EVALUATION OF PHYSICO-CHEMICAL PROPERTIES, FATTY ACID COMPOSITIONS AND BIODIESEL CHARACTERISTICS OF *Brucea javanica* AND *Hibiscus sabdariffa* SEED OILS AS SUSTAINABLE BIOFUEL

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KUALA LUMPUR

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Name of Candidate: **KHALILULLAH**

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Title of Project: **Evaluation of Physico-Chemical Properties, Fatty Acid Compositions and Biodiesel Characteristics of *Brucea javanica* and *Hibiscus sabdariffa* Seed Oils as Sustainable Biofuel**

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Evaluation of Physico-Chemical Properties, Fatty Acid Compositions and Biodiesel Characteristics of *Brucea javanica* and *Hibiscus sabdariffa* Seed Oils as Sustainable Biofuel

ABSTRACT

The biodiesel demand in worldwide is expected to rise sharply in the future because of its environmental friendly and renewable nature. Recently, biodiesel is mostly produced from edible feedstocks such as soybean, sunflower and palm oil. However, the competition of edible oil sources as food versus fuel makes edible oil not an ideal feedstock for biodiesel production. Biofuel production from non-edible plant seed oils is one of the effective way to anticipate the problems associated with fuel scarcity, food versus energy crisis and environmental pollution. In this study, exploration of the alternative non-edible feedstocks such as *Brucea javanica* seed oils (BJO) and *Hibiscus sabdariffa* seed oils (HSO) were investigated to be used for the production of biodiesel. Transesterification parameters such as methanol to oil ratio, temperature, catalyst and agitation were optimized by using Box-Behnken experimental design based on response surface methodology (RSM). The analysis of different oil properties, fuel properties and optimization of transesterification parameters for both feedstocks were investigated. The physicochemical properties of BJO investigated were found to be at the refractive index of 1.85, ultrasonic speed of 1.34 ms^{-1}, acid value of 4.546 mg KOH/g, oxidative stability of 3 h according to the rancimat test and kinematic viscosity was of 17.428 $mm^{2/s}$. On the other hand, HSO properties were refractive index of 2.16, ultrasonic speed of 1.52 ms^{-1}, acid value of 5.486 mg KOH/g, oxidative stability of 3.48 h according to the rancimat test and the kinematic viscosity of 14.228 $mm^{2/s}$. When converted into biodiesel using RSM experimental design, the optimum parameters found for converting *Brucea javanica* seed oils to biodiesel were 65 ^{o}C temperature, 1 % catalyst and 6:1 methanol to oil ratio with

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the highest yield of 94.34 %. In the case of *Hibiscus sabdariffa* seed oils, a methanol to oil ratio of 6:1, temperature 67.5 ^{o}C, catalyst 1% with a yield of 93.01 % were found to be the optimum parameters. The fuel properties of biodiesel produced were within the range of international standards such as EN14214 and ASTM standards of biodiesel except for the oxidative stability. To increase oxidative stability of the obtained biodiesel, the effectiveness of different antioxidants such as gallic acid (GA), tert-butyl-4-methylphenol (BHT), butylated hydroxyanisole (BHA), propyl gallate (PY) and t-butyl hydroquinone (TBHQ) were evaluated. All the antioxidants showed an improvement in the induction period of biodiesel with propyl gallate showing the highest efficiency in improving the oxidative stability of biodiesel up to 70 h. Both the feedstocks were successfully converted into biodiesel and a high biodiesel yield could be obtained using optimized transesterification parameters. Hence, it could be concluded in this study that non-edible feedstocks such as *Brucea javanica* and *Hibiscus sabdariffa* seed oils are suitable for biodiesel production that meets with the quality requirements of international standards as well as non-conflicting with food production.

Keywords: *Brucea javanica*, *Hibiscus sabdariffa*, Biodiesel, Response surface methodology, Antioxidants

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Penilaian terhadap Ciri-Ciri Fizikokimia, Komposisi Asid Lemak dan Ciri-Ciri Biodiesel bagi Minutesyak Bijian *Brucea javanica* dan *Hibiscus sabdariffa* sebagai Biobahan Api Lestari

ABSTRAK

Perminutestaan biobahan api global dijangka akan meningkat di masa hadapan kerana sifat mesra alam sekitar dan ciri-ciri boleh baharunya. Baru-baru ini, biobahan api seringkali dihasilkan daripada sumber boleh dimakan seperti minutesyak kacang soya, bunga matahari dan kelapa sawit. Walaubagaimanapun, persaingan di antara sumber-sumber boleh dimakan sebagai makanan dengan bahan api menjadikan minutesyak boleh dimakan sebagai sumber biodiesel yang tidak sesuai. Penghasilan biobahan api daripada minutesyak bijian tumbuhan yang tidak boleh dimakan adalah salah satu cara efektif untuk menyelesaikan masalah kekurangan bahan api, krisis makanan dan tenaga serta pencemaran alam sekitar. Dalam kajian ini, eksplorasi terhadap sumber-sumber alternatif dari minutesyak bijian *Brucea javanica* dan *Hibiscus sabdariffa* telah dijalankan untuk penghasilan biobahan api. Parameter-parameter transesterifikasi seperti nisbah metanol kepada minutesyak, suhu, pemangkin dan agitasi telah dioptimumkan menggunakan reka bentuk eksperimental Box-Behnken berdasarkan metodologi tindak balas permukaan (RSM). Analisis pelbagai ciri-ciri minutesyak, ciri-ciri bahan api dan proses mengoptimumkan parameter-parameter transesterifikasi untuk kedua-dua sumber biobahan api telah dijalankan. Ciri-ciri fizikokimia bagi BJO ialah 1.85 untuk indeks biasan, kelajuan ultrasonik 1.34 ms-1, nilai asid 4.546 mg KOH/g, kestabilan oksidasi 3 jam menurut ujian rancimat serta kelikatan kinematic 17.428 mm2/s. Bagi HSO pula, hasil analisis ialah nilai 2.16 indeks biasan, kelajuan ultrasonik 1.52 ms^{-1}, nilai asid 5.486 mg KOH/g, kestabilan oksidasi 3.48 jam menurut ujian rancimat serta kelikatan kinematic 14.228 $mm^{2/s}$. Selepas ditukarkan kepada biodiesel menggunakan reka bentuk

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eksperimental RSM, parameter optimum untuk minutesyak bijian *Brucea javanica* ialah 65 ^{o}C suhu, 1 % pemangkin dan 6:1 nisbah metanol kepada minutesyak dengan hasil tertinggi 94.34 %. Untuk minutesyak bijian *Hibiscus sabdariffa* pula, nisbah 6:1 metanol kepada minutesyak, suhu 67.5 ^{o}C, 1 % pemangkin dengan hasil tertinggi 93.01 % telah dijumpai. Ciri-ciri biodiesel yang dihasilkan adalah di dalam julat kod piawai antarabangsa seperti EN14214 dan ASTM kecuali untuk parameter kestabilan oksidasi. Bagi meningkatkan kestabilan oksidasi biodiesel yang dihasilkan, keberkesanan beberapa jenis antioksidan seperti asid galik (GA), tert-butyl-4-metilfenol (BHT), butylated hidroksianisol (BHA), propil gallate (PG) dan tert-butyl-hidrokuinon (TBHQ) telah diperiksa. Kesemua antioksidan dilihat menambahbaik tempoh induksi biodiesel dengan propil gallate menunjukkan keberkesanan tertinggi dalam meningkatkan kestabilan oksidasi biodiesel sehingga 70 jam. Kedua-dua sumber biobahan api ini telah berjaya ditukarkan kepada biodiesel dan hasil yang tinggi boleh dicapai menggunakan parameter-parameter optimum yang didapati. Oleh itu, dapat disimpulkan bahawa dalam kajian ini yang sumber-sumber biobahan api tidak boleh dimakan seperti minutesyak bijian *Brucea javanica* dan *Hibiscus sabdariffa* adalah sesuai untuk penghasilan biodiesel yang menepati keperluan kualiti kod piawai Antarabangsa serta tidak menimbulkan konflik dengan penghasilan makanan.

Katakunci: Melada pahit, Roselle, Biodiesel, Metodologi tindak balas permukaan, antioksidan

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LIST OF SYMBOLS AND ABBREVIATIONS

ASTM	:	American Society for Testing and Materials
AV	:	Acid Value
B100	:	Pure biodiesel
BJB	:	*Brucea javanica* Biodiesel
BJO	:	*Brucea javanica* Oil
CI	:	Compression Ignition
CN	:	Cetane Number
DG	:	Diglyceride
E	:	Alkyl esters
et al	:	et alia (and others)
EU	:	European Union
FAME	:	Fatty Acid Biodiesel
FFA	:	Free Fatty Acid
GC	:	Gas chromatography
h		hour
K	:	Kelvin
Max	:	Max
ME	:	Biodiesel
MG	:	Monoglyceride
Minutes	:	minutes
Mol	:	Molar Ratio
N/A	:	not available
N/S	:	not specified
ppm	:	Parts per million

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R	:	Universal molar gas constant (in Arrhenius equation)
R^2	:	Sum of error squares
Temp	:	Temperature
TG	:	Triglyceride
wt.%	:	Weight percentage (weight of solute/ weight of solvent*100)

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CHAPTER 1: INTRODUCTION

1.1 Background of Study

Life is dependent on energy as it is a basic need to power the generation sector, to run transportations and to smoothly carry on industrial work. Currently, petrochemical reservoirs are the major source of meeting the requirements of most of the world's energy such as natural gas, coal and petroleum (Suganthi & Samuel, 2012). Fossil fuels are used extensively for energy production especially petroleum but concern about its sustainability leads towards emphasis in alternative fuel resources (Verma et al., 2016a). Interests are diverted in to new alternative renewable sources for energy which can reduce environmental pollution and dependency on fossil fuel which are depleting and non-renewable such as biofuels (Ong et al., 2013).

Worldwide biofuel demand is increasing rapidly because of the shrinking of oil reservoirs and environmental concerns such as global warming and emission of toxic gases to the environment (Balat & Balat, 2009). Biofuel is a broad term which is produced from different biological sources and feedstocks such as plants, forestry by products, agricultural crops and wastes and animal fats. As far as Malaysia's oil producing capacity is concerned, it has dropped considerably to 13% from 2006 to 2008 and it is predicated that the under surface crude oil in Malaysia could diminished in 20 years' time (Oh et al., 2010). This will become a major concern to the country as two-thirds of the consumption in Malaysia is petro-diesel (Jayed et al., 2011).

Amongst biofuels, biodiesel is gaining much more importance due to the decreasing amounts of petroleum fuel reservoirs and its several advantages such as renewability, minimal degradation duration and its non-hazardous effects. Biodiesel is produced through transesterification from different sources such as waste cooking oil, animal fats and vegetable oil (Hincapié,et al., 2011). Biodiesel production have also been reported

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several times from feedstocks such as tea oil (Demirbas, 2010), cotton oil (Qian et al., 2010), tobacco oil (Usta et al., 2011), palm oil (Nur et al., 2014), palm kernel oil (Awalludin et al., 2015), canola oil (Kai et al., 2014) and soybean oil (Samart et al., 2010). Biodiesel is derived from bio-based resources and could be a potential alternative to petroleum diesel because of its renewable characteristics. Different solvents have been used in transesterification process of biodiesel production but methanol has advantages in reactivity and cost-effective. Biodiesel can be considered as completely renewable fuel (Verma et al., 2017). Edible plant oils and fats from animals are consumed as food and their usage as feedstock for biodiesel is questionable with great concern. As vegetable oil is used for food and its demand has increased in recent years especially in third world countries, it is difficult to justify its usage as biodiesel feedstock. Recent report revealed food versus fuel competition such as soybean oil in North America, palm and coconut oil in Malaysia, castor in Brazil and jatropha in India (Sanli & Canakci, 2008).

Non-edible oils such as *Jatropha curcas* (ratanjyot), *Madhuca longifolia* (mahua), *Azadirachta indica* (neem), *Pongamia pinnata* (karanj) and *Moringa oleifera* (moringa seed) are commercially available non-edible feedstocks for biodiesel (Verma et al., 2016b). Other non-edible oils such as *Nicotiana tabacum* (tobacco), *Acrocomia aculeate* (macaúba), *Crambeabyssinica* (hochst), linseed oil, rubber seed oil, *Sapium sebiferum* (chinese tallow), *Sapindus mukorossi* (soapnut), *Euphorbia tirucalli* (milk bush), *Calophyllum inophyllum* (polanga) and nahor oil have been used as feedstocks (Demirbas, 2007). Therefore, more emphasis is now given to non-edible seed oils as a source for biodiesel. There are various factors which effects the production of biodiesel such as methanol to oil ratio, catalyst percentage and reaction temperature. Therefore, transesterification optimization is of utmost importance to increase the biodiesel yield.

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Biodiesel oxidation stability is important because when the oxidative reaction occurs, biodiesel deteriorates. The major problem associated with biodiesel is its poor stability and storage performance (Verma et al., 2015). Oxidation of lipids are more or less the same in characteristic as biodiesel oxidation. In addition to the oxidation process, polymers may form when unsaturated fatty acids are present, which in turn will lead to a higher molecular weight product that will increase its viscosity. The oxidation of biodiesel could lead to many mechanical problems such as deposit formation, fuel system corrosion and filtering problems (Monyem & Gerpen, 2001).

At present, even though there is a large number of studies published in the literature pertaining to biodiesel, lack of studies focused on using non-edible feedstock such as *Brucea javanica* and *Hibiscus sabdrfiffa* and so far there is no optimization of biodiesel parameters for these feedstock and no literature describe improvement of biodiesel improving oxidative stability from *Brucea javanica* and *Hibiscus sabdariffa*. In this research, an effort has been made to study the production biodiesel from non-edible *Brucea javanica* and *Hibiscus sabdariffa* seed oils to optimize its transesterification parameters and to improve oxidative stability of biodiesel with antioxidants. *Brucea javanica* and *Hibiscus sabdariffa*, grows naturally in Sri Lanka and India to China, Indonesia, Malaysia, New Zealand and Australia. Its habitat includes open areas, secondary forest and sometimes sand dunes (Dai et al., 2007).

1.2 Problem Statement

There are different potential feedstocks for biofuel production. Globally, conventionally grown edible oils are the major feedstocks used for biodiesel production. This causes an imbalance between the utilization of energy sources and food consumption and contributes to the higher cost of biodiesel feedstocks. So, the non-edible plant seed oils which are also known as the second generation feedstocks could be considered as

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promising substitutions for the production of biofuel. The use of non-edible plant oils is very significant. Therefore, production of biofuel from non-edible oils is an effective way to overcome all the associated problems. The current study is intended to investigate the feasibility of producing biodiesel. The biodiesel production is a very complex process that is affected by variety of factors. It oxidized very quickly when expose to air or water and if stored for long time. It is a being a mixture of biodiesel of vegetable oil fatty acids, is more susceptible to oxidation than mineral diesel. The lower oxidative stability of biodiesel is caused by the higher level of unsaturation and possibly by the larger amount of dissolved oxygen. The storage stability of biodiesel is found to be deteriorated during storage, and it is suggested the addition of antioxidants to ensure storage stability. Synthetic and natural antioxidants were used previously which show good results (Mittelbach & Schober, 2003). But the synthetic antioxidants such as BHA produces higher hydrocarbon emission and BHT produces higher nitrogen oxide (Fattah et al., 2014).

1.3 Objectives of Study

1. To extract oil from *Brucea javanica* and *Hibiscus sabdariffa* seeds using solvent extraction in soxhlet apparatus.
2. To characterized Physico-chemical properties and fatty acid compositions of *Brucea javanica* and *Hibiscus sabdariffa* seed oils.
3. To investigate the optimum reaction conditions for biodiesel production from crude *Hibiscus sabdariffa* and *Brucea javanica* seed oils by parameter optimization.
4. To characterized biodiesel produced (cloud point, pour point, flash point, acid value, sulphur content, cetane number, density and kinematic viscosity) in accordance with several international standards.
5. To test antioxidant activity of *Brucea javanica* and to optimize oxidative stability of biodiesel with different antioxidants.

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1.4 Significance and Justification of the Study

Increasing uncertainty about global energy production and supply, environmental concerns due to the use of fossil fuels, high price and unsustainable nature of petroleum products are the major reasons to search for alternatives to fossil diesel. Having regard to the rate of actual energy consumption, petroleum resources are getting depleted as well as causing environmental hazards of global warming, natural disasters etc. Thus, there is the need to focus on new energy sources that can replace fossil fuel and meet emission standards. Biodiesel is proven to be one of the best available sources to fulfil the energy demand and to meet the emission standards (Agarwal & Agarwal, 2007). However, the continual use of edible oil for biodiesel production will cause food insecurity. In the current scenario, 95% production of biodiesel in the world is from edible oil because of their large scale availability from agricultural industry. On the other hand, if this continues without proper planning than there will be crisis of food vs energy in future (Gui et al., 2008). Thus, the need to focus on non-edible oil feeds such as waste vegetable oil and *Jatropha curcas*. The use of waste vegetable oil as biodiesel feedstock will help mitigate the environmental hazards posed by the improper handling of waste vegetable oil. Also, the use of waste and other non-edible oils as biodiesel feedstock will reduce the cost of biodiesel production since the feedstock costs constitutes approximately 70-95 % of the overall cost of biodiesel production. Hence, the use of non-edible seed oils need to be given higher priority over the edible oils as biodiesel feedstock.

1.5 Government Policy Relevance

Our project and research will relate the Malaysian government biofuel policy which clearly reflect and supports to promote biodiesel. Some of the policies of the government are diesel for land and sea transport will be a blend with 5% processed palm oil and 95% petroleum diesel. This ‘B5’ would be made available throughout the country, promote research, development and commercialization of biofuel technologies, encourage and

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facilitate the establishment of plants for producing biofuel for export and enhance the quality of the ambient air, reduce the use of fossil fuels and minimize emissions of greenhouse gases (mainly carbon dioxide), carbon monoxide, sulphur dioxide and particulates through increased use of biofuels (Adapted from National Biofuel Policy Malaysia, 2014).

1.6 Impact on Society, Economy and Nation

An established technology, if attained, will be able to contribute towards the alternative energy resources for Malaysia. Resolution of this issue, if achieved will have direct positive impact on the society and economy through improved environment and a more sustainable energy industry and overall economy of the nation.

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CHAPTER 2: LITERATURE REVIEW

2.1 Biodiesel

Petroleum products consumption is increasing and resources are limited. Since the prices of these products are rising very fast. The energy is needed to power transportation sector and the demand for energy is increasing with time and there is a need to look for an alternative source of fuel which should be economical and viable (Srivastava & Prasad, 2000). The diminishing of petroleum based energy and increasing cost of it forced us to think for an alternative source of energy (Meher et al., 2006). On one hand petro-diesel is depleting and on the other hand it also creates air and water pollutions catalyzing climates changes (Orecchini & Bocci, 2007). The biodiesel is thought to be the better option for the fulfilment of the need of energy of the world. The biodiesel is becoming more and more essential as fossil fuels are depleting with time (Basha et al., 2009). Malaysia's oil producing capacity has dropped considerably up to 13% from 2006 to 2008 and it is predicated that under surface crude oil in Malaysia could diminished in 20 year's time. This will become a huge concern to the country as two-third of consumption in Malaysia is petro-diesel (Jayed et al., 2011). Although the fossil fuel resources are declining, but the demands for energy is increasing. Thus, an alternative energy is needed. Due to biodiesel's potential as diesel substitute, the production of biodiesel has showed an increasing trend. However, the biodiesel tend to oxidize easily during storage when compared to the fossil fuel. Increasing environmental issues due to accumulation of wastes forced global measures to resolve these troubles. Various worldwide treaties, including European Union (EU) regulations and commands have been taken to normalize emissions of greenhouse gases to the air in order to increase the use of natural energy sources, and to ensure effective management and utilization of waste of all forms. The atmospheric pollution causes by greenhouse gases, majorly contributed by road transportation. European Union member states are supporting the production and use of

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biofuel in order to reduce the influence of these complications (Sendzikiene et al., 2005). The biodiesel has many advantages over petro-diesel when compared in term of performance as biodiesel such as lower exhaust emission, nontoxic, biodegradable essentially free of sulphur and renewable and hence considered as environmental friendly and sustainable energy (Knothe, 2010).

Biodiesel and diesel engine shares the same history and utilizing of vegetable oils were studied at the time when diesel engine was invented. The word biodiesel was actually derived from the word "bio" which means life and the word "diesel" referred to Rudolf Diesel, a famous German inventor that invented diesel engine in 1893 (Jayed et al., 2011). Rudolf diesel (1858-1913), the diesel engine inventor tested various oils for his engine such as cottonseed oil, palm oil, castor oil and soybean oil. The definition of biodiesel could be "it is a mono alkyl ester of long chain fatty acid". In vegetable oil these esters may be prepared from triglycerides through transesterification with alcohols. The biodiesel could be miscible with petro-diesel so it could be effectively used as natural biodiesel (B100) or mixed with petro diesel (Gerhard, 2005). The biodiesel fuel is renewable fats and oils from mono alkyl ester usually biodiesel that was created by the transesterification reaction to create long chain fatty acid ester. As biodiesel is naturally produce by chemical reaction of vegetable oil or animal fat, carbon in the fats/oil normally originated from carbon dioxide. Thus, it adds less influence mainly in global warming compared to byproduct emitted from fossil fuels such as petrol (Gerhard, 2005).

Biodiesel is also considered as sustainable for the society and the environment because it produce less exhaust emissions. Many methods and procedures are established in order to meet the standard specification of biodiesel. Triglycerides are chemically reacted with alcohol and converted to esters like biodiesel, ethyl esters using acid or alkali catalyst and the process is known as transesterification. Butanol, propanol, ethanol and methanol are

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commonly used alcohols in biodiesel production. In transesterification, triglycerides are converted fatty acid biodiesel and glycerol through consecutive reversible reactions. One molecule of triglycerides produces three ester molecules. There are many methods developed for the production of biodiesel such as acid-catalyzed method, enzymatic catalysis, alkali-catalyzed method and supercritical method (Fukuda et al., 2001). Different raw materials available for producing biodiesel are rapeseed oil, mahuva oil, linseed oil, soya bean oil, sunflower oil, beef tallow, lard, palm oil, cotton seed oil, jatropha oil, pongamia oil, olive oil, rice bran oil and guang-pi. The use of particular raw material depends upon the availability, price and policy (Sekhar, 2010).

2.2 Oil Extraction

Plant seeds are major oil sources that is promoting fuel market as well as food market. Oil extraction from plant seeds involve different processes which can be improved in order to get quality oil.

The modern and conventional oil extraction methods uses different solvent as extractor. In the section method is developed for extraction of oil from *Brucea javanica* and *Hibiscus sabdariffa* without damaging the oil quality.

2.2.1 Oil Extraction Processes

Drying is the initial step in oil extraction processes which reduce the moisture content minimum up to 11-12 %, followed by other physical impurities removal. Dehulling and flaked seeds aid in breaking seeds cells and make oil recovery easier by using solvent or pressing technique. The commonly used methods for extraction of oil are hard/mechanical pressing, solvent extraction and pre-press solvent extraction (Johnson & Lusas, 1983).

