EXTRACTION OF BIOACTIVE COMPOUND (MANGIFERIN) FROM MAHKOTA DEWA FRUITS USING SUBCRITICAL WATER EXTRACTION PROCESS

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I would like to dedicate this research work to my families, my supervisor Dr. Siti Kholijah Binti Abdul Mudalip and student master for my research supervisor, Dr. Siti Kholijah Binti Abdul Mudalip which is NorMaryam Aini binti Hashim as their work of encouragement and support for me in completing this undergraduate research. Besides that, I would like to thank personally to my research supervisor, Dr. Siti Kholijah Binti Abdul Mudalip and NorMaryam Aini binti Hashim who supported and always give me guidance throughout this journey of completing the undergraduate research study. Lastly, I dedicate this work to Universiti Malaysia Pahang for their given opportunity and experience to me to do my research and completing this thesis as an undergraduate chemical engineering student.

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ABSTRACT

Mahkota Dewa or scientifically known as *Phaleria Macrocarpa* is a popular herbal plant in Indonesia and Malaysia. The bioactive ingredient in this plant process antihistamine, antioxidant, anti-cancer compound. In this work, experimental studies were performed using subcritical water extraction process to extract the bioactive compound namely mangiferin from *Mahkota Dewa* fruits. The effect of process parameters which are solid to solvent ratios (30, 40, 50, 60, and 70 g/L), temperature (50, 75, 100, 125, 150 °C) and extraction time (3, 4, 5, 6, 7 h) on the extraction yields were investigated. The extraction yields were analysed and characterised using high performance liquid chromatography (HPLC) and 1,1-Diphenyl-2-picryl hydrazyl (DPPH) assay method, respectively. The highest mangiferin yield is 3.2027w/w % were obtained at 60 g/L of solid to solvent ratio, 100 °C of extraction temperature and 5 h of extraction time. The highest of antioxidant activity is 86.5377 % were obtained at 60 g/L of solid to solvent ratio, 125 °C of extraction temperature, 5 h of extraction time. Antioxidant is defined as a substance which significantly delays or inhibits oxidant process. The high value of antioxidant was obtained from the highest value of DPPH since the remaining DPPH in sample acts free radicals were increased in reactivity of unpaired electron and allowed the development of tissues damage in living organisms.

ABSTRAK

Mahkota Dewa atau nama saintifiknya dikenali sebagai *Phaleria macrocarpa* adalah pokok herba yang terkenal di Indonesia dan Malaysia. Kandungan bioaktif di dalam pokok ini proses kompenan antihistamine, antiokidan, anti kanser. Dalam proses ini, kajian ekperimen akan dijalankan menggunakan proses pengekstrakan subkritikal air untuk ekstrak kompenan bioaktif yang bernama mangiferin daripada buah Mahkota Dewa. Kesan daripada parameter proses yang mana melibatkan nisbah pepejal dan cecair (20, 40, 60, 80, 100) g/L, suhu (50, 75, 100, 125, 150 °C) dan tempoh masa pengekstrakan (3, 4, 5, 6, 7 jam) pada hasil perahan akan dikaji. Hasil perahan akan dianalisis dan dicirikan mengunakan kromatografi cecair prestasi tinggi (HPLC) dan and 1,1-Diphenyl-2-picryl hydrazyl (DHHP) kaedah cerakin , masing-masing. Hasil mangiferin tertinggi adalah 3.2027 w/w % telah diperolehi pada 60 g/L nisbah pepejal dan cecair, 100 °C suhu pengekstrakan dan 5 h masa pengekstrakan. Aktiviti antioksidan yang tertinggi adalah 86.5377 % telah diperoleh pada 60 g/L nisbah pepejal dan cecair, 125 °C suhu pengekstrakan dan 5 h tempoh masa pengekstrakan. Antioksidan ditafrifkan sebagai bahan yang ketara yang boleh melambatkan atau menghalang proses oksida. Nilai antioksidan yang tinggi diperoleh pada nilai tertinggi DPPH kerana baki DPPH dalam sample bertindak radikal bebas telah meningkat dalam kereaktifan electron berpasangan dan membenarkan perkembangan kerosakan tisu dalam organism hidup.

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LIST OF SYMBOLS

$^{\circ}C$	degree Celcius
g/L	gram per liter
h	hour
mg/L	milligram per liter
wt/wt %	weight per weight percent

LIST OF ABBREVIATIONS

CAM	Complementary and Alternative Medicine
DPPH	1,1-Diphenyl-2-picryl hydrazyl
HPLC	High Performance Liquid Chromatography
ROS	Reaction oxygen species
SCD	Supercritical carbon dioxide
SE	Soxhlet extraction
SWE	Subcritical water extraction
UAE	Ultrasonic assisted extraction

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Malaysia is rich with various biodiversity resources that can be used to treat several diseases. *Phaleria macrocarpa* (Scheff) Boerl. is known as 'God's Crown' or Mahkota Dewa, a plant from the family of Thymelaeaceae and this fruit come from the land of Papua, Irian Jaya, Indonesia and grow in tropical areas (Hendra et al., 2011). Mahkota Dewa is classified as plant capable of living in various conditions, from lowland to highland. It can grow in areas of 0-1000 meters above sea level. This plant can grow in the garden and also in the pot. It can live at height of approximately 3 meters and easily produces flower then grew as a fruit. Fruits from this plant are greenish and later turn to dark red color when matured whereby some of the native find them attractive. Although, almost all parts of this plant such as fruit, leaves, seeds, and stems can be used in treatment but the Mahkota Dewa cannot be consumed directly because it will may give swollen, numb and unconsciousness (Harmanto, 2003). The Mahkota Dewa fruit is empirically believed as a potent medicine to treat some diseases. Traditionally, Mahkota Dewa fruit is used by the locals as a herbal drink either singly or mixed other medicinal plant to cure several of illnesses (Kurnia et al., 2008).

Mahkota Dewa fruit contain chemical that good for human body such as flavonoid that help to improve flow of blood in our body then its capable to reduce of cholesterol and lastly reducing the chances of heart disease. Mahkota Dewa has been widely used as traditional treatment to treat cancer, heart disease, high blood pressure, various skin diseases, stroke, migraine and controlling kidney disorders. Mangiferin is an active compound in Mahkota Dewa that shows anti oxidative, antiviral, anticancer, immunomodulatory and analgesic effect. Mangiferin has been reported to reduce

plasma glucose and insulin level and increase insulin sensitivity, then reduced hyperglycemia and prevents diabetes (Raihan et al., 2013).

