agreement with the value in literature and this value is the best value. This helps the seed from deterioration by bacterial activities.

4.1.2 Proximate Composition Analysis of the Seed

Proximate composition is important in determining the quality of raw materials. The analysis was determined according to the methods described by AOAC which was mentioned in the Table below. For this type of analysis, the moisture content, total ash, crude protein, crude fat, crude fiber and carbohydrate were analyzed and their values and the corresponding test methods were summarized as shown in Table 10.

Table 10 Proximate Composition of Jatropha curcas l. seed

№	**Characteristic**	**Experimental Value**	**Test Method Used**
1	Moisture Content (%)	6.42	AOAC 930.15,2005
2	Total Ash (%)	5.25	AOAC 923.03
3	Crude Protein (%)	21.59	AOAC 2001.11.2016
4	Crude Fat (%)	46.57	AOAC 2003.06
5	Crude Fiber (%)	16.77	AOAC 962.09,2016
6	Carbohydrate (%)	3.4	By difference

The moisture content obtained for the seed curcus is 6.42. High amount of moisture in seeds makes them vulnerable to microbial attack, hence, spoilage. The moisture content of any seeds is an index of its water activity and is used as a measure of stability and susceptibility to microbial contamination. Then since the moisture content of the seed is very small, then the result was good.

The crude fat content of the seed was found to be relatively high (46.57% wt.) suggesting that the seed is potentially useful as oilseeds.

4.1.3 Mineral Composition Analysis of the Seed

Mineral composition indicated that potassium is dominant (1159mg/100g) followed by sodium (31.99mg/100g). The values of calcium (8.650 ppm) and zinc (0.308 ppm) were obtained.

Findings of present research regarding characterization of proximate and mineral were in close conformity with the values described in the literature with slight differences. Environmental factors like climate and location might be a possible reason for these variations. Moreover, difference in genetic makeup could also be a contributing factor.

Table 11 Mineral Composition of Jatropha curcas l. seed

No	Name of Mineral	Experimental Value	Test Method Used
1	Sodium (Na)	31.99 mg/100g	Flame Photo Meter
2	Potassium (K)	1159 mg/100g	Flame Photo Meter
3	Calcium (Ca)	8.650 ppm	AOAC 999.11,2005 AAS
4	Zinc (Zn)	0.308 ppm	AOAC 999.11,2005 AAS
5	Phosphorous (P)	0.03 g/100g	AOAC 995.11:2016

4.2 Solvent Extraction Using Hexane

The oil content (dry basis) of the seeds was determined by Soxhlet extraction method. 50 g of the milled seeds, with particle sizes of 0.66—1.59 mm, using standard methods (ASTM E11-13) was wrapped in filter papers and the oil was extracted using a Soxhlet extractor with n-hexane as the solvent. The solvent to solid ratio was varied from 9:1—11.7:1 and the extraction temperature was carried out from 62—78ºC. The hexane in the solvent-oil mixture was evaporated using a rotary vacuum evaporator. The oil was then collected and weighed. The percentage oil yield was calculated using the expression listed below.

4.2.1 Percentage Oil Yield Calculation

The percentage oil yield (% OY by wt.) was calculated by equation 3.2and was prepared as follows:

Table 12 Factors and oil yield of Jatropha seed oil extraction

Run	Temperature (°C)	Solvent to Solid Ratio (ml/g)	Particle Size (mm)	Oil Yield (% by wt.)
1	70	10	0.66	55.30
2	70	10	1.13	48.32
3	70	10	1.59	52.40
4	70	8.3	1.13	41.92
5	75	9	0.85	47.24
6	75	11	1.4	40.18
7	62	10	1.13	0
8	70	10	1.13	47.08
9	70	10	1.13	49.46
10	65	9	1.4	0
11	70	11.7	1.13	50.32
12	70	10	1.13	47.57
13	65	11	1.4	0
14	70	10	1.13	48.54
15	65	11	0.85	0
16	78	10	1.13	47.20
17	65	9	0.85	0
18	70	10	1.13	49.29
19	75	9	1.4	52.50
20	75	11	0.85	53.20

4.3 Optimization of Solid Liquid Extraction of Jatropha Seeds

Optimization was carried out after observing all the interaction effects between the extraction variables. The proficiency of 'point optimization' was used for all the variables. Table 12 showed the optimum working conditions (ultimate goals, high and low limits) of the response (percent oil yield) and factors (temperature, solvent to solid ratio, and particle size) employed during the optimization analysis. Due to the high cost of production/extraction of oil, extraction of the highest percentage oil yield was maximized while the factors values were set in the range studied. A set of solutions were generated by the Design-Expert 7.0.0 version software, to determine the optimum conditions of the process which would select the values have high composite desirability and high extraction yields. The software searched for a combination of factors that simultaneously satisfied the requirements placed on the ultimate goals of response and each of the factors. Table 13presents the optimum conditions in uncoded units (temperature, solvent to solid ratio and particle size) which give the highest composite desirability (0.998) from the Design expert software. The predicted (%) and experimental (%) values of percent oil yield under the optimum conditions were also presented.

Table 13 Working conditions of response and factors for optimization

Variables	**Ultimate Goal**	**Experimental Range**	
		Lower Limit	**Upper Limit**
Temperature (°C)	In the range	62	78
Solvent to solid ratio (ml/g)	In the range	9	11.7
Particle size (mm)	In the range	0.66	1.59
% Oil yield	Maximize	0	55.30

4.4 Effects of Individual Factors on Oil Yield

4.4.1 Effect of Temperature on Oil Yield Extraction

Using Soxhlet extraction, the effect of extraction temperature on the amount of extracted oil is greatly pronounced. A range of temperature values from 62°C to 78°C were used for oil extraction. Unlike leaching, solid liquid extraction using Soxhlet, the temperature should be near and above the boiling point of the solvent. For temperatures below the boiling point of hexane like 62°C, 65°C, extraction has not been happened. This is due to the solvent has not been vaporized and condensed for extraction. However, as the temperature increases from 65°C to near the boiling point of hexane (69°C), then the solvent begins vaporization and starts extraction. The maximum oil yield (55.30% by wt.) was obtained at 70°C. However, the oil yield declines afterwards as most of the solvent had vaporized. By increasing the temperature approaching to the boiling point of the solvent, both the diffusion coefficient and the solubility of the oil in the solvent are enhanced, thus improve the extraction rate [80].

4.4.2 Effect of Solvent to Solid Ratio on Oil Yield Extraction

Increasing solvent to solid ratio (v/w) up to a specific limit increases the oil yield since the concentration gradient between the solid and the liquid phase becomes greater which favors good mass transfer. Therefore, as the ratio has increased from 9:1 to 11.7:1, the total amount of extracted oil using hexane first increased from 52.50 to 55.30%, and then decreased to 50.32%. Then based on the results, the solvent to solid ratio of 10:1 was good to extract the maximum amount of oil (55.30%).

4.4.3 Effect of Particle Size on Oil Yield Extraction

The extraction was carried out using three different meal sizes namely 0.66mm—0.85mm, 0.85—1.40 mm, and between 1.4 and 1.59mm. The highest percentage of oil yield was obtained for the particle size of 0.66mm.Less oil is extracted from the larger particles (>1.40 mm) compared to the smaller size particles. The reason is that larger particles with smaller contact surface areas are more resistant to solvent entrance and oil diffusion. Therefore, less amount of oil will be transferred from inside the larger particles to the surrounding solution in comparison with the smaller ones. Nevertheless, when the particle is too small, that is, much below 0.85 mm, only small amount of oil can be extracted even though the contact surface area for small particle is supposed to be significantly higher than the larger particles. This could have been due to the agglomeration of the fine particles which reduced the effective surface area available for the free flow of solvent to solid [83, 84].This problem is more apparent when oily Jatropha seeds are in use since the oily particles stick together easily to form paste material which prevents free interactions between solid and solvent. Based on the result, the highest oil yield (55.30%) was obtained at 0.66 mm particle sizes.

4.5 Statistical Data Analysis

Response surface methodology (RSM) was used for the statistical analysis of experiments conducted for oil extraction using hexane as solvent. The percentage oil extracted was entered as a response in the selected design lay out. The major statistical analyses of the oil extraction process were model generation; model fitness test and ANOVA analysis which were presented and discussed in detail as following sections.

4.5.1 Estimation of Quantitative Effects of the Factors

As discussed in the previous section, the ultimate objective of CCD method used in this study was to find out the significant effects of the process parameters, namely; extraction temperature, solvent to solid ratio, and particle size on the percentage oil yield. 3D response surface and contour plots were used to investigate the effect of all the factors on the responses. The 3-D response surface plots are graphical representations of the interactive effects of any two variables. The nature of the response surface curves shows the interaction between the variables. An elliptical shape of the curve indicates good interaction of the two variables and circular shape indicates no interaction between the variables.

1. Effect of Temperature and Solvent to Solid Ratio on Oil Yield

The combined effect of temperature and solvent to solid ratio on percentage oil yield at constant particle size (1.13 mm) is depicted in Figure 9.The percentage oil yield has been increased as both the temperature and solvent to solid ratio increased. Increasing solvent to solid ratio may increase the oil recovery since the concentration gradient between the solid and the liquid phase becomes greater which possibly favors good mass transfer. As extraction temperature increased, the diffusivities of the solvent increased, which resulted in higher oil recovery. By increasing the temperature approaching to the boiling point of the solvent, both the diffusion coefficient and the solubility of the oil in the solvent were possibly enhanced, thus improved the extraction rate.

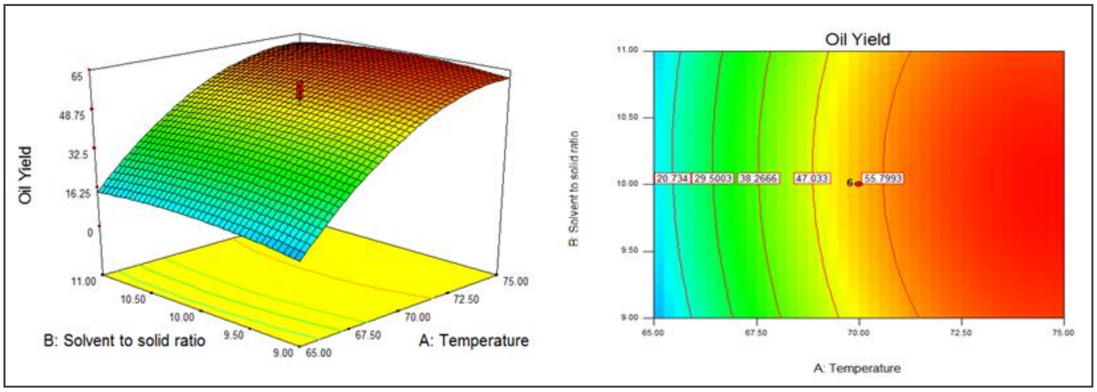

Figure 9 Response surface plot of temperature and solvent to solid ratio and their interaction effect on the oil yield showing (Left) 3D effect; (Right) Contour plot

2. Effect of Temperature and Particle Size on Oil Yield

The combined effect of temperature and particle size on the percentage oil yield is shown in Figure 10.As can be seen from the plot (Contour plot), it was observed that, the increase in temperature from 65 to say 70^{o}C, and an increase of particle size did not have effect on the extraction oil yield. But later when the temperature increases and the particle size decreases, the yield was observed to increase.

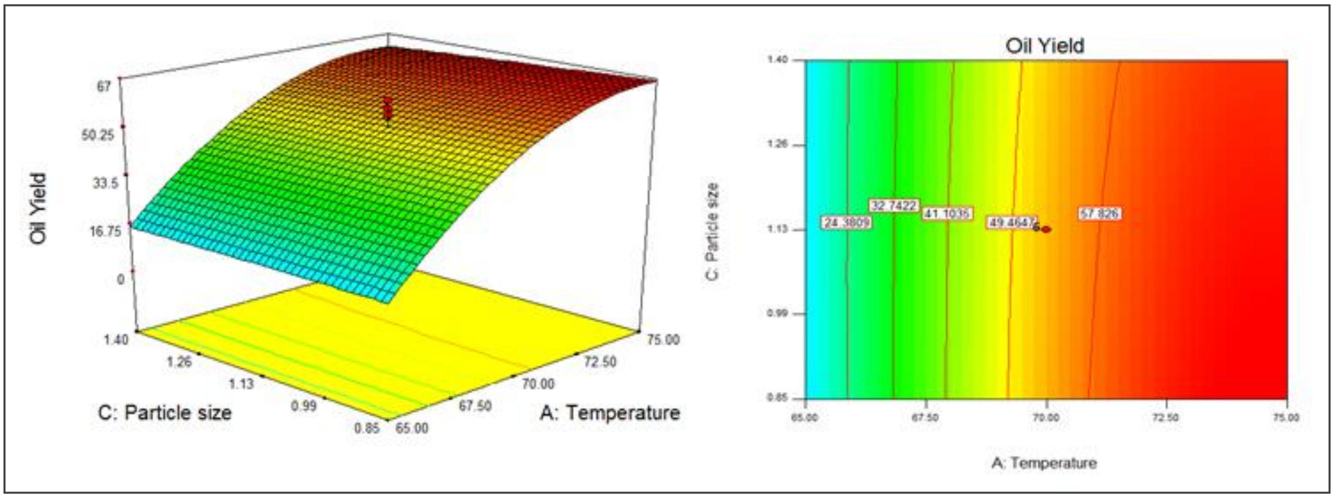

Figure 10 Response surface plot of temperature and particle size and their interaction effect on the oil yield showing (Left) 3D effect; (Right) Contour plot

3. Effect of Solvent to Solid Ratio and Particle Size on Oil Yield

The interaction between solvent to solid ratio and particle size on extraction oil was presented in Figure 11.From the plot, it can be seen that as solvent to solid ratio and particle size increase, the oil yield increases. However, whenever, the amount of solvent to solid ratio and particle size increases much, the yield declines.