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Oil could be extracted by squeezing and pressing and this was the oldest mechanical method used for extraction from seed. Seeds was cooked before pressing in order to reduce their moisture content.

Previously, people used squeezing method to extract oil from plants seeds. Squeezing method involve physical pressure and consist of screw press, the advantages of that method was practically embodiment and energy efficient. It was bounded by a slot cages screw conveyor and plant seed material pass through pitch of the screw. Mechanical method consist of a screw for feed and a barrel cage through which feed moves and subjected to increasing pressures. The movement of material in the barrel generates friction which produce heat and break the protein in the material. Usually, oil residual content of 6–12 % is achieved in plant oils such as soybean and cotton seeds this could be reduced to 2–4 % by hard pressing (Johnson & Lusas, 1983). But, the free place to contain oil was less and oil squeezed via slot and out of material and half of the oil wasted in the process of extraction. The method also required high voltage energy, high maintenance and wear plus higher capacity machines (Johnson & Lusas, 1983).

Prepress solvent is another method used for oil extraction which extract around 30 % oil from plant seeds. At this time, less intensity pressing of the seeds is performed than the hard press method and up to18 % reduction in oil content, earlier using solvent to remove the residual oil (Johnson & Lusas, 1983).

Furthermore, there is another method of oil extraction from plant seeds known as the direct solvent extraction method where a specific solvent is used to extract oil from plant seeds. The most commonly used solvent is hexane. Flaking can help in rupture of cells and optimize oil extraction from seeds by minimizing distance need for oil transfer into solvent. Dialysis, washing, leaching and diffusion are performed to extract residual oil and oil bodies in the solvent (Johnson & Lusas, 1983).

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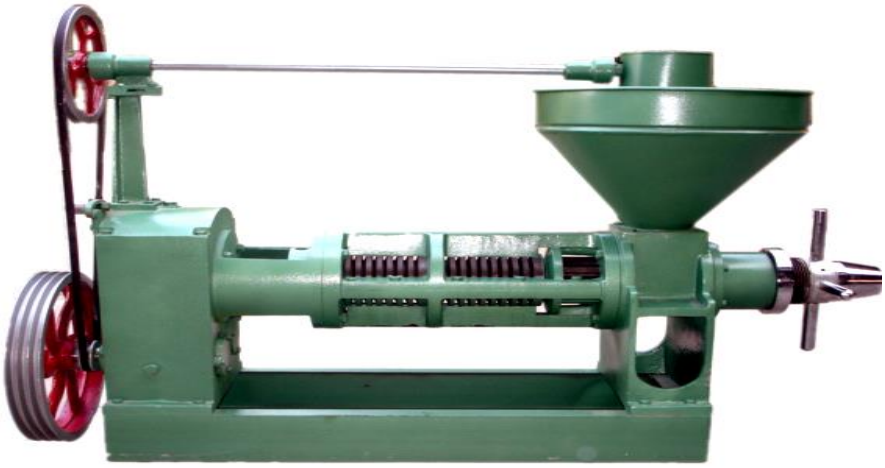

Figure 2.1: Mechanical oil extractor

The advantage of using direct solvent extraction with hexane are mostly used worldwide industries, need lesser maintenance and voltage. An efficient method with reliability and this is rational reason of using solvent method which is considered as initial and quick way of extracting oil from materials in tons.

Table 2.1: Hexane and its isomers properties

Properties	Hexane isomers	Solvent (hexane)
Pressure of vapors at 37.9 °C	39.4	38.1
Density of vapours	~3	~3
Gravity at 15.6 °C	0.66	0.68
Pounds per gal at 15.6°C	5.52	5.63
Molecular weight(Mt)	86.2	86.2
Melting point (°C)	-154	-94
Heat vaporization (kcal/kg)	N.A.	79.6
Temperature of ignition °C	264	225
Flash point °C	-18	-26
Flammable limit (vol. percent)	1.0-7.0	1.2-7.7
Boiling temperature (°C)	56-60	67-69
Specific heat liquid (kcal per kg °C at 15.6 °C)	N.A.	0.531
Specific heat vapour (kcal per kg-°C at 15.6 °C)	N.A.	0.339
Water solubility [moles per L at 60°F (15.6 C)]	negligible	negligible

N.A. - data not available. Adapted from NFPA-36, 2009

Hexane is used widely in oil extraction especially direct solvent method and it is also known as commercial and extraction hexane. The properties of hexane are listed in Table

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2.1. Hexane consists of different isomers which make it strong extractor of oil from seeds as compare to pure hexane. The best solvent used over years with better result in extraction. It has a boiling point of 69 °C and is a liquid in all. With a fairly high a low sensible and volatility heat of 144 Btu/lb (335 kJ/kg), a low voltage is required to easily evaporated hexane from oils.

Figure 2.2: A common soxhlet solvent extraction apparatus

This solvent has an azeotrope, a slightly reduced 61.6 °C (bt) when in the presence of water or steam and resulting in a vapor coming off at about 95% by weight hexane and 5% by weight water. Because of azeotrope property of hexane, it is easy and efficient to remove residual hexane from oils using direct steam. When compared with other solvent hexane did not show any severe effect or toxicity to human that's why it is preferred to be used. Hexane did not blend with water, allowing fairly simple processes to keep it in the system while water passes through the extraction process as moisture in the oil, air and seeds. To mix and dissolve seed oils, an aggressive capable solvent is required that could wash-down the desired oils out of a fibrous or solid material and hexane has this

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property. On the other hand, hexane odor is tolerable and cause minimum dis-comfort when someone is exposed to it.

2.3 Transesterification

The oil extracted from plant seeds cannot directly be used in diesel engine because of their viscosity which is normally higher in oils and could halt engine performance (Pali et al., 2015). The plant oils must be treated and viscosity should be reduced before using in diesel engine in order to avoid problem in performance of engine, various methods available for oil viscosity reduction. Mostly used processes to reduce viscosity of oils are pre-heating, blending/dilution, pyrolysis, micro-emulsion and transesterification (Ashraful et al., 2014; Atabani et al., 2013).

Transesterification is used widely among all the processes for biodiesel production as it has advantages such as economical and quality method to reduce the viscosity of plant oils (Mofijur et al., 2013). The chemical process of transesterification is shown in Figure.1. Transesterification occurs when plant oil react with an alcohol in presence of catalyst which in turn reduce the viscosity of oil by converting it to biodiesel which is termed as biodiesel. Mostly used solvents are ethanol and methanol and potassium and sodium hydroxides as catalyst in the reaction (Atabani et al., 2013).

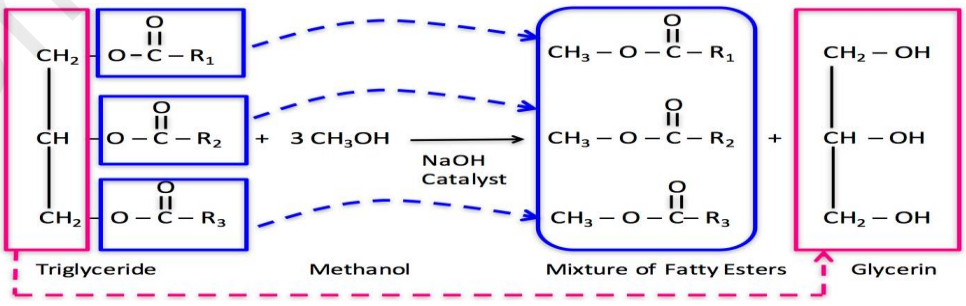

Figure 2.3: Transesterification of triglycerides to biodiesel (John, 2017)

Transesterification depends on various factors such as solvent ratio, mixing of solution, temperature and catalyst which plays vital role in conversion of oil to biodiesel.

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Once oil is converted to its biodiesel then it could be used in diesel engine as it has reduced viscosity and in a more pure form or blended with diesel. The biodiesel quality could be determined by many factors such as feed-stock, fatty-acid composition, oil percentage, purifying process and pre-treatment parameters (Atabani et al., 2013). Different countries follows and applied standards for biodiesel production. The important standards used for biodiesel production ate listed in Table 2.2. The most important material used in biodiesel production is alcohol which converts triglyceride into biodiesel. It comprises a mono-hydric aliphatic chain up to 1-8 carbons (Ma & Hanna, 1999).

Table 2.2: Different international standards for biodiesel (Chen et al., 2013)

Serial No	Properties	BIS	ASTM-D-6751	France	Germany	Italy	Austrian	EN14214	Taiwan
1	Oxidation stability (h)	6	3	-	-	-	-	6	6
2	Kinematic viscosity	1.9 - 6	1.9-6	3.5-5	3.5-5	3.5-5	3.5-5	3.5-5	3.5-5
3	Flash point ^{0}C	130	130	100	110	100	100	101	101
4	Density (gm/cm$^{3)}$	0.87-0.8	-	0.87-0.89	0.875-0.89	0.86-0.89	0.85-0.89	860-890	860-890
5	Cetane number (minutes)	40	47	49	49		49	51	51
6	Carbon residue (max)	0.05	0.05	0.05	0.05	0.05	0.05	0.03	0.03
7	Acid number(KOH/gm) (max)	0.5	0.8	0.5	0.5	0.5	0.8	0.5	0.5

Various types of alcohol has been used for the production of biodiesel such as methanol and ethanol. Many short chain alcohol has also been explored for the production of biodiesel like butanol, branch alcohol, octanol, tert butanol and propanol but these solvents are very costly (Yusuf et al., 2011).

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The widely used solvent for biodiesel in the industries are methanol and ethanol. The chemical and physical properties of methanol put it on the priority to be used as alcohol. Methanol dissolves easily in sodium hydroxide and make quick conversion of triglycerides into biodiesel (Ma & Hanna, 1999). Methanol is easier to get as compare to ethanol that's termed as wood-alcohol (Demirbas, 2008b). As previous stated, to produce biodiesel through transesterification, methanol is the most common and best alcohol to be used.

But, water content presence in any alcohol make the reaction unsuccessful and unable to produce biodiesel properly, the reason is that presence of water could cause triglyceride hydrolysis and leads to formation of soap and low quality yield. The whole short chain alcohols are hygro-scopic and can definitely absorb atmospheric water (Gao et al., 2009). In contrast, alcohols with long chains are commonly sensitive water contamination (Gerpen, 2005).

However, if any alcohol used for biodiesel production and its does not make any change chemically and biodiesel meet ASTM standard then that alcohol is acceptable (Gerhard, 2005). Because of steric hindrance effect, higher molecular alcohols are not likely to be used in biodiesel production reaction.

Other alcohols used in transesterification as well such as ethanol is used as an alternative to methanol sometime in the reaction, because it's cheap and completely bio based. Additionally, butanol can also be extracted from biological sources and using it could achieved entirely bio-based biodiesel yield. On the other hand, alcohol obtained from petro-chemical sources and methane derived natural gas such as methanol isopropanol and propanol. Ethanol is a renewable alcohol, environmental friendly, toxicity is less which makes it a good substitute to be used in transesterification (Hameed et al., 2009). But, methanol is cheaper than ethanol in term of cost and methanol is more

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reactive than ethanol (Yao & Hammond, 2006). Some studies also suggest that it is difficult to use alcohols such as isopropanol and ethanol for production of biodiesel because these alcohols form azeotrope with water. Although, to take out water a molecular sieve is mostly used (Gerpen, 2005).

2.3.1 Transesterification Parameters

Transesterification involves different parameters which directly affect efficiency of conversion, percent yield, quality and cost of biodiesel production (Ma & Hanna, 1999). The connection between triglyceride and alcohol and their miscibility need higher molar ratio of methanol to oil than normal 3:1 ratio for transesterification and it is a reversible reaction. In order to complete transesterification, a higher ratio is required as compare to stoichio-metric ratio which is 3:1 (Lee & Saka, 2010).

Excess methanol ratio help in breaking the link between fatty acid and glycerin in transesterification and convert them to biodiesel(Miao & Wu, 2006). Hence, to achieve higher conversion of alkyl esters in short time, a higher ratio is preferred (Helwani et al., 2009).

Additionally, biodiesel purity and yield could be increases with increase in methanol to oil ratio up to a certain extent. This is in line with the results reported based on neat vegetable oils (Eevera et al., 2009). Non-edible oil such as neem and pongamia produced higher ester yield in presence of more alcohol, which could be reason that oils have low viscosity than non-edible oils.

Nonetheless, when compared to edible oil, non-edible oils esters content yield was lower but yield of glycerol yield was higher in non-edible oil when compared to edible oils (Eevera et al., 2009).

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Catalyst concentration, temperature, time, alcohol ratio, water content, pressure and FFAs are the main parameters of transesterification which affect the yield of biodiesel produced. The range acceptable for methanol to oil ratio in transesterification is 3:1 to 30:1 (Balat & Balat, 2008).

In addition to molar ratio, it is also stated that mass transfer, kinetic and equilibrium control are the stage which determines the transesterification reaction overall. And the slower stage among these three is the transfer of mass stage due to immiscibility of triglycerides and methanol (Halim et al., 2009).

Transesterification could be influenced significantly by alcohols ratio to oils is reported by another studies. These studies suggested that an increase in the yield depends on increase in alcohol ratio. They reported that the process yield is increased with increase in the alcohol to oil molar ratio. Hence, a maximum biodiesel yield was obtained for corn and canola oil when molar ratio increased to 9:1(Patil & Deng, 2009).

Furthermore alcohol ratio of 3:1-15:1 were tested in the transesterification of Cynara oil using ethanol. The biodiesel yield of was improved with a 12:1 alcohol molar ratio to oil. Thus, alcohol ratio between 6:1 and 12:1 presented the best results. When alcohol ratio was decreased especially below 6:1, the reaction was incomplete and glycerol separation was difficult at ratio of 18:1. The higher alcohol ratio leave glycerol in biodiesel which in turn effect the yield and decreased output. Hence, a suitable alcohol ratio for transesterification 6:1-9:1 (Lee & Saka, 2010). Ting et al., 2008 studied the effects of various feedstocks to alcohol molar ratios (1:9-1:39) on production of biodiesel. And a molar ratio of 1:15 gave conversion of 99 % after 12 h of reaction at 50 ^{o}C.

Low levels of methanol to oil molar ratio could create an incomplete reaction. The optimum methanol to oil ratio was 6:1 with highest yield of biodiesel. Literature suggests

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that due to reactivity of methanol it is opted for using as solvent in transesterification process but its excessive usage could increase polarity which in turn increases glycerol solubility and make it difficult to separate from alkyl ester (Verma et al., 2017). The molar ratio has substantial effect on biodiesel among different reaction parameters as described previously. Therefore, both catalyst concentration and methanol to oil molar ratio displayed optimum value whilst too high or too low catalyst concentrations or methanol ratio results in a reverse trend. When the concentration of catalyst turns out to be too high, soap can be rapidly formed creating a problem in separating the biodiesel, thus yield may decrease as well. On the other hand, excess of methanol could) increase yield but could also increase the solubility of glycerol resulting in a reaction by reducing the yield and this could be relate to Le Chatelier's principle, which states that further proceed of the transesterification may lead to the reverse of reaction to the reactant side and hence, biodiesel yield may be lowered (Wong et al., 2015). The optimal molar alcohol ratio to oil play a major role among reaction parameters to improve biodiesel yield because lower ratio can cause incomplete reaction and higher ratio would decrease the yield (Verma & Sharma, 2016) .

Catalyst around 1.25 % showed the highest yield up to 94% (Hasni et al., 2017). After that a decreasing pattern was seen in the yield when the concentration of catalyst was outside that range. Since methanol and triglyceride in the esters are immiscible, adding a catalyst could help the transesterification reaction, and increases the yield rapidly. However, when the concentration of catalyst was too low or too high, soap formation could occur which makes glycerol separation from biodiesel more difficult, this may decrease yield. In contrast, inadequate usage of catalyst could result in an incomplete reaction and a lower yield. Biodiesel yield increased with the increase in amount of catalyst. When the catalyst amount reached a certain value, a reversed trend was observed (Dwivedi & Sharma, 2015). Previous literature explained the effect of catalyst

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concentration on biodiesel yield, an increase in concentration of catalyst can increases yield up to a certain point that may be due to giving more alkyl ester in higher reaction but after that certain point yield decreased significantly (Verma et al., 2017).

Temperature play a vital role in transesterification and its effect could be observed in the range of 60-70 ^{o}C. When temperature raises, biodiesel yield increases but once the temperature crosses 65 ^{o}C, a reverse trend is seen and yield decreases. The effect could be explained using the Arrhenius equation which states that steady increases in reaction rate constant by temperature increase which might increase the yield. Methanol vaporizes when temperature is too high resulting in a two phase interface by decreasing yield. Optimum yield for alkali transesterification was acquired at 65 ^{o}C temperature (Sun et al., 2014). Temperature at 65 ^{o}C could produce the highest yield of biodiesel to up to 94.34 % and of 98.4% was achieved (Dwivedi & Sharma, 2015; Hasni et al., 2017). A decreased pattern is seen in the yield when concentration and temperature was outside the range. Inadequate usage of high or low temperature catalyst could result in an undesirable reaction with a lower yield. Previous literature revealed that biodiesel yield increase as temperature increases up to 60 to 65 ^{o}C and thereafter a significant drop in the yield. The maximum yield obtained at the mentioned range is due to better mixing of oil with methanol and glycerol can also be easily separated at that range. On the other hand when temperature increases after that yield could decrease yield and reason might be that a higher temperature, side reaction such as alkyl ester hydrolysis could occur and produce acids. The other possible reason is methanol polarity which decreases when temperature is higher (Verma et al., 2017).

Fuel properties of biodiesel indicates the quality of fuel and their impact on engine. Acid value is an important property, which shows the free fatty acid value in fuel. Generally, high acid value is not desirable as it could produce corrosion in fuel systems

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(Ghadge & Raheman, 2005). Higher kinematic viscosity (KV) causes poor atomization which creates operational problems when injected into chamber therefore, leads to deposits in engines. Studies showed that if KV is high then such problems would exist in the engine (Gerpen et al., 2004).

Another important property of biodiesel is its cold property, which has essential parameters such as cloud point (CP) and pour point (PP). Whenever the temperature falls, crystal nuclei's appear and become visible in biodiesel, which makes it cloudy and in turn stops fluid pouring. The nuclei crystal form is termed as CP and when temperature further decreases and pouring halts and that point is termed as PP (Verma & Singh, 2014).

Oxidative stability plays a major role in protecting biodiesel from oxidation after its exposure to air or oxygen. It depends on fatty acid compositions and presence of antioxidants in the oil (Dunn & Knothe, 2003). Antioxidant can improve oxidative stability to an extent (Dunn, 2008) that's why antioxidants were used to improve oxidative stability of biodiesel. Ignition quality of any fuel is determined by its cetane number. Both high and low CN results in incomplete combustion (Gerpen et al., 2004). Biodiesel purity and fatty acid profile suggests the density of fuel, which depends on the atoms of carbon. If carbon atoms increase, density decreases to a certain extend and vice versa. Higher density creates viscosity problems in the engine (Demirbas, 2008a).

2.4 Edible Feedstocks

Biodiesel production have also been reported several feedstocks such as tea oil (Demirbas, 2010), cotton oil (Qian et al., 2010), tobacco oil (Usta et al., 2011) palm oil (Nur et al., 2014), palm kernel oil (Awalludin et al., 2015), canola oil (Kai et al., 2014) and soybean oil (Samart et al., 2010). Edible plant oils and fats from animals are consumed as food and their usage as feedstock for biodiesel is questionable with great concern. As vegetable oil is used for food and its demand has increased in recent years

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especially in third world countries, it is difficult to justify its usage as biodiesel feedstock. Recent report revealed food versus fuel competition such as soybean oil in North America, palm and coconut oil in Malaysia, castor in Brazil and jatropha in India (Sanli & Canakci, 2008). A list of edible oil used for biodiesel production is presented in Table 2.3.

Table 2.3: Edible feedstock for Biodiesel production (Balaji & Cheralathan, 2013)

Serial no	Edible oil	Countries
1	Rapeseed oil	EU, Canada, US, China and India
2	Soybean oil	USA, Brazil and Argentina
3	Sunflower oil	India
4	Palm and palm kernel oil	Malaysia and Indonesia
5	Peanut oil	South America, Mexico and Central America
6	Rice bran oil	India, China and Japan
7	Sesame oil	India, China, Bangladesh, Sri Lanka, Afghanistan, Thailand and Saudi Arabia
8	Coconut oil	India and Philippines
9	Corn oil	USA
10	Cotton seed oil	USA, India, Greece and Turkey
11	Passion fruit seed oil	Brazil and South America
12	Moringa oil	India, Arabia and Africa

2.5 Non-Edible Feedstocks

Currently, edible feedstocks are considered as major sources for biodiesel as developed countries are rich in edible sources and 95% feedstock used for biodiesel is edible. But, the use edible plants can be a major risk in future for food scarcity and a competition will start between foods versus energy (Gui et al., 2008). To overcome food vs energy crisis, research interests are diverted to second generation biofuel such as, non-edible plant seed oilss. Non-edible plant seeds contains toxic ingredients which make them unfavorable for human use (Banković et al., 2012)

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Non-edible plant oils are thus considered as potential source for biodiesel production and also not competing with food. Other advantages of using these oils are higher bio-degradability, aromatic in nature, contain low sulphur, renewable, available easily and liquid in nature (Shikha & Chauhan, 2012). But, these oils have high FFAs (free fatty acid) which could increase production cost a bit (Banković et al., 2012).

2.6 Advantages and disadvantages of Biodiesel

In broad-spectrum, it means a fuel derived from biological source as a substitute of the conventional energy sourcing. The advantages of biodiesel are its higher boiling point, lower emission, biodiesel is an oxygenated fuel, so it contributes to a more complete fuel burn and a greatly improved emissions, can be used as blend with petroleum, there is no need of installing special equipment, a good substitute for petroleum, no need to buy special vehicles or engines to run on biodiesel, less carbon monoxide, less sulphur dioxide emissions reducing public health risk. It will reduce the country's dependence on imported oil and it is safe to handle, store, and transport (Sekhar, 2010). Apart from being green, biodiesel oxidative stability is low, especially when it is produced from polyunsaturated fatty acids. Storage time of biodiesel is reduced if it consists of unsaturated fatty acid and degraded easily by the attack of free radical which possess unpaired electron (Umamaheswari & Chatterjee, 2008). Other drawbacks could be its power, torque and fuel economy is less as compared to diesel due to its lower energy content. NO_x emission are higher in biodiesel. Since its cloud and pour point is around -10, it solidifies at that temperature during winter in European and American Countries (Sekhar et al., 2010). The oxidation of biodiesel could lead to many mechanical problems such as deposits formation, fuel system corrosion and filtering problem .These issues could be overcome by either adding antioxidants or altering the fatty acid chain via hydrogenation (Monyem & Gerpen, 2001).