Mahkota Dewa is a one of native Indonesian plants, contains various bioactive compounds and thus possesses antioxidant, antimicrobial, or even anti cancer attributes. Antioxidant is defined as a substance which significantly delays or inhibits oxidant process. The antioxidant activity is measured by determining the rate of oxidant process in the presence of an antioxidant (Antolovinch et al., 2002). Antioxidant also defined as substances that even at low concentration significantly prevent oxidation of easy oxidisable substrates (Halliwell et al., 1995). Free radicals are molecules with an unpaired electron. The unpaired electron results in high level of reactivity because the free radical seeks another electron to fulfill a pair. Free radicals are naturally by-product of cellular metabolism, but they are also generated by external action of ultraviolet radiation, toxic substances and even intensive exercise (Kanter 1998). Free radicals play an important role in the development of tissues damage and pathological events in living organisms (Anggraini et al., 2015). According to Raihan, Mahkota Dewa showed anti oxidative by the function of bioactive compound in that fruits. Antioxidants are the substance that present in low concentration to prevent oxidation of that substance (Halliwell et al., 1998). Generation of free radical known as reaction oxygen species (ROS) and other activities beyond the antioxidant capacity of biological system gives rise to oxidative stress during metabolism (Zima et al., 2001). Oxidative stress plays role in aging process, heart disease, neurodegenerative diseases, cancer and age-related degenerative disease. The risk of these diseases can be lower by dietary antioxidant.

Extraction method can be divided into conventional and non-conventional method. Conventional method is a classical existing technique based on the extracting power of different solvents in use and also application of heat and mixing towards the target plant. Example of the conventional technique is soxhlet extraction (SE). Meanwhile, non-conventional methods such as supercritical carbon dioxide (SCD), ultrasonic assisted extraction (UAE) and subcritical water extraction (SWE) are give more advantages compared to conventional methods in term of environmental friendly, cost, due to its less of synthesis and organic chemical usage in the operation to produced the high yield and extract quality in short operational time (Azmir et al., 2013).

Subcritical water extraction is a method that offers an efficient, non-toxic, and environment friendly alternative to extract polar compounds by using water under external pressurization above its boiling points as an extraction solvent (Kim et al., 2010). This process is fastest by combination pressure and temperature and does not require the use of expensive and toxic organic solvent, decreased energy consumption and reduced thermal degradation effect of extract. SWE also defined as extraction with water as solvent at temperature ranging from boiling point to the critical temperature and at pressure high enough to keep the water in a liquid state throughout the extraction process. Water as green solvent that has many advantages when used in extraction since it is environmentally friendly and sustained, and some its physical and chemical properties can be modified by heating (Herrero et al., 2013).

The subcritical water extraction is used to produce pharmaceutical extracts such as flavonoids, mangiferin and alkaloids from medicinal plant (Liang and Fan, 2013). These bioactive compounds can be found in different parts of Mahkota Dewa. Bioactive widely uses for pharmaceutical, nutraceutical and biomedical application (Thiruvenkadam et al., 2015). Since many support the medicinal values of Mahkota Dewa, the demand for this plant is gradually increasing. Many products contain Mahkota Dewa fruit extracts are currently sold in market, for example in instant coffee, herbal tea, cosmetics, food and medicine.

1.2 Motivation

Cancer is one of the number one silent killers that caused by the presence of free radicals that hold responsibilities for the damage of proteins, lipids, and nucleic acids in the cells. The current treatment of cancer using commercial drugs is quite expensive. Natural bioactive compounds derived from plants and synthetic derivatives are expected in creation of novel and improved therapies for cancer management. *Phaleria macrocarpa* commonly used as medicinal plant in Papua Indonesia (Faried et al., 2016). Reactive oxygen species (ROS) have deleterious effects on the cellular membranes and internal structures that might contribute to the onset of cardiovascular disease, cancer, and impairment of the immune function by altering the metabolism (Thiruvenkadam et al., 2015).

There is an increasing interest in natural food additives which can function as natural antioxidants besides seasoning the food. Health applications have been stimulated by the observations that free radicals and oxidation are involved in many physiological functions and can cause pathological condition. Selection of a suitable extraction procedure can increase the antioxidant concentration relative to the plant material, and differences in antioxidant activity between the extracts indicate the polarity of the compounds mediating antioxidant effect. In fact, antioxidant capacity has been related to various different disease processes and their prevention such as cancer, neurological degeneration, and aging (Madhavi et al., 1996). Natural antioxidants have generated considerable interest in preventive medicine, offer food, pharmaceutical, nutraceutical, and cosmetic manufacturers, and the possibility of multiple actions that improve and extend food and pharmaceutical stabilization (Schaich, 2006).

Mangiferin were reported have ability to inhibit the cancer cells by inducing apoptosis and also prevention of arthritic diseases. It has been observed in various animal models that it could act as a potent antioxidant by reducing free radical species and preventing the potential DNA damage (Zhang et al., 2007). Mangiferin is known to scavenge ROS generated in the cells. It is also known to inhibit xanthine oxidase, the enzyme responsible for oxidation. Antioxidant and anti-inflammatory activities of mangiferin are quite useful for treating gastrointestinal spasm.

Recently, the used of complementary and alternative medicine (CAM) have gained popularity among cancer patients. The prevalence to utilize CAM the therapies is driven by the eagemess to strengthen the immune system and minimize adverse affect due to conventional treatment. The used of CAM have less or no side effect to human body because that are made from renewable resources of raw materials and it is easily available as well as cost effective. Therefore, the potential use of using Mahkota Dewa extracts as anticancer can be explored as a complementary alternative medicine.

1.3 Problem Statement

The conventional extraction methods such as soxhlet extraction and reflux extraction that are used to extract the bioactive compounds from the plant matrix are typically performed using organic solvent, i.e. hexane, ethanol, and methanol. The conventional extraction process are time consuming since its required about 2 to 7 days and these involved bulk amount of solvent (Kim et al., 2010). Due to long extraction time, low extraction yield, and residual toxic problem, the urge to develop new non organic solvent-based extraction methods with higher extraction efficiency is needed.

Organic solvent are difficult to remove completely, costly, low extraction yield and required high purity of solvent (Easmin et al., 2014). Organic solvent is flammable, the recovery of the organic solvent for re-use after the purification (Wheatley et al., 2010). The organic solvent produce greenhouse gases in the environment that are hazardous and toxic to health, environment and dangerous for humans, agriculture and microorganisms (Easmin et al., 2014). Recently, the researchers are exploring a safer and greener extraction approach including green solvent. This study will investigate the potential use of green solvents which is water as a solvent for the extraction process.

