CHEMICAL PROFILE, PHYSICOCHEMICAL- SENSORY PROPERTIES, TRANS-ESTERIFICATION AND BIOLOGICAL EFFICACY OF AGRO-WASTE SEED OILS IN MALAYSIA

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UNIVERSITI PENDIDIKAN SULTAN IDRIS

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ABSTRACT

This study aims to investigate the potential use of biochemical seed oils of agro-waste as anti-trypanosomal, anti-oxidant, and anti-bacterial edible oil, sun-protection oil, and biodiesel. A comparative study was conducted to examine the chemical profile, physicochemical-sensory properties, and trans-esterification of seed oils collected from agro-waste in the Mualim district. Five drying techniques were tested before extracting the seed oils. Standard methods were then used to determine the chemical compounds, physicochemical-sensory properties, and bio-activity of the extracted seed oils. The analysis performed on such oils showed there were significant differences in the amount of seed oils between drying techniques, $F\ (df_{b,}\ df_{w}) = 39.89$, $p = 0.00$. Specifically, the analysis showed the microwave-drying technique produced the highest oil yield except for *Carica papaya. L* and *Cucurbita sp*, and the seed oils extracted from Mangifera *indica* had the best antioxidant property. Also, significant variations were detected in the bacterial inhibition zones depending on the species and different oil concentrations, with the highest inhibition zone being found in the seed oils extracted from *M. indica* and *C. papaya.* L. Furthermore, the highest and lowest phenolic contents were obtained from *C. papaya.* L and *Durio zibethinus*, respectively. Meanwhile, the highest and lowest flavonoid contents were observed in *C. papaya.* L and *Cucurbita sp*, respectively. In addition, the percentages of biodiesel obtained from *Nephelium lappaceum*, *Cucurbita sp*, *M. indica,* and *C. papaya. L* were 94.13%, 96.81%, 93.24%, and 76.14%, respectively. In conclusion, the findings indicate that seed oils extracted from *C. papaya. L*, *Cucurbita sp*, *M. indica*, *C. papaya. L*, *D. zibethinus,* and *Artocarpus heterophyllus Lam* have excellent anti-oxidant and anti-bacterial properties that can be manufactured into edible oil and sun-protection oils, whereas seed oils extracted from *C. papaya. L*, *Cucurbita sp*, *M. indica*, and *C. papaya. L* can be efficiently made into biodiesel. As such, these findings will have a significant implication on the use of *C. papaya. L*, *Cucurbita sp*, *M. indica,* and *C. papaya. L* in the energy, medical, and food industries.

PROFIL KIMIA, SIFAT FIZIKOKIMIA-SENSORI, TRANS-ESTERIFIKASI DAN EFIKASI BIOLOGI TERHADAP BIJI BENIH SISA PERTANIAN DI MALAYSIA

ABSTRAK

Kajian ini bertujuan mengenal pasti potensi penggunaan minyak biji benih biokimia dari sisa pertanian sebagai minyak boleh makan, minyak pelindung sinaran matahari, dan minyak biodiesel yang mempunyai sifat anti-tripanosoma, anti-oksidan, dan anti-bakteria yang baik. Kajian perbandingan dijalankan untuk mengkaji profil kimia, sifat fizikokimia-sensori, dan trans-esterifikasi antara minyak biji benih yang diperoleh dari sisa pertanian di daerah Mualim. Lima teknik pengeringan diuji sebelum mengekstrak minyak biji benih. Kaedah piawai kemudiannya digunakan untuk menentukan kandungan kimia, sifat fizikokimia-sensori, dan bio-aktiviti minyak biji benih berkenaan. Analisis yang dijalankan terhadap minyak berkenaan menunjukkan terdapatnya perbezaan yang signifikan dalam jumlah minyak biji benih antara teknik pengeringan $F\ (df_{b,}\ df_w) = 39.89$, $p = 0.00$. Terutamanya, analisis menunjukkan teknik pengeringan gelombang mikro menghasilkan jumlah minyak biji benih yang tinggi, kecuali *Carica papaya. L* dan *Cucurbita sp*, dan minyak biji benih dari *Mangifera indica* mempunyai sifat anti-oksida yang terbaik. Tambahan pula, variasi yang signifikan dikesan dalam zon perencatan bakteria mengikut spesies dan kepekatan minyak biji benih, terutamanya, zon perencatan bakteria dikesan dalam minyak biji benih yang diekstrak dari *M. indica* and *C. papaya. L*. Kandungan fenolik tertinggi dan terendah diperoleh dari *C. papaya. L* dan *Durio zibethinus*, masing-masing. Sementara itu, kandungan flavanoid tertinggi dan terendah dikesan dalam *C. papaya.* L dan *Cucurbita sp*, masing-masing. Juga, peratusan biodiesel diperoleh dari *Nephelium lappaceum*, *Cucurbita sp*, *M. indica*, dan *C. papaya. L* adalah 94.13%, 96.81%, 93.24%, and 76.14%, masing-masing. Kesimpulannya, dapatan menunjukkan minyak biji benih yang diekstrak dari *C. papaya. L*, *Cucurbita sp*, *M. indica*, *C. papaya. L*, *D. zibethinus*, dan *Artocarpus heterophyllus Lam* mempunyai sifat anti-oksidan dan anti-bakteria yang tinggi yang boleh dijadikan sebagai minyak makan dan minyak pelindungan sinaran matahari, manakala minyak biji benih yang diekstrak dari *C. papaya. L*, *Cucurbita sp*, *M. indica*, dan *C. papaya. L* boleh dijadikan dengan efisien sebagai minyak biodiesel. Dengan ini, dapatan kajian mempunyai implikasi yang signifikan terhadap penggunaan *C. papaya. L*, *Cucurbita sp*, *M. indica,* dan *C. papaya. L* dalam industri tenaga, perubatan, dan makanan.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