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Table 2.4: List of non-edible plants and their oil percentage

S.No	Name	Botanical name	Oil (%)	References
1	Karabi	Thevetia peruviana	60-65	(Atabani et al., 2013; Bora, 2009; Yarkasuwa et al., 2013).
2	Poon	Sterculia foetida	50-60	(Atabani et al., 2013; Devan & Mahalakshmi, 2009).
3	Jojoba	Simmondsia chinensis	40-50	(Hamamre & Salaymeh, 2014; Ashraful et al., 2014; Atabani et al., 2013).
4	Sal	Shorea robusta	12-13	(Pali et al., 2015).
5	Kusum	Schleichera oleosa	60-70	(Atabani et al., 2013).
6	Soapnut	Sapindus mukorssi	42.7	(Atabani et al., 2013).
7	Castor	Riccinus communis	40-60	(Atabani et al., 2013).
8	Putranjiva	Putranjiva roxburghii	42	(Haldar et al., 2009).
9	Karanja	Pongamia pinnata	30-40	(Ashraful et al., 2014; Atabani et al., 2013).
10	Tobacco	Nicotiana tabacum	35-49	(Ashraful et al., 2014; Atabani et al., 2013).
11	Moringa	Moringa oleifera	33-41	(Hamamre & Salaymeh, 2014; Atabani et al., 2013)
12	Mahua	Madhuca indica	35-40	(Ashraful et al., 2014; Atabani et al., 2013).
13	Jatropha	Jatropha curas	50-60	(Ashraful et al., 2014; Atabani et al., 2013).
14	Rubber	Hevea brasiliensis	50-60	(Ashraful et al., 2014; Atabani et al., 2013)
15	Cotton	Gossipium hirusutum	17-25	(Ashraful et al., 2014)
16	Croton	Croton megalocarpus	40-45	(Hamamre and Salaymeh, 2014; Atabani et al., 2013)
17	Seamango	Cerbera odollam	54	(Atabani et al., 2013)
18	Kapok	Ceiba pentandra	25-28	(Vedharaj et al., 2013)
19	Polanga	Calllophylum inophylum	65-75	(Ashraful et al., 2014; Atabani et al., 2013)
20	Neem	Azadirachta indica	40	(Ashraful et al., 2014; Atabani et al., 2013)

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In addition to oxidation, polymers may form in the presence of unsaturated fatty acid which in turn will lead to higher molecular weight products that will increases its viscosity (Neff et al., 1997). The disadvantage of the above mentioned methods is that it utilizes a part of total energy developed in the engine and in few cases engine modification is required which is not at all desired.

2.7 Biodiesel Degradation

The two central nemesis of biodiesel degradation are thermal stability and oxidative stability. The thermal stability is initiated by the exposure of high temperatures usually exceeding 250°C whereas the oxidative stability is affected by oxygen either in the gas form or that are been dissolved that come in connection with the fuel during a sufficiently extended period of time (Velasco et al., 2009). The oxidative stability also known as storage stability because of the reason of oxygen in air interaction with the fuel under storage conditions which likely to interfere with the fuel stability (Dunn, 2008).

Whenever, biodiesel is exposed to oxygen or air, there is a chance for hydrolysis to occur because it is an ester molecule. The flash point of biodiesel will be reduced if there is a presence of alcohol and total acid number will increase with alcohol presence. The above mentioned factors will make biodiesel unstable when stored for long duration and damage the chemistry of it. The oxidative strength of biodiesel is less than petro-diesel. Therefore, mixing it into petro-diesel will affect fuel strength considerably. The double bond in biodiesel is reason of its poor stability due to that gum formation occur (Knothe & Dunn, 2003). Biodiesel degradation is caused by a free radical. A free radical is any atom or molecule having un-paired electrons. Free radicals are classified as primary oxygen derived free radical, superoxide anion (O2·), hydroxyl (OH·), hydroperoxyl (OOH·), peroxyl (ROO·) and alkoxyl (RO·) radicals while hydrogen peroxide (H2O2), hypochlorous acid (HOCl), ozone (O3) and singlet oxygen (1O2) are non-free radical.

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These reactive intermediates are together known as reactive oxygen species (ROS) (Umamaheswari & Chatterjee, 2008).

The additional minutesor causes of biodiesel degradation are light, water and metal presence in the fuel that will speed up the oxidation process (Jain & Sharma, 2010). The light exposure cause photo-oxidation mechanism, though it is unlikely to occur in biodiesel since it needs the exposure to ultraviolet and the occurrence of a photo-sensitizer. Similarly photo-oxidation and auto-oxidation usually take place in biodiesel (Knothe, 2007; Lapuerta et al., 2012). The oxidation strength of vegetable oil is more stable in comparison to animal oil even though a large amount of polyunsaturated fatty acid is present. This is due the lack of natural antioxidant of fatty acid biodiesel in animal chubby (Sendzikiene et al., 2005). These issues could be overcome by either adding antioxidants or altering the fatty acid chain via hydrogenation.

2.8 Antioxidants

2.8.1 Introduction

Antioxidants are compounds which can slow down or inhibit oxidative stress of fatty acids by breaking oxidative chain of propagation (Velioglu, 1998). The plants consist of secondary metabolites which have the ability to inhibit oxidation process. It is suggested that secondary metabolites could be used as natural antioxidants to boost up oxidative stability (Kranl et al., 2005). The plants contain a large amount of natural antioxidant compounds, vitaminutess and carotenoids (Velioglu, 1998). A lot of experimental work had been carried out to check the amount of phenolic antioxidant compounds in plant extracts through usage of qualitative and quantitative determination (Nakatani, 2000). The plant crude extracts are rich in phenolic compounds which possess strong antioxidant activity that can inhibit the oxidative stress of lipids (Javanmardi et al., 2003). There are various methods available to test the antioxidants activities of the plants. The most

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commonly used assays are ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity, metal chelating activity, 2,2-dphenyl-1-picrylhydrazyl (DPPH) and trolox equivalent antioxidant capacity (TEAC) (Cao & Prior, 2001).

It is usually believe that antioxidant can play a vital role in combating with oxidation of fats /oils and to reduce free radical in the oxidation process. The antioxidants are group of molecules that are capable of preventing and slowing down the oxidation of other molecule, antioxidant is also termed as radical scavengers. The antioxidant can also play major role in human by preventing human body from free reactive oxygen species which are the cause of numerous diseases such as anemia, asthma, arthritis, aging process and dementias (Borrelli & Izzo, 2000).

Currently antioxidants related studies are flourishing and had almost multiplied in the previous era (Prior et al., 2005). From Webster's Third New International Dictionary, the antioxidants are defined as "a substance that compete against oxidation or hinders reactions stimulated by oxygen or peroxides, various of these substances being used as additives in many products such as in gasoline to delay the expansion of rancidity, in fuel manufactured goods to retard the gum development and in rubber to slow down the aging process". In order for a compound to perform as antioxidant, it essentially has the capability to stabilizing the formed phenoxyl radical after reaction with lipid radicals and formed delocalized unpaired electrons. This let the molecule to act as hydrogen donor, singlet oxygen donor and reducing agents (Matthäus, 2002).

2.8.2 Mechanism of Antioxidants

The initial study on reaction mechanism of antioxidants stated that it is a reaction chain process for free radical terminutesator, it contain a highly liable hydrogen which is quickly offered to peroxy radical which later interfere with oxidation (reaction (2.1) and

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(2)) (Yang et al., 2013). The chain propagation reaction shown in (3) and (4), the reaction is exothermic in nature.

$$R. + O \rightarrow ROO. \quad (1)$$
$$ROO + RH \rightarrow R\text{-}OOH + R. \quad (2)$$
$$R. + R. \rightarrow R + R \quad (3)$$
$$ROO. + R. \rightarrow R\text{-}OO\text{-}R. \quad (4)$$

Figure 2.4: Antioxidant reaction mechanism

Antioxidants uses various mechanisms to interrupt oxidation chain reaction such as scavenging activities, can chelate metal ions so that cannot generate reactive oxygen species, peroxides formation preventing, decreasing oxygen concentration, and inhibiting autoxidation chain reaction. The reactive oxygen species (ROS) are the free radicals that include and hydroperoxyl (HOO), hydroxyl radical (OH), peroxy radical (ROO) and super anions (O_2-). Hydrocholorous acid (HOCl) and hydrogen peroxide (H_2O_2) are also member of ROS family but usually not considered as free radical as there electron pairing is complete. Reactive nitrogen species are also included in reactive oxygen species like peroxynitrate (ONOO-), nitrogen dioxide (NO_2) and nitric oxide (NO) (Dusting & Triggle, 2005).

The phenolic antioxidants (Butylated hydroxytoulene (BHT), butylated hydroxyanisole (BHA),2,5-di-tert-butylhydroquinone(DTBHQ), tert-butyl hydroquinone (TBHQ) and propyl gallate (PG) are considered as valuable antioxidants based on liability of hydrogen, flavonoids and aminuteses are used as well. The antioxidant concentration effect on oxidation be determined by factors like prevailing conditions, structure of antioxidant and nature of sample being oxidized (Shahidi & Wanasundara, 1992).

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The factors that can affect antioxidant activity are oxidation-reduction capability, rate constant and energy of activation (volatility, heat susceptibility and solubility) of antioxidants. The antioxidants that can inhibit or break free radical chain reaction are considered as potential antioxidants, these donates hydrogen (H) to free radical composed during oxidative process and act as radicals themselves. These antioxidants contains phenolic and aromatic rings.

2.8.3 Different Classifications of Antioxidants

Antioxidants into two key classes of non-enzymatic and enzymatic (Jeong et al., 2004). The enzymatic antioxidants are those which are created endogenously while non-enzymatic are those which are created as exogenously. The antioxidants are divided into two groups known as primary and secondary antioxidant that vary in term of action mechanism (Hue et al., 2012). Primary antioxidants stabilize the free radical by scavenging it and give a hydrogen atoms or electron and secondary antioxidants suppress the formation of free radicals and avoid the oxidative damage (Prior et al., 2005).

Mostly antioxidants are classified into two groups, the synthetic and the natural antioxidants. The synthetic antioxidants have been used for maintaining the oxidative stability of lipids/oils but it doesn't get so much importance because of its toxic and oncogenic nature (Jeong et al., 2004). The antioxidants are also classified into different groups on the basis of activity performed such as metal ion chelator, oxygen scavengers and free radical terminators (Hue et al., 2012)

2.8.4 Antioxidants and Biodiesel Oxidative Stability

Many scientists studied the effects of artificial and natural antioxidants on biodiesel oxidative strength from the different sources. The caffeic acid (CA), ferulic acid and TBHQ were tested using the Rancimat test and other techniques in which it was found that CA meet European standard (EN 14214) specifications limit (Damasceno et al.,

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2013). BHA and TBHQ which are synthetic antioxidants were used to check their efficiency on soybean biodiesel oxidation, both shows higher potential to prevent the oxidation of biodiesel (Maia et al., 2011). The synergistic effect of BHA and BHT on rapeseed biodiesel had been studied at various concentrations and the result was promising at 400 ppm. A study had also been done on Jatropa biodiesel oxidation stability in which propyl gallate (PY) was used as antioxidant at various concentrations (200 ppm to 800 ppm) and induction period (IP) was retained for 6 hours up to six months (Jain & Sharma, 2013). PG, PY and BHA were used on biodiesel produced from Croton megalocarpus oil at different concentration and their effect was determined. PG and PY showed the higher potential as compare to BHA (Kivevele et al., 2011). Three different antioxidants BHA, TBHQ and PG were applied on linseed oil biodiesel, among them TBHQ was most effective antioxidant (Pantoja et al., 2013). Synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoulene that are commercially available in industry have caused several problems. Synthetic antioxidant such as butylated hydroxytoulene (BHT) yield greater nitrogen oxide and butylated hydroxyanisole (BHA) produces higher hydrocarbon emission (Fattah et al., 2014).

2.9 Structures of Natural Antioxidants

The naturals antioxidants found in plants as secondary metabolites. Some of the most important natural antioxidants structures are shown below;

2.9.1 Antioxidant Activity Assays

Though numerous *in vitro* techniques detection are available to permit quick screening and investigating antioxidant activity but their limits and advantages are still being debate and no agreement has been reached to set a standard including all the characteristic features of different classes of antioxidants because each method offer different idea and way of stating the result. Indirect *in vitro* method such as 2,2'-azino-bis, 3-

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ethylbenzothiazoline-6-sulphonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) , and Ferric reducing antioxidant power assay (FRAP) that include electron transfer reaction are simple to apply but have some limitations. For example, free radical scavenging capability of antioxidant compounds that are assessed by these indirect approaches are not essentially mirrored the real oxidative degradation even though in some amount the donation of hydrogen atoms or electrons compares with antioxidant activity (Tiveron et al., 2012).

Natural antioxidants can be isolated from plants and used for the protection of biodiesel from oxidation. Plants are considered as the major source of antioxidants and most of them are plants secondary products (Sikora et al., 2008).

2.9.2 Antioxidants in Plants

The plant extracts are extremely effectively and potential antioxidants due to their strong H-donating property. The plants mostly contains phenols (caffeic, rosmarinic acids and gallic acids), phenolic diterpenes (rosmanol, carnosol and carsonic acid) flavonoids (catechin, quercetin and kaempferol) and volatile oils, thus can be used as a potential antioxidants against oxidation. The lipids /fatty acids contain polyunsaturated fatty acids chains (double bond) and prone to oxidation whenever oxidation stress occur which in turn greatly affect the quality. Adding antioxidants to reduce the oxidation is the better option (Brewer, 2011). Butylated hydroxytoulene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PY) are synthetic antioxidants which can break oxidative stress chain and thus reduce oxidation process effectively. Some chelating agent can also avert oxidation such as ethylene diaminutese tetra acetic acid (EDTA). A huge quantity of antioxidant compounds are present in plant extracts, herbs and spices (Hinneburg et al., 2006).

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CHAPTER 3: METHODOLOGY

3.1 Biodiesel Production from *Brucea javanica* Seeds Oil

3.1.1 Instruments and Materials

The seeds were then grinded into powder form using biomass blender and the moisture content was confirmed to be below 1 wt% (weight percentage) using Karl-Fischer method. Batch process setup includes three neck round bottom flask, heating mantle, hot plate agitator, temperature probe and digital temperature control unit. Weigh balance (Metier Toledo), Gas chromatography (Shimadzu), Fourier transform infrared spectroscope (Perkin Elmer Spectrum GX), distilled water unit, digital thermo-meter, water bath, magnetic stirrer, heating coils. Sampling bottles, volumetric flask (100 mL to 500 mL), conical flask (100 to 250 mL), pipette, burette, separating funnel (200 to 500 mL), viscometer, beaker (100 to 500 mL), measuring cylinder (50 to 200 mL), condenser with standard joints, heating mantle, round bottom flask, gas burner, desiccators, dean and stark condenser with standard joints.

All chemicals and reagents used for this extraction work such as hexane and methanol (99.9 % purity), sodium hydroxide pellet (99 % purity), sulphuric acid (98.9 purity), extraction thimbles and filter papers were of analytical grades and supplied by Sigma Aldrich, Kuala Lumpur, Malaysia.

3.1.2 Oil Extraction

A 500 ml Soxhlet apparatus was used with hexane as a solvent. A known amount of powdered (grinded) seeds of *Brucea javanica* and *Hibiscus sabdariffa* were put in a thimble and placed in a condenser. A flask containing a known volume of hexane was stationed at the end of the soxhlet apparatus and a condenser was fixed tightly at the bottom end. The whole set up was heated up in a heating mantle at a constant temperature of 70 ^{o}C and the *Brucea javanica Hibiscus sabdariffa* oil were collected in the flask. This

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was done in accordance to the standard method for the Analysis of Fats, Oils. The yield was calculated according to the equation,

Oil yield = Weight of oil produced / Weight of oil used x100

Figure 3.1: Different steps of oil extractions from plant seeds

3.1.3 Biodiesel Production

3.1.3.1 Acid Esterification

Esterification process was applied as pre-treatment by using 1 % H_2SO_4 (v/v) to reduce the free fatty acid content to less than 2 % and followed by base catalyst transesterification using sodium hydroxide (Dwivedi & Sharma, 2015). After the completion of reaction, alcohol-catalyst were separated from upper layer. Next, the esterified oil is washed with distilled water to remove the remaining catalyst and heated to remove water content.

3.1.3.2 Transesterification

The transesterification process was conducted in a 500 mL three-neck round bottom flask. An oil quantity of 40 to 50 g were used for 17 experimental runs. For each experiment, oil was carefully transferred into the reaction flask and preheated in an oil bath up to its reaction temperature. Sodium hydroxide, a homogeneous catalyst was used. A sodium hydroxide and methanol solution were freshly prepared and added to the

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preheated oil, and the mixture was agitated. At the completion of the transesterification process, the mixture undergoes gravity settling in a separating flask for 6 to 8 h to separate the methanol-water and the biodiesel phases. The top phase containing the biodiesel was collected and mixed with distilled water at 40 ^{o}C to remove residual impurities. Methanol and water were removed using rotary evaporator at 70 ^{o}C.

3.1.4 Design of Experiments (DoE)

Three factors design which applied with generated 17 experimental runs. Selected extraction parameters were methanol to oil molar ratio (mol): A, catalyst (%): B, and temperature (^{o}C) were *Brucea javanica* and four factors design which applied with generated 29 experimental runs for *Hibiscus sabdariffa*. Selected extraction parameters were methanol to oil molar ratio (mol), catalyst (%), agitation (rpm) and temperature (^{o}C). The randomization of experiments was used to minimize the effects of unexplainable variability in the observed response. Overall methodology flow is shown in Figure 3.2.

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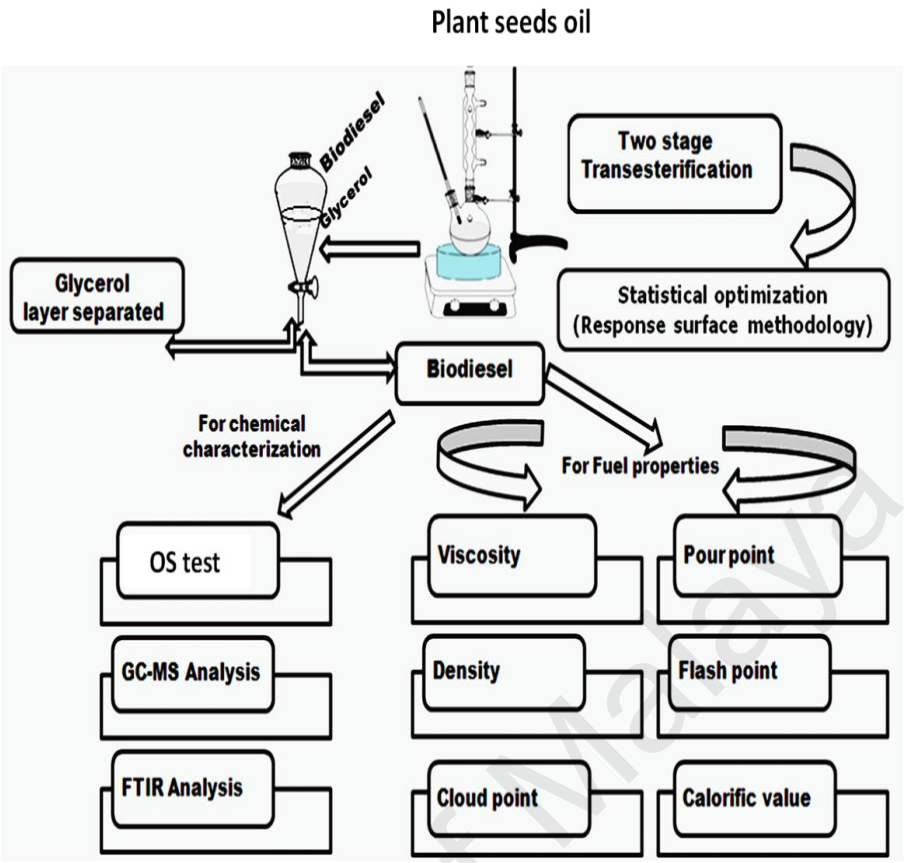

Figure 3.2: Methodology flow of research project

3.1.5 Statistical Analysis

The results obtained from 17 and 29 experimental runs were analyzed statistically using Response Surface Methodology for *Brucea javanica* and *Hibiscus sabdariffa* biodiesel, so as to fit the quadratic polynomial equation by the Design Expert 10.1 (Stat-Ease Inc., Minneapolis, USA). To correlate the response, variable Y (biodiesel yield) to the independent variables molar ratio, agitation, catalyst and temperature, multiple regressions were used to fit the coefficient of the polynomial model of the response. The quality of the fit of the model were evaluated using test of significance and analysis of variance (ANOVA). The model as drawn and result were achieved using the equation which is referred as polynomial equation.

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The fitted polynomial equation is as follows,

$$y = b_0 + \sum_{i=1}^{k} biXi + \sum_{i=1}^{k} biiX^2 i + \sum_{i<j}^{k} bijXiXj + e.....(2)$$

- *Y = Biodiesel yield*
- *bo = intercept value. (i = 1,2,3….k)*
- *bi = model coefficient (first order)*
- *bij = effect of interaction*
- *bii = qaudratic coefficients of Xi*
- *e = error (random).*

3.1.6 Chemical Characterization

3.1.6.1 Fourier Transform Infrared Spectrum (FTIR) Analysis

Gas chromatography analysis was performed using gas chromatograph (GC-2014, Shimadzu, Japan) equipped with flame ionization detector (FID) and capillary column (Omega wax, 30 m x 0.25 mm x 0.25 µL). The detector and injector were set at 250 ˚C and 260 ˚C, respectively. The oven program (200 ˚C-5 minutes, 20 ˚C/minutes to 260 ˚C, kept constant 260˚C-6minutes) with Helium as carrier gas at 2 mL/minutes flow-rate.

3.1.6.2 Gas Chromatography

Gas chromatography mass spectrophotometry analysis was performed by using Trace GC 2000 gas chromatograph coupled to a Polaris-Q Ion trap mass spectrometer (Thermo Finnigan, Austin, TX, USA). Zebron ZB-5 ms (Phenomenex, Torrance, CA, USA) fused silica capillary column (30m long x 0.25mm I.D. x 0.25 film thickness) was used as the column. The oven temperature was programmed to hold the initial temperature of 40 °C. It was maintained for 5 minutes before being increased gradually every 10 °C for 2-10

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minutes up to 280 °C. Helium was used as carrier gas at 1 mL/minutes in constant flow mode with injection temperature of 200 °C and auxiliary temperature of 250 °C.

3.1.7 Fuel Properties

Karl Fischer moisture titrator MKC-520 (Kyoto Electronics MFG. Co. Ltd.) was used to check water content and Automatic Kinematic Viscosity Measuring System AKV-201 was used to test kinematic viscosity at 40 ˚C. Iodine value was measured according to Wijs method while density was determined using I-type hydrometer.

Oxidation stability was determined by Rancimat 743 (Metrohm, Herisau, Switzerland). Tocopherol, iodine value, water content and kinematic viscosity were measured using the same method with oil properties determination. Acid value was determined using AOCS Official Method AOCS Cd 3d-63 (AOCS 1976). The pour point and cloud point tests were done on a Minutes Pour/Cloud Point Tester MPC- 102. Automated Cold Filter Plugging Point Tester AFP-102 was used to determine cold filter plugging point while Pensky-Martens Closed Cup Automated Flash Point Tester APM-7 was used to determine the flash point.