During recent years consumers have been more concerned about the addition of synthesis additives to food and the two most commonly used antioxidants, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) that shown DNA damage introduction (Prakash et al., 2007). The imbalance between the production of bodily antioxidant defense system and free radical formation results in oxidative stress. Oxidative stress has been implicated in the alteration of generation material that inducing oxidation and caused membrane lipid peroxidation, decreased membrane fluidity and inducing metabolic injury and death. This may lead to accelerated aging, cancer, cardiovascular diseases and inflammation (Wong et al., 2006).

1.4 Objectives

The main objective of this work is to extract bioactive compound (mangiferin) from Mahkota Dewa using subcritical water extraction process.

1.5 Scopes of Study

The following are the scopes of this research:

1) To study the effect of different solid to solvent ratios of 30, 40, 50, 60, and 70 g/L on extraction yield. The extraction temperature and extraction time was fixed at 100°C and 5 h respectively.

2) To investigate the effect of different extraction temperature (50, 75, 100, 125, 150 °C) on extraction yield at solid to solvent ratio obtained from (i). The solid to solvent ratio obtained from (i) and extraction time will be fixed at 60 g/L and 5 h, respectively.

3) To investigate the effect of different extraction times (3, 4, 5, 6 and 7 h) on extraction yield at fixed solid to solvent ratio obtained from (i) and extraction temperature from (ii). The extraction temperature and extraction time will be fixed at 60 g/L and 100° C, respectively.

4) To analyze the extraction yield using high performance liquid chromatography (HPLC) analysis and measured the antioxidant activity using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay method.

CHAPTER 2

LITERATURE REVIEW

2.1 Mahkota Dewa

Mahkota Dewa are from the family of Thymelaeaceae which is family of flowering plants of cosmopolitan distribution (Fajerska et al., 2011). It is a complete tree, including stem, leaves, flowers and fruits. The leaves are green and width and tapering with length from 3 cm to 5 cm and 7 cm to 10 cm respectively. The color flowers are from green to maroon. The fruit is of eclipse shape with diameter of 3 cm and are green when un-ripened and become red on ripening as showed in Figure 2.1. Although the herb is being used in both un-processed and processed form, however, the former can be poisonous and toxic (Yosie et al., 2011).

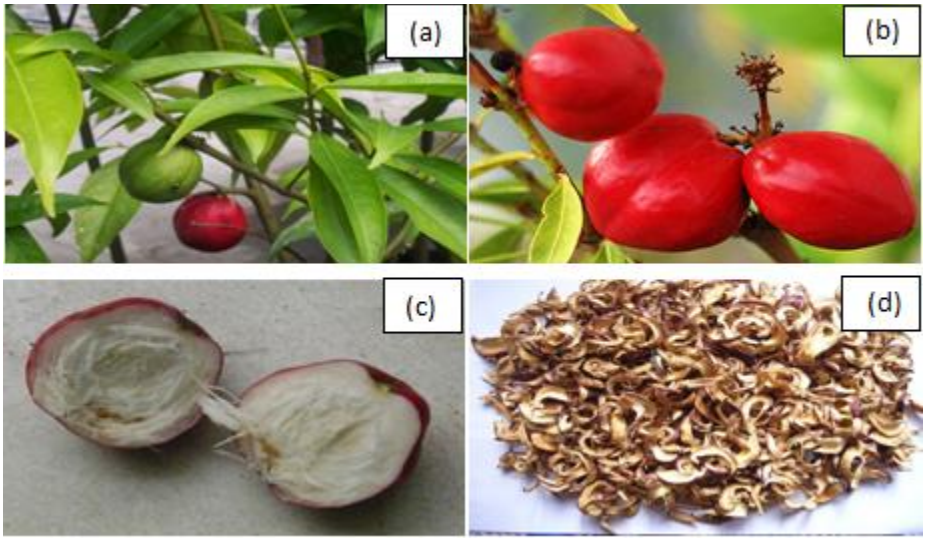


Figure 2.1: Mahkota Dewa showing a typical: (a) green tapering leaves (b) fruits; (c) wet pulps; and (d) dried pulps

All part of Mahkota Dewa plant including fruits, seeds, stem and leaves have specific bioactive compound that used in tradisional medicine for treatment of various diseases. In Table 2.1 shows the phytoconstituents isolated from Mahkota Dewa with their respective biological activity.

Table 2.1: Phytoconstituents isolated from Mahkota Dewa with their respective biological activity.

Mahkota Dewa	**Bioactive compounds**	**Biological activity**	**Author**
Leaves, stem, seed and fruits	Phalerin, gallic acid	Anticancer	Saufi et al. 2008
Pericarp	Phalerin	Antidiabetic	Ali et al. 2012
Fruits	Gallic acid	Antihyperlipidemic	Chong et al. 2011
Leaves, seed, fruits	Flavanoids, saponins, polyphenols, tannins	Antibacterial and antifungal	Shodikin 2009
Fruits	Terpenoids, saponins, tannins, flavanoids and phenols such as rutin and cathecol, phalerin,benzophenone glucoside	Anti inflammatory	Hendra et al. 2011
Bark, leaves, mesocarp, pericarp and seed	Flavanoids, phenolics,gallic acid, 6, hydroxyl-4-methoxy-benzophenone, -2-O-β-D-glucoside	Antioxidant	Yosie et al. 2011
Fruits, leaves and seed	Flavonoids (Kaempferol), icaricide	Vasorelaxant	Oshimi et al. 2008
Fruits	Saponin	Increase male fertility	Parhizkar et al. 2013
Fruit, seed	Des-acetylfevicordin A, and its derivatives	Toxicity	Kurnia et al. 2008

Mahkota Dewa	Bioactive compounds	Biological activity	Author
Whole plant	Mahkoside A, dodecanoic acid, palmitic acid, desacetyl flavicordin A, flavicordin A, flavicordin D, flavicordin A glucoside, ethyl, stearate, lignans, alkaloids, saponins, sucrose	Anti-microbial	Hendra et al. 2011

Source: (Easmin et al. 2014)

2.1.1 Mangiferin

Mangiferin dissolves well in water, so it can be easily extracted into infusions and decoctions. In mangiferin molecules as shown in Figure 2.2 contain four aromatic hydroxyl groups determine its strong antiradical and antioxidant properties. Mangiferin is also an efficient in preventing the generation of hydroxyl radical. other activities of mangiferin: analgesic, antidiabetic, antisclerotic, atimicrobial and antiviral, cardio-, hepato-, and neuroprotective, antiinflammatory, antiallergic, and memory improving, as well as radioprotective against X-ray, gamma, and UV radiation (Matkowski et al., 2013).