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CHAPTER 1

INTRODUCTION

1.1 Agriculture in Malaysia

Malaysia is a tropical country suitable for agriculture and produces various agricultural crops. Agriculture is a vital industry in this country and has established its economy by providing village items for household applications and serving as an abundant source of foreign currency (Dardak, 2015; Basri et al., 2015). The agricultural sector has vital contribution to the national Gross Domestic Product (GDP) and provides work opportunity for the general population, particularly those from rural areas. The agricultural sector employs more than 1.6 million individuals or 10.9% of the total workforce, contributing to more than 23% of all foreign trade profits and approximately 7.2% of Malaysia's GDP in 2013 (Dardak, 2015; Raihana Marikkar, Amin & Shuhaimi, 2015). Since 1816, Malaysia has manufactured rubber and participated in the recently upgraded promotional marketing sector for agricultural goods. This country has also entered the worldwide agricultural market by manufacturing palm tree oil in 1971, and this economy is still dynamic to date. According to Oudejans (1999), other agriculture crops, including bananas, rice, and coconuts are also produced in Malaysia.

Wong (2007) mentioned that several crops, such as banana, durian, pineapple, coconut, rambutan, and rice, are produced for household consumption. In Malaysia, 12% of the country's GDP is attained from the agricultural sector, and 16% of the population is familiar with different kinds of agricultural chores. Malaysia have expanded the plantation scale after a certain period of time. For instance, its rubber plantation started in 1876, palm oil in 1917, and cocoa in 1950.

The climate in Malaysia is appropriate for cultivating unique crops. The influence of hurricanes and drought is considerably low in this country. Similarly, Malaysia has approximately 90% humidity because of its proximity to the equator. The climate remains hot and damp throughout the year (Majid et al., 2000). Approximately 24% of Malaysia's territorial zone comprises agricultural land, 43,000 distinctive agricultural machines and tractors are in use, and 7,605,000 hectares of arable and everlasting cropland are available. Malaysia also produces 535,000 tons of bananas annually (Basri et al., 2015). The Malaysian government aims to expand the production of agricultural crops by improving different advancement programs. Wong et al., (2007) indicated that the Integrated Agricultural Development Project screens, reviews, coordinates, and actualizes these projects.

In Southeast Asia, many countries have rich plant biodiversity with thousands of various types of fruit-bearing trees. Several of these fruits are already commercialized, and some trees are cultivated traditionally by villagers or local farmers. Therefore, indigenous tropical fruits with potential to be commercialized should be identified by researchers, farmers, or the industry to generate opportunities. Indigenous fruits are rich in phytochemicals, specifically phenolic compounds, carotenoids, terpenes, and other terpenoids. Phytochemicals of the medicinal fruits provide protective effects against chronic diseases, such as cancers, cardiovascular diseases, and diabetes and have inflammatory and antimicrobial effects (Khoo et al., 2016).