3.1.8 Rancimat Test

Rancimat (Metrohm, Herisau, Switzerland) was used to test the oxidative stability of biodiesel in accordance with European biodiesel standard EN14112. The equipment operates under the following conditions, the air flow rate was 10 L/h, a total of 3 g biodiesel placed in a heating block with a temperature range of 100 to 120 oC, the vapors discharged to a flask containing 0.06 L distilled water and the conductivity change was recorded by a computer simultaneously. The induction period was determined to see the time duration and antioxidant concentration was 0 to 5000 ppm.

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3.2 Biodiesel Production from *Hibiscus sabdariffa* Seeds oil

- **Please refer to 3.1 methodology portion**

3.3 *Brucea javanica* Seeds Antioxidant Activity Assays

3.3.1 Materials

Biodiesel, butylated hydroxyanisole (BHA), ferrozine, sodium nitroferricyanide (III) dehydrate, sodium acetate trihydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid monohydrate, 2,4,6-tripyridyl-S-triazine (TPTZ), Folin Ciocalteu reagent and sodium phosphate monobasic were obtained from Sigma Chemical Company (USA). Acetic acid glacial, ascorbic acid, ferric sulfate, ferric chloride hexahydrate, sodium chloride, aluminum chloride, potassium acetate, quercetin, ethylene-diaminutese-tetra-acetic-acid (EDTA) and sodium bicarbonate were purchased from Merck Chemical Co. (Malaysia). Ethyl acetate, methanol and hexane (Systerm), all the grade solvent for liquid chromatography mass spectrometry (LCMS) were purchased from Fisher Scientific (Malaysia). All chemicals used are of analytical grade and were used without further purification.

3.3.2 Sample Collection

Brucea javanica seeds were collected from vicinity of University of Malaya's Glami Lemi Biotechnology Research Centre in Jelebu District, Negeri Sembilan, Malaysia. The seed powder was completely dried and stored at 2-8 ^{o}C in capped bottles filled with nitrogen.

3.3.3 Extraction Preparation

Brucea javanica seeds were grounded into powder form. The extraction was done with three different solvents. A total of 1kg of powder was extracted with methanol, ethyl acetate and hexane solvent. The extraction was done by using soxhlet apparatus and was

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filtered with Whatman filter papers. The methanol, ethyl acetate and hexane crude extracts were later tested for their antioxidant activities.

3.3.4 Polyphenol Contents Determination

3.3.4.1 Total Phenolic Contents

The total phenolic content was evaluated by using the Folin Ciocalteu method (Singleton & Rossi, 1965) 100 µl sample extract was added to 1 mL of 0.5 M Folin–Ciocalteu reagent, and it was then stirred .1 mL of 75 gL-1 sodium bicarbonate was added and the mixture was shaken again for 30 seconds. The incubation period was 2 hours and the sample was placed in the dark and 96 well microplate were used to measure its absorbance at 765 nm. Gallic acid was used as standard. The total phenolic content was assessed utilizing the standard curve of Gallic acid and it was expressed as gallic acid equivalents (GAE) mg/g of dry extract.

3.3.4.2 Total Flavonoid Contents

Total flavonoid content determination was evaluated according to a modified method. 100 mL of 10 % aluminum chloride with 1M of potassium acetate was prepared as reagent. Then 3.8 mL of methanol was added at room temperature for 40 minutes. 20 µl of sample extract was mixed with the reagent and analyzed using 96 well microplate at 510 nm. The total flavonoid contents were determined by plotting the quercetin calibration curve with 5 different concentrations and expressed as quercetin equivalent (QAE) mg/g of dry extract.

3.3.5 Spectrometry Analysis

3.3.5.1 Gas Chromatography Mass Spectrometry

Gas chromatography mass spectrophotometry (GCMS) analysis was performed by using Trace GC 2000 gas chromatograph coupled to a Polaris-Q Ion trap mass spectrometer (Thermo Finnigan, Austin, TX, USA). Zebron ZB-5ms (Phenomenex,

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Torrance, CA, USA) fused silica capillary column (30m long x 0.25mm I.D. x 0.25 film thickness) was used as the column. The oven temperature was programmed to hold the initial temperature of 40°C. It was maintained for 5 minutes before being increased gradually every 10°C for 2-10 minutes up to 280°C. Helium was used as carrier gas at 1 mL/minutes in constant flow mode with injection temperature of 200°C and auxiliary temperature of 250 °C.

3.3.5.2 Liquid Chromatography Mass Spectrometry

A broad and rapid LC/MS with MS/MS data collection based analysis is done for *Bruce javanica* seeds. Ionization mode was negative. Dilution of sample was done with water and nylon filter (0.2uM) was used for filtration. The column was Agilent Zorbax (C18-50mm × 5uM × 2.0mm). The buffer used were Acetonitrile (5mM ammonium format and with 0.1% formic acid) and urine. AB Sciex instrument was used for detection in LCMS. The identification of known compounds was done by crosschecked with the extension library.

3.3.6 Antioxidant Activity Assays

3.3.6.1 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

Donating hydrogen ability was used to measure free radical assay of extracts by using DPPH radical (Mărghitaş et al., 2009) with a modification. Concisely, 40μL sample extracts with varying concentrations were blended with 50 μM DPPH (200 μL) solution in methanol. The mixture was shaken vigorously and incubated at room temperature for 20 minutes. Measurement of absorbance was done at 517 nm in photo-spectrometer. Ascorbic acid (5–80 μg/mL) was used as a standard and ethanol as the control. Tests were performed in triplicates (n=3) and expressed as μg/mL.

Inhibition percentage was calculated as,

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Percentage Inhibition= Absorbance of Control - Absorbance of Sample /Absorbance of Control x100

3.3.6.2 Metal Chelating Activity Assay

The metal chelating activity based on ion chelating activity of ferrous was done (Srivastava et al., 2012) by looking at Fe+ ferrozine complex based on the method as described by Welch (1990). Crude extracts with various concentrations were mixed with dH2O (120 μL) and FeCl2 (2 mM) 10μL in 96-well microplate. Ferrozine (5 mM, 20 μL) was added to the mixture to initiate the reaction. The incubation period of reaction mixture was 20 minutes and the measurement of absorbance at 562 nm was done. EDTA Na2 (5 to 80 μg/mL) was used as metal chelator with ethanol (100 μL) was used as control. Percentage inhibition was calculated using the standard equation.

The following formula was used to express the result,

Percentage Inhibition= Absorbance of Control - Absorbance of Sample /Absorbance of Control x100

3.3.6.3 Ferric Reducing Antioxidant Assay

The activity of FRAP assay was determined as stated previously (Müller et al., 2010), 20 μL of extracts in methanol were mixed with 200 μL of FRAP assay reagent which was prepared on daily base (5 mL 10 m MTPTZ in 40 mM HCl, 5mL of 20 mM FeCl3, and 50 mL of 0.3 M acetate buffer (pH 4) in micro-well plate reader. A 10 minutes incubation period was used. TPTZ-Fe2+ complex formation was measured at 595 nm in the presence of antioxidant compounds with a 96 well microplate and methanol was used as blank. Standard curve calibration was plotted using ferrous sulphate (FeSO4) as standard. The linear regression line was used to evaluate FRAP value. The absorbance at 595 nm was measured and the results were stated as mmol Fe2+/g of dry extract from triplicated test.

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3.3.7 Stability Tests of Biodiesel

3.3.7.1 Thermal Stability Test

A total of 2 g of biodiesel were placed individually in a 10 mL test tube. 200 to 600 ppm of *Brucea javanica* seed crude extract were added into each tube and duration was 2 h for 8 h and temperature was 80 ^{o}C. A DPPH is used as free radical in the experiment.

In DPPH method, a total 2 g of oil along with a total of 2 mL of DPPH (0.01 mM) were mixed and kept in caped tubes along with crude extracts. DPPH was used as free radical and crude extract were used as natural antioxidant. The reduction capability was determined by examining the decrease and increase in DPPH absorbance at 517 nm induced by antioxidants.

3.3.7.2 Oxidative Stability Test

Rancimat (Metrohm, Herisau, Switzerland) was used to test the oxidative stability of biodiesel in accordance with European biodiesel standard EN14112. The equipment operates under following conditions, the air flow rate was 10 L/h, a total of 3g biodiesel placed in heating block with a temperature range of 100 to 120 ^{o} C, the vapours discharged to a flask containing 0.06 L distilled water and the conductivity change was recorded by a computer simultaneously. The induction period was determined to see time duration and antioxidants concentration was 0 to 10,000 ppm.

3.3.8 Statistical Analysis

All the data were statistically analyzed using SPSS to determine whether there are significant differences between the samples that contribute to the oxidation activity. The results expressed as ± SEM (standard error mean). Statistical package for the social sciences (SPSS) software was used in statistical analysis applying ANOVA test with $P < 0.05$.

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CHAPTER 4: RESULTS

4.1 Biodiesel Production from *Brucea javanica* Seeds oil

4.1.1 Oil Physico-chemical Properties

Brucea javanica seeds used in this study contain 42 (weight percentage) wt% oil. Physio-chemical properties are the most important criteria to check the quality of oils. The physio-chemical properties were evaluated and the result obtained are shown in the Table 4.1. The oil was yellowish in color at room temperature with a refractive index of 1.85 and an ultrasonic speed of 1.34 ms-1. The acid value of the oil was 4.546 (mg KOH/g) showing the presence of free fatty acids in the oil. Oxidative stability of the BJO was tested on rancimat which was 3 h and the kinematic viscosity of the oil was 17.428 ($mm^{2/s}$).

Table 4.1: Physico-chemical properties of *Brucea javanica* seeds oil

Properties	*B. javanica* oil
Physical State at 25 ^{o}C	yellowish in color
Acid Value	4.546 (mg KOH/g)
Kinematic Viscosity	17.428 ($mm^{2/s}$)
Refractive Index at 25 ^{o}C	1.85
Oil Percentage	42 %
Ultrasonic Speed (m/m^{-1})	1.34 ms^{-1}
Oxidation Stability	3.0 h

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4.1.2 Optimization of Transesterification Parameters using Response Surface Methodology.

The results obtained from all the variables (methanol to oil ratio, catalyst and temperature) which were studied in this research are showed in Table 4.2. As the table displays the set of 17 experiments which were developed using RSM. The table also shows the experiment‘s biodiesel yields, predicated biodiesel yields and residual values. The results indicated that the variation in the parameter variables were clearly effecting the *Brucea javanica* biodiesel yield.

Table 4.2: Prediction & experimental yield of *Brucea javanica* biodiesel (BJB) using Box- Behnken experimental design

Run	Molar ratio (mol)	Catalyst (%)	Temperature (^oC)	Experimental yield	Predicted yield	Residual value
1	4	1	70	84.59	83.33	1.25
2	6	1.5	65	89.27	89.28	-0.01
3	5	0.5	60	86.36	86.18	0.18
4	5	1	65	93.51	93.03	0.47
5	5	0.5	70	83.29	84.55	-1.26
6	5	1.5	70	84.84	85.22	-0.38
7	4	0.5	65	89.19	89.37	-0.18
8	6	1	70	85.54	85.54	-0.00
9	6	1	60	82.75	84.20	-1.45
10	6	0.5	65	91.96	90.88	1.07
11	4	1	60	85.71	85.90	-0.19
12	4	1.5	65	89.01	90.28	-1.27
13	5	1	65	90.1	93.03	-2.93
14	5	1.5	60	85.88	84.81	1.06
15	6	1	65	94.34	93.03	1.30
16	5	1	65	93.88	93.03	0.84
17	5	1	65	92.85	93.03	-0.18

ANOVA for 17 experimental runs was obtained from RSM based on Box-Behnken design with 3 level operating parameters and indicating three replicate at the center point is used for fitting a quadratic equation model (Equation 3). The design is checked by considering its p-value and the determination coefficient R^2 of the model. The analysis of

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variance ANOVA shown in Table 3.3 indicates that the model is highly significant with a p-value of less than 0.05.

Table 4.3: Analysis of variance (ANOVA) from obtained results

Source	Sum of squares	df	Mean Square	F Value	p-value	Remarks
Model	219.16	9	24.35	8.3	0.0054	Significant
A-Methanol	0.13	1	0.13	0.044	0.8392	
B-Catalyst	0.4	1	0.4	0.14	0.7212	
C-Temperature	0.74	1	0.74	0.25	0.6299	
AB	1.58	1	1.58	0.54	0.4875	
AC	3.82	1	3.82	1.3	0.2912	
BC	1.03	1	1.03	0.35	0.5721	
A^2	13.07	1	13.07	4.46	0.0727	
B^2	7.3	1	7.3	2.49	0.1587	
C^2	179.36	1	179.36	61.14	0.0001	
Residual	20.53	7	2.93			
Lack of Fit	9.29	3	3.1	1.1	0.4456	Insignificant

- *R-Squared 0.91*
- *Adj R-Squared 0.80*
- *Pred R-Squared 0.30*
- *Adeq Precision 7.38*

In order to observe performances of the developed model, comparison was made and the results obtained from the experimental runs were compared with the predicted values. A regression analysis model was used to obtain the prediction and accuracy on a scattered diagram, where the predicted yield and experimental yield were compared. The regression co-efficient (R^2) using linear model was 0.91 indicated a good fit between experimental

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and predicted results. *Brucea javanica* biodiesel yield including all the run and outlier t plot is shown in Figure 4.1 (a, b, c).

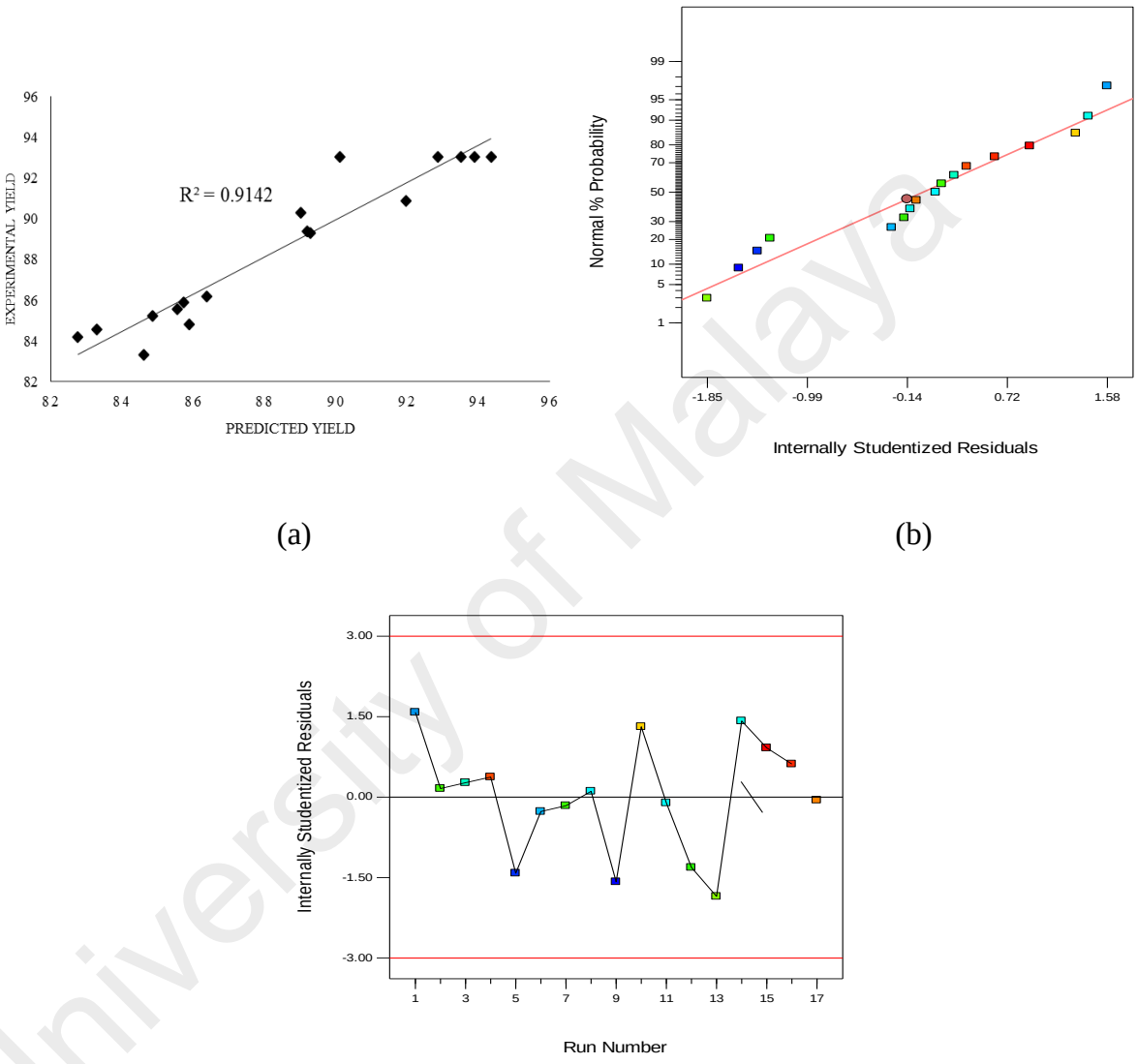

Figure 4.1: Residuals plots (a) predicted biodiesel yield vs experimental biodiesel yield. (b) Studentized residual normal probability (c) experimental run vs residual *t* plot

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4.1.3 Effect of Independent Parameters on Biodiesel Yield

To indicate the effects of independent parameters on dependent one, a 3-D response curves were drawn shown in Figure 4.2 (a, b, c). The optimum parameters found for converting *Brucea javanica* seed oils to biodiesel were 65 ^{o}C temperature, 1 % catalyst and 6:1 methanol to oil ratio with the highest yield of 94.34 %.

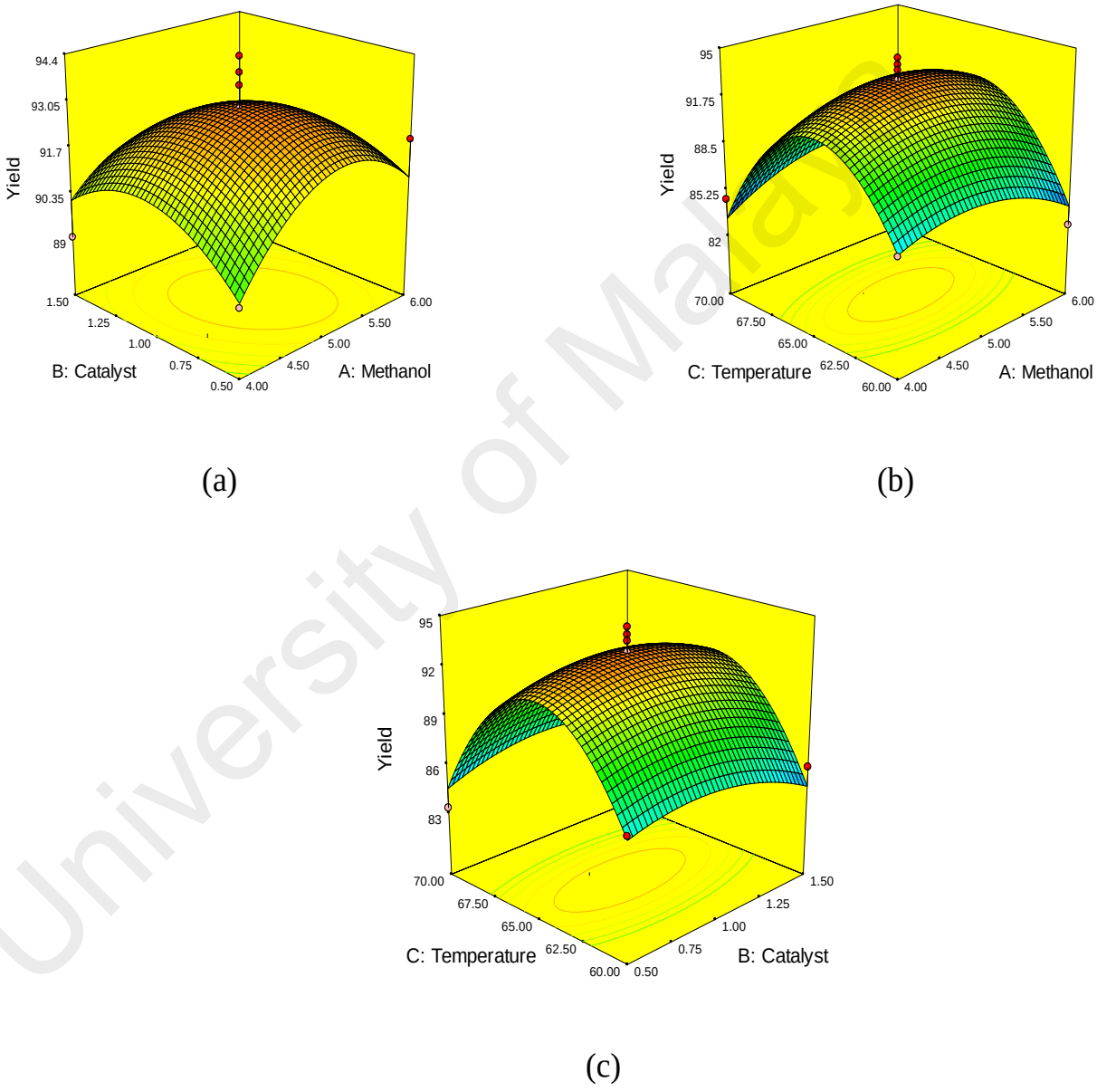

Figure 4.2: 3-D surface plot showing parameters effect on biodiesel yield: (a) methanol ratio and catalyst (b) temperature and methanol ratio (c) temperature and catalyst

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4.1.4 Gas Chromatography Analysis and FTIR Analysis (Fourier transform infrared spectrum) of *Brucea javanica* Biodiesel

In the functional group –CO–O–CH_3 of *Brucea javanica* biodiesel (BJB), which was generated during the transesterification process, 2853 cm^{-1} and 2923 cm^{-1} were attributed to the stretching and asymmetric stretching vibrations of –CH_3. The FTIR result is shown in Figure 4.3.