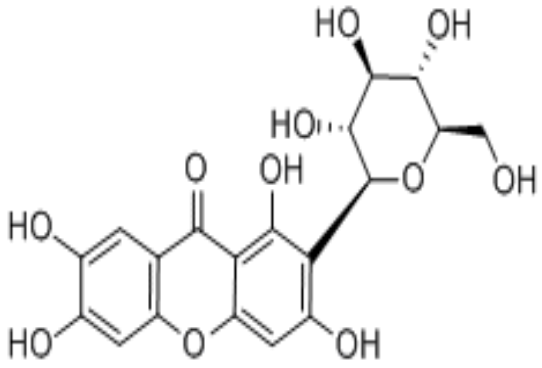


Figure 2.2: Chemical structure of mangiferin

2.1.2 Antioxidant compound

Ionizing radiation leads to the production of ROS. The molecules of antioxidant compound showed in Figure 2.3 caused damage to DNA, proteins, lipids, and finally, to tissue injury and death. Antioxidant compound can protect against injury to the lungs, gastrointestinal tract and the eye in a mouse model of acute exposure to ionizing radiation in reducing the chronic ROS production and inflammatory cascade that mediates the radiation-induced tissue injury (Health, N. J. 2011).

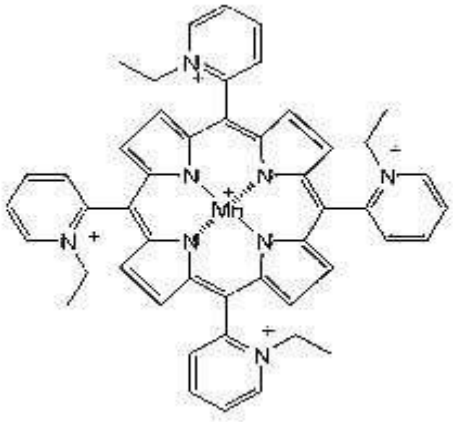


Figure 2.3: Antioxidant compound

2.2 Extraction Method

2.2.1 Soxhlet Extraction

Soxhlet extraction works by boiling a solution that has a solute of limited solubility and the impurity was insoluble in that solvent, then cooling and collecting the condensate in a reservoir from which the concentrated solute can be extracted. The solvent were used and were heated up until it reached its boiling point. The extraction process was conducted for several of extraction time. Easmin et al., 2014 reported the conventional soxhlet extraction (CSE) used toxic organic solvent as a extraction solvent and required the time consuming 2 to 7 day and also organic solvent that used in extraction process are difficult to remove completely in extract. Disadvantage of CSE are not acceptable for industrial applications due to long extraction time which can

achieved longer time than 24 hours, large consumption of hazardous solvent and azeotropic mixture or constant mixture was required for ensure efficient extraction (Bimakr et al., 2011).

2.2.2 Supercritical Carbon Dioxide

The extraction of polar components is highly limited to the poor solvent power of supercritical carbon dioxide thus polar nature of mangiferin is difficult to soluble in the non polar supercritical carbon dioxide medium (Kim et al., 2010). Carbon dioxide has low polarity which makes it ideal for lipid, fat and non polar substance, but that not suitable for most pharmaceuticals and drug samples (Azmir et al., 2013). According to Azmin et al. (2016) carbon dioxide has unique properties which content non-toxicity, non-flammability, and lack of reactivity with extraction materials and equipment then, used of carbon dioxide can be replaced organic solvent in extraction process and also can reduced the environmental and health concern that caused of organic solvent.

2.2.3 Ultrasonic Assisted Extraction

Ultrasonic assisted extraction (UAE) is provide efficient contact between solid and solvent by increase the pressure and temperature but the extraction used large volume of organic solvent (Yolando Picó , 2013). The extraction of organic compound from various plant materials can be improved by using ultrasound to achieve higher product yields at reduced processing time and solvent consumption (J.Wu et al., 2001). The application of ultrasound in extraction which breaks the plant cells to facilitate penetration of solvent into the cells by foams cavitations bubbles (Ilghami et al., 2015). According to Azmin et al. (2016) sonication extraction is inexpensive, easy to use, reduced working time, increase the extraction yield, improved solvent consumption. Then, a sonic wave creates regions of alternating compression expansion among the molecules of the medium by changing pressure and cavitation of formed gas bubbles occurs. Therefore, the controlling mechanism of ultrasound-assisted extraction is generally attributed to mechanical, cavitation, and thermal effects which can result in disruption of cell walls, particle size reduction, and enhanced mass transfer across cell

membranes, which lead to target compounds dissolving in the solvent, hence increasing yield with shorter time (Shirsath et al., 2012).

2.2.4 Subcritical Water Extraction

Subcritical water extraction (SWE) technique required small amounts of solvents because of the combination of high pressure and temperature which provides faster extraction. The higher extraction temperature can promote higher analyte solubility by increasing both solubility and mass transfer rate and decrease the viscosity and surface tension of the solvents then improving the extraction yield (Ibanez et al., 2012). According to Azmir et al. (2013) SWE has been successful technique to extract bioactive compounds from different plant materials at high pressure to remain solvent liquid beyond their normal boiling point and by development of SWE are from automation technique along the decreased extraction time and solvents requirement. The water used above its normal boiling point can facilate in situ sterilization of the extract, similar to the experienced using thermal retorting (King et al., 2003). SWE provides the ability to dielectric constant and solvent strength by increasing the temperature below the critical point and water as a extraction solvent is the most cost-efficient and least hazardous material to use for extraction process (Luong et al., 2015). SWE is also useful for extraction of organic pollutants from the environmental matrices which are stable at high temperature (Wang and weller, 2006). Ghoreishi et al. (2008) reported the higher extraction yield in SWE than by using Soxhlet method. The advantages of SWE are used green solvent extraction, environmentally friendly technology that produce higher quality of extraction yield and less time consuming in extraction process. All extraction methods are summarized in Table 2.2.