Malaysia has colorful, tropical, and natural products with agro-industrial potential. These products include mango, pumpkin, papaya, jackfruit, durian, and rambutan with high prospect for consumption, particularly as industrialized items. However, a considerable volume of waste is produced daily in the agricultural industry in Malaysia. Majority of such waste is utilized for animal nourishment industry or is discarded in nature, causing genuine ecological harms (Raihana et al., 2015; Jayakumar et al., 2017).

1.2 Agro-Waste Scenario in Malaysia

Agro-waste, also called agricultural residue, is generally defined as a vital product from various agricultural processes and contains materials of economic value (da Silva & Jorge, 2014). Nevertheless, this waste can be converted to human food and processed to achieve horizontal agriculture, protect the environment from pollution, improve agricultural products, and provide employment opportunities in the agricultural sector. Thus, agro-waste utilization can improve the economy, environment, health, and society, especially in rural areas (Rinaldi et al., 2017). Agro-waste production is highest during harvesting, crop collection, and marketing preparation. These wastes are rich in recyclable materials and contain organic matter and minerals, thus can be possibly converted into biofuels and oils (Jayakumar et al., 2017; Sabiiti et al., 2004).

Given its high economic development among tropical countries, Malaysia has encouraged industrialization to attain the status of a developed country by 2020 (Basri et al., 2015). Malaysia's rapid economic growth and high GDP resulted in 5.1%, 5.6%, and 4.7% growth rates in 2011, 2012, and 2013, respectively. However, its population has also increased from 18 million in 1990 to 30 million in 2014 (Mofijur et al. 2012). Therefore, the energy demand in Malaysia has been amplified in the past two decades. For example, different sectors in Malaysia (i.e., industry, transport, agriculture, and private and business sectors) have developed. The growing agricultural production has increased the livestock waste, agricultural crop residues, and agro-industrial byproducts. A remarkable increment in agrowastes could be reached when developing nations continuously intensify their cultivating systems. A total of 998 million tons of agro-waste is produce yearly. In addition, 80% of overall solid wastes from farms are organic wastes (Wang et al. 2015). The waste created is subjected to the type of completed agricultural activities.

In Malaysia, 1.2 million tons of wastes are annually produced from agricultural activities and disposed in landfills (Agamuthu, 2009). Approximately 15% of the entire waste produced in various Asian countries is agrowaste. Rural waste production in Malaysia was approximately 0.122 (kg/top/day) in 2009 and is anticipated to increase by up to 0.210 (kg/top/day) in 2025 (Agamuthu, 2009). In view of the Intergovernmental Panel on Climate Change Guidelines for National Greenhouse Gas Inventories in 2006, agro-based industry delivers a remarkable amount of post-preparation wastes and residues in Malaysia, such as tropical fruit palm oil and businesses that manufacture rubber–wood items. Although agro-based industries produce different types of wastes, these wastes are usually organic matter that can be converted into useful products. Tropical fruits have a substantial amount of skin, rind, and seed that are considered as waste as shown in Table 1.1. Durian, mangosteen, and jackfruit have more than 50% of rind and seeds, high moisture content, and abundant biodegradable organic ingredients. Hence, a waft of unbearable stench is produced during their decomposition (Wang et al., 2014). This long-term disposal of waste causes greenhouse effect (Dhillon et al., 2013) and serves as breeding ground for bacteria, mice, and pests, resulting in the spread of plague. Thus, researchers aim to recover the health beneficial compounds of fruits and transform these wastes to biomass to minimize their effects. Given the growing demand for the health benefits of phenolic compounds from natural plants, their recovery from fruit wastes is an urgent concern.

Table 1.1

Percent of Flesh, Rind/Skin, and Seed in Tropical Fruits

Types of Fruits	Flesh (%)	Rind/skin (%)	Seed (%)	Reference(s)
Durian	20–35	55–66	5–15	Siriphanich, 2011
Rambutan	34–54	37–62	4–9	Issara et al., 2014
Mango	60–75	11–18	14–22	Mitra et al., 2010
Jackfruit	30–35	55–62	8–10	Cheok et al., 2018
Papaya	80–90	10–20	10–20	Parni and Verma, 2014; Lee et al., 2011

Wastes from fruits possess high potential because they contain sugar, nutrients, and minerals and are rich in fibers, oils, and different compounds with useful properties. The examination of exceptional oils present in common food items and their role in food quality and human well-being is an emerging exploration field; however, this topic is seldom investigated. Using wastes to generate oils with unique qualities and to extract compounds for nourishment and in pharmaceutical and restorative industries can enhance item accessibility to satisfy the requirements for new oil sources (Wu et al., 2015; Dias, et al., 2013). Hence, raw materials containing oils with abundant phytosterol and lipid profiles similar to common oils and phenolic mixes must be examined.