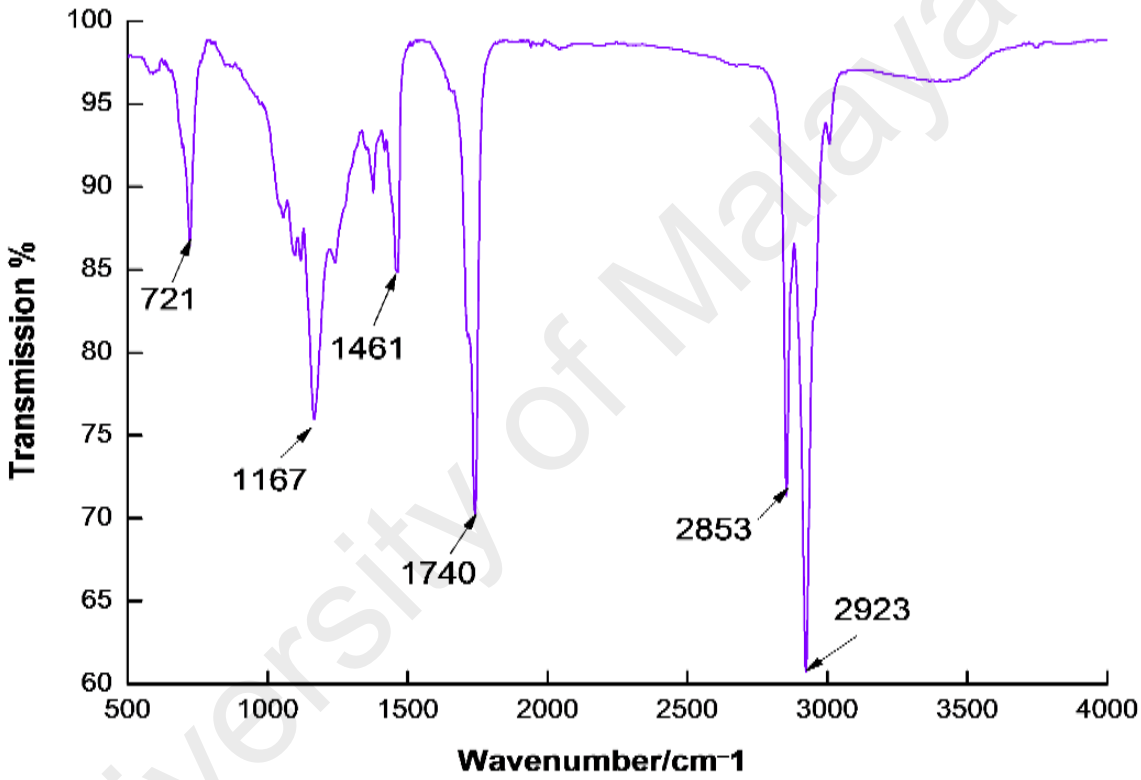

Figure 4.3: FTIR analysis of *Brucea javanica* biodiesel

Gas chromatography was used to determine the fatty acid composition of BJB which is shown in Table 4.4. The dominutesant fatty acids in BJB was methyl palmitate (6.57%), methyl oleate (10%), methyl stearate (49.74), linoleic (25.37%), Methyl arachidate (2.21%), Methyl erucate (2.94%) and others (2.97%).

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Table 4.4: Fatty acid composition of *Brucea javanica* biodiesel using gas chromatograph

Fatty acid	Name	BJB (%)
16:0	Methyl palmitate	6.20
C18:1	Methyl oleate	9.59
C18:0	Methyl stearate	46.93
C18:2	Methyl linoleate	23.94
C20:0	Methyl arachidate	2.09
C22:1	Methyl erucate	2.78
Others	--------	2.81
Total	--------	94.34

4.1.5 Fuel Properties of *Brucea javanica* Biodiesel (BJB)

Fuel properties of biodiesel indicates the quality of fuel and their impact on engine. Acid value of BJB falls in desirable ranges according to American Standard (ASTM) and European standard (EN14214) which was 0.027 mg KOH/g. The present study indicated that kinematic viscosity (KV) of BJB as 3.556 mm2/s which is acceptable and in accordance with American and European standards. The cold properties and pour point of BJB complies with ASTM, which was 2 and 1, respectively. Oxidative stability plays a major role in protecting biodiesel from oxidation after its exposure to air or oxygen. Oxidative stability of BJB was 3.0 h which complies with ASTM but less than the requirement of EN14214. Cetane number (CN) of BJB falls in the range of both ASTM and EN 14214 which is 51 minutes. Both high and low CN results in incomplete combustion. The density of BMJE falls within range of both American and European standards, which was 871.3 kg/m3.

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Table 4.5: Comparison of *Brucea javanica* with other non-edible oils

Non-Edible oil	Density(mg KOH/g)	Oxidative stability (hour)	Viscosity (mm2/s)	Cloud point (^{o}C)	Cetane (minutes)	Flash point (^{o}C)
Tobacco	888	0.8	4.23	--	51.6	--
Neem	884	7.1	5.21	14.4	57.83	--
Mahua	850	--	3.98	--	--	208
Castor	899	1.1	15.25	-13.4	--	--
Jatropha	880	2.3	4.80	2.7	52.31	135
Karanja	--	--	4.80	--	55.84	150
Brucea javanica	871	3.0	3.55	2	51	164
EN14214	900	6	5	--	55	120
ASTM	--	3	6	-3 to 12	47	170

4.1.6 Biodiesel Oxidative Stability with Antioxidants

Low oxidative stability could affect the quality of biodiesel. *Brucea javanica* biodiesel (BJB) had oxidative stability of 3 h which fall in range of ASTM but EN14214 has a standard of 6 to 8 h. This might be due to its high content of polyunsaturated fatty acid. Antioxidants were used to increase the oxidative stability of BJB. Figure 4.4, shows the induction period (IP) of BJO-based biodiesel as a function of the concentration of antioxidant added. The antioxidants were added to the BJO-based biodiesel in a concentration range from 1000 and 5000 parts per millions (ppm). Generally, the induction period of samples were observed to increase with increasing antioxidant concentrations.

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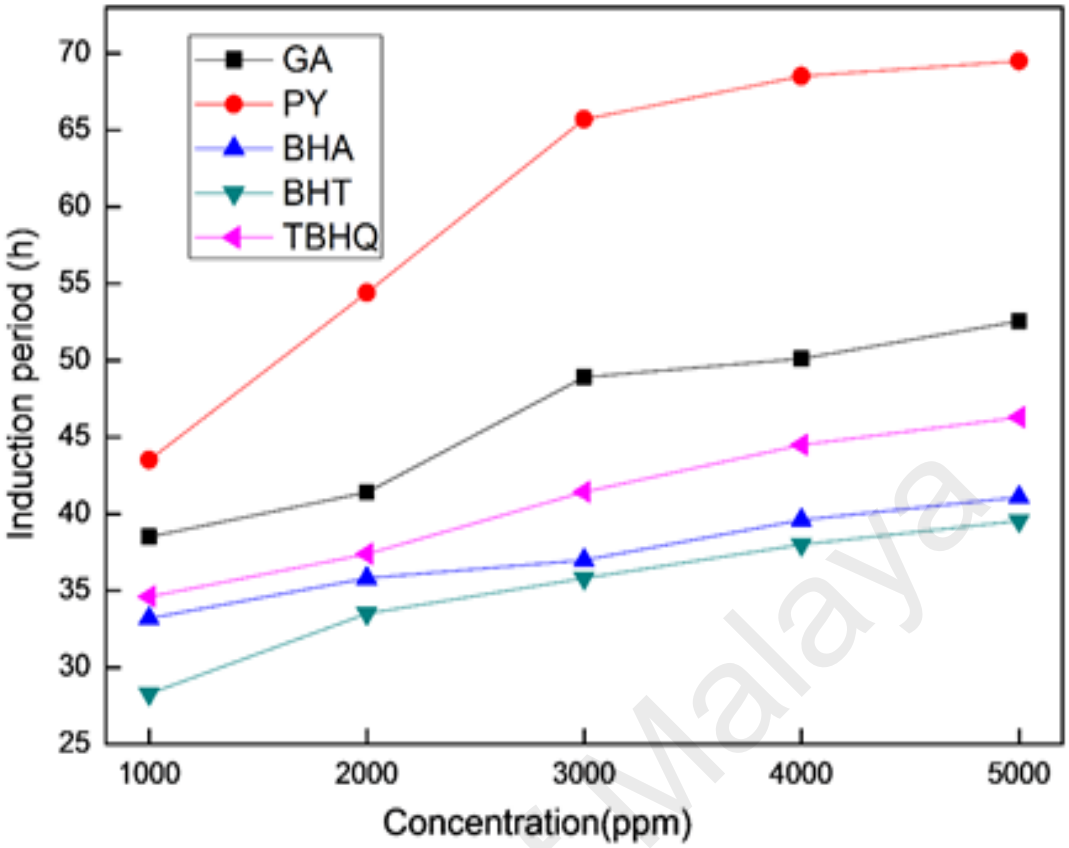

Figure 4.4: Biodiesel oxidative stability improvement with antioxidants

4.2 Biodiesel Production from *Hibiscus Sabdariffa* Seeds oil

4.2.1 *Hibiscus Sabdariffa* Oil Properties

Hibiscus sabdariffa seeds used in this study contain 36 weight percentage (wt %) oil. Physio-chemical properties are the most important criteria to check the quality of oils. The physio-chemical properties were evaluated and the result obtained are shown in the Table 4.6. The oil was yellowish in color at room temperature with a refractive index of 2.16 and an ultrasonic speed of 1.52 ms-1. The acid value of the oil was 5.486 mg KOH/g showing the presence of free fatty acids in the oil. Oxidative stability of the HSO was tested on rancimat which was 3.48 h and the kinematic viscosity of the oil was 14.228 $mm^{2/s}$.

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Table 4.6: *Hibiscus sabdariffa* seed oils physico-chemical properties

Properties	*B. javanica* oil
Physical State at 25 ^{o}C	yellowish in color
Acid Value	5.486 (mg KOH/g)
Kinematic Viscosity	14.228 ($mm^{2/s}$)
Refractive Index at 25 ^{o}C	2.16
Oil Percentage	36 %
Ultrasonic Speed (m/m^{-1})	1.52 ms^{-1}
Oxidation Stability	3.0 h

4.2.2 Variable Optimization using Response Surface Methodology (RSM)

The regression for the determination of predicted values of output parameter *Hibiscus safdariffa* Biodiesel (i.e. HSB yield) is given as follows. The experimental and predicted HSB yield indicate that both the value are very close to each other shown in Figure 4.5.

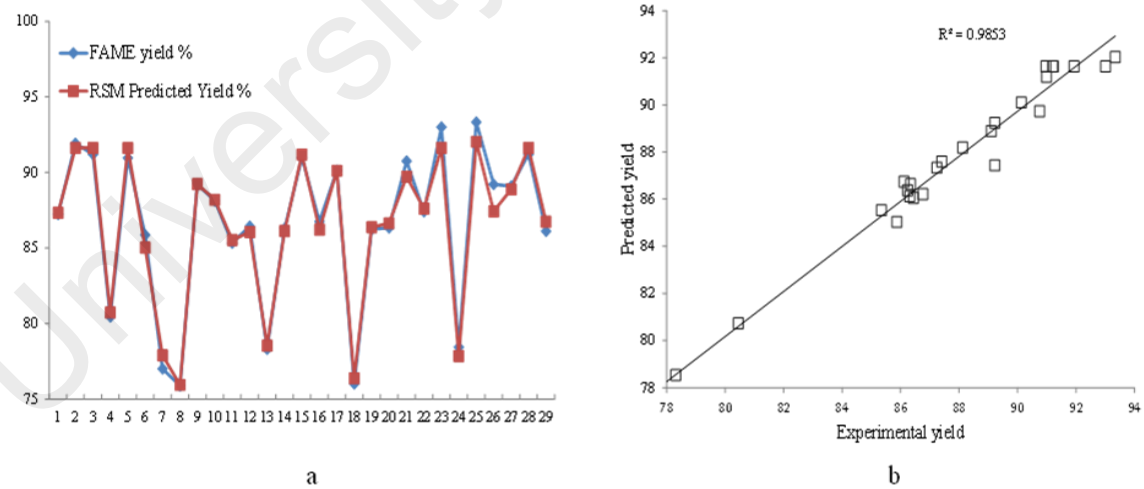

Figure 4.5: Showing experimental vs predicted biodiesel yield

RSM Box-Behnken design with 4 level operating variables and 29 experimental run's ANOVA is shown in Table 4.7. The validity is tested by looking at model p and R-square values. The ANOVA displayed a p-value of > 0.05 and shows its significance. AB, AC,

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BC, A^2, B^2, C^2 and D^2 are model terms in this model. A quadratic model was used which shows p-value less than 0.05 conferring significant at 95 % confidence level. R-square (regression) with value of 0.99 and Adj r-square = 0.98.

Table 4.7: Analysis of Variance (ANOVA)

ANOVA for Response Surface Quadratic Model Analysis of variance table						
Source	Sum of Squares	df	Mean Square	F Value	p-value	
Model	736.79	14	52.63	106.21	< 0.0001	Significant
A-Molar ratio	7.25	1	7.25	14.64	0.0019	
B-Temperature	274.85	1	274.85	554.7	< 0.0001	
C-rpm	0.57	1	0.57	1.15	0.3008	
D-catalyst	20.44	1	20.44	41.24	< 0.0001	
AB	2.36	1	2.36	4.76	0.0468	
AC	1.84	1	1.84	3.71	0.0748	
AD	9.09	1	9.09	18.35	0.0008	
BC	0.27	1	0.27	0.54	0.4765	
BD	0.57	1	0.57	1.15	0.3016	
CD	15.52	1	15.52	31.33	< 0.0001	
A^2	36.07	1	36.07	72.8	< 0.0001	
B^2	373.74	1	373.74	754.27	< 0.0001	
C^2	38.44	1	38.44	77.58	< 0.0001	
D^2	6.61E-03	1	6.61E-03	0.013	0.9097	
Residual	6.94	14	0.5			
Lack of Fit	4.17	10	0.42	0.6	0.765	Insignificar

- $R^2 = 0.99$
- $Adj\ R^2 = 0.98$
- $Pred\ R^2 = 0.96$

The predicted and experimental values for *Hibiscus sabdariffa* biodiesel (HSB) yield responses at the design points and all variable shown in Table 4.8.

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Table 4.8: Biodiesel yield versus predicted yield

Run	A:Molar ratio	B:Temperature °C	C:Agitation rpm	D:catalyst %	Biodiesel %	Predicted %
1	6	60	750	0.5	87.24	87.34
2	6	67.5	750	1	91.93	91.63
3	6	67.5	750	1	91.21	91.63
4	6	75	750	1.5	80.45	80.74
5	6	67.5	750	1	90.97	91.63
6	9	67.5	750	0.5	85.87	85.03
7	6	75	900	1	77.01	77.89
8	9	75	750	1	75.88	75.94
9	3	67.5	750	1.5	89.22	89.24
10	6	67.5	600	1.5	88.12	88.19
11	9	67.5	600	1	85.34	85.54
12	6	67.5	900	0.5	86.44	86.05
13	3	75	750	1	78.31	78.53
14	6	60	900	1	86.31	86.12
15	9	67.5	750	1.5	90.99	91.19
16	9	60	750	1	86.76	86.21
17	3	67.5	750	0.5	90.13	90.10
18	6	75	600	1	76.01	76.37
19	9	67.5	900	1	86.24	86.37
20	6	60	600	1	86.34	86.63
21	6	60	750	1.5	90.76	89.73
22	3	67.5	900	1	87.41	87.60
23	6	67.5	750	1	93.01	91.63
24	6	75	750	0.5	78.44	77.84
25	6	67.5	900	1.5	92.34	92.03
26	3	67.5	600	1	89.22	87.43
27	6	67.5	600	0.5	89.1	88.88
28	6	67.5	750	1	91.22	91.63

4.2.3 Effects of Parameters on Biodiesel Yield

The independent variable's impact on yield was drawn with help of response curve using 3-D surface. The peak point was obtained when independent variables interact perfectly and optimal yield was achieved, which lead to the conclusion that response

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variable depends on relationship and interaction between independents variables. For *Hibiscus sabdariffa* biodiesel production , a methanol to oil ratio of 6:1, temperature 67.5 ^{o}C, catalyst 1% with a yield of 93.01 % were found to be the optimum parameters. The fuel properties of biodiesel produced were within the range of international standards such as EN14214 and ASTM standards. The 3-D surface response is shown in Figure 4.6.

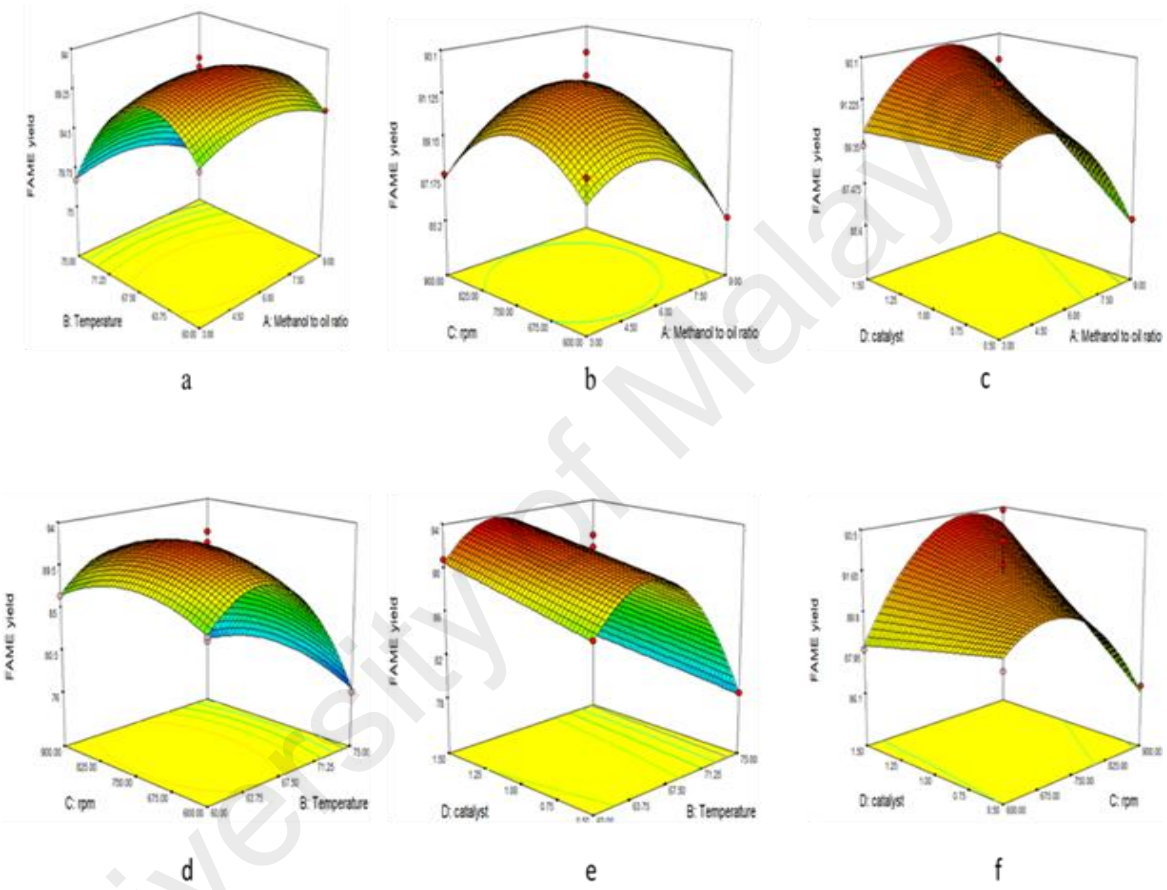

Figure 4.6: 3-D surface plot showing parameters effect on biodiesel yield: (a) methanol ratio and temperature (b) rpm and methanol ratio (c) methanol ratio and catalyst (d) temperature and rpm (e) temperature and catalyst and (f) rpm and catalyst

4.2.4 Fourier Transform Infrared Spectroscopy Analysis of Biodiesel

Identification of spectrum peaks of *Hibiscus sabdariffa* biodiesel are displayed in Figure 4.7. The spectrum 720.55 cm-1 – 970 cm-1 showed =C-H functional group presence. At low frequency and low energy a bending type of vibration is found in those groups and these are all double bounded.

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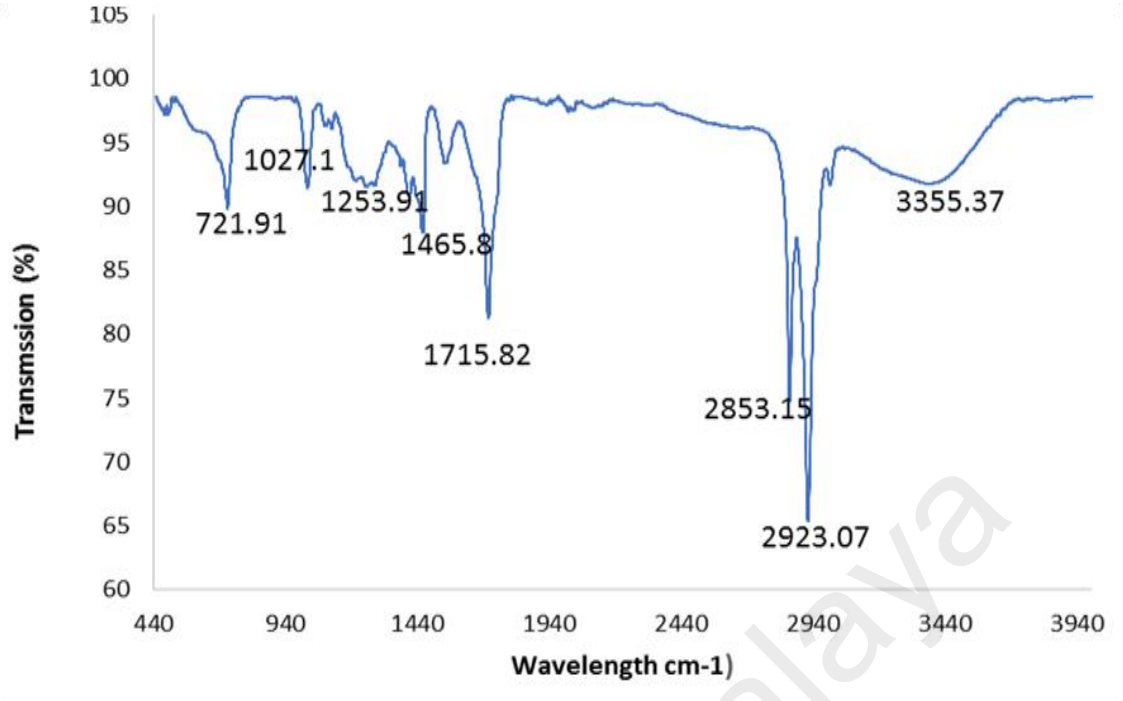

Figure 4.7: FTIR analysis of fatty acid biodiesel

4.2.5 Biodiesel Physicochemical Properties

The EN 14214 and ASTM D6751 are the international standard used to compare fuel properties. *Hibiscus sabdariffa* biodiesel (HSB) physicochemical are shown in Table 4.9. The kinematic viscosity (kv) of HSB biodiesel as (4.55 mm2/s,) which falls within international standards such as ASTM D6751 and EN 14214 (1.9 – 6.0 mm2/s and 3.5 – 5.0 mm2/s). The density of biodiesel depends on its purity and composition of fatty acid in it. The HSB density found was (856 kg/m3), which follows the range of specifications. Injection system of fuel could be affected if the density is increased in turn it become more viscous. The acid value (AV) measured for HSB biodiesel was 0.054 KOH/g and falls within range of international standards.