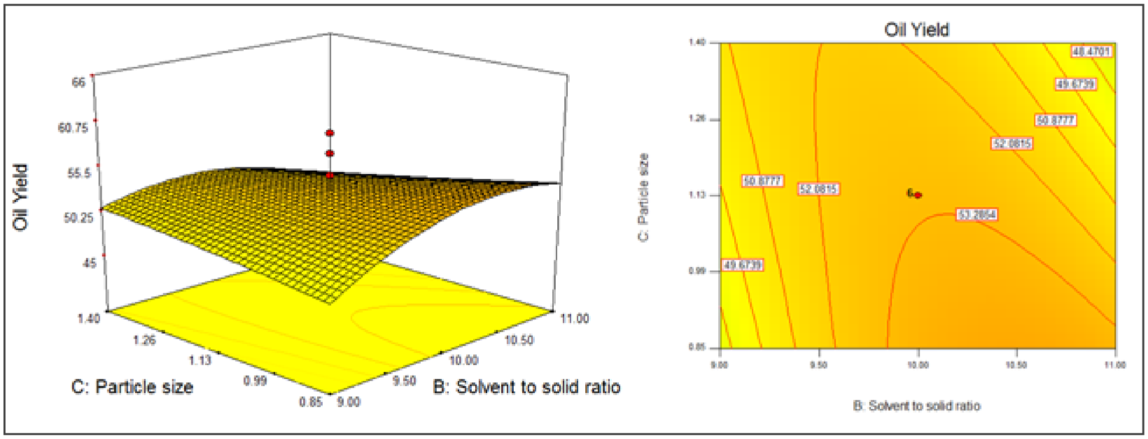

Figure 11 Response surface plot of solvent to solid ratio and particle size and their interaction effect on the oil yield showing (Left) 3D effect; (Right) Contour plot

4.5.2 Modeling and Model Analysis for Jatropha Oil Extraction

The design of the experiments carried out in this work was based on the fact that oil yield and its properties are functionally related to three factors: temperature, solvent to solid ratio and particle size. The experiments to be carried out were designed with the aid of Design Expert 7.0.0 using CCD of Response Surface Methodology (RSM). The maximum and the minimum levels used for the factors considered were as given in Table 14.Using the levels of the three factors given in this table, the design of experiment gave twenty (20) runs to be carried.

Table 14 Minimum and maximum levels of the experimental factors

Variable	**Unit**	**Minimum**	**Maximum**
Temperature (A)	°C	62	78
Solvent to solid ratio (B)	ml/g	9	11.7
Particle size (C)	Mm	0.66	1.59

According to the CCD of response surface methodology, the response (% oil yield) was analyzed using the three interaction factors which were used for the model generation. The output of different model summary statistics in the table below focused on the model maximizing, the Adjusted R-

Squared and the Predicted R-Squared values to be large. Therefore, the quadratic model was suggested.

Table 15 Model Summary Statistics

Source	**Std. Dev.**	**R-Squared**	**Adjusted R-Squared**	**Predicted R-Squared**
Linear	16.69	0.6410	0.5737	0.3846
2FI	18.40	0.6454	0.4817	-0.4030
Quadratic	9.51	0.8725	0.9167	-0.1608
Cubic	15.54	0.8833	0.6304	-22.1680

A second order quadratic regression was performed to estimate the response function as a second order polynomial after the examination of the model fit summery revealed that quadratic model was statistically significant for the response percent oil yield. To examine the combined effect of the three different independent process parameters on percentage oil yield, 20 experiments were performed. The experimental design was given in Table 16, along with experimental data and predicted responses.

Table 16 CCD matrixes for the experimental design and predicted responses for % oil yield

Run	**Factors**			**Oil Yield (%)**	
	Temperature in^{o}C (A)	**Solvent to solid ratio in ml/g (B)**	**Particle size in mm (C)**	**Experimental Value**	**Predicted Value**
1	**70**	**10**	**0.66**	**55.30**	**55.28**
2	70	10	1.13	48.32	48.53
3	70	10	1.59	52.40	52.48
4	70	8.32	1.13	41.92	40.86
5	75	9	0.85	47.24	47.03
6	75	11	1.40	40.18	40.15
7	62	10	1.13	0	0
8	70	10	1.13	47.08	47.01
9	70	10	1.13	49.46	49.44
10	65	9	1.40	0	0
11	70	11.7	1.13	50.32	50.22
12	70	10	1.13	47.57	46.55
13	65	11	1.40	0	0
14	70	10	1.13	48.54	47.32
15	65	11	0.85	0	0
16	78	10	1.13	47.20	47.00
17	65	9	0.85	0	0
18	70	10	1.13	49.29	38.13
19	75	9	1.40	52.50	52.50
20	75	11	0.85	53.20	53.21

Regression analysis was performed to fit the response functions, that is, percentage oil yield. The second order polynomial equation developed represent responses as functions of extraction temperature (A), solvent to solid ratio (B), particle size (C). An empirical relationship between the response and the input test variables in coded units can be expressed by the following equation:

Final Equation in Terms of Coded Factors:

$$\begin{aligned}\text{OIl Yield (\% wt.)} &= 53.12 + 24.11\text{A} + 0.57\text{B} - 0.93\text{C} - 0.79\text{AB} - 0.97\text{AC} - 2.28\text{BC} - 12.70\text{A2} \\ &\quad - 2.97\text{B2} - 0.24\text{C2} \quad (4.1)\end{aligned}$$

The above equation described how oil yield was affected by the individual variables (linear and quadratic) or double interaction. Negative coefficient values indicate that individual or double interactions factors negatively affect oil yield while positive coefficient values represented that factors increase percentage of oil yield. For instance, among all linear factors solvent to solid ratio and particle size had a negative effect but temperature had a positive effect on percent oil yield.

Table 17 Analysis of variance (ANOVA) for the quadratic model

Source	Sum of Squares	Df	Mean Square	F value	p-value prop>F	
Model	10399.57	9	1155.51	5.74	0.0058	Significant
A-Temperature	7941.12	1	7941.12	39.42	< 0.0001	
B-Solvent to solid ratio	4.42	1	4.42	0.022	< 0.001	
C-Particle size	11.69	1	11.69	0.058	< 0.001	
AB	5.06	1	5.06	0.025	< 0.001	
AC	7.53	1	7.53	0.037	< 0.001	
BC	41.77	1	41.77	0.21	< 0.001	
A^2	2325.07	1	2325.07	11.54	0.0068	
B^2	127.30	1	127.30	0.63	< 0.001	
C^2	0.82	1	0.82	4.089E-003	0.0073	

Residual	2014.47	10	201.45			
Lack of fit	1869.43	5	373.89	12.89	0.0070	Insignificant
Pure error	145.03	5	29.01			
Cor Total	12414.04	19				

$R^2 = 0.8725$

Adjusted $R^2 = 0.9167$

Predicted $R^2 = -0.1608$

Adequate precision ratio = 8.832

The adequacy and significance of the quadratic model was justified by the analysis of variance (ANOVA). The ANOVA summary is given in Table 17and the analysis was done by means of Fisher's 'F'-test. The model F-value observed was 5.74 enlightening that the model was significant. The parameters having an F-statistics probability value less than 0.05 are said to be significant [85]. In this present study the probability of model F statistics value was <0.0001indicating that the model suggested by the software was highly significant. In this case A, B, C, AB, AC, BC, A^2, B^2, and $C^2$are statistically significant ($P<0.05$) model terms at the 95% confidence level. The non-significant value of lack of fit (F-value of 12.89) for the model showed that the developed model is valid [86].Furthermore the fitting of the experimental data to the regression model was checked and suitably explained by the value of the adjusted determination coefficient (R^2Adj= 0.9167). This result means that 91.67 % of the total variation on oil yield data can be explained by the selected model. The ratio determined was greater than 4, representing that the quadratic model can be used to navigate the design space and to find the optimal conditions of this process. Furthermore, the relationship between internally studentized residuals and normal % probability values (Figure 12(Left)) showed that the values are distributed relatively near to the straight line, indicating good fitness of the model. Again in Figure 12 (Right), the plot of predicted versus studentized residuals was tested and the residuals were scattered randomly around ±3.00.This was an indication of better fitment of the model with the experimental data.

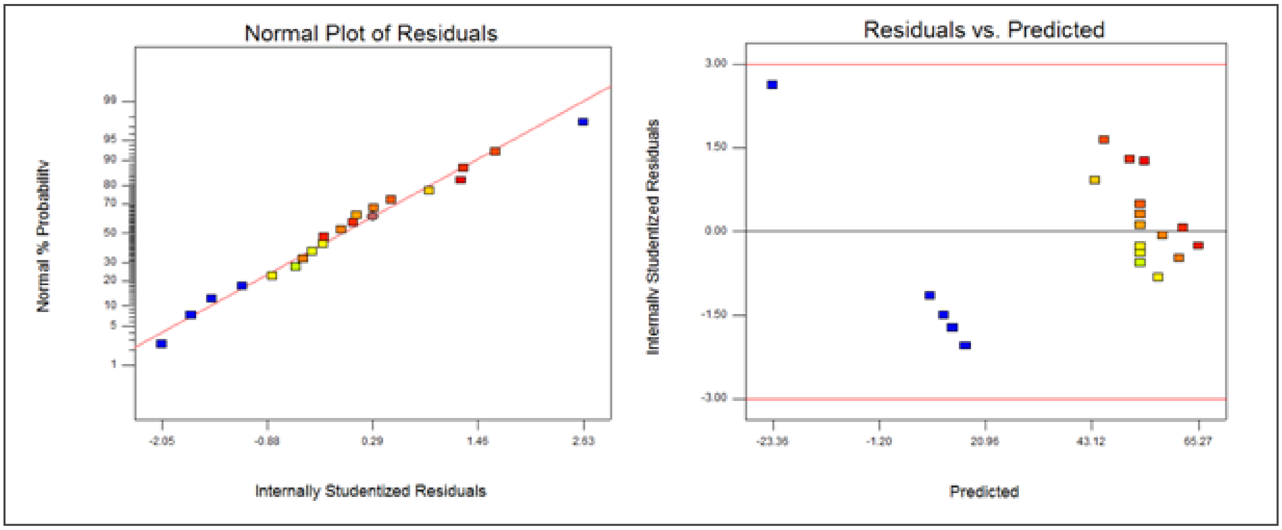

Figure 12 Plot of (Left) Internally studentized residuals and normal % probability values; (Right) Predicted versus studentized residuals

4.6 Validation of the Predictive Models

In order to verify the optimization results, an experiment was performed under the predicted conditions by the developed model. The model predicted 55.28 % by wt. oil yield at a temperature of 70°C, solvent to solid ratio of 10:1 and 0.66mm as particle size. An experiment was conducted using these optimal conditions and a value of 54.81% by wt. oil yield was found which is closely in agreement with the result obtained from the model and hence validated the findings of the optimization.

4.7Physicochemical Characterization of Jatropha Oil

Physicochemical characteristics provide a base line for suitability of oils [79]. The physicochemical properties of the oil determined were moisture and volatile matter of oil, density, refractive index, specific gravity, kinematic viscosity, pH, acid value, iodine value, saponification value, free fatty acid, ash content and flash point.

4.7.1 Determination of Physical Properties

A. Determination of moisture and volatile matter of oil

According to the procedure shown in the methodology, the moisture and volatile matter of the was shown in the following Table:

Table 18 Moisture and volatile matter determination of the oil

№	Time	Weight of Sample (g)
1	Initial weight	10.1560
2	After 2 hours	8.7589
3	After 4 hours	8.6292
4	After 6 hours	8.6036
5	After 8 hours	8.5834
6	After 10 hours	8.5830
	Final Moisture Content	**15.4883**

The moisture content of the extracted oil was found to be 15.4883% by weight. Since the solvent residue found in the oil has not been completely removed, then the value obtained experimentally was a little bit higher than that found in literature.

B. Determination of Kinematic Viscosity

The dynamic/absolute viscosity was measured three times and the average value was obtained to be 12.063 mpa.s at 40 °C using vibro viscometer. The kinematic viscosity was then calculated using equation (3.4).The kinematic viscosity (m^2/s) was calculated as follows:

$$v = \frac{12.063\ \mathrm{mPas}}{855.20\ \mathrm{kg/m3}} = 0.014\mathrm{mm2}=0.00014\ \mathrm{stokes}$$

C. Determination of Specific Gravity

The specific gravity and density of the oil were measured using digital density meterat 20°Cand were found to be 0.8567 and 855.20 kg/m3, respectively.