Enthusiasm for the polyphenol, flavonoid, and vitamin C and E content of organic products, teas, vegetables, grains, and restorative plants with antimicrobial and cancer prevention properties has recently increased (Hseu et al., 2008; Sufya et al., 2014).

Seeds are one of the important wastes produced from agricultural crops and contain a high proportion of oil that can be used in human consumption. Biofuels may also be extracted from agro-waste seeds (Chatterjee & Ghose, 2016).

1.3 Chemical and Oil Composition of Seeds

Seeds are a crucial element in the reproduction and development of gymnosperm and angiosperm plants. Angiosperms are related to primitive plants, such as ferns, liverworts, and mosses. These plants do not have seeds and depend solely on water for propagation. Seed plants dominate the biological niches on land starting from forests to grasslands in warm and cold climates (Bialek et al., 2016). A “seed” is generally known as anything that can be sown, such as potato, corn, or sunflower seeds (Awan et al., 2001).

Rinaldi & Morena (2017) highlighted that certain seeds contain epoxy-unsaturated fatty acids known as epoxy acid oils. Acetic acid is part of the triglycerides in plant families, such as Celastraceae, Lardizabalaceae, Ranunculaceae, and Rosaceae, which contain monoaceto triglycerides. 2-or 3-Acetyl glycerides (liliosides) have been isolated from *Lilium longiflorum*. Rasor and Duncan (2014) mentioned that the seed oil of *Impatiens glandulifera* and *Euonymus verrucosus* contain real triglyceride species sn-glycerol-1,2-diacyl-3-acetins.

Durian seeds contain approximately 20%–25% oil with phytosterol, the main constituents of edible oil. Papaya seed oil contains more than 37% triacylglycerols, such as triolein, and more than 70% monounsaturated fatty acids, such as oleic acid. Therefore, papaya seed oil can be consumed (Samaram et al., 2014). Pumpkin seeds are rich in vitamin E tocopherol (620 mg/kg), essential fatty acids, especially linoleic (high) and linolenic acid (low), and other nutrients and thus are a good source of edible oils. (Aziz & Kalek, 2011). Mango seed is also considered as a good source of edible oil (11.5%) (Yatnatti et al., 2014). Babu et al. (2017) found that jackfruit seed oil is exceptionally abundant in basic unsaturated fatty acids, such as linoleic acid and alpha-linolenic acid. Rambutan seeds possess 38.9% total triacylglycerol and fatty acid (Harahap et al., 2012**).**

Numerous seeds are consumable, and most of human calories originate from seeds, particularly those from grains, vegetables, and nuts. Seeds are an additional source of most cooking oils, numerous beverages and flavors, and vital food-added substances. Bewley & Black (1994) suggested that seeds stand out among the most imperative sources of financial income because of their importance in food. Seeds are the wellspring of a few prescriptions, such as castor oil, tea tree oil, and malignancy drugs. The United States Food and Drug Administration has classified castor oil seeds as “generally recognized as safe and effective” for over-the-counter use as a diuretic. The small digestive tract is its significant site of activity where it is processed into ricinoleic acid. Razdi (2012) suggested that castor oil seed or a castor oil subordinate (e.g., Kolliphor EL, polyethoxylated castor oil, a nonionic surfactant) is added to numerous advanced medications, such as miconazole, an antifungal operator, and paclitaxel, a mitotic inhibitor utilized in malignancy chemotherapy.

Finding new antimicrobial compounds with different concoction structures and novel systems of activity is needed because of the new and re-emerging irresistible ailments. Another concern is improving the protection from antibiotics in current clinical use. To date, tranquilizing protection from human pathogenic microbes has been normally detailed worldwide. The medication against bacterial and contagious pathogens has also complicated the treatment for illnesses in AIDS and malignancy patients. Nevertheless, numerous endeavors have been directed to find new antimicrobial mixes from microorganisms, animals, and plants. Subbaiah, Dakappa, & Lakshmikantha (2017) propounded the use of folk medicine as a new source. Deliberate screening of such folk medicine may result in the disclosure of a novel powerful compound.