Table 2.2: Advantages and Disadvantages of Extraction Process

Method	**Description**	**Author**
Soxhlet extraction	The conventional soxhlet extraction (CSE) used toxic organic solvent as a extraction solvent and required the time consuming 2 to 7 day and also organic solvent that used in extraction process are difficult to remove completely in extract.	Easmin et al., 2014
	Disadvantage of CSE are not acceptable for industrial applications due to long extraction time, large consumption of hazardous solvent	(Bimakr et al., 2011).
Ultrasonic assisted extraction (UAE)	UAE is provide efficient contact between solid and solvent by increase the pressure and temperature but the extraction used large volume of organic solvent.	Yolando Picó , 2013
	Ultrasound method achieved higher product yields at reduce solvent consumption and processing time and thermal damage to extract are avoided by carried out at lower temperature for recovery of temperature-sensitive ingredient of natural product and loss volatile component in boiling.	Wu et al., 2001
	The application of ultrasound in extraction which breaks the plant cells to facilitate penetration of solvent into the cells by foams cavitations bubbles.	Ilghami et al., 2015
	The controlling mechanism of ultrasound-assisted extraction is generally attributed to mechanical, cavitation, and thermal effects which can result in disruption of cell walls, particle size reduction, and enhanced mass transfer across cell membranes, which lead to target compounds dissolving in the solvent, hence increasing yield with shorter time.	Shirsath et al., 2012

Method	Description	Author
Supercritical carbon dioxide	Carbon dioxide has unique properties which content non-toxicity, non-flammability, and lack of reactivity with extraction materials and equipment then, used of carbon dioxide can be replaced organic solvent in extraction process and also can reduce the environmental and health concern that caused of organic solvent.	Azmin et al., 2016
	Carbon dioxide is low polarity which makes it ideal for lipid, fat and non polar substance, but that not suitable for most pharmaceuticals and drug samples.	Azmir et al., 2013
	The extraction of polar components is highly limited to the poor solvent power of supercritical carbon dioxide. Polar nature of mangiferin difficult to soluble in the non polar supercritical carbon dioxide medium.	Kim et al., 2010
Subcritical water extraction (SWE)	Subcritical water extraction technique required small amounts of solvents because of the combination of high pressure and temperature which provides faster extraction. The higher extraction temperature can promote higher analyte solubility by increasing both solubility and mass transfer rate and decrease the the viscosity and surface tension of the solvents then improving the extraction yield.	Ibanez et al., 2012
	The water used above its normal boiling point can facilate in situ sterilization of the extract, similar to the experienced using thermal retorting.	King et al., 2003
	Subcritical water extraction provides the ability to dielectric constant and solvent strength by increasing the temperature below the critical point.	Luong et al., 2015

Method	Description	Author
	SWE is also useful for extraction of organic pollutants from the environmental matrices which are stable at high temperature.	Wang and weller, 2006
	SWE has been successful technique to extract bioactive compounds from different plant materials at high pressure to remain solvent liquid beyond their normal boiling point.	Azmir et al., 2013
	Development of SWE are from automation technique along the decreased extraction time and solvents requirement.	
	The higher extraction yield in SWE than by using Soxhlet method. The advantages of SWE are used green solvent extraction, environmentally friendly technology that produce higher quality of extraction yield and less time consuming in extraction process.	Ghoreishi et al., 2008

2.3 Parameter Affecting Subcritical Water Extraction

2.3.1 Extraction Temperature

SWE can be carried out at the temperature between 125°C to 175°C. The increase of temperature can increase the diffusion rate, increase mass transfer rate and high solubility of bioactive compounds and also decrease viscosity as well as surface tension of the water, thus increase the extraction yield (Asl and Khajenoori, 2013). However, too high temperature may degrade the phytochemicals compound. Due to evaporation or reaction with other compound, phytochemical compound may losses and that will affects the bioactivity (Fariza et al., 2014). According to Ghoreishi et al. (2008) the increasing in temperature from 100°C to 150°C reduced the extraction yield from 76.75 to 64.68 wt/wt %. The increasing in temperature during SWE process can cause

changes of quantitative and qualitative composition of extract due to acidic hydrolysis (Lekar et al., 2013). Reaction temperature is the important factor affecting SWE efficiencies because temperature significantly affects physiochemical properties of water (Jintana and Shuji, 2008).

2.3.2 Solid to Solvent Ratio

The amount of water used show significant affect on the extraction efficiency. When the amount of water was lower, water and solid particles would simple inundated, then, it will resulted in low extraction efficiency (Gong et al., 2013). According to Cardenas-Toro et al. (2014) and Tunchaiyaphum et al. (2013) large amount of water can easily dissolved the extract than little quantity of water. According to Fariza et al. (2014) at 1:20 g/ml of solid to solvent ratio were produce highest concentration of Mahkota Dewa (297.28 ± 5.41 ppm).

2.3.3 Extraction Time

Extraction time in subcritical water very short compared to conventional extraction technique (Erland Björklund and Tobias Nilsson, 2000). According to Kim et al. 2010 the most suitable extraction time is 5 hours, since it produce the highest extraction yield which is 25.0 mg/g). All parameters were effected the subcritical water extraction process are summarize in Table 2.3.

Table 2.3: Summary of Parameters Affecting Subcritical Water Extraction

Parameter	**Description**	**Author**
Solid to solvent ratio	Large volume of water can easily dissolved the extract.	Cardenas-Toro et al., 2014 Tunchaiyaphum et al., 2013
	The amount of water used show significant affect on the extraction efficiency.	Gong et al., 2013

Parameter	Description	Author
	The solid to solvent 1:20 (g/ml) produce highest concentration of Mahkota Dewa (297.28 ± 5.41 ppm).	Fariza et al., 2014
Temperature	Subcritical water extraction can be carried out at the temperature between 125°C to 175°C. The increase of temperature can increase the diffusion rate and decrease viscosity as well as surface tension of the water, thus increase the extraction yield.	Asl and Khajenoori, 2013
	The increasing in temperature from 100°C to 150°C reduced the extraction yield from 76.75 to 64.68 wt/wt %.	Ghoreishi et al., 2008
	The increase in temperature during subcritical water extraction process can cause changes of quantitative and qualitative composition of extract due to acidic hydrolysis.	Lekar et al., 2013
Extraction time	The most suitable extraction time is 5 hours, since it produce the highest extraction yield which is 25.0 mg/g).	Kim et al., 2010
	Extraction times in subcritical water are very short compared to conventional extraction technique.	Erland Björklund and Tobias Nilsson, 2000

CHAPTER 3

METHODOLOGY

3.1 Materials and Methods

The dried pulp of Mahkota Dewa fruit was purchased from Ethno Resources Sdn. Bhd, Selangor, Malaysia. The average size of dried pulp is 1.0 mm. The mangiferin standard was purchased from Permula Chemical Sdn. Bhd Malaysia. HPLC grade methanol (99.9 wt% purity), ortho-phosphoric acid (85.0 wt% purity) and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) were purchased from Merck (Selangor, Malaysia). Deionized water was prepared using Milli-Q, Ultrapure Water Purification System (Massachusetts, USA) with a 0.22mm filter. The 0.2 µm nylon filter and 0.45 µm nylon syringe filter that used in preparation of sample for HPLC analysis were supplied from MHY Energy Resources (Kuantan, Malaysia).