D. Determination of pH

The probe was first washed by distill water and was immersed in to the sample. After carefully calibrating the pH meter, the pH was found to 5.74 which acidic.

E. Determination of Refractive Index

The refractive index was measured three times and the average value was obtained to be 1.4512 using digital refractometer.

Table 19 Refractive index of Jatropha oil at 20 °C

No	Refractive Index of Experimental Runs			Average Refractive Index
	Run 1	Run 2	Run 3	
1	1.4494	1.4463	1.4580	1.4512

Table 20 Summary for physical properties of Jatropha oil

No	Physical Property	Experimental Value
1	Moisture and volatile matter of oil (%)	15.4883
2	Kinematic viscosity (mm^2/s) at 40°C	0.014
3	Specific gravity at19.97°C	0.8567
4	Density (kg/m^3) at 19.97°C	855.20
5	pH	5.74
6	Refractive index at 20°C	1.4512

4.7.2 Determination of Chemical Properties

1. Determination of Acid and Free Fatty Acid Values

Titration method was used to determine the acid value. The required solutions were prepared with the required concentration as follows.

- **Preparation of 0.1 N standard alkolic potassium hydroxide solutions: -**This was done by dissolving a known quantity of KOH pellet in 250 ml of 95% ethanol. The mass of KOH was first calculated as follows: To find the mass of KOH, the following equation was used.

$$0.1 \text{ N KOH} = \frac{\text{Number of moles KOH}}{\text{Volume in liter of solution}}$$

$$0.1 \text{ N KOH} = \frac{\text{Mass of KOH}}{\text{Molecular Wt.} * \text{Volume in liter of solution}}$$

$$\text{Mass of KOH} = 0.1 \text{ N KOH} * \text{Molecular Wt.} * \text{V in litre of solution}$$

$$\text{Mass of KOH} = 0.1\frac{\text{mol}}{\text{L}} * \frac{56.1056\text{g}}{\text{mol}} * 0.25\text{L}$$

$$\text{Mass of KOH} = 1.4026 \text{ g}$$

- **Preparation of a solution of diethyl ether with ethanol (1 to 1 ratio in v/v)**:- the solution was prepared using 100 ml of diethyl ether and 100 ml of 95% ethanol
- **Preparation of phenophitalin indicator:-** this indicator was prepared using 1 g of phenophitalin and 100 ml of 95% ethanol
- **Preparation of 0.1 N KOH solution: -**1.4026 g of KOH was dissolved in 250 mlof distill water instead of 95% ethanol. The solution was stored in brown bottle for 5 days in dark place.

Next, the mixture was carefully neutralized with 0.1N KOH. Five gram of the oil was dissolved in the mixed neutral solvent and titrated with aqueous 0.1N ethanolic KOH, shaking constantly until the pink color which persisted for 15 seconds was obtained. The volume of 0.1 N ethanolic KOH (V) for the titration was obtained.

The total acidity (acid number) in mgKOH/g oil was calculated using equation 3.5 (a).

$$AcidNumber = \frac{V * N * 56.1}{M}$$

Where:-

V - Volume expressed in milliliter of 0.1N solution of ethanolic KOH

M - Mass in gram of test portion

N - Concentration of ethanolic KOH in mol/L

$AcidNumber = \frac{11.6 * 0.1 * 56.1}{5}$= 13.0152 mg KOH g^{-1} oil

The FFA value was calculated from the acid value using the relation:

Free fatty (% by weight) = Acid Value/ 2 = 13.0152/2 = 6.5076

2. Determination of Saponifcation Value

According to the steps described in the methodology, the following steps were conducted:

- **Preparation of 0.5 N alcoholic potassium hydroxide solutions: -** the 0.5N of Ethanolic KOH solution was prepared by dissolving 35 g of KOH pellets in 20 ml of distill water and mixing the solution in 1000 ml of 95% ethanol. This solution was allowed to stand for 12 hrs at room temperature and kept in dark.
- **Standardization of 0.5 M HCl:** first the molarity of HCl was found as follows:

$$\text{Molarity of HCl} = \frac{\text{Moles of solute}}{\text{liter of solution}}$$

$$\text{Molarity of HCl} = \frac{\text{Mass of solute} * \%\text{purity}}{\text{Molecular wt.} * \text{liter of solution}}$$

$$\text{Molarity of HCl} = \frac{\text{Mass of solute} * \%\text{purity}}{\text{Molecular wt.} * \text{liter of solution}}$$

$$\text{Molarity of HCl} = \frac{1.18\frac{\text{g}}{\text{ml}} * 35.4\%}{36.46\text{g/mol}} = 1.14569\%\frac{\text{mol}}{\text{ml}} = 11.456\text{ M}$$

Then using the dilution law, the initial volume (V_1) of HCL was found as follows: $\text{C1V1} = \text{C2V2}$

Where C_1=molarity of HCl C$_2$=molarity of solution (0.5 M)

V_1=initial volume of HClV_2=volume of solution in ml (250 ml)

$$\text{V1} = \frac{\text{C2V2}}{\text{C1}} = \frac{0.5\text{ M} * 250\text{ml}}{11.456\text{ M}} = 10.9113\text{ml of HCl}$$

- **Preparation of Na_2CO_3 solution (0.1N):** After carefully weighing 0.53 g of anhydrous Na_2CO_3, dissolve itin 100 ml of distill water.

After 2 gm of oil was dissolved in alcoholic KOH and heated gently for 1 hour. It was titrated with HCl to the end point. The end point was reached when pinkish coloration changed to colorless. Similar titration was done for the blank where blank determination was conducted along with that of sample, using the same reagents minus the sample. In both cases the value of HCl was recorded.

Using equation (3.7), the saponification number was calculated as follows:

$$SaponificationNumber = \frac{(Vb - Va) \ * N * 56.1}{W}$$

Where:-

W - Weight of oil taken in gram
N - Normality of HCl solution
Va - Volume of HCl solution used in the test in milliliter
Vb- Volume of HCl solution used in black in milliliter

$$SaponificationNumber = \frac{(23.5 - 13.5) * 0.5 * 56.1}{2}$$

Saponification Number=140.25 mg KOH g^{-1} oil

3. Determination of Iodine Values

The iodine value of vegetable oils and FAME can be determined using the empirical formula suggested by researchers for determination of higher heating value by using equations 4.2 to 4.4. After rearrangement, the iodine value was calculated from equation 4.5. Equation 4.2 was developed for the calculation of the Higher Heating Value (HHV) of vegetable oils and FAME from their viscosity (ν). Relationships derived from physical properties of vegetable oil and biodiesel fuels [80].The equations between viscosity and higher heating values are:

For vegetable oils,

$$\mathrm{HHV} = 0.0317\mathrm{v} + 38.053 \tag{4.2}$$

For FAME,

$$HHV = 0.4625v + 39.450 \tag{4.3}$$

$$HHV = 49.43 - [0.041\ (SN) + 0.015\ (IV)] \tag{4.4}$$

$$IV = \frac{-HHV + 49.43 - 0.041\ (SN)}{0.015} \qquad (4.5)$$

Where : SN – Saponification Numer

Therefore, since the sample is a vegetable oil, then equation (4.2) was applied and hence,

The iodine value is found to be104 g I2/100 g. The limitation of unsaturation is necessary due to the fact that heating higher unsaturated fatty acid results in polymerization of glycosides. This can lead to the formation of deposits or to deterioration of the lubricating properties. However, this experimental value is within the range of the literature.

4. Determination of Ash Content

The weight of ash was weighed and found to be 0.0156 g.

Table 21 Determination of ash content

N<u>o</u>	**Weight of Crucible (g)**	**Weight of Crucible and Oil (g)**	**Weight of Sample (g)**	**Weight of Crucible and Ash (g)**	**Weight of Ash (g)**
1	36.8720	56.1013	19.2293	36.8876	0.0156

The ash content of the oil was obtained using equation (3.12) as follows:

$$\mathrm{As} = \frac{0.0156}{19.2293} * 100 \ \ = 0.0811\ \%\ \mathrm{w/w}$$

Table 22 Summary for chemical properties of Jatropha oil

N<u>o</u>	**Chemical Property**	**Experimental Value**
1	Acid value (mg KOH g^{-1} oil)	13.0152
2	Free fatty acid value (% by wt.)	6.5076
3	Saponifcation value (mg KOH g^{-1} oil)	140.25
4	Iodine value (g I_2 100 g^{-1} oil)	104
5	Ash content (% by wt.)	0.0811

4.7.3 FT-IR Analysis of Jatropha curcas l. Seed Oil

Infra-red (IR) spectrum was recorded on Spectrum 65 FT-IR (Perkin Elmer) spectrometer. About 1 mg of the isolated compound was prepared as KBr pellets and employed for recording the IR spectrum (frequencies between 4000 and 400 cm^{-1}).

FT-IR is used to determine the different functional group such as alcohol, alkane, alkynes, alkenes and other such groups present in the substance which here is Jatropha oil as shown in Figure 13below. Interpreting infrared (IR) spectra is of immense help to structure determination. Not only will it tell you what functional groups and structural elements are there, it will also clarify which ones are not there, and also concentration of bands by using values of absorbance as shown in Table 23.The IR spectrum of Jatropha oil is shown below. This graph is obtained from the result after the oil was analyzed using FT-IR method.

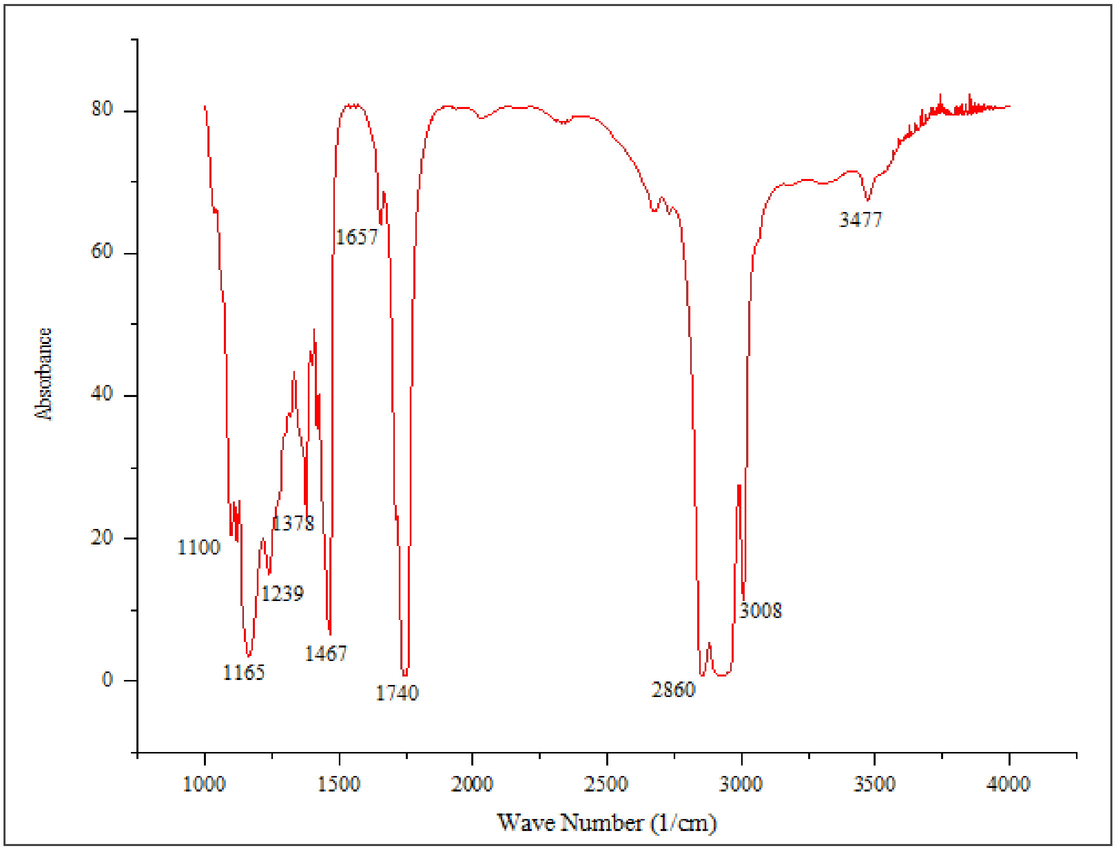

Figure 13 FT-IR result for Jatropha oil

Alcohols have characteristic IR absorptions associated with both the O-H and the C-O stretching vibrations. When run as a thin liquid film, or "neat", the O–H stretch of alcohols appears in the region 3600-3200 $cm^{-1}$and is a very intense, broad band. The C–O stretch shows up in the region 1250-1000 cm^{-1}.

All carbonyl compounds absorb in the region 1820-1630 cm^{-1} due to the stretching vibration of the C=O bond. This distinctive carbonyl band is particularly useful for diagnostic purposes because it has a characteristic high intensity and few other functional groups absorb in this region. Different carbonyl compounds absorb in narrow ranges within the general carbonyl region. The exact wave number of the C=O stretch can give you clues as to whether the compound is a ketone, aldehyde, ester, or carboxylic acid; furthermore it can tell you whether it is an alpha, beta- carbonyl.