Different from those of artificial medications, the antimicrobial effects of agents from plants have no side effects and have massive therapeutic potential to heal many infectious diseases. Iwu et al., (1999) suggested that the potential for developing antimicrobials from higher plants is rewarding because it will lead to the discovery of phytomedicine against microbes. In light of these certainties, the plant kingdom with an assortment of natural compounds has received considerable attention. Ebrahimabadi et al., (2010) indicated that discovering natural plant materials with antioxidant and antibacterial effects is a goal in the research of food additives.

In 2001, 319 million metric tons of seed oil worldwide was reported. Some portions of the total generation of seed oil are utilized specifically as food, but most are squashed to extract oil. A squashed seed usually contains 26% oil (i.e., 15% minimum in cottonseed and maximum at 62% in copra). Puangsri et al. (2005) reported that the worldwide vegetable oil utilization has dramatically doubled from 1980 to 2000 and continuously increases up to approximately 76 million tons.

1.4 Bioactive Compounds in Seed Oil

Seed oils are important in a well-balanced daily diet because of their high content of energy, vitamins, unsaturated fatty acids, phytosterols bioactive antioxidants, and triacylglycerols. In addition to vitamins A and E, seed oils contain phenolic compounds (i.e., phenolic acids and flavonoids), tocopherols, phytosterols, carotenoid pigments, and phospholipids. All these compounds protect the unsaturated fatty acids in oil from oxidation (Kuraisak et al., 2019). When these bioactive compounds are consumed without being processed, they are beneficial to human health. Thus, polyphenols are important components of oil because they have multidirectional functions and provide spontaneous protection by activating endogenous defense systems and regulating cellular signaling processes. Furthermore, polyphenols affect taste and color, stabilize fats and other labile food ingredients, and act as antioxidants by providing oils with high concentration of mono-unsaturated fatty acids that prevent cardiovascular diseases via several mechanisms (Chen et al., 2000; López-Miranda et al., 2006; Kuraisak et al., 2019). In addition, sterols have serum cholesterol-lowering properties and potential antioxidant properties. Their antioxidant activity is associated with the production of allylic free radicals after isomerization with other stabilized free radicals (Kozłowska et al., 2016). Common sterols (phytosterols) include avenasterol, campesterol, sitosterol, and stigmasterol. Sitostanol in its saturated form called stanols is the most representative example. Phytosterols are utilized as food ingredients because they have biological properties that can reduce blood cholesterol level. Food products, such spreads, milk, cheese, or chocolate products with 2–3 g of daily consumption are rich in phytosterols and phytostanols. Kmiecik et al., (2011) claimed that this amount is sufficient to reduce cholesterol and LDL fraction in a human body. Plant seed oils with squalene provide protection against oxidative DNA damage of mammary epithelial cells in humans (Kozlowska et al., 2016).

Plant oils are known for their antimicrobial properties because of their ability to control plant diseases caused by bacteria and eradicate them from the seeds.

Nowadays, a growing demand exists for natural products due to their availability, few side effects, and enhanced biodegradability compared with currently available antibiotics and preservatives. Thus, extensive investigation on the naturally occurring antimicrobial substances for food preservations has increased due to the consumers' awareness on natural food products (Belal et al., 2017).

Kaur et al., (2010) suggested that mango seed kernels have antimicrobial effects, and their powder exhibits various inhibitory effects. Mango kernels produce 12%–15% edible oil and have been studied to confirm their potential as a source of bioactive compounds and antioxidants. Nadeem, Imran, & Khalique (2016) scientifically proved their cardio- and hepatic-protective effects and anticarcinogenic and anti-aging influences.

Samaram et al. (2015) asserted that because of the high annual utilization, the amount of seeds increases during preparation and is viewed as squanders. Papaya seeds account for approximately 15%–20% of the natural product weight (Mohammad, et al., 2019; Samaram et al., 2014). These seeds can be utilized as a potential wellspring of oil and contain abundant triacylglycerols and oleic acid. Seeds are an excellent renewable source of biodiesel given their high lipid content, high accessibility, and minimal cost.