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Table 4.9: Comparison of *Hibiscus sabdariffa* biodiesel with other non-edible seed oils

Plant Seed oils	Density (mg KOH/g)	Cloud point (^oC)	Flash point (^oC)	Oxidative stability (hour)	Viscosity (mm2/s)	Cetane number (minutes)
Roselle	856	3	161	3.48	4.55	49
Neem	884	14.4	--	7.1	5.21	57.83
Mahua	850	--	208	--	3.98	--
Castor	899	-13.4	--	1.1	15.25	--
Jatropha	880	2.7	135	2.3	4.80	52.31
Karanja	--	--	150	--	4.80	55.84
Melada pahit	871	2	164	3.0	3.55	51
EN14214	900	--	120	6	5	55
ASTM	--	-3 to 12	170	3	6	47

4.3 *Brucea javanica* seeds as source of potential natural antioxidants to improve biodiesel thermal and oxidative stability

4.3.1 Total Phenolic and Flavonoid Content of *Brucea javanica* Seed Extracts

The extractable phenolic compound content in *Brucea javanica* seed extract was determined by using the linear gallic acid standard curve (R2=0.966). Total flavonoid and phenolic contents amount in *Brucea javanica* seeds extract is listed in Table 4.10. Among the three extracts of *Brucea javanica* seed, ethyl acetate extract exhibits the highest capacity of total phenolic at 98.5±0.1 mg GAE/g of dry extract. The data implies that ethyl acetate extract is the more extractable solvent to extract phenolic content.

The total flavonoids content suggests that ethyl acetate extract exhibits the highest total flavonoid content at 20.0 ±0.4 mg QAE/g of dry weight and methanol extract exhibits at 12.9 ± 0.3mg QAE/g of dry weight. Hexane extract presented the lowest amount of flavonoid content at 10.0 ±0.4 mg QAE/g of dry weight. Ethyl acetate extracts showed highest TPC and TPC because of its polarity to extract more compounds as compared to methanol and hexane.

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Table 4.10: Total Flavonoid and Phenolic Contents in *B. javanica* Seed

Fractions	Ethyl Acetate	Methanol	Hexane
TFC (QAE/g dry extract)	20 ± 0.4^{a}	12.9 ± 0.3^{b}	10 ± 0.4^{c}
TPC (GAE/g dry extract)	98.5 ± 0.1^{a}	85.3 ± 0.6^{b}	36.9 ± 0.8^{c}

Data are mean ± SD (n=3). Mean with different lower cases (a, b, c) in the same column are significantly different at $P < 0.05$ using ANOVA

4.3.2 Mass Spectrometry Analysis

GCMS identification and analysis was carried out in order to identify compounds that are possibly present inside *Brucea javanica* seed extracts shown in Table 4.11.

To identify the chemical compounds LCMS was conducted and hence confirmation of antioxidant compounds present in *Brucea javanica* seed was achieved.

The analysis was performed using LCMS technique for methanol and ethyl acetate extracts of *Brucea javanica* seed and chromatogram. The compound with their retention time and molecular formula are listed in Table 4.12.

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Table 4.11: GCMS Analysis of *B. javanica* seed extract

Peak No	Retention Time	%Area	Chemical Compound	Reference
1	5.1239	0.0521	2-Decene,6-methyl	27063
2	7.3211	0.1454	2-Decenal	26669
3	7.7503	0.0588	2,4-Decadienal	25129
4	8.0707	0.0775	2,4-Decadienal, (E,E)	25126
5	8.8775	0.7042	n-Decanoic acid	39473
6	10.102	0.3125	Cyclopentane, decyl	69587
7	10.6113	0.0564	Dodecanoic acid, biodiesel	72688
8	10.9889	0.039	Cyclohexene, 1-nonyl	67950
9	11.2693	0.1815	Cyclohexadecane	81247
10	11.8243	0.0204	11-Hexadecyn-1-ol	92516
11	12.4022	0.5184	8-Heptadecene	92565
12	12.9401	0.2045	Tridecanoicacid,12-biodiesel	95899
13	13.4837	0.7112	3-Octadecene	104188
14	13.9815	0.0318	Naphthalene	16914
15	14.5651	0.1061	1-Nonadecene	115904
16	14.7769	0.1189	cis-9-Hexadecenal	92517
17	15.0858	6.0428	Hexadecanoic acid, biodiesel	119407
18	15.8068	5.2165	n-Hexadecanoic acid	107549
19	16.8082	7.6304	9-Octadecenoic acid, biodiesel, (E)	141310
20	17.5234	22.5493	Oleic Acid	129337
21	17.9697	3.5004	Octadecanoic acid	131262
22	18.2386	8.5806	9-Eicosyne	126189
23	19.2171	4.9914	10-Heneicosene (c,t)	139793
24	19.755	4.6028	9,17-Octadecadienal, (Z)	114272
25	20.6075	8.2593	1,13-Tetradecadiene	56485
26	20.7792	5.6151	(Z)-14-Tricosenyl formate	194460
27	21.317	1.179	9,12-Octadecadienoic acid (Z,Z)	127648
28	21.689	0.456	cis-Vaccenic acid	129339
29	22.1525	0.6666	Z,E-2,13-Octadecadien-1-ol	115874
30	22.244	0.7581	Triacontyl acetate	232691
31	23.1195	1.6968	Docosane	153223
32	24.7388	9.6426	Heneicosane	141424
33	25.1107	4.1019	Stigmastan-3,5-diene	210541
34	26.8044	0.7648	Erucic acid	175491
35	27.2336	0.4068	9,12-Octadecadienoic acid	127647

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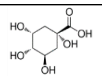
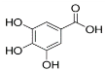
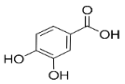
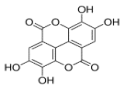
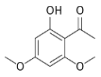
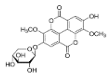
Table 4.12: LCMS Analysis of *Brucea javanica* seed extracts

Peak	R. Time	Compound	Molar Mass g/mol	Structure
1	1.97	Quinic acid	192.1	
2	3.02	Gallic acid	170.1	
3	3.82	Protocatechuic acid	154.1	
4	4.62	Ellagic acid	302.1	
5	4.63	Brevifolin	196.2	
6	5.40	Ellagic Acid Isomers	--------	------
7	7.54	Di-O-methyl Ellagic acid	462.3	

4.3.3 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

The summary of DPPH assay is shown in Figure 4.8. The results indicate that ethyl acetate extract of *Brucea javanica* seed shows the highest DPPH inhibition (90 % with IC_{50}= 31.2 μg/mL) using different concentrations of seeds extract followed by methanol extract with 82 % inhibition (IC_{50}= 184 μg/mL) using different concentrations of seeds extract (62.5, 125, 250, 500 and 1000 μg/mL) and the lowest inhibition is shown by n-hexane extract, up to 78 % DPPH inhibition with (IC_{50}= 319μg/mL) using different concentrations of seeds extract. Ascorbic acid was used as control.

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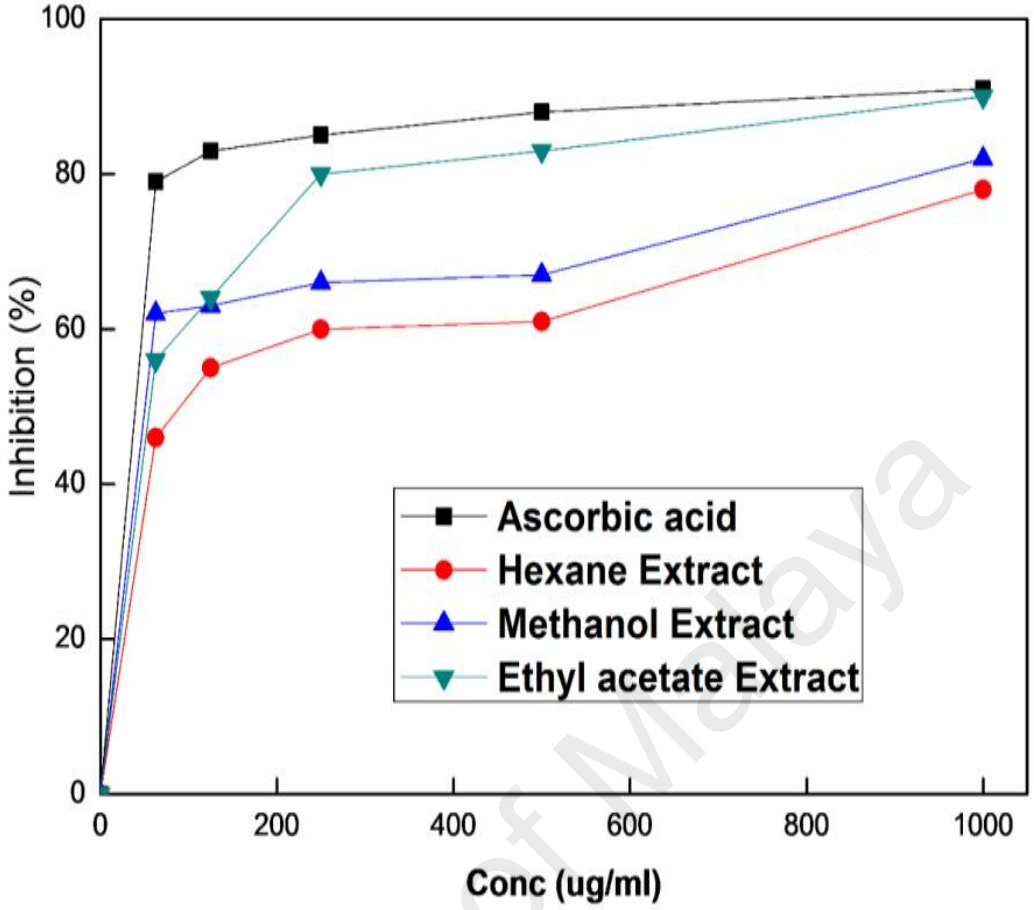

Figure 4.8: DPPH test of ethyl acetate, methanol and hexane extract of *Brucea javanica* seed

4.3.4 Metal Chelating Activity Assay

The procedure used to determine metal chelating activity was the ion chelating activity of ferrous shown in figure 4.9. The resolution suggests that the ethyl acetate extract of seed shows the highest inhibition up to 59% with (IC50=299 μg/mL) followed by methanol extract with 27 % inhibition with (IC50=656 μg/mL) and the lowest activity is shown by hexane extract with 22 % inhibition (IC50= 1100 μg/mL) at different concentrations of seeds extract.

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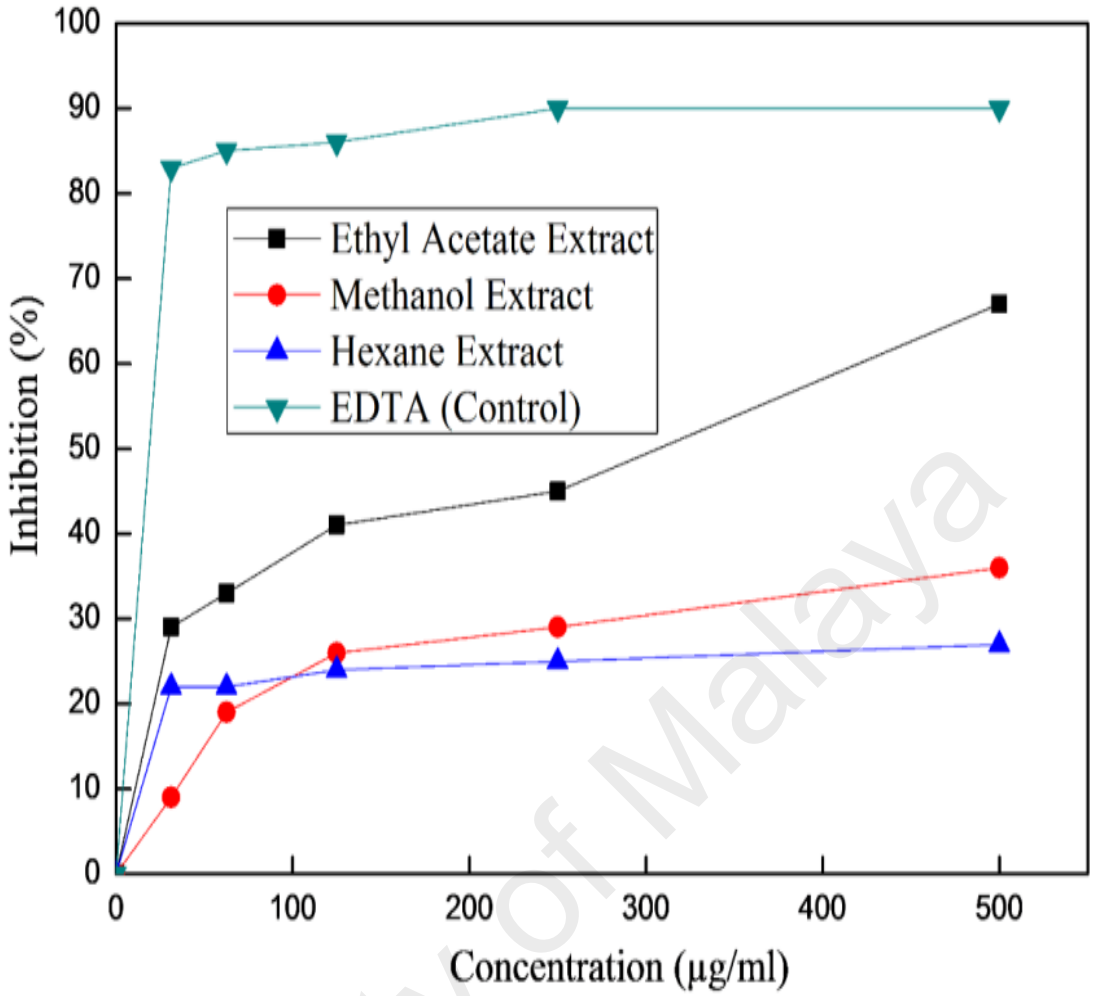

Figure 4.9: Metal Chelating activity assay of ethyl acetate, methanol and n-hexane extracts of *Brucea javanica* seed

4.3.5 Ferric Reducing Antioxidant Power Assay (FRAP)

Frap assay react with ferric tripyridyltriazine [Fe3+-TPTZ] complex and measure the reducing potential of antioxidant by producing [Fe2+-TPTZ]. Frap assay treats the antioxidants in the sample as a reductant. In the proposed study, the trend for ferric ion reducing activities of *Brucea javanica.*

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Table 4.13: FRAP assay of *Brucea javanica* seed extract

Seed Extracts	FRAP (mmol Fe2+/g extract)
Methanol Seed Extract	0.180 ±0.003
Ethyl acetate Seed Extract	0.163 ±0.002
Hexane Seed Extract	0.15 ±0.004

4.3.6 Addition of *Brucea javanica* Seed Crude Extract to Biodiesel

The DPPH assay has been shown to be a good predictor of the oxidative stability of oils as determined using thermal oxidations of oil. Biodiesel was tested for the antioxidant activity at 80 ºC. Absorbance of DPPH in methanol with adding of *Brucea javanica* seed extract residue in different concentrations (200, 400 and 600 mgL-1) to biodiesel is shown in Figure 4.10.

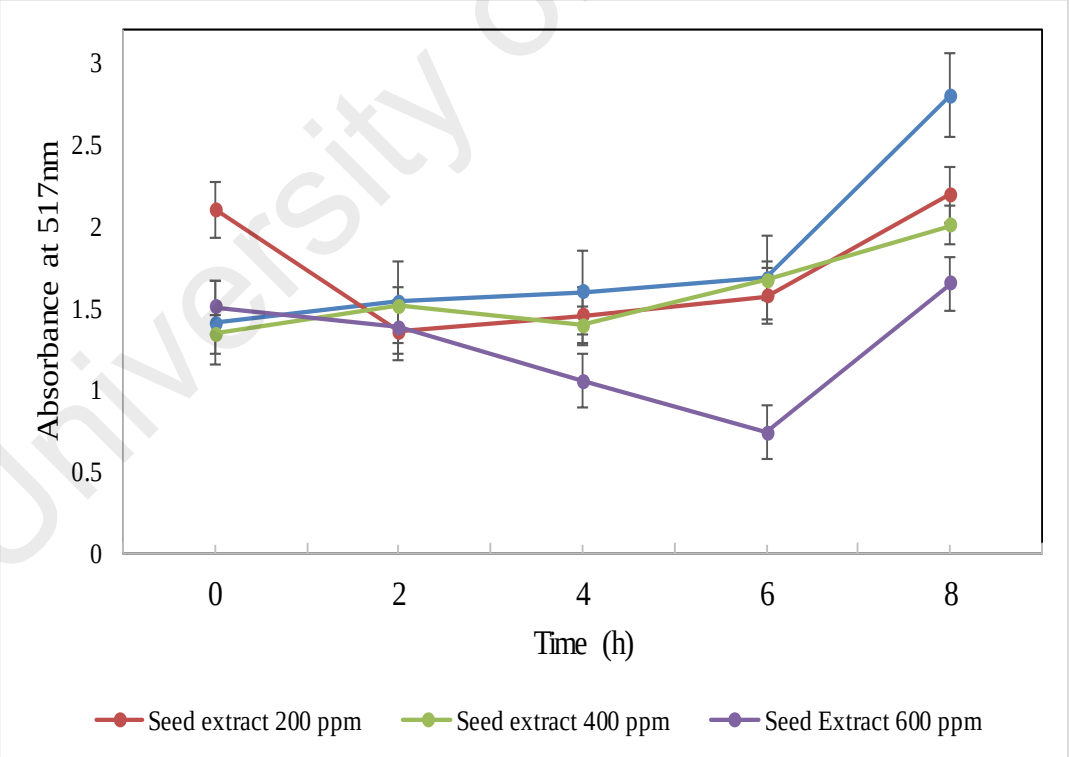

Figure 4.10: Effect of *Brucea javanica* seed crude extracts on Biodiesel

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4.3.7 Rancimat Test

Plants and plant derived product are rich sources of natural antioxidants Plant extracts are considered as a potential natural antioxidants because of the presence of phenolic, flavonoids and anthocyanin compounds. In order to confirm autoxidation effect of phenolic compounds of *Brucea javanica* seed, gallic acid was identified and detected in both extracts of *Brucea javanica* seed confirmed by LCMS profiling. After confirming the presence of gallic acid as clear peak in LCMS. The gallic acid was applied as a natural antioxidant for biodiesel to test its oxidative stability in the Rancimat machine with different concentrations from 2000 to 10,000 ppm is shown in Figure 4.11.

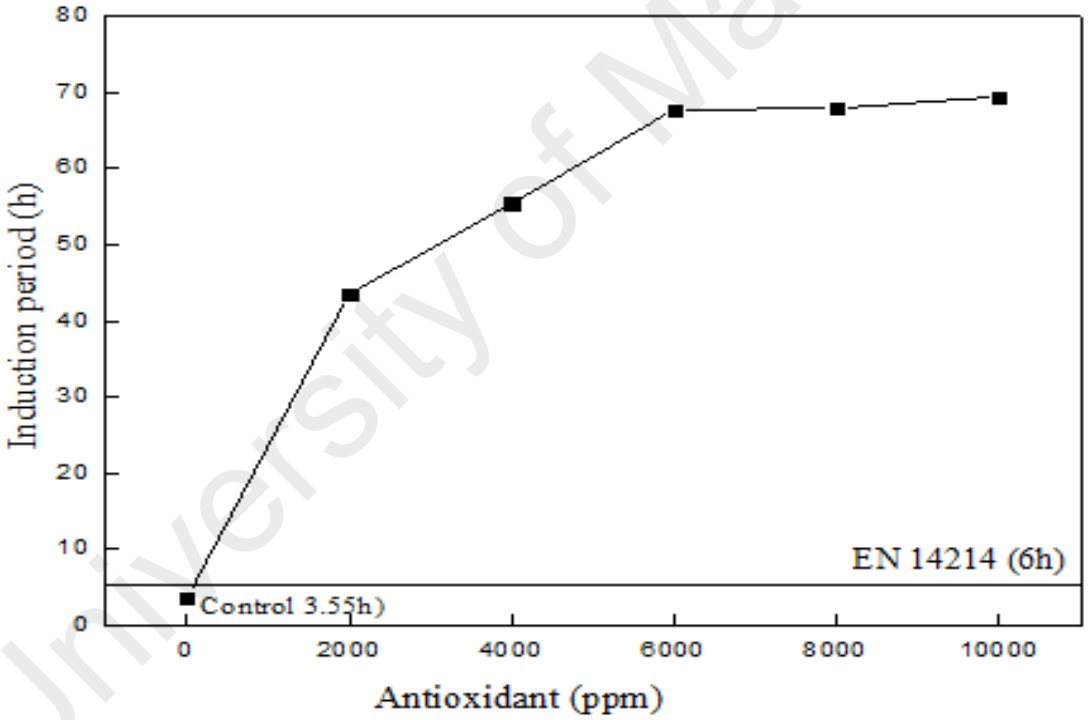

Figure 4.11: Influence of natural antioxidant on oxidative stability of biodiesel at 110 ^{0}C with concentration (2000-10,000 ppm) in Rancimat

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CHAPTER 5: DISCUSSION

5.1 Biodiesel Production from *Brucea javanica* Seeds oil

Life is dependent on energy as it is a basic need to power the generation sector, to run transportations and to smoothly carry on industrial work. Currently, petrochemical reservoirs are the major source of meeting the requirements of most of the world's energy such as natural gas, coal and petroleum (Suganthi & Samuel, 2012). Fossil fuels are used extensively for energy production especially petroleum but concern about its sustainability leads towards emphasis in alternative fuel resources (Verma & Sharma, 2016). Interests are diverted in to new alternative renewable sources for energy which can reduce environmental pollution and dependency on fossil fuel which are depleting and non-renewable (Ong et al., 2013).

Worldwide biofuel demand is increasing rapidly because of the shrinking of oil reservoirs and environmental concerns such as global warming and emission of toxic gases to the environment (Balat & Balat, 2009). Biofuel is a broad term which is produced from different biological sources and feedstocks such as plants, forestry by products, agricultural crops and wastes and fats (Demirbaş, 2002). Malaysia's oil producing capacity has dropped considerably to 13% from 2006 to 2008 and it is predicated that the under surface crude oil in Malaysia could diminished in 20 years' time (Oh et al., 2010). This will become a major concern to the country as two-thirds of the consumption in Malaysia is petro-diesel (Jayed et al., 2011).

Amongst biofuels, biodiesel is gaining much more importance due to the decreasing amounts of petroleum fuel reservoirs and its several advantages such as renewability, minimal degradation duration and its non-hazardous effects. Biodiesel is produced through transesterification from different sources such as waste cooking oil, animal fats and vegetable oil (Hincapié et al., 2011). Biodiesel production have also been reported

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several times from feedstocks such as tea oil (Demirbas, 2010), cotton oil (Qian et al., 2010), tobacco oil (Usta et al., 2011) palm oil (Nur et al., 2014), palm kernel oil (Awalludin et al., 2015), canola oil (Kai et al., 2014) and soybean oil (Samart et al., 2010). Biodiesel is derived from bio-based resources and could be a potential alternative to petroleum diesel because of its renewable characteristics. Different solvents have been used in transesterification process of biodiesel production but methanol has advantages in reactivity and cost-effective. Biodiesel can be considered as completely renewable fuel (Verma et al., 2017). Edible plant oils and fats from animals are consumed as food and their usage as feedstock for biodiesel is questionable with great concern. As vegetable oil is used for food and its demand has increased in recent years especially in third world countries, it is difficult to justify its usage as biodiesel feedstock. Recent report revealed food versus fuel competition such as soybean oil in North America, palm and coconut oil in Malaysia, castor in Brazil and jatropha in India (Sanli & Canakci, 2008).