The spectra of simple alkanes are characterized by absorptions due to C–H stretching and bending (C–C stretching and bending bands are either too weak or of too low a frequency to be detected in IR spectroscopy). In simple alkanes, which have very few bands, each band in the spectrum can be assigned. The above discussions were clearly indicated as in Table 23 below.

Table 23 FTIR studies of Jatropha oil

No	Peaks (cm^{-1})	Stretching Frequency (cm^{-1})	Bond	Name of Compound	Intensity
1	3477	3200-3600	O-H	Alcohols, phenols	Weak to strong
2	3008	2700-3300	C-H	Alkanes	Weak to strong
3	2860	2700-3300	C-H	Alkanes	Weak to strong
4	1740	1630-1820	C=O	Esters, saturated, aliphatic, aldehyddes	Strong
5	1657	1630-1820	C=O	Esters, saturated, aliphatic, aldehyddes	Strong
6	1467	1300-1373	Sulfones, sulfonyl chlorides, sulfates, sulfon amides	Sulfones, sulfonyl chlorides, sulfates, sulfon amides	Strong
7	1378	1300-1373	Sulfones, sulfonyl chlorides, sulfates, sulfon amides	Sulfones, sulfonyl chlorides, sulfates, sulfon amides	Strong
8	1239	1000-1250	C-O	Alcohols	Strong
9	1165	1000-1250	C-O	Alcohols	Strong
10	1100	1000-1250	C-O	Alcohols	Strong

4.7.4 GC-MS Condition for Analysis of Fatty Acid Profile

The component identification was achieved by the GC-MS analysis using HP 7820 series GC equipped with mass selective detector (MSD), HP 5977 series (German) in Addis Ababa University, Department of Chemistry. Column detector and MSD DB-5ms (60m x 250um, 1.4µm thickness), Helium was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 1 µl was employed, injector temperature 250°C; Ion-source temperature of 280°C. The oven temperature was programmed from 50 °C (isothermal for 4min.), with an increase of 3^0C/min, to 280 ^{0}C and held for 10min at 3^0C/min, isothermal at 280 °C and Mass scan range from 29-800amu.

Qualitative identification of the constituents was based on comparison of elution or the retention time on the HP-597 column keeping minimum quality of each component 90% and quantitative analysis was done using electronic integration of peak area percentages. The following database system and previous work were used for the analysis and its comparison: MassHunter\Library\ NIST14.L was used.

A. Identification of Components by GC-MS

A total of more than thirteen different components, whose minimum quality is above 90,of the Jatropha oil with different retention times were eluted from the GC column as indicated by the chromatogram shown in Appendix A and were further analyzed with an electron impact MS voyager detector. Identification of constituents was done on the basis of their retention times and mass spectra library search. The mass spectrographs of the identified constituents were indicated in Appendix A. The relative amount of individual components was calculated based on GC peak areas.

The instrument`s data bank identified the presence of the following different components:(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene (0.43%), Bicyclo[3.1.0]hexane (0.2%), Bicyclo[3.1.1] heptanes (0.3%), Eucalyptol (0.69%), Pentadecane (1.25%), Undecanoic acid (0.17%), Hexadecane (0.26%), Methyl tetradecanoate (2.63%), 11-Hexadecenoic acid (1.85%), Hexadecanoic acid (22.55%), 9, 12-Octadecadienoic acid (13.64%),9-Octadecenoic acid (44.61%), Methyl stearate (8.64%). The retention times, peak areas and the minimum qualities of the components were indicated in the Table shown below (Table 24).

Table 24 Chemical composition of Jatropha oil

No	**Name of Compound**	**Retention Time (Minute)**	**Area %**	**Minimum Quality**
1	(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	5.356	0.43	94
2	Bicyclo[3.1.0]hexane	6.015	0.2	91
3	Bicyclo[3.1.1] heptanes	6.084	0.30	90
4	Eucalyptol	6.999	0.69	99
5	Pentadecane	13.636	1.25	98
6	Undecanoic acid	13.952	0.17	91
7	Hexadecane	14.847	0.26	95
8	Methyl tetradecanoate	16.296	2.63	99
9	11-Hexadecenoic acid	18.624	1.85	99
10	Hexadecanoic acid	18.904	22.55	97
11	9, 12-Octadecadienoic acid	21.696	13.64	99
12	9-Octadecenoic acid	21.797	44.61	99
13	Methyl stearate	22.241	8.64	99

A chromatogram is shown below. However, most of them were listed in Appendix A.

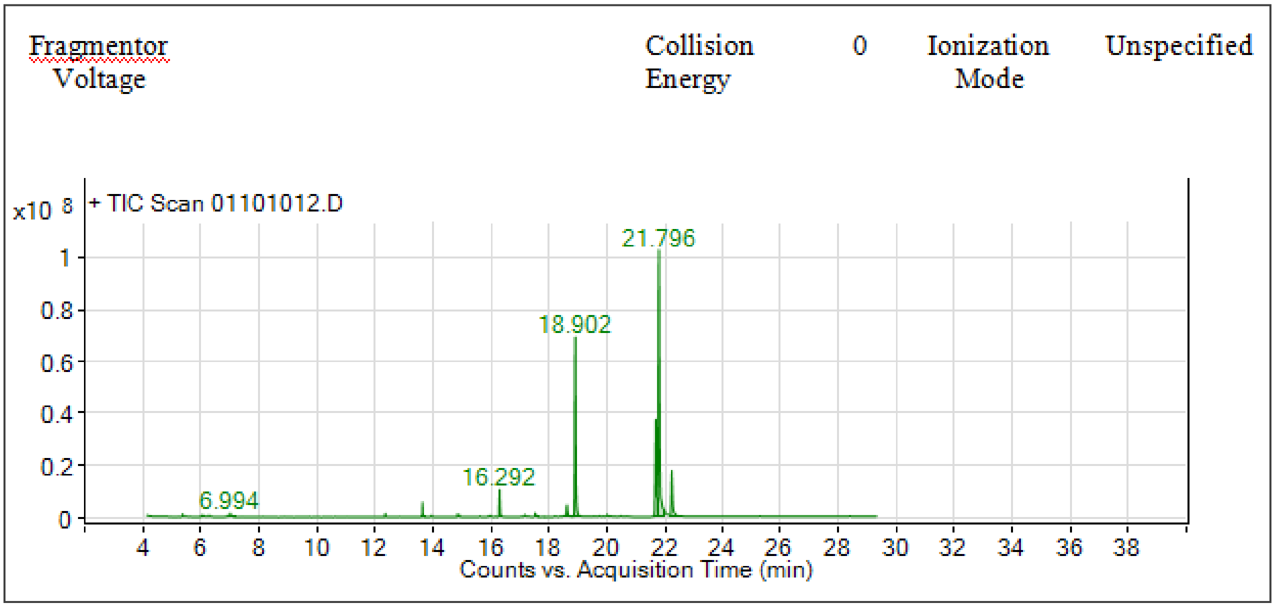

Figure 14 Chromatogram of Jatropha oil

Table 25 Integration Peak List

Peak	Start	RT	End	Height	Area	Area %
1	6.967	6.994	7.182	1520773.29	4882101.01	1.53
2	13.574	13.627	13.735	5570198.95	9269094.36	2.9
3	16.252	16.292	16.386	10398016.58	19131283.4	5.99
4	17.476	17.516	17.658	1463455.28	3432533.45	1.07
5	18.566	18.62	18.781	4392635.79	12374371.19	3.87
6	18.842	18.902	19.077	68809136.38	166203304.5	51.99
7	21.607	21.688	21.742	37348025.5	105265166.4	32.93
8	21.742	21.796	21.97	102168547.5	319652702	100
9	22.172	22.24	22.415	17482218.22	63475148.15	19.86

Fatty Acid Composition:

Fatty acid composition determination was another important characteristic carried out on this study. The major saturated fatty acids in Jatropha curcas seed oil were palmitic (22.55%) and stearic acids (8.64%); the main unsaturated fatty acid were oleic acid (44.61%) and linoleic acid (13.64%) with small amounts of other acids listed in the following table (Table 26).

Table 26 Fatty acid composition of Jatropha oil

№	**Name of Fatty Acid**	**Experimental Value (%)**	**Reported Value**(%)[48, 81]
1	Oleic acid	44.61	43.5
2	Palmitic acid	22.55	11.5
3	Linolic acid	13.64	33.5
4	Stearic acid	8.64	8
5	Myristic acid	2.63	
6	11-Hexadecenoic acid	1.85	
7	Other acids	6.08	3.5

There are little variations between the composition of some of the fatty acids in the present study and values from literature. This is mainly due to the differences between the environmental conditions (temperature, altitude, rainfall, sunlight, and soil conditions), genetics, plant age and plant management (use of pesticide and fertilizer, irrigation, plant density), etc.

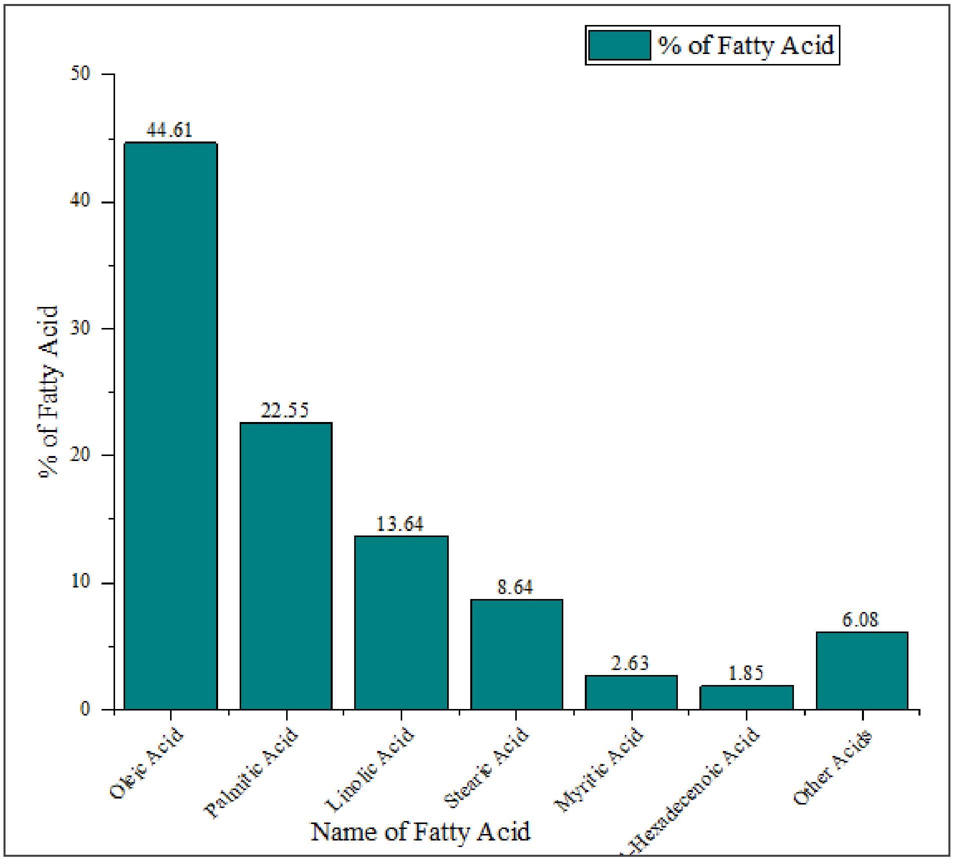

Figure 15 Fatty acid composition of Jatropha oil

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Jatropha curcas l. seed is a good source of oil due to its high percentage of oil yield obtained in the present study. The proximate analysis is a quantitative method used in determining the macronutrient content of the Jatropha seeds. For this analysis, the moisture content, crude protein, crude fat, ash content, crude fiber and carbohydrate were conducted. Since moisture content of a feed sample is related to the shelf life of the sample, low-moisture content may imply higher shelf life. The low moisture content of the seed (6.3621% wt.) is expected to last longer on storage conditions. The crude fat content of the seed was found to be relatively high (46.57% wt.) suggesting that the seed is potentially useful as oilseeds.

Three main operating parameters affecting the solid liquid extraction of Jatropha seeds were optimized based on the maximum oil yield extracted from the seeds. The optimum conditions for the lab scale solid liquid extraction were obtained at temperature of 70°C, solid to solvent ratio of 10:1, 0.66 mm particle size and hexane as a solvent. A central composite design was used in order to investigate the individual and combined effects of the process parameters. The highest oil was found to be 55.30% by weight. Soil condition and improper processing techniques such as prolong exposure of harvested seeds to sunlight can impair the oil yield considerably [87].

The GC-MS analysis result showed that Jatropha curcas oil is predominantly made up of the following major saturated fatty acids: palmitic (22.55%) and stearic acids (8.64%); the main unsaturated fatty acid were oleic(44.61%) and linoleic acids(13.64%) with small amounts of other acids. This result is relatively in agreement with the values in the literature indicated in Table 2.The prevalence of the unsaturated fatty acids indicates that the J. curcas oil is of the unsaturated type. Oil containing high amount of polyunsaturated fatty acids tend to exhibit poor oxidation stability, and may not be useful at low temperatures due to a high pour points, but can find an application in the surface coating industries.