1.5 Trans-esterification

Trans-esterification is a process whereby the glycerides exist in fats and oils including an alcohol with the existence of catalyst to develop esters and glycerols (Shahid & Jamal, 2008), as presented in Figure 1.1. The catalysts will raise the reaction rate and the yield. Trans-esterification process is reversible which allow excess alcohol to be utilized in a forward direction. For this purpose, alcohols that are being used are primary and secondary monohydric aliphatic alcohols that have 1 to 8 carbon atoms (Benerjee & Chakraborty, 2009). For biodiesel production, these modifications are said to be ideal and suitable. Henriques (1898) attained a complete conversion based on the reaction of vegetable oils with methanol along with the presence of potassium hydroxide that act as a catalyst. In 1930s and 1940s, these reactions were frequently applied in the fat and soap industry. From 1941, Bradshaw's patent application is still being used as a model for the production of biodiesel plants around the world. Meanwhile, Chavanne (1942) mentioned that the Belgian patent 42287 in the production of palm oil ethyl esters acids was used as catalysed trans-esterification to be the main usage of fuel known to be as biodiesel.

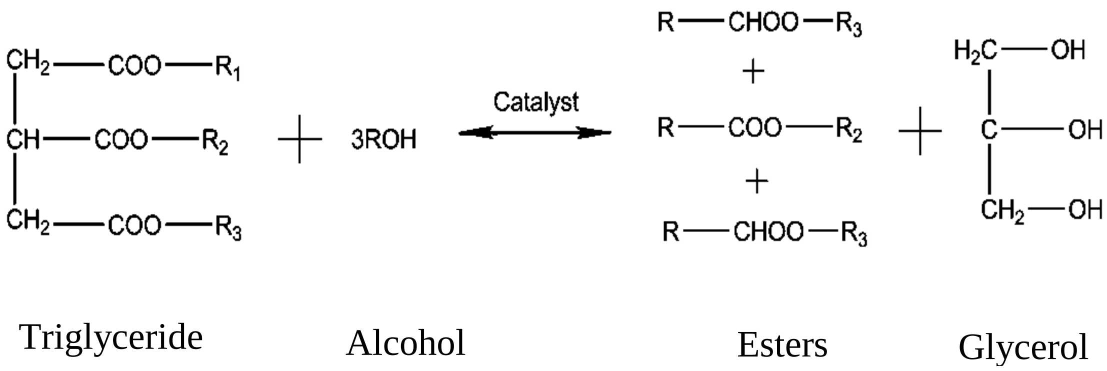

Figure 1.1. Trans-esterification Reaction of Triglycerides with Alcohol

Biodiesel is produced after the vegetable oils, leftover oils, and fats undergo the trans-esterification process. Furthermore, it is also a substitute of diesel (Banos et al., 2011) and this substance can be labelled as a full diesel substitute with minor modifications. Several investigations have been done determined that the properties of biodiesel is the same as diesel fuel which can be utilized in motor's diesel which has no modification. In 2001 and 2006, the global generation of biofuels were measured with 43% and 23% respectively. Canakci (2007) asserted that biodiesel with higher number of cetane than diesel fuel will have no aroma and sulphur and tend to contain of 10% to 11% of oxygen by weight.

In 2007, 45 billion liters of bioethanol was produced in a year globally while 5 billion liters was the annual production of biodiesel (Panwar et al., 2011). It was listed that the main ozone-harming greenhouses gases are carbon dioxide, nitrous oxide, and methane. The mixture of these gases can lead to a serious impact that is greater than the carbon dioxide. In the biodiesel advancement, interest on the exhaustion of fossils supplies is crucial. Due to this reliance on fossil fuels, it leads to the depletion of oil supply and negative greenhouse impacts. Therefore, the authority should immediately develop an alternative energy to overcome the issue of petroleum products. Jayakumar et al., (2017) asserted that non-renewable energy will not last long and suggested that supplanting early can be the earliest opportunity when there is a critical situation. Now, Malaysia is planning to go against the requests for the present and future oil supply by using the sustainable power supply. In Malaysia, the requirement for the oil is expanding colossally.

Malaysia is a nation that is rich with various resources. It is said to be a country with promising energy supply that needs to be immediately associated to the fossil fuels problem with the alternative strategies. In Malaysia, petroleum products are expected to be spent within 14 years time frame, while the world's supply can last for an additional of 35 years (Muda & Pin, 2012; Jayakumar et al., 2017).

Meanwhile, biofuels utilize agro-waste or plant matter to create energy with low amount of ozone-depleting substance discharges. In developed countries, current innovations and proficient bioenergy were utilized as they are cost-effective than petroleum products (Demirbas, 2008).