Non-edible oils such as *Jatropha curcas* (ratanjyot), *Madhuca longifolia* (mahua), *Azadirachta indica* (neem), *Pongamia pinnata* (karanj) and *Moringa oleifera* (moringa seed) are commercially available non-edible feedstocks for biodiesel (Verma et al., 2016a). Other non-edible oils such as *Nicotiana tabacum* (tobacco), *Acrocomia aculeate* (macaúba), *Crambeabyssinica* (hochst), linseed oil, rubber seed oil, *Sapium sebiferum* (chinese tallow), *Sapindus mukorossi* (soapnut), *Euphorbia tirucalli* (milk bush), *Calophyllum inophyllum* (polanga) and nahor oil have been used as feedstocks (Demirbas, 2007). Therefore, more emphasis is now given to non-edible seed oils as a source for biodiesel. *Brucea javanica* belongs to the Simaroubaceae family, which is an evergreen plant, with its seed also known as yadanzi, was first cited in the Chinese medical monograph called *Compendium of Materia Medica*, published in the sixteenth century. *Brucea javanica* seeds are the potential source of oil which could be utilized in pharmaceuticals and biofuel. *Brucea javanica* grows up as a shrub or small tree to 5

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meters (20 ft) tall. The tiny flowers (1.5--2 mm in diameter). The flower anthers are typically red. It typically flowers in June and July and sets fruit in July and August. Each fruit, which are a drupe, measures up to 0.5 cm (0.2 in) long. When ripe they are a black gray color that becomes wrinkled when dry. The seeds are intensely bitter, yellowish and covered with an oil membrane. Three hectare of plantation could produce approximately 17.0 tonnes seeds annually. Oil content of the seeds are reported to be in the range of 35-42 weight percentage wt%. *Brucea javanica* grows naturally from Sri Lanka and India to China, Indonesia, Malaysia, New Zealand and Australia. Its habitat includes open areas, secondary forest and sometimes sand dunes (Dai et al., 2007). This plant, its seeds and seeds oil is shown in Figure 5.1.

Figure 5.1: *Brucea javanica* plant (plant, seeds and oil)

5.1.1 Optimization of Transesterification Parameters using Response Surface Methodology

The transesterification parameter parameters were optimized using response surface methodology (RSM) based on Box-Behnken experimental design which has not been reported yet for this plant. For the optimization of variables, response surface methodology is a great technique which includes models building, design of experiment and statistics (Kalil et al., 2000). RSM has the capability of minimizing the experimental run with suitable proof for the result to be accepted statistically (Boey et al., 2013). RSM is a useful tool to be applied in investigating the effect of different independent parameters

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or combine effect of independent variable on dependent parameter of an experiment and suggesting that where the model borders should be, and treatment combinations could be avoid (Boey et al., 2013). It is an iterative investigational method focused precisely on discovering the role of singular process parameter and also the influence of their interaction with one another in carrying out the responses. Independent variables such as the methanol to oil molar ratio, temperature and catalyst concentrations were the operating parameters which effects the process significantly (Chellamboli & Perumalsamy, 2014). RSM is based on mathematical model which stimulate the optimization of parameters and the outcome is the maximum yield of biodiesel. RSM could address the problem of where the experimental limits should be, what should be the range and help in setting the optimal range where the maximum yield can be achieved. Other advantages are cost saving, reduce the need of running large number of experiments which save money (Ghadge & Raheman, 2006), time and efforts. The experimentation design for synthesis of biodiesel is capable of stimulating transesterification condition with good estimations of errors (Ghadge & Raheman, 2006). A large numbers of researchers implement RSM for optimization process of biodiesel production using different feedstocks. For example, up to 98% biodiesel yield from neem oil by RSM optimization. RSM based on the Box-Behnken also effectively optimized biodiesel yield from waste cooking oil up to 99.5% (Hamze et al., 2015). Optimization of operating parameters methanol to oil ratio, temperature and catalyst concentration could improve biodiesel yield significantly (Dwivedi & Sharma, 2015). An equation was developed after the results were analysed for FAME. The model achieved using the experiments results were designed using Box Behnken design shown in Equation (3). The relationship between variables (input) and the response (output/yield) were studies using the model equation. A (molar ratio), B (catalyst) and C (temperature) are labelled as A, B and C in equation 3. The equation is given below;

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$$Biodiesel\ yield = 92.94 + 0.13 \times A - 0.22 \times B - 0.3 \times C - 0.63 \times A \times B + 0.98 \times A \times C + 0.5 \times B \times C - 1.76 \times A^2 - 1.32 \times B^2 - 6.53 \times C^2 \quad \text{.....(3)}$$

In this case AB, AC, BC, A2, B2 and C2 are model terms. The quadratic model coefficients developed and response. As stated above the model was found significant at 95% confidence level as the value of p-value is less than 0.05. The regression statistics goodness of fit (R^2 = 0.91) and goodness of prediction (Adjusted R^2= 0.80) for the response.

Note that the R^2 value indicates the total variability of model response after considering all the significant parameters and terms. Both the p-value and the R^2 of the model indicate that the model fits the data very well. Adequate Precision was (7.38)' indicates the signal to noise ratio.

Normally the ratio greater than 4 is desirable (Patel et al., 2016). Statistical test and quadratic model was developed in order to bring the adequacy of the model. The results of ANOVA proposed that the developed 'Regression Model' was best fit with R^2 of 0.91 and p >0.05. Furthermore, R^2 of the model was calculated which was used to see how well data is represented. The model had value of *R*-square (0.9143) which was close to unity 1, suggesting that the developed model equation for the yield of BJB was a good representative of the system. There was also a good agreement in experimental and predicted yield. Majority of the value of residual standard should lie in intervals ± 3. Any value outside this interval renders is an operational error in the data or model (Noshadi et al., 2012). The figure indicated that there is no data beyond this interval ± 3, which suggests that the model is consistent with the experimental data with no error recorded.

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5.1.2 Effects of Independent Variables on Biodiesel Yield

5.1.2.1 Effect of Methanol to Oil Ratio

To indicate the effects of independent parameters on dependent one, a 3-D response curves were drawn shown and obtained by using RSM shows the optimum value of methanol to oil molar ratio effect on yield. It is observed that too low or too high values of the methanol to oil ratio have undesirable effects. This could be described by the fact that the transesterification process was an equilibrium reaction in which excess alcohol could increase reaction and in turn increase yield or decrease the yield by increasing solubility of glycerol. Low levels of methanol to oil molar ratio could create an incomplete reaction. The optimum methanol to oil ratio was 6:1 with highest yield of biodiesel. Literature suggests that due to reactivity of methanol it is opted for using as solvent in transesterification process but its excessive usage could increase polarity which in turn increases glycerol solubility and make it difficult to separate from alkyl ester (Verma et al., 2017). The results indicated that molar ratio has substantial effect on biodiesel among different reaction parameters as described previously. Therefore, both catalyst concentration and methanol to oil molar ratio displayed optimum value whilst too high or too low catalyst concentrations or methanol ratio results in a reverse trend. When the concentration of catalyst turns out to be too high, soap can be rapidly formed creating a problem in separating the biodiesel, thus yield may decrease as well. On the other hand, excess of methanol could) increase yield but could also increase the solubility of glycerol resulting in a reaction by reducing the yield and this could be relate to Le Chatelier's principle, which states that further proceed of the transesterification may lead to the reverse of reaction to the reactant side and hence, biodiesel yield may be lowered (Wong et al., 2015). The optimal molar alcohol ratio to oil play a major role among reaction parameters to improve biodiesel yield because lower ratio can cause incomplete reaction and higher ratio would decrease the yield (Verma & Sharma, 2016).

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5.1.2.2 Effect of Catalyst Concentration

It clearly shows that the concentration of catalyst around 1.25% would produce the highest yield of biodiesel. After that a decreasing pattern was seen in the yield when the concentration of catalyst was outside that range. Since methanol and triglyceride in the BJB are immiscible, adding a catalyst could help the transesterification reaction, and increases the yield rapidly. However, when the concentration of catalyst was too low or too high, soap formation could occur which makes glycerol separation from biodiesel more difficult, this may decrease yield. In contrast, inadequate usage of catalyst could result in an incomplete reaction and a lower yield. Biodiesel yield increased with the increase in amount of catalyst. When the catalyst amount reached a certain value, a reversed trend was observed (Dwivedi & Sharma, 2015). Our finding were in agreement with previous literature that explain effect of catalyst concentration on biodiesel yield, an increase in concentration of catalyst can increases yield up to a certain point that may be due to giving more alkyl ester in higher reaction but after that certain point yield decreased significantly (Verma et al., 2017). The catalyst concentration effect after certain amount added was present in BJB as yield starts decreasing significantly.

5.1.2.3 Effect of Temperature

The temperature effect was observed by temperature variation in the range of 60 to 70 ^{o}C. Results indicated that when temperature raises, biodiesel yield increases but once the temperature crosses 65 ^{o}C, a reverse trend is seen and yield decreases. The effect could be explained using the Arrhenius equation which states that steady increases in reaction rate constant by temperature increase which might increase the yield. Methanol vaporizes when temperature is too high resulting in a two phase interface by decreasing yield. Optimum yield for alkali transesterification was acquired at 65 ^{o}C temperature (Sun et al., 2014). Optimum temperature, 65 ^{o}C, which produced the highest yield of biodiesel to up to 94.34 % which are in agreement with a previous study which showed a biodiesel

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yield of 98.4% was achieved by optimizing parameters using response surface methodology (Dwivedi & Sharma, 2015). A decreased pattern is seen in the yield when concentration and temperature was outside the range. Inadequate usage of high or low temperature catalyst could result in an undesirable reaction with a lower yield. The best optimum conditions obtained were 6:1 methanol to oil ratio, 1 % catalyst and 65 ^{o}C temperature with highest yield of 94.34 % biodiesel. Our finding also in agreement with the previous literature which reveals that biodiesel yield increase as temperature increases up to 60 to 65 ^{o}C and thereafter a significant drop in the yield. The maximum yield obtained at the mentioned range is due to better mixing of oil with methanol and glycerol can also be easily separated at that range. On the other hand when temperature increases after that value could decrease yield and reason might be that a higher temperature, side reaction such as alkyl ester hydrolysis could occur and produce acids. The other possible reason is methanol polarity which decreases when temperature is higher (Verma et al., 2017). The results suggested that response surface methodology could be effective in the optimization of *Brucea javanica* biodiesel by optimizing the parameters for transesterification such as methanol ratio, catalyst and temperature.

5.1.3 Gas Chromatography Analysis and FTIR Analysis (Fourier transform infrared spectrum) of *Brucea javanica* Biodiesel.

In the functional group –CO–O–CH_3 of *Brucea javanica* biodiesel (BJB), which was generated during the transesterification process, 2853 cm^{-1} and 2923 cm^{-1} were attributed to the str*etc*hing and asymmetric str*etc*hing vibrations of –CH_3,. On the other hand, 1461 cm^{-1} corresponded to the asymmetric bending vibration of –CH_3, whilst 1167 cm^{-1} was due to the str*etc*hing vibration from O-CH_3; and 1740 cm^{-1} was assigned to the vibration of −C=O. The stretching C=O peak is was 1742 cm^{-1} and were encountered commonly in FAME which is located in the range of 1700-1800 cm^{-1} and is an ester (Soares et al., 2008). The fingerprint region (1500-900 cm^{-1}) which is a major spectrum from biodiesel

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has a peak at 1461 cm^{-1} corresponded to bending vibrations of $–CH_3$ (Rabelo et al., 2015). These results reflect the conversion of triacylglycerols which could be converted into biodiesel (Siatis et al., 2006). Gas chromatography was used to determine the fatty acid composition of BJB which is shown in Table 3.4. The dominant fatty acids in BJB was methyl palmitate (6.57%), methyl oleate (10%), methyl stearate (49.74), linoleic (25.37%), Methyl arachidate (2.21%), Methyl erucate (2.94%) and others (2.97%).

5.1.4 Fuel Properties of *Brucea javanica* Biodiesel (BJB)

Fuel properties of biodiesel indicates the quality of fuel and their impact on engine. Acid value is an important property, which shows the free fatty acid value in fuel. Generally, high acid value is not desirable as it could produce corrosion in fuel systems (Ghadge & Raheman, 2005). Acid value of BJB falls in desirable ranges according to American Standard (ASTM) and European standard (EN14214) which was 0.027 mg KOH/g. Higher kinematic viscosity (KV) causes poor atomization which creates operational problems when injected into chamber therefore, leads to deposits in engines. Studies showed that if KV is high then such problems would exist in the engine (Gerpen et al., 2004). The present study indicated that KV of BJB as 3.556 mm2/s which is acceptable and in accordance with American and European standards.

Another important property of biodiesel is its cold property, which has essential parameters such as cloud point (CP) and pour point (PP). Whenever the temperature falls, crystal nuclei's appear and become visible in biodiesel, which makes it cloudy and in turn stops fluid pouring. The nuclei crystal form is termed as CP and when temperature further decreases and pouring halts and that point is termed as PP (Verma & Singh, 2014). The cold properties and pour point of BJB complies with ASTM, which was 2 and 1, respectively.

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Oxidative stability plays a major role in protecting biodiesel from oxidation after its exposure to air or oxygen. Oxidative stability of BJB was 3.0 h which complies with ASTM but less than the requirement of EN14214. It depends on fatty acid compositions and presence of antioxidants in the oil (Dunn & Knothe, 2003). Antioxidant can improve oxidative stability to an extent (Dunn, 2008) that's why antioxidants were used to improve oxidative stability of biodiesel. Ignition quality of any fuel is determined by its cetane number. The cetane number (CN) of BJB falls in the range of both ASTM and EN 14214 which is 51 minutes. Both high and low CN results in incomplete combustion (Gerpen et al., 2004). The density of BMJE falls within range of both American and European standards, which was 871.3 kg/m^3. Biodiesel purity and fatty acid profile suggests the density of fuel, which depends on the atoms of carbon. If carbon atoms increase, density decreases to a certain extend and vice versa. Higher density creates viscosity problems in the engine (Demirbas, 2008a). Physico-chemical properties of BJB were compared with different non-edible oils properties.

5.1.5 Oxidative Stability with Antioxidants

Biodiesel oxidation stability is important because when the oxidative reaction occurs, biodiesel deteriorates. The major problem associated with biodiesel is its poor stability and storage performance. Oxidation of lipids are more or less the same in characteristic as biodiesel oxidation. In addition to the oxidation process, polymers may form when unsaturated fatty acids are present, which in turn will lead to a higher molecular weight product that will increase its viscosity. The oxidation of biodiesel could lead to many mechanical problems such as deposit formation, fuel system corrosion and filtering problems (Gerpen, 2004). At present, even though there is a large number of studies published in the literature pertaining to biodiesel, lack of studies focused on using non-edible feedstock such as *Brucea javanica*, so far there is no optimization of biodiesel parameters using response surface methodology for this feedstock and no literature

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describe improvement of biodiesel improving oxidative stability from *Brucea javanica*. In this research, an effort has been made to improve oxidative stability of biodiesel with antioxidants. The outcomes of this research showed that biodiesel oxidative stability improved significantly.

Low oxidative stability could affect the quality of biodiesel. BJB had oxidative stability of 3 h which fall in range of ASTM but EN14214 has a standard of 6 to 8 h. This might be due to its high content of polyunsaturated fatty acid. Antioxidants were used to increase the oxidative stability of BJB. The induction period (IP) of BJO-based biodiesel as a function of the concentration of antioxidant added. The antioxidants were added to the BJO-based biodiesel in a concentration range from 1000 and 5000 parts per millions (ppm). Generally, the induction period of samples were observed to increase with increasing antioxidant concentrations.

Five different antioxidants were used to increase oxidative stability of BJB. The effectiveness of different antioxidants such as gallic acid (GA), butyl-4-methylphenol (BHT), butylated hydroxyanisole (BHA), Propyl gallate (PY) and tert-butyl hydroquinone (TBHQ) on induction period (IP) of biodiesel were evaluated. Results showed that the induction period (IP) of BJB significantly increased with increasing concentrations of antioxidant. Moreover, PY was the most effective antioxidant to improve the oxidative stability of BJB-based biodiesel and results agreed that previous findings show antioxidants can inhibit oxidation in biodiesel and prolong biodiesel oxidation and storage stability (Tang et al., 2009). PY improved biodiesel oxidative stability significantly followed by GA> TBHQ > BHA > BHT. Propyl gallate is very effective in improving oxidative stability of biodiesel (Dwivedi & Sharma, 2015).

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5.2 *Hibiscus sabdariffa* Biodiesel

5.2.1 Conversion of Hibiscus Sabdariffa Seed Oils to Biodiesel

World is looking for the alternative fuels as the fossil fuel are degrading rapidly and also causing problems such as toxic compound emission and global warming (Balat & Balat, 2009). Biofuel is gaining more and more importance as it is bio-based product produced from agricultural and plant bio-products, fats and animal wastes (Demirbaş, 2002). As far as Malaysia is concern its fossil fuels are expected to be diminished in next 20 years and every year its capacity drop by 14 % (Oh et al., 2010). This may lead to a serious problem as the most countries depends on their fossil fuels to run the transportation industry (Jayed et al., 2011).

Reusability, renewability and non-toxic nature of biofuel especially biodiesel makes it the favorable fuel to be produced from biological products. Animal fats, wastes cooking oils and plant seeds oil are trans-esterified and biodiesel is produced as output product (Hincapié et al., 2011). Various feedstocks has been used for biodiesel production such as soybean (Samart et al., 2010), palm kernel (Awalludin et al., 2015), tobacco (Usta et al., 2011), palm (Nur et al., 2014), cotton (Qian et al., 2010) and canola oil (Kai et al., 2014). In the past, biodiesel was produced mostly from edible oils and fats which creates a competition between energy and food. Third world countries faced serious concern as they use edible oil for food consumption especially vegetable oil. Recently report edible oils used for fuel are soybean oil usage in America , coconut and palm oil in Malaysia, Brazil uses castor oil and India use jatropha (Sanli & Canakci, 2008). Non-edible oils such as tobacco, macaúba, hochst, linseed oil, rubber seed oil, chinese tallow, soapnut, milk bush, polanga and nahor oil have been used as feedstocks (Demirbas, 2007). Therefore, more emphasis is now given to non-edible seed oils as a source for biodiesel. *Hibiscus sabdariffa* belongs to herbaceous, commonly known as Roselle (family Malvaceae) it has various pharmaceutical and industrial usage all over the world in many

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countries. Over the globe more than 300 species of roselle can be found. It is mostly found in tropical region such as in Philippines, Australia, Malaysia and Indonesia, Tropical Africa and also could be cultivated in Hawaii, Brazil and Florida. The seeds, stem and leaves of this plant are cultivated and used in different medical and industrial application. Its seeds shape is similar to kidney and are brown in color. Tropical climate need for its cultivation (humid and warm), 20 ^{o}C to 35 ^{o}C temperature needed for growth. Preferred soil pH and it shows photo-periodism. It can tolerates wind and floods. The moistures contents of roselle seeds is 9.8 %, it consists of 22.12 gm of Fats. The polyphenolic compound found are saponins, steroids, alkaloids, tannins, flavonoids in the seeds. The roselle seeds oil mostly consist of unsaturated fatty acids and a source of antioxidants rich seeds. Its most abundant fatty acids found in roselle seeds is of linoleic and oleic category. The research during 2000 to 2015 showed its usage industrial and medicinal (Padmaja et al.,, 2014).

Figure 5.2: *Hibiscus sabdariffa* plant (flower, seed and oil)

5.2.2 *Hibiscus sabdariffa* oil Properties

Hibiscus sabdariffa seeds used in this study contain 36 weight percentage wt% oil. Physio-chemical properties are the most important criteria to check the quality of oils. The physio-chemical properties were evaluated and the result obtained. The oil was yellowish in color at room temperature with a refractive index of 2.16 and an ultrasonic speed of 1.52 ms^{-1}. The acid value of the oil was 5.486 mg KOH/g showing the presence

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of free fatty acids in the oil. Oxidative stability of the HSO was tested on rancimat which was 3.48 h and the kinematic viscosity of the oil was 14.228 mm2/s.

5.2.3 Variable Optimization

The predicted and experimental values for *Hibiscus sabdariffa* biodiesel (HSB) yield responses at the design points and all variable. The regression for the determination of predicted values of output parameter (i.e. HSB yield) is given as follows. The experimental and predicted HSB yield indicate that both the value are very close to each other. Hence, model reliability is valid which is developed to see a correlation between the process parameters and the HSB yield. The results achieved from all four variables (methanol to oil ratio, temperature, rpm and catalyst) which were investigated. As the table shows the set of 29 experiments which were developed by RSM. For the optimization of variables, response surface methodology is a great technique which includes models building, design of experiment and statistics (Kalil et al., 2000). RSM has the capability of minimizing the experimental run with suitable proof for the result to be accepted statistically (Boey et al., 2013). RSM is a useful tool to be applied in investigating the effect of different independent parameters or combine effect of independent variable on dependent parameter of an experiment and suggesting that where the model borders should be, and treatment combinations could be avoid (Boey et al., 2013; Chellamboli & Perumalsamy, 2014). It is an iterative investigational method focused precisely on discovering the role of singular process parameter and also the influence of their interaction with one another in carrying out the responses. Independent variables such as the methanol to oil molar ratio, temperature and catalyst concentrations were the operating parameters which effects the process significantly (Chellamboli & Perumalsamy, 2014). RSM is based on mathematical model which stimulate the optimization of parameters and the outcome is the maximum yield of biodiesel. RSM could address the problem of where the experimental limits should be, what should be the

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range and help in setting the optimal range where the maximum yield can be achieved. Other advantages are cost saving, reduce the need of running large number of experiments which save money, time and efforts. The experimentation design for synthesis of biodiesel is capable of stimulating transesterification condition with good estimations of errors (Ghadge & Raheman, 2006). A large numbers of researchers implement RSM for optimization process of biodiesel production using different feedstocks. For example, up to 98% biodiesel yield from neem oil by RSM optimization. RSM based on the Box-Behnken also effectively optimized biodiesel yield from waste cooking oil up to 99.5% (Hamze et al., 2015). Optimization of operating parameters methanol to oil ratio, temperature and catalyst concentration could improve biodiesel yield significantly (Dwivedi & Sharma, 2015). An equation was developed after the results were analysed for HSB. The model achieved using the experiments results were designed using Box Behnken design. RSM Box-Behnken design with 4 level operating variables and 29 experimental run's ANOVA. The validity is tested by looking at model p and R-square values. The ANOVA displayed a p-value of > 0.05 and shows its significance. AB, AC, BC, A^2, B^2, C^2 and D^2 are model terms in this model. A quadratic model was used which shows p-value less than 0.05 conferring significant at 95 % confidence level. R-square (regression) with value of 0.99 and Adj r-square = 0.98. The relationship between input that is variables and the output/yield that is response were studied using the model equation of molar ratio, temperature, agitation and catalyst are labelled as A, B, C and D in the equation 3 which is displayed below,

$$Biodiesel\ yield = 91.6 - 0.78 \times A - 4.79 \times B + 0.22 \times C + 1.31 \times D - 0.77 \times A \times B + 0.68 \times A \times C + 1.51 \times A \times D + 0.26 \times B \times C - 0.38 \times B \times D + 1.97 \times C \times D - 2.36 \times A^2 - 7.59 \times B^2 - 2.43 \times C^2 - 0.032 \times D^2 \quad (3)$$

Note that the R^2 value indicates the total variability of model response after considering all the significant parameters and terms. Both the p-value and the R^2 of the

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model indicate that the model fits the data very well. Adequate Precision was (34.18)' indicates the signal to noise ratio. Normally the ratio greater than 4 is shown desirable in the literature (Hasni, et al., 2017).