The increasing emphasis on the use of quality Jatropha oil for the production of biodiesel and for direct use required a rapid and reliable analysis, so as to reveal some properties that are so insensitive to manual analysis. Fourier transform infrared spectrometry (FTIR) was used to evaluate the possible functional groups present in the Jatropha oil. Jatropha curcas oil was run through FTIR, the bonds as well as functional groups present are found to respond differently to the incoming radiation, due to variation in their molecular vibration of stretching and bending. Based on the analysis, functional components like alcohols, phenols, alkanes, easters, aldehydes were identified.

5.2 Recommendations

Jatropha curcas provides great opportunities for human as an alternative source of energy reducing fossil fuel. Although chemical extraction method gives the highest oil yield, the high cost of the solvents and the consequences of these chemicals to the environment make the process less likely to be profitable. The problems associated with the high production costs and environmental issues are less common when using mechanical extraction method even though the extraction of oil yield is very small. Further research on low cost solid-liquid extraction must be further developed

The country, Ethiopia, should make very strong policies on how to promote, source and coordinate Jatropha development and investment opportunities in all geographic locations of the country towards the purpose of social economic development and the alleviation of poverty.

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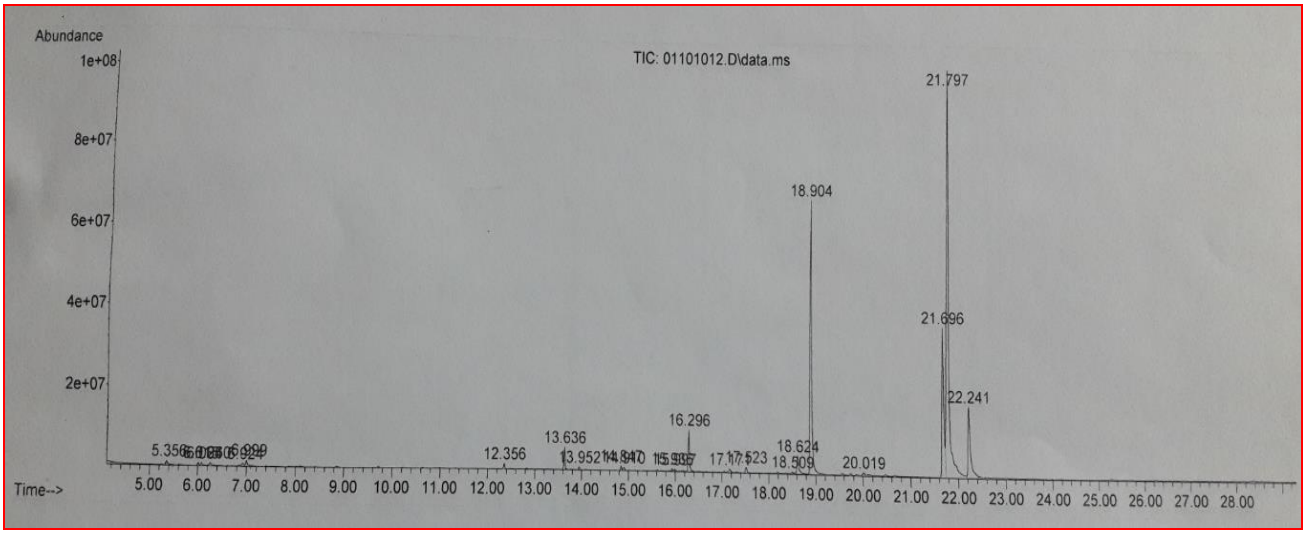
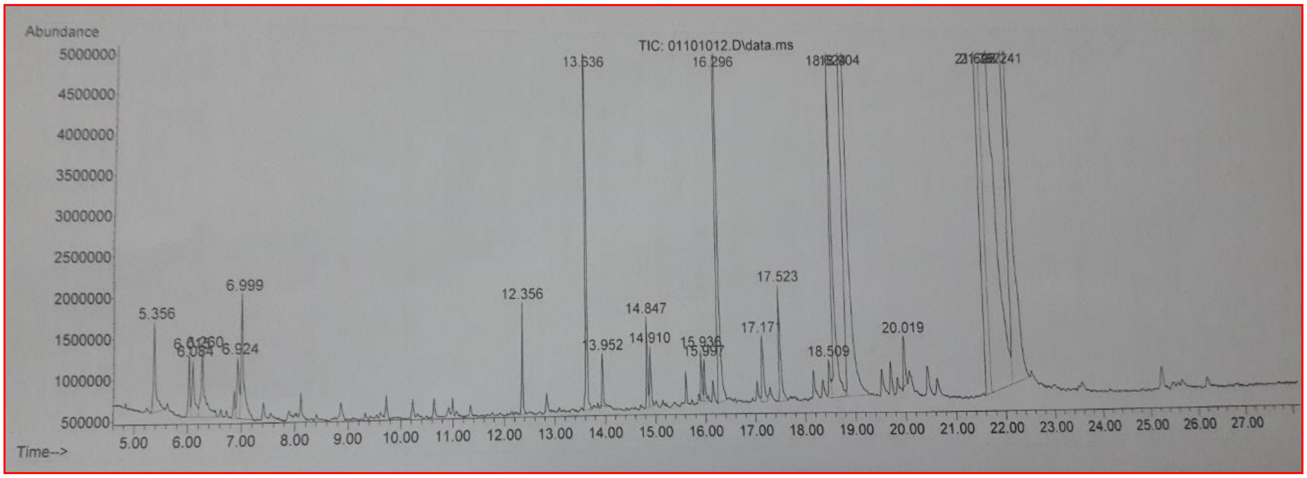
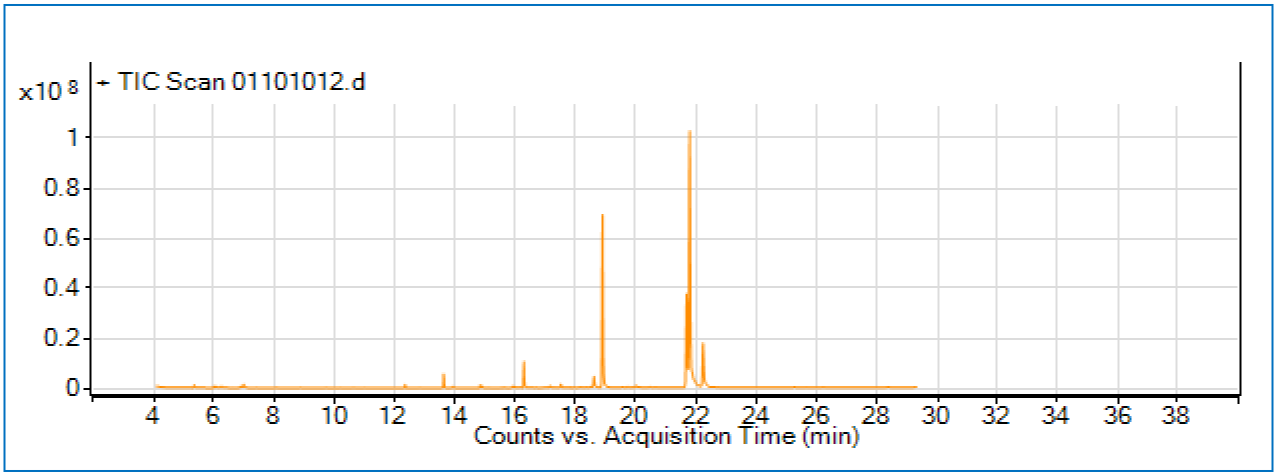
LIST OF APPENDIXES

Appendix A

GC-MS Analysis Result







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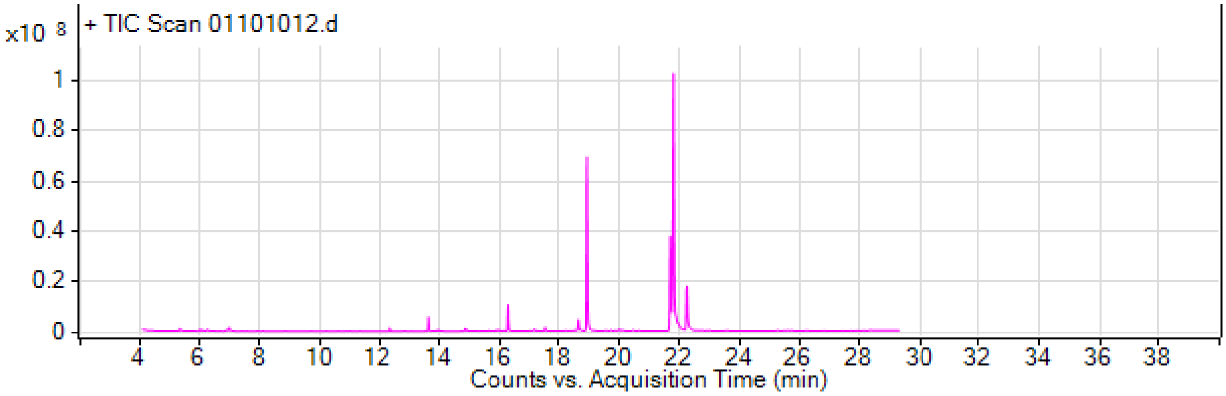

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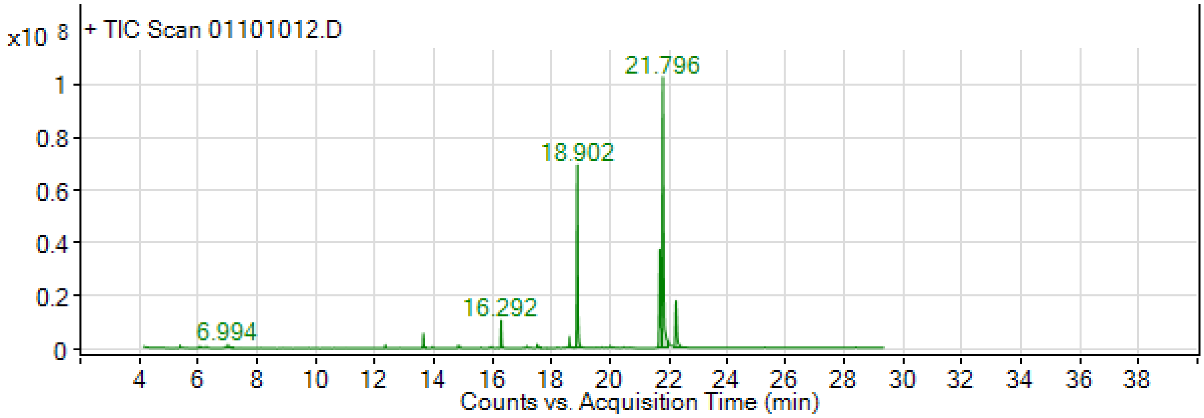
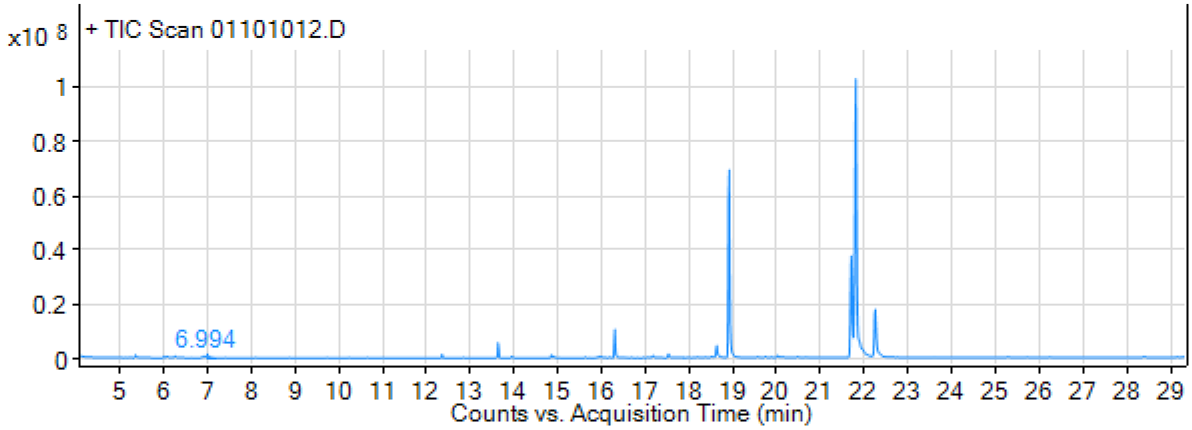