Utilizing vegetable oils as elective sources of diesel requires considerable endeavor because seed oils must be extracted, and gainful plant species with high return of oil should be grown progressively. Oils from plants are abundant and unlimited and thus are practical to use in biodiesel. Furthermore, *M. indica*, *Cucurbita sp*., *C. papaya L*, and *N. lappaceum* are organic product plants that are effectively grown in Malaysia. Oil yields from rambutan, mango, pumpkin, and papaya seeds have been previously explored (Mahisanunt et al., 2017; Mei et al., 2014; Kansedo & Lee, 2013; Malacrida, Kimura & Jorge, 2011; Kansedo, Lee & Bhatia, 2009). Oils have been extracted from the seeds of rambutan (30%–43%), mango (11%–14%), pumpkin (32%–41%), and papaya (27%–29%). Given that these seeds are regularly relinquished as squanders, their free and high accessibility as oil source for biodiesel production is worth researching.

1.6 Problem Statements

Agro-waste accounts for more than 30% of agricultural production worldwide. Vast quantities of renewable agro-residues are generated annually as a result of extensive agricultural practices. Their direct use as food is non-existent. Most of the wastes from agricultural production, such as crop roots and straw, will induce harmful gas contamination in air and soil. Burning agrowastes produces a substantial amount of harmful gases, smoke, and residue that contaminate the air environment (Varma et al., 2015; Karak et al., 2015; Westerman & Bicudo, 2005).

A portion of agricultural residues is rapidly released into water, causing serious contamination of aquatic ecosystems (Udeigwe et al., 2015). Agro-wastes vary in diverse zones. Resource utilization, ecological crisis, and other problems caused by agrowastes should be addressed. Inappropriate agricultural practices may result in negative impacts, that is, death of fish and invertebrates and hazards to human health mainly due to the entry of agrowastes and excessive nutrition (nitrogen, phosphorus) into the surface waters. These phenomena will lead to algal growth that depletes oxygen and promotes the eutrophication of water bodies. Nitrate and pathogen entry in surface water and groundwater sources results in risks to human health.

Water quality is decreased because soil erosion and run-off carry suspended solids and sediments into the surface water. Agricultural emissions, such as ammonia and particulate matter from manure, air ventilation, and smoke from incinerators, pass into the air and cause respiratory problem in humans (Udeigwe et al., 2015). By contrast, Wang et al. (2016) denoted that the amount of agro-waste increases every year at a normal rate of 5%–10%. The technique, cost, and benefits of agro-waste recourses remain unknown. Despite the abundance of agro-waste on the ground, a worldwide concern exists regarding their nutritive value and pharmacological action. Therefore, profiling of these wastes is necessary to determine their chemical profile and bioactive compounds. These wastes are part of agricultural industry and hence can be improved and incorporated into the human diet.

Edible oils can be extracted from agrowastes and used in the medical field due to their antioxidant, antibacterial, and cosmetic effects and in the extraction of chemical compounds, such as tocopherol. Therefore, identifying agro-waste seeds in Malaysia and their potential antimicrobial and antioxidant effects is important. This step is crucial before compounds can be proposed as an agent with medical purpose. Vegetable oils have similar heating value to diesel fuel and can be used to produce biodiesel fuels. However, the physicochemical characteristics of oils depend on the type of oil, climate, and plant locality and influence the quality of biodiesel. As such, biodiesel yield and stability and other properties may be affected.

Agro-wastes generated through the escalated utilization of non-conventional crude materials, such as oil palm, coconut, pineapple, and bagasse, are not used properly. Malaysia and Indonesia are the world's biggest producer of agricultural waste, particularly oil palm and various waste products. However, the agro-waste material can be used for paper production, composites, and engineered materials. In addition, agro-based waste materials already have numerous applications. For example, natural fiber composites are utilized for automotive and building parts. Nanocellulose fiber biocomposites are also used to make free fill packing and in biomedical applications. Seeds are agro-waste products that are not properly explored. Therefore, biodiesel oil production and its nutritive value and pharmacological action should be assessed in future studies.