5.2.4 Effect and Impact of Variables on Biodiesel Yield

The independent variable's impact on yield was drawn with help of response curve using 3-D surface. The peak point was obtained when independent variables interact perfectly and optimal yield was achieved, which lead to the conclusion that response variable depends on relationship and interaction between independents variables.

Methanol to oil ratio is considered as an important parameter in transesterification to get an optimum yield of biodiesel. Varying ratio of methanol to oil was used ranging from 3:1 to 9:1. To see the methanol oil molar ratio effects on yield of *Hibiscus Sabdariffa* biodiesel, a molar ratios (3:1, 6:1, 9:1) were employed on transesterification runs. Methanol to oil ratio of 6:1 showed highest yield of biodiesel (93.01 %). As the ratio increase further, a slight decline is seen in the yield. It has been observed that at the start of the reaction, the biodiesel yield increased with increasing methanol oil molar ratio, peak point was achieved and then a decrease is seen in yield as the ratio cross a certain range. It could be explained in way that whenever methanol exceeds the optimal value then it can by slight difficult to separate esters from glycerol. In other words, gravity decantation is hindered whenever methanol is in excess that could be due to presence of glycerol and contaminants in biodiesel (Leung & Guo, 2006). Literature also reveals that no substantial effects can been seen in biodiesel production when the range cross the 6:1 methanol to oil ratio and methanol addition excess could lead to serious problem in separation phase and increase the cost of process. A molar ratio less than 6:1 may showed a dilution effect. Molar ratio of 6:1 offered a highest biodiesel yield of 94.34 % (Hasni et

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al., 2017) . Hence, it can be said that ideal molar ratio is required of methanol to oil ratio for transesterification.

The results indicated that an increase pattern was observed in yield when temperature increase up to certain extend then yield drops after if temperature increase or decrease from that range. The optimum biodiesel yield (93.01 %) was achieved at a temperature of 67.50 ^{o}C. Temperature increase can lower down the viscosity and help converting oil into biodiesel which are in agreement with previous finding. As afar catalyst variable is concern, it follows the similar pattern as molar ratio.

Furthermore, a maximum biodiesel yield (93.01 %) was achieved at a temperature of 67.50 ^{o}C and catalyst 1 %. Excessive catalyst percentages result in emulsions which eventually reveals on high viscosity. Moreover, biodiesel recovery become difficult as side products formed during the reaction (Rubio et al., 2013). It can be observed that a catalyst concentration of 1 % showed highest biodiesel yield of (93.01 %) displayed in figure that when catalyst amount cross the range above 1 %, a declined pattern was see. Meanwhile, fatty acid and methanol in the HSB are immiscible, transesterification reaction need addition of catalyst to be occurred and the yield increases rapidly. But, whenever the amount of catalyst was too high or too low, the formation of soap occurred which may lead to difficulty in separation phase and lower the yield as well. On the other hand, insufficient use of catalyst may result lower biodiesel yield and offered an incomplete transesterification. Both increase and decrease in amount of catalyst affect reaction and show reverse pattern (Hasni et al., 2017).

A 3-D surface plot of agitation speed effects on the HSB yield, agitation speed used a range of different variation at 600 rpm to 900 rpm. It could be observed that the agitation speed also an important independent variable which effect on the yield, the HSB biodiesel yield somewhat increases with an increase in the agitation speed to certain value. Mixing

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intensity or agitation is important parameter which directly affect solution homogeneity in this case transesterification when catalyst is present in the reaction. Increasing agitation periods facilitate transesterification and help in mixing the solution and increases the contact area between methanol, oil and NaOH solution. Reaction without agitation speed occur only at interface of 2 layers and this could slow don transesterification process. Previous literature revealed that increasing the agitation speed in transesterification process stimulates the homogeneity of reactants and hence yield of biodiesel increased significantly (Meher et al., 2006; Rashid & Anwar, 2008). Moreover, this showed that agitation speed of 750 rpm was enough to achieve the maximum biodiesel yield in the transesterification reaction.

5.2.5 Fourier Transform Infrared Spectroscopy and Gas Chromatography Analysis of Biodiesel.

Identification of spectrum peaks of *Hibiscus sabdariffa* biodiesel was done. The spectrum 720.55 cm-1 – 970 cm-1 showed =C-H functional group presence. At low frequency and low energy a bending type of vibration is found in those groups and these are all double bounded (Jimoh et al., 2012; Saifuddin & Rafel., 2014). The specific peak at 721.91 cm-1 has rocking vibration overlap by =C-H group. This group point out the presence of methyl functional groups in fatty acid biodiesel. Rocking vibrations mode states about fatty acid biodiesel basin structure in biodiesel which ensure the presence of aliphatic chain in biodiesel (Saifuddin. & Rafel., 2014).

The spectrum region peaks at 1027.1 cm^{-1} to 1253.9 cm-1 showed the stretching vibration of C-O-C and C-O and also O-CH3 in that region. Methyl groups (C-H) in biodiesel could be in the spectrum range of 1367 – 1466 cm-1(Shuit et al., 2010). The spectrum band at 1715.82 cm-1 which is ascribed to stretching mode of C=O group and indicated carbonyl presence in fatty acid biodiesel. The groups showed that oil

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triglycerides are converted to biodiesel (Jimoh et al., 2012). The asymmetric and symmetric vibrations of C-H group (stretching) could be seen in the band peaks at 2853.50 cm-1 and 2923.07 cm-1 , respectively (Jimoh et al., 2012; Shuit et al., 2010). Gas chromatography was used to determine the fatty acid composition of HSB. The dominant fatty acids in HSB was methyl palmitate (6.55 %), methyl oleate (9 %), methyl stearate (49.12 %), linoleic (25.43 %), Methyl arachidate (2.61 %), Methyl erucate (2 %) and others (2.77 %). The results are in agreement of previous findings on *Hibiscus sabdariffa* biodiesel.

5.2.6 Physicochemical Properties of Obtained Biodiesel.

The EN 14214 and ASTM D6751 are the international standard used to compare fuel properties. *Hibiscus sabdariffa* biodiesel (HSB) physicochemical. The kinematic viscosity (kv) of HSB biodiesel as (4.55 mm2/s,) which falls within international standards such as ASTM D6751 and EN 14214 (1.9 – 6.0 mm2/s and 3.5 – 5.0 mm2/s). The density of biodiesel depends on its purity and composition of fatty acid in it. The HSB density found was (856 kg/m3), which follows the range of specifications. Injection system of fuel could be affected if the density is increased in turn it become more viscous. The acid value (AV) measured for HSB biodiesel was 0.054 KOH/g and falls within range of international standards (Nehdi et al., 2014). Usually, if the AV is high then it's not considered as a good fuel because corrosion occur severely in the fuel system which disturb internal engine. The oxidative stability is the property which shows the strength of fuel to be stored longer. The oxidative stability for HSB was found to be 3.48 h which should be minimum of 3 hours according to ASTM and 6 hours for EN 14214. Though, the HSB oxidative stability was found to be far from diesel which has an oxidative stability of 15.2 h, the flash point was 161 ^{o}C, cloud point 3 ^{o}C and cetane number 49 minutes. But, still was within the range of specified standards.

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The results suggested that HSB physicochemical properties were fall in the range of EN14214 and ASTM standards and transesterification parameters optimization helps in achieving higher yield.

5.3 *Brucea javanica* seeds Potential Source of Antioxidants for Biodiesel

5.3.1 Introduction

Biodiesel can be produced from various sources by transesterification of oil/triglycerides with alcohols (Meher et al., 2006). Biodiesel is produced from plant seed oils, Jatropha curcas is most well-known plant for biodiesel production (Demirbas, 2010). Some other known oil crops are palm macaw (Ciconini et al., 2013), andriba and chest-nut tree (Iha et al., 2014), cardoon (Bouriazos et al., 2014), Ilama (Reyes et al., 2014), crambe (Wazilewski et al., 2013), parasol tree china (Zhang et al., 2015) and babassu (Da etal., 2014). A major problem associated with biodiesel is that it is vulnerable to oxidative stress. Hence, the biodiesel oxidation is very important because when the oxidative reaction occurs, biodiesel deteriorates. The oxidation of lipids is more or less same in characteristic as biodiesel oxidation. In addition to the oxidation process, polymers may form when unsaturated fatty acids present, which in turn will lead to higher molecular weight products that will increase their viscosity. The oxidation of biodiesel could lead to many mechanical problems such as deposit formation, fuel system corrosion and filtering problems (Monyem & Gerpen, 2001).

This plant is used as traditional medicine (Kim et al., 2004) and used for anti-diabetic treatment (Noor et al., 2009). Several secondary metabolites have been isolated from *Brucea javanica* seed such as alkaloids, lignans, quassinoids, triterpenoids and flavonoids. The literature search suggested that *Brucea javanica* plant contains 74 quassinoid compounds, in which 33 of them are glycosides (Kim et al., 2004). The

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objective of the study was to investigate antioxidants activities of *Brucea javanica* seeds and determine its potential to boost up the oxidative stability of biodiesel.

5.3.2 Total Phenolic and Flavonoid Content

The extractable phenolic compound content in *Brucea javanica* seed extract was determined by using the linear gallic acid standard curve *(R^2=0.966)*. Total flavonoid and phenolic contents amount in *Brucea javanica* seeds extract were calculated. Among the three extracts of *Brucea javanica* seed, ethyl acetate extract exhibits the highest capacity of total phenolic at 98.5±0.1 mg GAE/g of dry extract. The data implies that ethyl acetate extract is the more extractable solvent to extract phenolic content.

The total phenolic content extracted from methanol extract was 85.3±0.6mg GAE/g of dry weight. Among the three extracts, hexane has the smallest total of phenolic content at 36.9±0.8mg GAE/g of dry weight. The data indicate that the quantity of total phenolic content varies among the three selections. The deviation may be expected due to the different phenols present in the extracts. This can be supported up by the previous finding which stated that different solvents extract different phenolic compounds and presents a different reaction in the Folin-Ciocalteu method (Heinonen et al., 1998).

The total flavonoids content suggests that ethyl acetate extract exhibits the highest total flavonoid content at 20.0 ±0.4 mg QAE/g of dry weight and methanol extract exhibits at 12.9 ± 0.3mg QAE/g of dry weight. Hexane extract presented the lowest amount of flavonoid content at 10.0 ±0.4 mg QAE/g of dry weight. Ethyl acetate extracts showed highest TPC and TPC because of its polarity to extract more compounds as compared to methanol and hexane.

The most commonly distributed antioxidant groups found in plants are flavonoids which are characterized by a ring structure known as benzo-y-pyrone, which is distributed in

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most fruits and vegetables (Yumrutas & Saygideger, 2010). Flavonoids contain an aromatic ring which lets them to take over and donate electrons from free radicals (Kanner, 1994). Flavonoids have been proven to protect lipids against oxidation. In the proposed work, the total flavonoids content in *Brucea javanica* seed extracts was assessed using linear quercetin standard curve (R^2=0.996). The flavonoids content found were little in total compared to the total phenolic content.

5.3.3 Mass Spectrometry Analysis

GCMS identification and analysis was carried out in order to identify compounds that are possibly present inside *Brucea javanica* seed extracts. Hence, information was provided on key compounds that may be responsible for the antioxidant activity such as phenols and flavonoids. Phytochemical profile was determined using mass analysis through direct infusion technique in n-hexane extract of *Brucea javanica* seed. Chemical compounds suggested and detected in n-hexane extract along with the corresponding values for comparison determined with the reference library.

To identify the chemical compounds LCMS was conducted and hence confirmation of antioxidant compounds present in *Brucea javanica* seed was done. The compounds detected via LCMS was gallic acid, ellagic acid, quinic acid, brevifolin and strictinin determined with reference library.

5.3.4 Antioxidants Assays

5.3.4.1 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

The DPPH assay results indicate that ethyl acetate extract of *Brucea javanica* seed shows the highest DPPH inhibition (90 % with IC_{50}= 31.2 μg/mL) using different concentrations of seeds extract followed by methanol extract with 82 % inhibition (IC_{50}= 184 μg/mL) using different concentrations of seeds extract (62.5, 125, 250, 500 and 1000 μg/mL) and the lowest inhibition is shown by n-hexane extract, up to 78 % DPPH

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inhibition with (IC_{50}= 319μg/mL) using different concentrations of seeds extract. Ascorbic acid was used as control. These findings are similar in some ways with previous findings which suggested that variations in extract polarity alter extract ability to extract specific phenolic and flavonoid compounds (Zhou et al., 2004).

5.3.4.2 Metal Chelating Activity Assay

The procedure used to determine metal chelating activity was the ion chelating activity of ferrous. The resolution suggests that the ethyl acetate extract of seed shows the highest inhibition up to 59% with (IC_{50}=299 μg/mL) followed by methanol extract with 27 % inhibition with (IC_{50}=656 μg/mL) and the lowest activity is shown by hexane extract with 22 % inhibition (IC_{50}= 1100 μg/mL) at different concentrations of seeds extract.

5.3.4.3 Ferric Reducing Antioxidant Power (FRAP) Assay

Frap assay react with ferric tripyridyltriazine [Fe^{3+}-TPTZ] complex and measure the reducing potential of antioxidant by producing [Fe^{2+}-TPTZ]. Frap assay treats the antioxidants in the sample as a reductant. In the proposed study, the trend for ferric ion reducing activities of *Brucea javanica*. The increase in the absorbance of *Brucea javanica* is due to the formation of the Fe2+-TPTZ complex with increasing concentration of seeds extracts. The methanol extracts of *Brucea javanica* seeds showed increased ferric reducing power with the increased concentration as standard antioxidants.

Hence, The outcome indicates that the methanol extract of *Brucea javanica* seed showed the highest FRAP activity up to 71 % inhibition followed by ethyl acetate and n-hexane. The result is expressed as mill mole of iron per gram of extract. Therefore, they should be able to donate electrons to free radicals stable.

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5.3.5 Addition of *Brucea javanica* Seed Crude Extract to Biodiesel

The DPPH assay has been shown to be a good predictor of the oxidative stability of oils as determined using thermal oxidations of oil. Biodiesel was tested for the antioxidant activity at 80 ^{o}C. Absorbance of DPPH in methanol with adding of *Brucea javanica* seed extract residue in different concentrations (200, 400 and 600 mgL-1) to biodiesel.

For thermally oxidized biodiesel with adding of 200 mgL^{-1} residue (*Brucea javanica* seed extract) at 0 h to 2 h a decrease in DPPH absorbance indicating biodiesel with little radical scavenging compounds. As the time of oxidation increased from 2 h to 8 h, a gradual increase in DPPH absorbance is shown indicating lack of oxidative stability. Changes of DPPH absorbance due to thermally oxidized Palm biodiesel with addition of 400 mgL^{-1} residue (*Brucea javanica* seed extract) at 0 h to 6 h were insignificant showing a steady state pattern indicating stability of Palm oil biodiesel. As the time of oxidation increased at 6 h to 8 h an increase in DPPH absorbance is shown indicating a reverse pattern. A steady state pattern is shown at 0 h to 8 h thermally oxidized Palm biodiesel with 600 mgL^{-1} addition of residue (*Brucea javanica* seed extract). The pattern indicated the equivalent antioxidant dose to be used in Palm biodiesel for oxidative stability needed. Adding 200 mgL^{-1}, 400 mgL^{-1}, and 600 mgL^{-1}, residue (*Brucea javanica* seed extract) with Palm biodiesel showed the optimum oxidation stability needed from natural extract to be 600 mgL^{-1}. Results indicated that residue adding of (*Brucea javanica* seed extract) can be used as antioxidant to improve the oxidation stability of biodiesel. The results agreed with previous stated finding that Jatropha plant methanol crude extract shows stability up to 6 hours when added to biodiesel in crude form (Diwani et al., 2009).

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5.3.6 Rancimat Test

The oxidation stability of biodiesel can be extended by adding antioxidants (Luo etal., 2012). Plants and plant derived product are rich sources of natural antioxidants Plant extracts are considered as a potential natural antioxidants because of the presence of phenolic, flavonoids and anthocyanin compounds (Brewer, 2011). In order to confirm autoxidation effect of phenolic compounds of *Brucea javanica* seed, gallic acid was identified and detected in both extracts of *Brucea javanica* seed confirmed by LCMS profiling. After confirming the presence of gallic acid as clear peak in LCMS. The gallic acid was applied as a natural antioxidant for biodiesel to test its oxidative stability in the Rancimat machine with different concentrations from 2000 to 10,000 ppm.

The effect was substantial and positive. It was noted that a substantial increment in the initiation period with gallic acid as antioxidant and which in turn prolong the oxidative stability of biodiesel. The induction period (IP) of Sample I was 43 hours with 2000 ppm gallic acid as antioxidants followed by Sample II (IP: 55 hour, 4000 ppm) followed by Sample III (IP: 67 hour, 6000 ppm) which is followed by Sample IV (IP: 67 hour, 8000 ppm) and maximum peak was obtained with Sample V (IP: 69 hour, 10,000ppm.).

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CHAPTER 6: CONCLUSION

6.1 Concluding Remarks

Green technology concept is boosting up and getting more and more importance in the recent past decades. Green energy is one of the forms of green technologies which is replacing fossil fuels slowly and gradually because of the advantages like renewability and environmental friendly. In the recent times non-renewable resources for production of energy as compared to renewable sources are favoured. Biofuel in general and biodiesel in particular is a type of green energy which is produced from different renewable and biological sources such as vegetable/plant oils and animal fats. In this study, biodiesel is produced from two different non-edible and renewable feedstocks which are *Brucea javanica* and *Hibiscus sabdariffa* seed oils. Furthermore, the oxidative stability of biodiesel was improved using different antioxidants. The significant conclusions and major findings achieved in the proposed study are as follows;

Brucea javanica and *Hibiscus sabdariffa* seed oils could be possible non-edible feedstocks for the production of biodiesel. Our study suggested that obtained *Brucea javanica* and *Hibiscus sabdariffa* biodiesel was better fuel in term of quality, yield and conversion carried out by transesterification process. The physico-chemical properties of the obtained biodiesel fall within range of ASTM and EN14214 international standards.

Different factors/variables are investigated to see their effects on biodiesel yield. It was observed that with the rise in temperature and molar ratio, the biodiesel yield/output was increased significantly. For *Brucea javanica* biodiesel the highest conversion attained was 94.34% at 65 °C temperature, 1% catalyst concentration and 1:6 methanol to oil molar ratio. On the other hand, for *Hibiscus sabdariffa* biodiesel the maximum conversion achieved was 93.01 % at 67.5 ^{o}C, 1 % catalyst and 6:1 molar ratio. As the variables ranged crossed these value/points, the biodiesel yield decreased for both the

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feedstocks suggesting that a further increase or decrease directly affects the output and yield.

To optimize transesterification process and variables, an optimized design was followed known as Box-Behnken experiment design for biodiesel production using response surface methodology (RSM). Different variables ranges were set in order to achieve optimized variables and highest yield. The ANOVA test implied that temperature, catalyst and methanol to oil molar ratio had the great significant factors affecting the conversion of biodiesel. RSM is a useful statistical tool used for optimization of process variables in different industries.

All physico-chemical properties of biodiesel was within range of ASTM and EN14214 except its induction period/oxidative stability. Biodiesel oxidative stability is a major problem because whenever it is exposed to air or oxygen it deteriorates. Antioxidant are the compounds that could stop oxidation reaction and hence, protecting biodiesel from degrading quickly. Different antioxidants used in this study to improve biodiesel oxidative stability and all the antioxidant showed significant results. However, propyl gallate showed the highest induction period of 70 hours.

The proposed research was aimed at the usage of cheap, less popular and novel feedstocks for the biodiesel production with a critical analysis. Variable optimization was successfully achieved and higher biodiesel yield was obtained. The process facilitated an easy production route with an appreciable degree of conversion, yield and product quality. Though, it is important to mention here that it is too early to be able to exactly define what may be a practical level for the production of biodiesel, but a rough scale could be achieved by investigative a comprehensive process analysis of this proposed research work.

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6.2 Future Recommendations

The proposed study in this thesis was to produce biodiesel from non-edible feedstocks, transesterification optimization and to improve the biodiesel oxidation stability. But, there are always rooms for improvement so some issues still need attention and further research to be done in future. Some of the future recommendation are given below for future research,

Firstly, in this study homogenous catalyst was used but there is need of investigating different catalysts for biodiesel production in the future such as biologically derived enzymes and heterogeneous catalyst like calcium oxide derived from different sources and their comparison with homogenous catalyst.

Secondly, there is a need to study on the chemical kinetics of transesterification to see the detail mechanism of the process and to set a scale for further research plus getting detail of the study. The experimental works on the biodiesel kinetic studies should be carried out. Therefore, it is suggested and recommended for upcoming experimentations.

Thirdly, glycerol which is produced as by-product from the process could be purified and utilised in the industry. This directly help in reducing cost of the production of biodiesel and could be utilised as useful product. Otherwise, a biodiesel production that produces no glycerol is worth to be explored.

The fourth recommendation is *Brucea javanica* crude extract were used to improve oxidative stability of biodiesel along with other five antioxidants. So, for future research it is recommended to fractionate and isolate plants seeds extract into several partition by using other solvent with different polarity. Thus, by testing content of extract with different polarity such as acetone, chloroform and water, it will enable close identification

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of compounds responsible for antioxidant activity rather than a combination of miscellaneous compound acting together.

It is also recommended to do a pre-analysis test using HPLC to analyse an individual compound and do a downstream test with antioxidant assay. The reason of that study should be to identify which compound is responsible for the antioxidation activity as the extract is a mixture of compounds. This could also help in determine which compound could contribute in combating the free radical and which is the one promoting it. Thus, further test should be done by purification and separation of the compound individually and testing it with antioxidation and oxidative test.

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LIST OF PUBLICATIONS AND PAPER PRESENTED

Manuscripts published

1. Hasni, K., Ilham, Z., Dharma, S., & Varman, M. (2017). Optimization of biodiesel production from *Brucea javanica* seeds oil as novel non-edible feedstock using response surface methodology. *Energy Conversion and Management*, *149*, 392-400.
2. Hasni, K., Ilham, Z., Dharma, S., M. Jamaludin & Varman, M. (2017). *Brucea javanica* seeds as source of potential natural antioxidants to improve biodiesel thermal and oxidative stability. *Malaysian Journal of Fundamental and Applied 13*, 207-212.

International conferences accepted

1. Mass Spectrometry Analysis and Antioxidant Activities of *Brucea javanica* Seed used to prevent Lipid Oxidation *International Sustainable Technology, Energy and Civilization Conference (ISTECC 2016), February 13th 2016, Kuala Lumpur, Malaysia.*
2. Natural antioxidants and biodiesel storage. *20th Biological Science Conference, Chulalongkorn University, December 9-11th 2015, Bangkok Thailand*

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