3.2 Subcritical Water Extraction Unit

The subcritical water extraction (SWE) experiments were performed in a 2L stainless steel Kiloclave laboratory pressure vessel reactor system (Buchiglasuster, Uster, Switzerland) illustrated in Figure 3.1. Subcritical water extraction brand Buchiglasuster does not support pressure to be manipulated. So, the extraction yields of mangiferin are weakly dependent on extraction pressure because the polarity of the subcritical water does not change much with pressure. The pressure reactor is made up from stainless steel and designed for the use of large steel pressure vessel. The vessel can operated within temperature range from -20°C to 250°C.

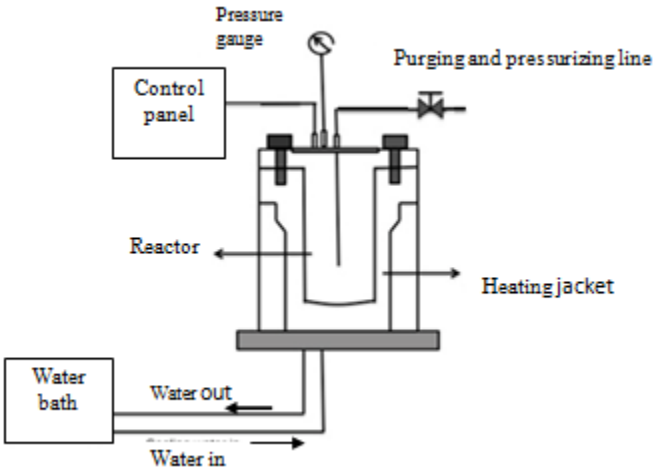


Figure 3.1: Schematic diagram of Kiloclave laboratory pressure vessel reactor system, Buchiglasuster

3.3 Subcritical Water Extraction Process

The grinded pulp of Mahkota Dewa was weighted and mixed with deionized water in 2 L beaker. The solution was charged into extraction vessel. The reactor was tightly closed and secured with 12 nuts and bolts. The temperature and time were set at desired value on the control panel. After extraction time was completed, the reactor was cooled about 1 hour before switch off the machine. The cooling water was transferred for cooling the reactor using Stuart recirculating cooler RE300RC (Staffordshire, UK). Then, the sample was poured into 40 mL in centrifuges and kept in -4°C refrigerator prior to analysis. The same processes were repeated with different solid to solvent ratio, extraction temperature and extraction time as tabulated in Table 3.1.

Table 3.1: Summary of process parameter

Parameter	Manipulated Parameter	Fixed Parameter
Solid to solvent ratio	30, 40, 50, 60, 70 g/L	100°C, 5 h
Temperature	50 °C , 75°C, 100°C, 125°C, 150°C	60 g/L, 5 h
Time	3, 4, 5, 6, 7 h	60 g/L, 100°C

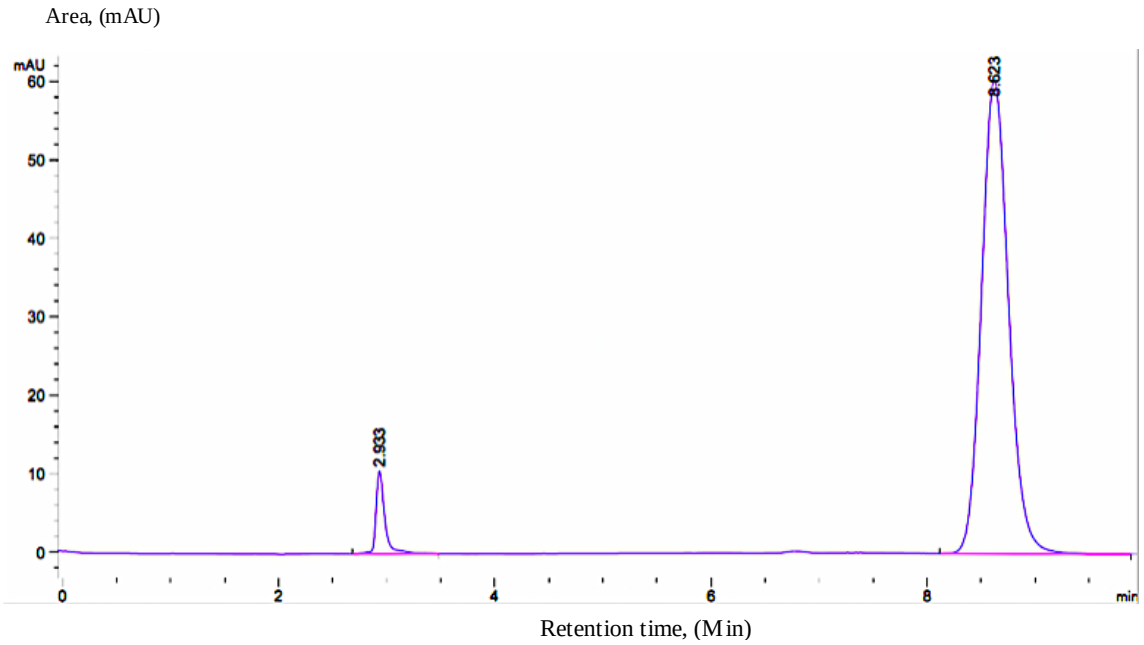
3.4 High Performance Liquid Chromatography

Agilent Technologies 1100 HPLC (Agilent Technologies 1100, California, USA) was used to quantify amount of mangiferin in the extracts. About 40 mL of sample was centrifuged for 20 minutes at 5000 rpm. Then, 20 mL of the sample was poured into vial and sonicated for 30 minutes at 35°C. 5mL of the sonicated samples were dissolved in 10 ml of methanol by using syringes. The mixtures then filtered by using 0.45 µm nylon syringe filter and the sample were stored in Agilent MS analyzed vial kits (California, USA) to analysis using HPLC. The HPLC system was comprised of solvent delivery pump, a column (Zorbax Eclipse Plus C18). Mobile phase 0.1% phosphoric acid (69%) and methanol HPLC grade (31%) at flow rate of 1.0 mL/min. The amount of sample injection was set at 10 µL. Standard solution was prepared by dissolving 5 mg of mangiferin in 10 mL methanol. The mixture was then diluted to obtain desired concentrations range from 5 ppm - 60 ppm, and was used for constructing calibration curves of mangiferin was illustrated in Figure 3.2. The peak at retention time of 8.623 min was used to estimate the mangiferin yield in Mahkota Dewa extract. The relationship between peak area of bioactive compound (mangiferin), x and concentrations of mangiferin (mg/L), y can be represented by linear equation as follows:

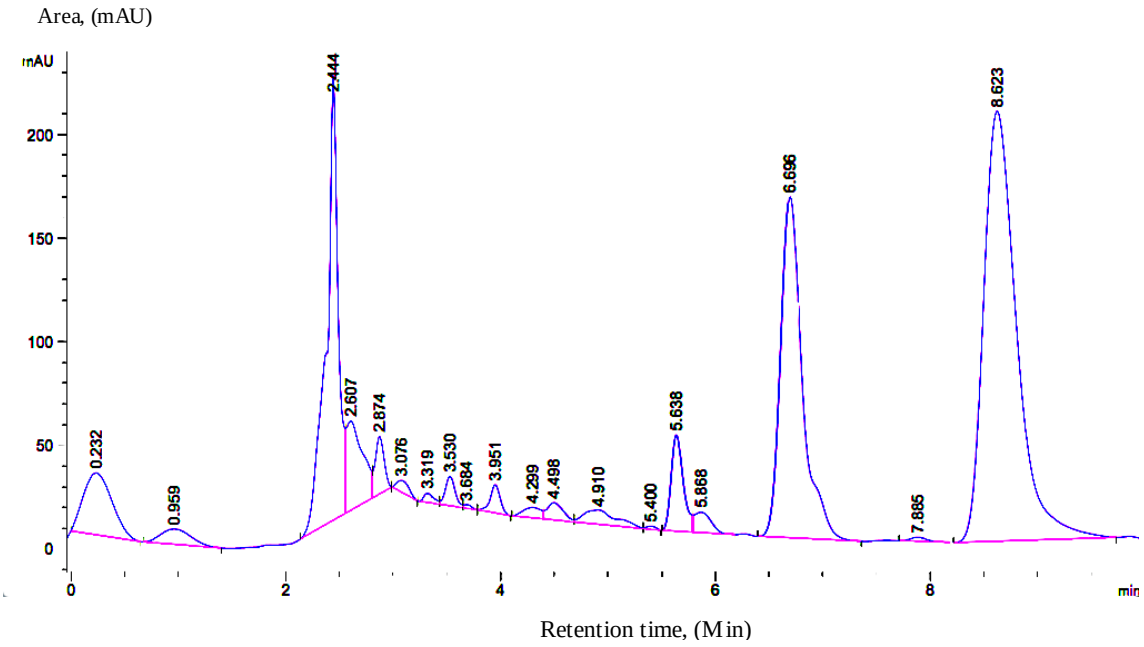
$$y = 15.806x \ (R^2 = 0.99482) \quad \textbf{(3.1)}$$

Extraction yield (wt / wt %) =

$$\frac{(concentration\ from\ HPLC)(volume\ water)(dilution\ factor)}{weight\ of\ sample} x100\% \quad \textbf{(3.2)}$$



(a) Mangiferin standard



(a) Mahkota Dewa extract

Figure 3.2: HPLC chromatogram of (a) Mangiferin standard and (b) Mahkota Dewa extract.

3.5 1,1-Diphenyl-2-picryl hydrazyl (DPPH)

A DHHP assay method was used to determine the antioxidant activity of the extracts. About 23.5 mg of DPPH diluted in 100 mL methanol were prepared for stock solution. Mahkota Dewa extract of 1820 µL and 180 µL of methanol in 20 mL centrifuges were prepared for each parameter as sample solution. An aliquot of 50 µL of sample solution for each parameter were added with 1950 µL of stock DPPH in others 20 mL centrifuge and covered with aluminium foil and cover with cap. Shake the sample and saved in dark place and room temperature for 4 hours. After 4 hours, the sample was transferred into cuvette and was measured absorbance at 517 nm using UV-Vis spectrometer. The absorbance of the samples was recorded and the % total of DPPH inhibition will be calculated using the following equation:

$$y = 0.1584x - 0.1602 \ (R^2 = 0.99642) \quad \textbf{(3.3)}$$

$$\text{DPPH inhibition} = \frac{Abs\ control - Abs\ sample}{Abs\ control}\ x\ 100\% \quad \textbf{(3.4)}$$

Abs = absorbance

CHAPTER 4

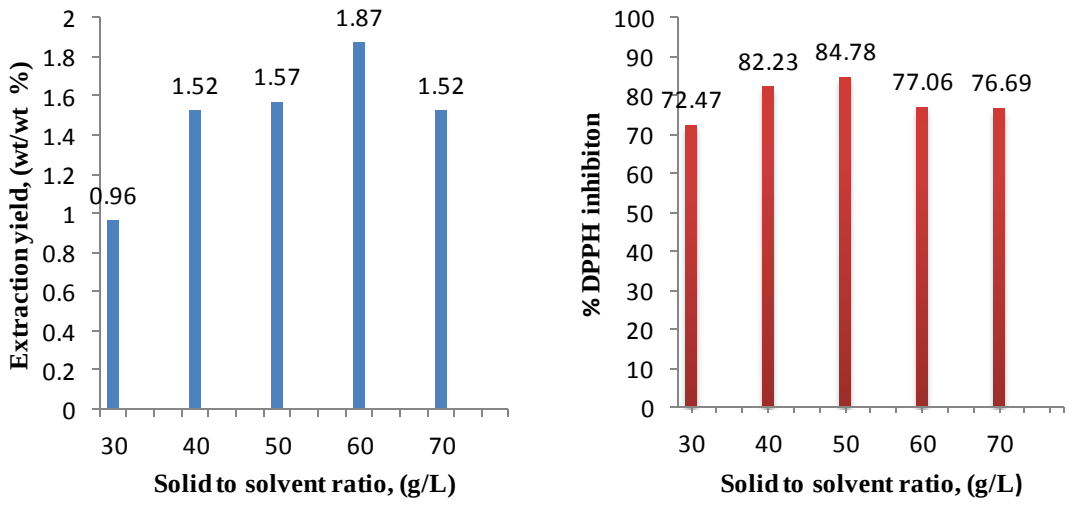
RESULTS AND DISCUSSION

4.1 The Effect of Solid to Solvent Ratio

Figure 4.1 shows the effect of solid to solvent ratio on the mangiferin yield of Mahkota Dewa and antioxidant activity. It can be seen that extraction yield is highly dependent on the amount of solid in a solvent. The extraction yield of mangiferin was increased from 0.96 wt/wt % to 1.87 wt/wt % at solid to solvent ratio of 30 g/L to 60 g/L. However, further increase of the solid to solvent ratio decreased the mangiferin yield to 1.52 wt/wt %. Particles sizes offer greater surface area for mass transfer and it does not guarantee a higher yield. Finer particle sizes are more prone to agglomeration which could hinder the extraction process, but larger particle size also ineffective. Thus, the most effective particle size for extraction should be able to maximize the surface area for mass transfer, yet prevent agglomeration. This is because of the mass transfer principle where it's driving force consideration to the concentration gradient between solid to and the solvent.