Compounds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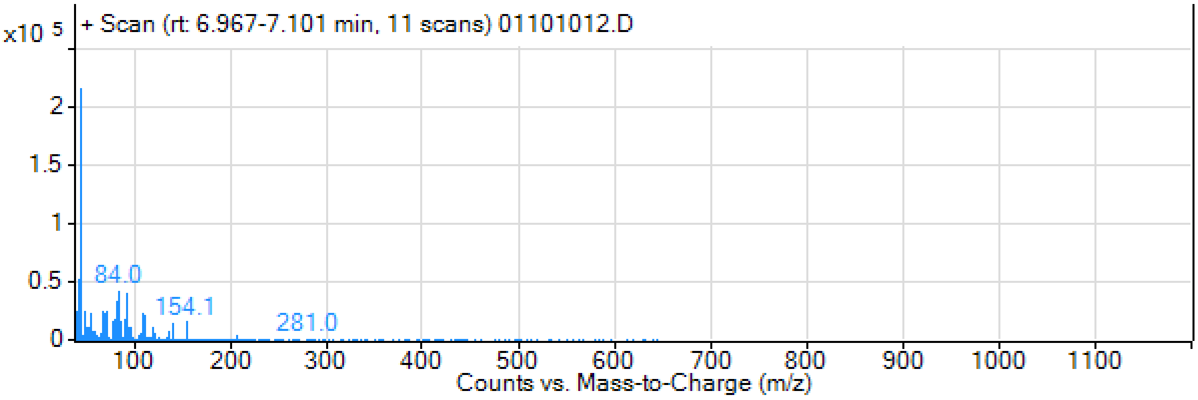
Counts vs. Acquisition Time (min)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281.0
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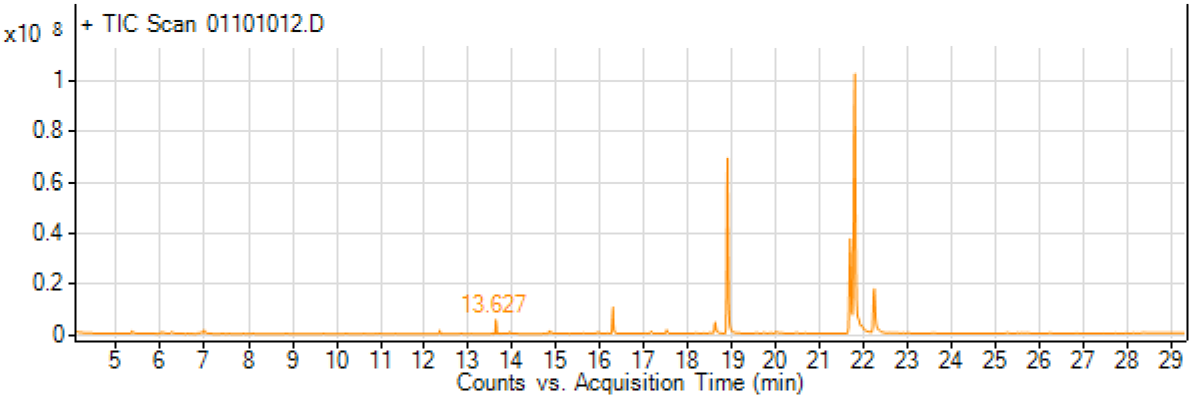
Counts vs. Mass-to-Charge (m/z)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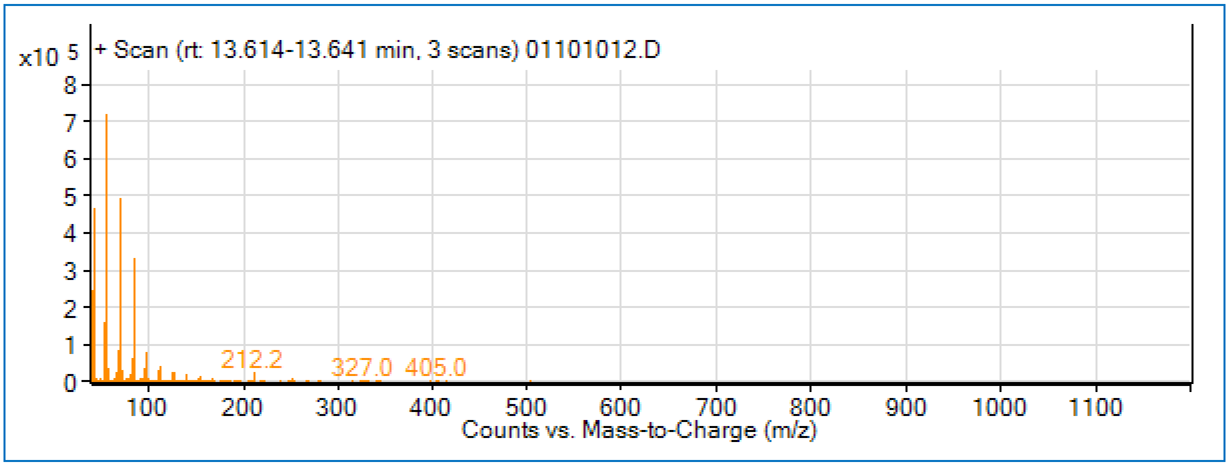
Counts vs. Acquisition Time (min)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8
7
6
5
4
3
2
1
0
212.2
327.0
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200
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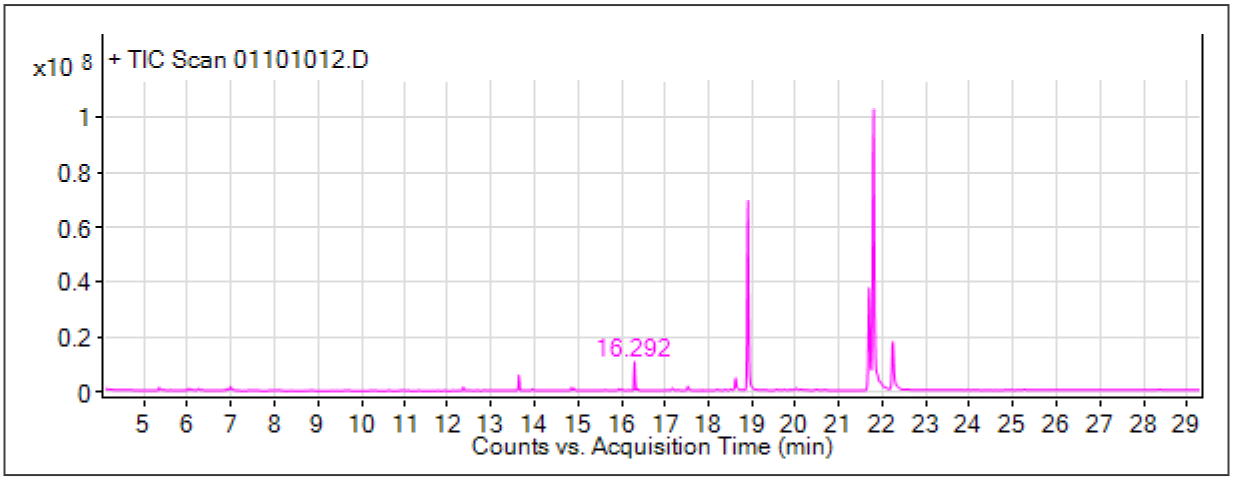
400
500
600
700
800
900
1000
1100
Counts vs. Mass-to-Charge (m/z)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5
6
7
8
9
10
11
12
13
14
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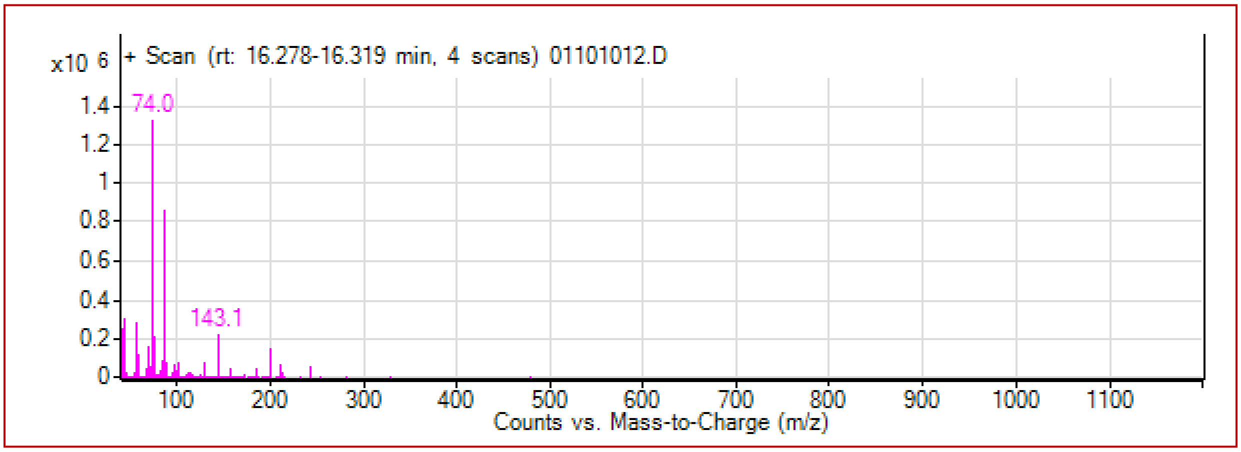
16
17
18
19
20
21
22
23
24
25
26
27
28
29
Counts vs. Acquisition Time (min)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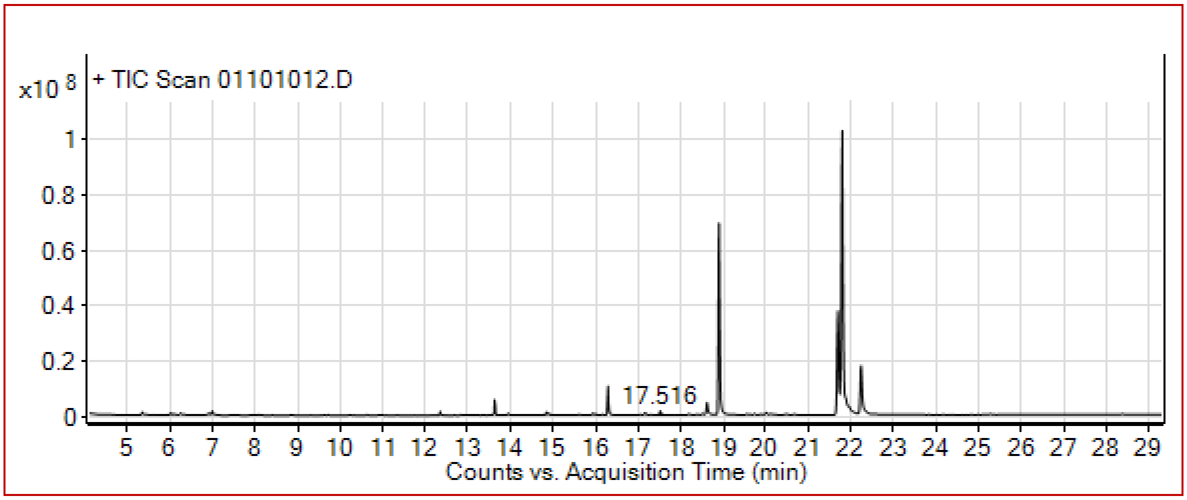
1.2
1
0.8
0.6
0.4
0.2
0
74.0
143.1
100
200
300
400
500
600
700
800
900
1000
1100
Counts vs. Mass-to-Charge (m/z)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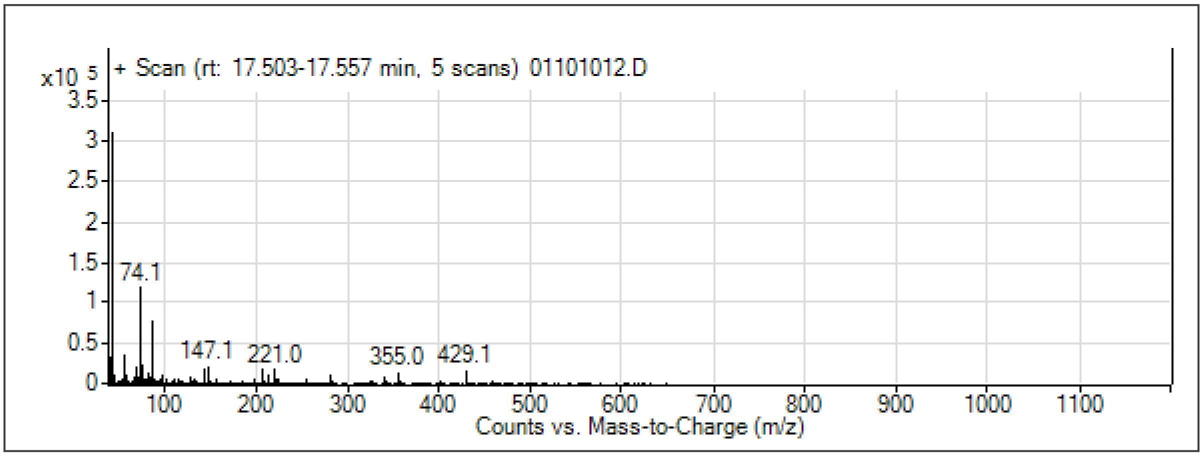
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2
1.5
1
0.5
0
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147.1
221.0
355.0
429.1