The presence of bioactive compounds, such as flavonoids, alkaloids, carbohydrates, cardiac glycosides, tannins, steroids, terpenoids, and coumarins, enables fruit seed oil extracts to become alternative sources of oils with functional properties and ability to reduce disease risk when ingested frequently. In this context, we need to convert the agro-wastes into beneficial products by maximizing the use of agro-waste seeds as an additional national income for Malaysia. Therefore, there is an urgent need to identify the potentials of agro wastes seeds by determining the chemical profile, physicochemical and sensory properties, the potential as biodiesel resources and edible oil in Malaysia. Thus, the data can be used to create or innovate new product that can conserve the environment, securing the health of human being and create wealth. This study aimed to evaluate the oils extracted from the seeds of *Cucurbita sp.*, *Carica papaya L*, *N. lappaceum*, *M. indica*, *D. zibethinus* and *A. heterophyllus Lam*.

1.7 Research Objectives

The objectives were to:

1. determine the drying technique for seeds that will produce the highest amount of oil.
2. determine the anti-trypanosomal, antioxidant, antibacterial activity and sun protection factor in seed oils.
3. estimate the amount of bioactive compounds.
4. identify the chemical profiles of agro-waste seeds.
5. determine the potential production of edible oil from agro-waste seeds.
6. optimize biodiesel production from agro-waste seeds.

1.8 Research Questions

1. Which drying technique will produce the highest amount of oil?
2. What are the anti-trypanosomal, antioxidant, and antibacterial activities and UV protection factor activity of seed oils?
3. What is the estimated amount of bioactive compounds?
4. What are the chemical profiles of agro-waste seeds?

5. What is the production potential of edible oils in agro-waste seeds?

6. What is the production potential of biodiesel from agro-waste seeds?

1.9 Research Hypothesis

Ho_1 No significant difference in the amount of seed oil among drying techniques.

1.10 Significance of the Study

Utilizing vegetable oils as elective sources of diesel requires considerable endeavor because seed oils must be extracted, and gainful plant species with high return of oil should be grown progressively. Oils from plants are abundant and unlimited and thus are practical to use in biodiesel. Furthermore, *M. indica*, *Cucurbita sp*., *C. papaya L*, and *N. lappaceum* are organic product plants that are effectively grown in Malaysia. Oil yields from rambutan, mango, pumpkin, and papaya seeds have been previously explored (Mahisanunt et al., 2017; Mei et al., 2014; Kansedo & Lee, 2013; Malacrida, Kimura & Jorge, 2011; Kansedo, Lee & Bhatia, 2009). Oils have been extracted from the seeds of rambutan (30%–43%), mango (11%–14%), pumpkin (32%–41%), and papaya (27%–29%). Given that these seeds are regularly relinquished as squanders, their free and high accessibility as oil source for biodiesel production is worth researching. That agro-waste seed oil can also be used in medical, and food industries.

The chemical profile, physicochemical–sensory properties and trans-esterification of agro-waste seeds oil and its importance in eliminating environmental pollution were determined. This study aims to maximize the use of agro-waste seeds as an additional national income for Malaysia. Seeds are one of the important wastes produced from agricultural crops. This material contains a high proportion of oil that can be used for human consumption. Biofuels can also be generated from the agro-waste seeds.

1.11 Scope and Limitation of the Study

This study was limited to characterizing oil extracts from the seeds of the following six plants: pumpkin (*Cucurbita* sp.), papaya (*Carica papaya* L), rambutan (*Nephelium lappaceum*), mango *(M.indica*), durian (*D.zibethinus*), and jackfruit (*A. heterophyllus* Lam). The seeds were obtained from the agro-waste in the district of Muallim, Perak, Malaysia. The effects of drying on oil yield were determined by using different techniques (microwave exposure, ultrasonication, oven, autoclave, and sun dry). The physical and sensory properties of these oils were also investigated.

The chemical profile of seed oils were determined using Gas chromatography mass spectrometry (GCMS), and tocopherol content was measured using highperformance liquid chromatography (HPLC)-UV method. This study was focused on the analysis of anti-trypanosomal, anti-oxidant, anti-bacterial, sun protection, edible oil and biodiesel. Agar disc diffusion was used to determine antibacterial activity against two bacterial strains: *S. aureus* and *E. coli*. The optimum condition for the production of biodiesel parameters was limited to catalyst loading, reaction temperature, and methanol-to-oil molar ratio. The oil and biodiesel products were analyzed using Fourier transform infrared (FTIR) spectroscopy.

CHAPTER 2

LITERATURE REVIEW

2.1 Agro-Waste