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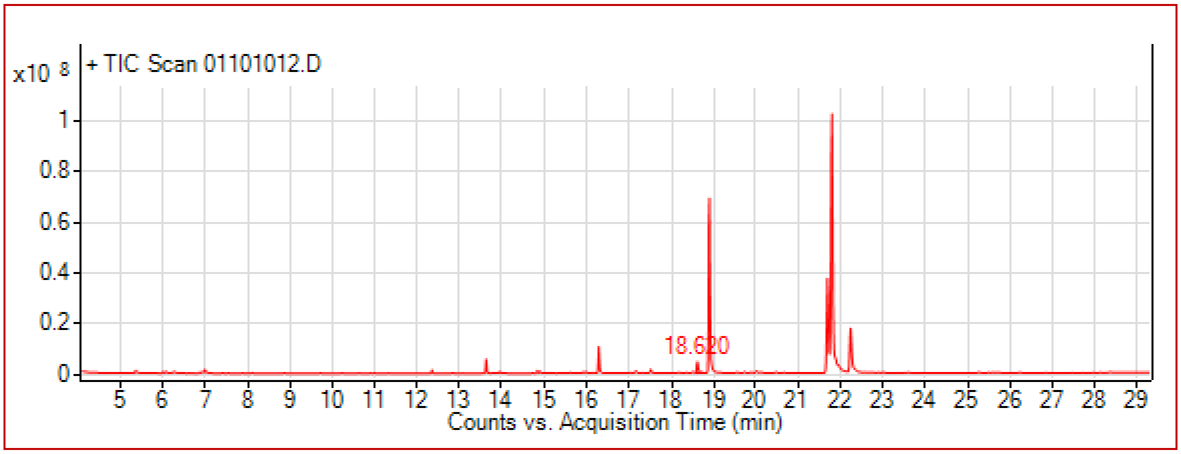
Counts vs. Mass-to-Charge (m/z)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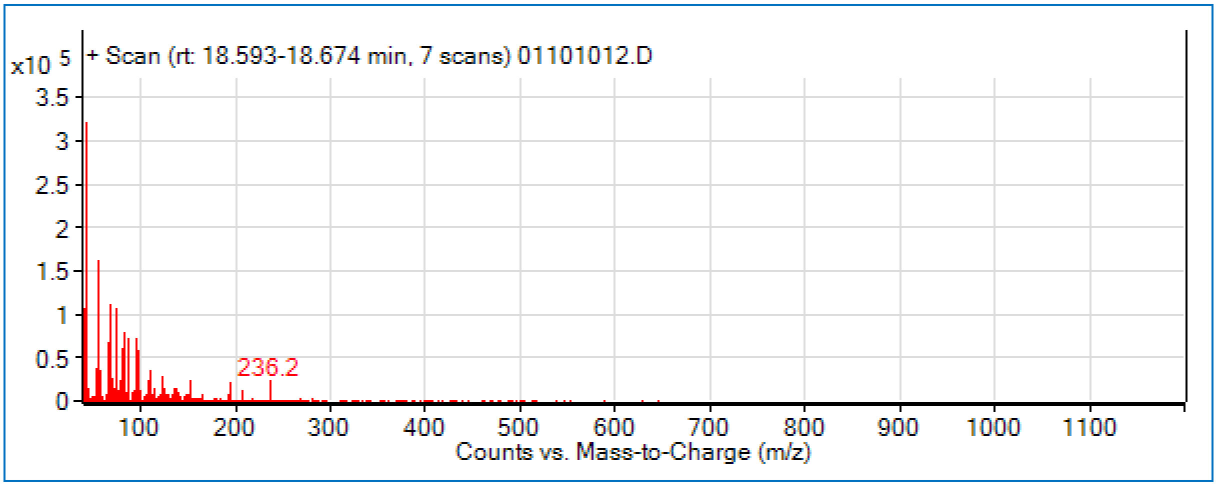
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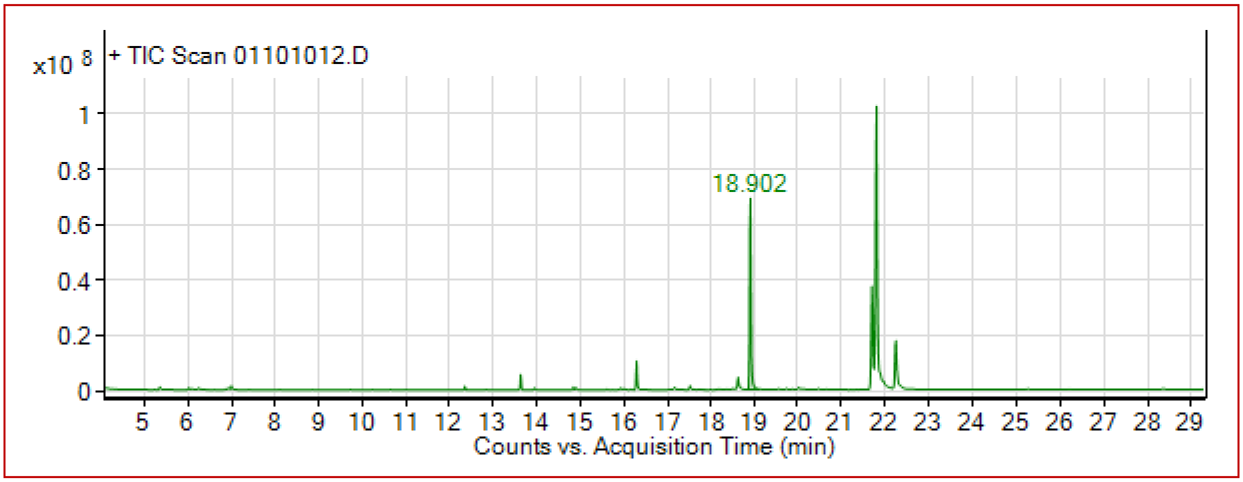
300
400
500
600
700
800
900
1000
1100
Counts vs. Mass-to-Charge (m/z)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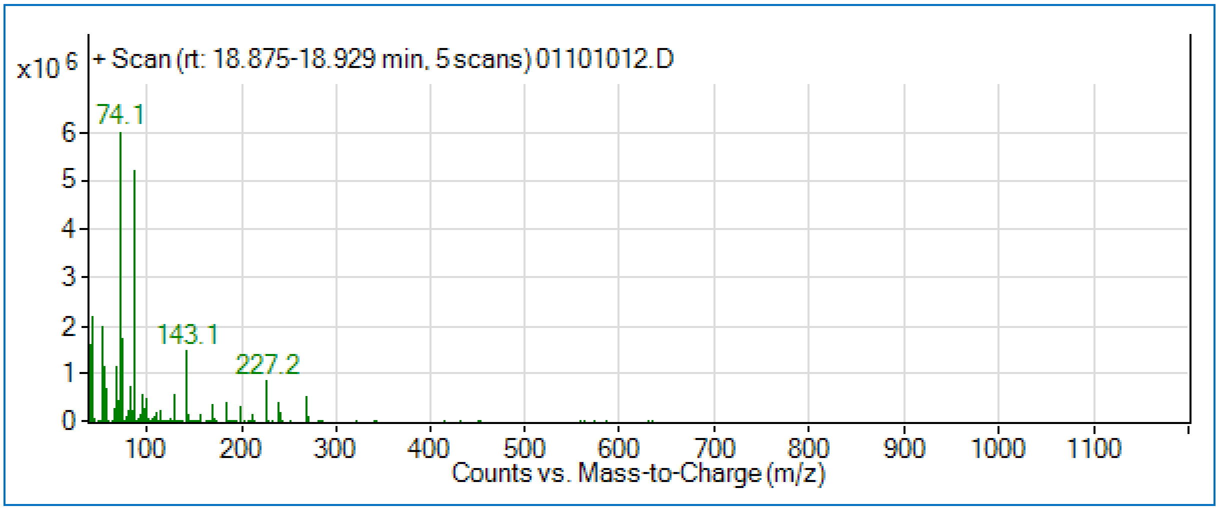
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
Counts vs. Acquisition Time (min)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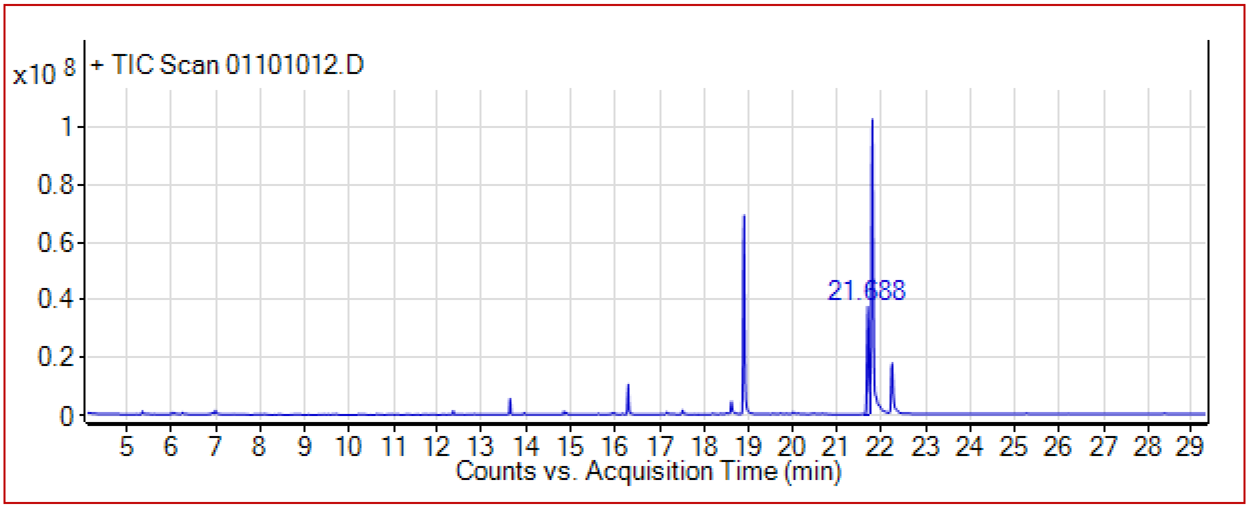
900
1000
1100
Counts vs. Mass-to-Charge (m/z)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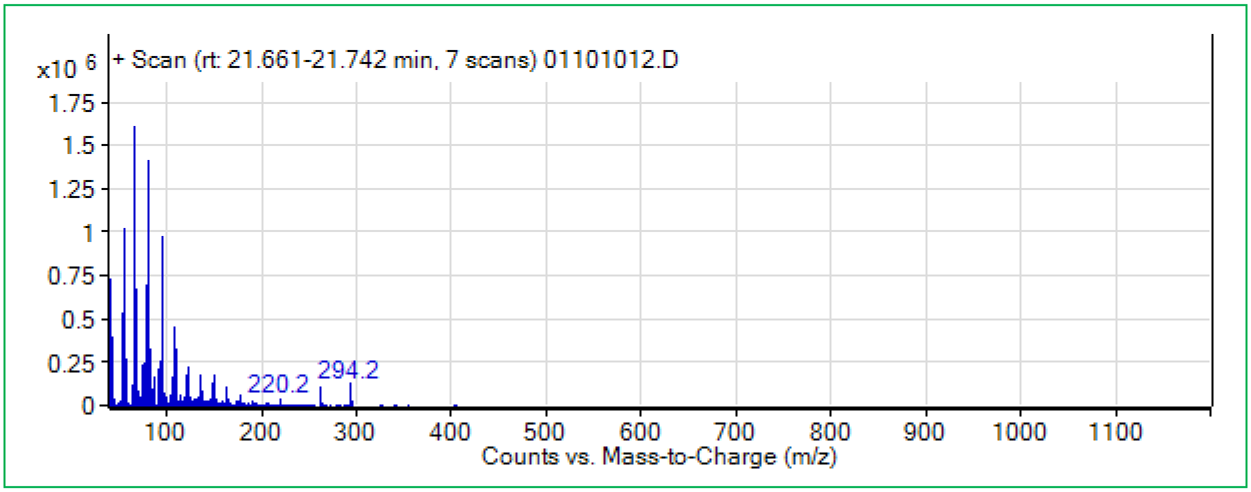
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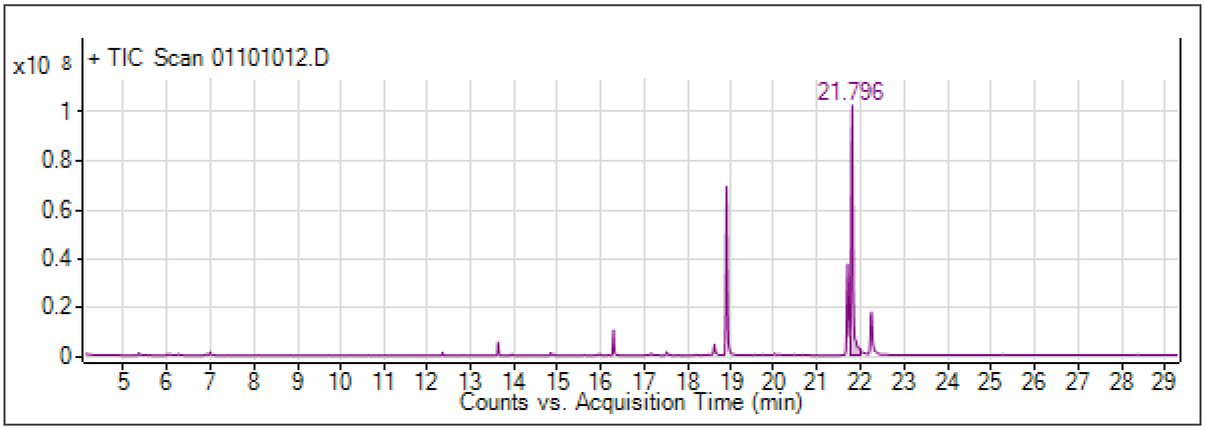
Counts vs. Mass-to-Charge (m/z)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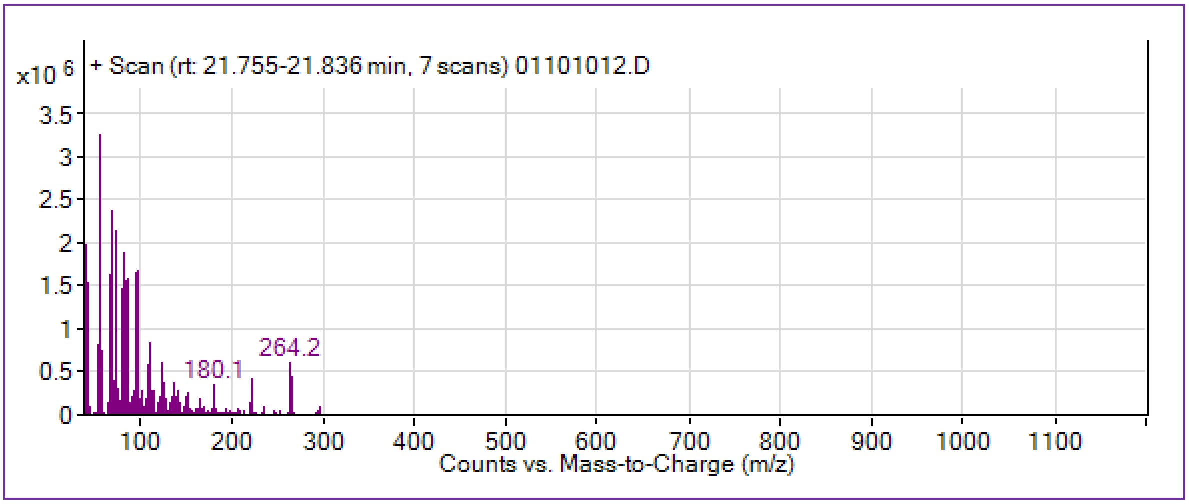
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2
1.5
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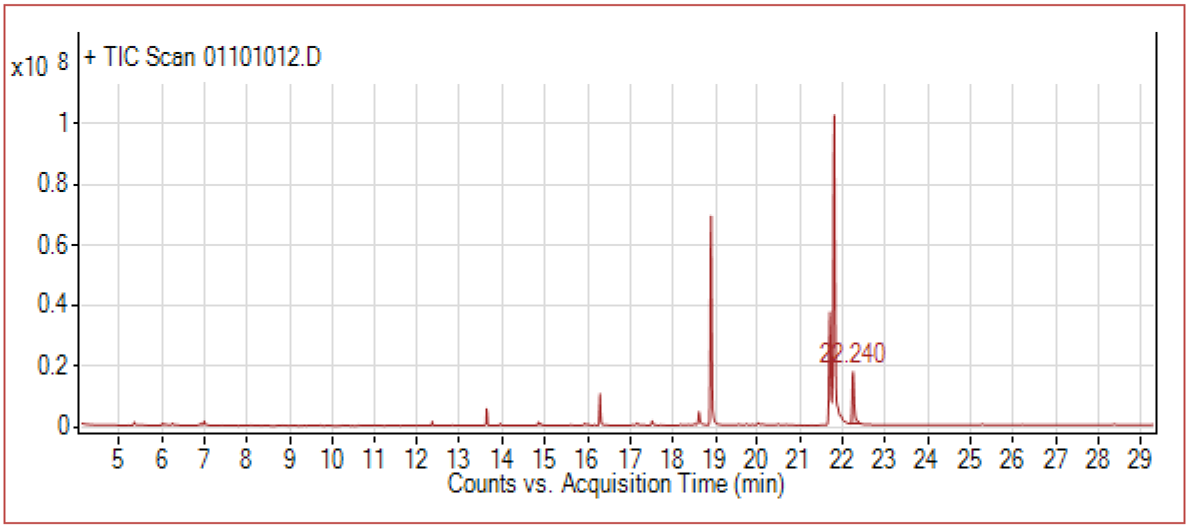
800
900
1000
1100
Counts vs. Mass-to-Charge (m/z)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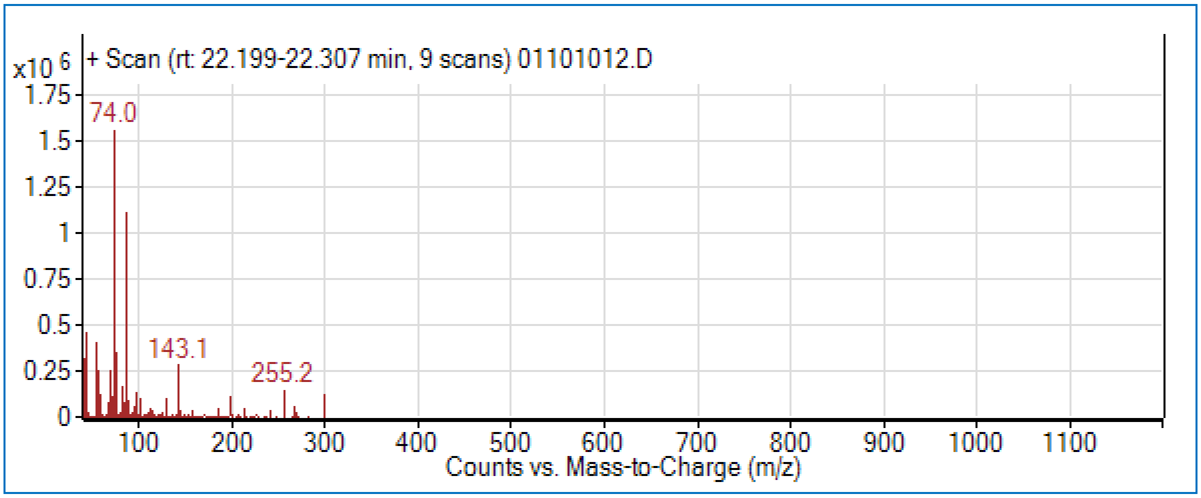
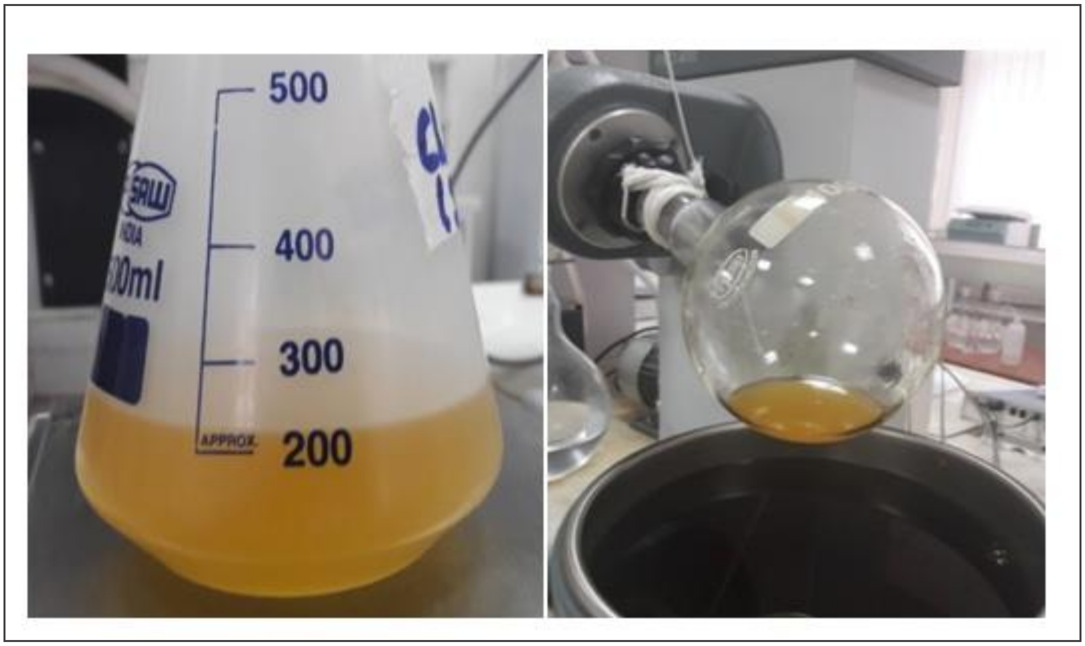
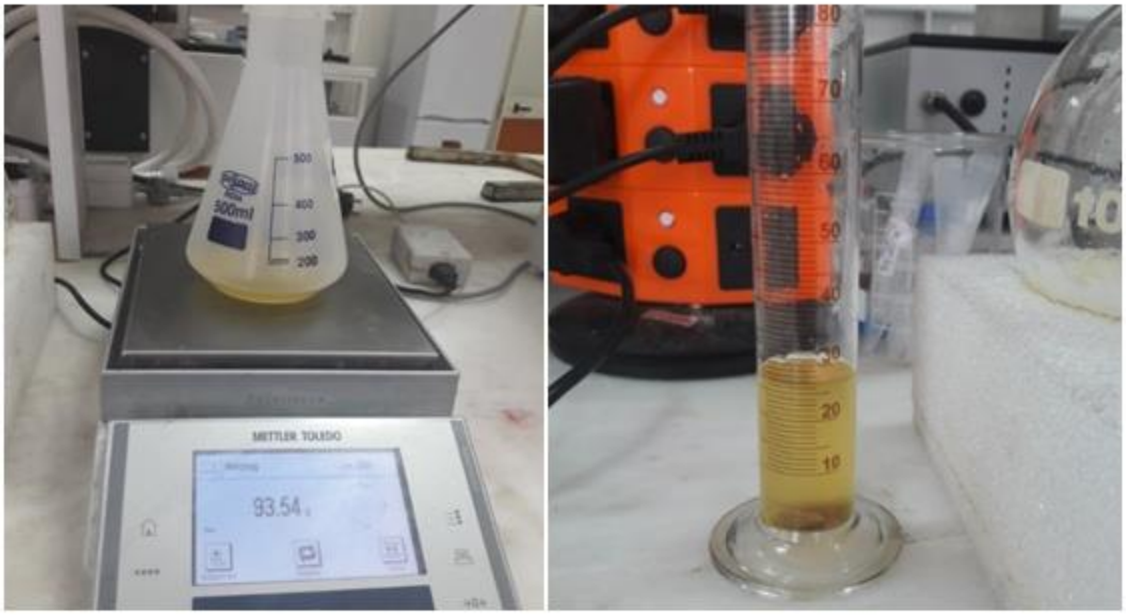
0.6
0.4
0.2
0
22.240
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
Counts vs. Acquisition Time (min)

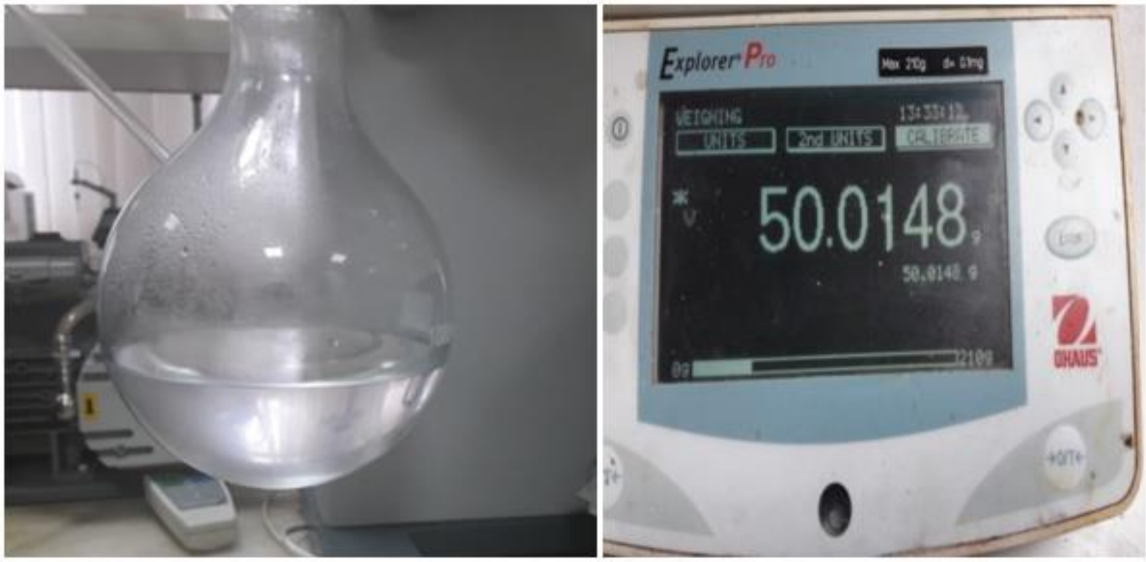

x10 6
+ Scan (rt: 22.199-22.307 min, 9 scans) 01101012.D
1.75
1.5
1.25
1
0.75
0.5
0.25
0
74.0
143.1
255.2
100
200
300
400
500
600
700
800
900
1000
1100
Counts vs. Mass-to-Charge (m/z)

Appendix B

Different Pictures Used in the Methodology




500ml
METTLER TOLEDO
93.54 g
80
70
60
50
40
20
10


Explorer Pro
Max 210g d= 0.1mg
WEIGHING
13:33:12
UNITS
2nd UNITS
CALIBRATE
50.0148 g
50.0148 g
0g
210g
OHAUS


PSAW
INDIA
500ml
500
400
300
200
100