A high solid to solvent ratio could promote an increasing concentration gradient, thus resulting in increasing of diffusion rate, which allows greater extraction of solid by solvent (Eikani et al., 2007). The decreasing of mangiferin yield caused by the solubility of the solute and the equilibrium of solid and solvent occur at 60 g/L. No further changes in solute concentration occur when amount of solvent is inadequate to dissolve all the solute present in solution (Khajenoori et al., 2015). The changing of bioactive compound coming into contact with extraction solvent by increasing the amount of solid and when equilibrium achieved the data will not continue to increase (Zhang et al., 2007).

The increasing of % DPPH inhibition from 72.47 % at 30 g/L of solid to solvent ratio to 84.78 % at 50 g/L of solid to solvent ratio. Then, the reading of DPPH inhibition was decreased to 76.69 % at 70 g/L of solid to solvent ratio. This result showed remaining of DPPH in sample were reduced 50 g/mL of solid to solvent ratio were given the highest of antioxidant activity. DPPH radical scavenging capacities were increased gradually with the increase of the solid-to-solvent ratio and achieved the highest DPPH radical scavenging. Thus, it can be deduced that antioxidant capacity increase with the increase of solid-to-solvent ratio until reaching an optimum level. A number of cellular defence systems have evolved to counteract the accumulation of ROS. While DPPH can accept an electron or hydrogen radical to become a stable, diamagnetic molecule, it can be oxidized only with difficulty, and then irreversibly. Antioxidant analysis may be limited to those compounds soluble in the selected solvents. The advantage of this method is that DPPH is allowed to react with the whole sample and sufficient time given in the method allows DPPH to react slowly even with weak antioxidants (Prakash et al., 2007). DPPH method may be utilized in aqueous and nonpolar organic solvents and can be used to examine both hydrophilic and lipophilic antioxidants (Prior et al., 2005). DPPH can only be soluble in organic solvent and the interference of absorbance from the sample compounds could be a problem for the quantitative analysis (Arnao 2000).



(a) (b)

Figure 4.1: Effect of different solid to solvent ratio on (a) mangiferin yield and (b) antioxidant activity. This extraction temperature and time were fixed at 100°C and 5 h, respectively

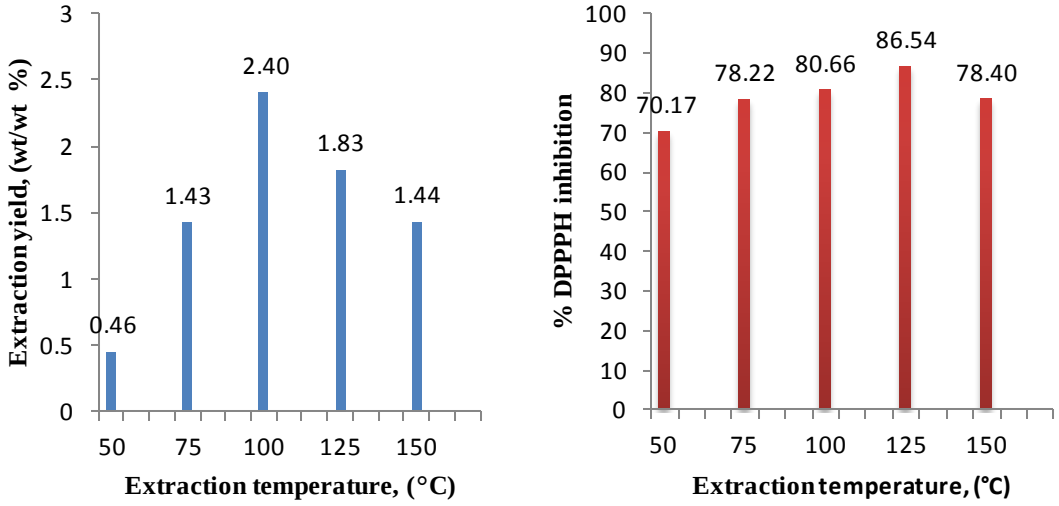
4.2 The Effect of Extraction temperature

Temperature plays a vital role in influencing the extraction yield in SWE. Water should ideally be at temperature where it can enhance its chemical and physical properties to become an efficient processing medium. Figure 4.2 illustrates the effect of extraction temperature on the mangiferin yield of Mahkota Dewa. The yield of mangiferin was increased from 0.46 wt/wt % to 2.40 wt/wt % at extraction temperature from 50°C to 100°C. Then, the extraction yield was decreased to 1.44 wt/wt % at extraction temperature 150°C. The increased of mangiferin yield is due to the mangiferin solubility at higher temperature of 100°C that the increase of temperature may decreased the polarity of subcritical water and thus increasing the rate of mass transfer. Al-Farsi et al. (2007) reported that increased temperature could promote the mangiferin extraction by increasing both diffusion coefficient and solubility of mangiferin compounds in extraction solvent.

The mass transfer kinetics were also favorably improved by the disruption of intermolecular forces such as van der Waals forces, hydrogen bonds and dipole attractions) in the sample matrix. Nevertheless, the most important effect of the increment of liquid water temperature is the weakening of hydrogen bonds, resulting in a lower dielectric constant. As the result of increment of the solubility of the compounds present on the matrix being extracted and decrease in the surface tension of the water, which allows the improved penetration into the sample matrix. The temperature chosen must be enough high for desired reaction can take place at a significant rate (Sealock et al., 1993). However, the temperature cannot be too high as it may cause breakdown of analytes mangiferin because reducing of surface tension and viscosity of solvent and may cause degradation of vital compounds.

The DPPH inhibition increased from 70.17 % at extraction temperature of 50°C to 86.54 % at 125°C of extraction temperature. The decreased of DPPH inhibition from 86.54 % to 78.36 % at 125°C to 150°C. The highest of DPPH inhibition showed highest of antioxidant activity. The antioxidant efficiency is measured at ambient temperature so that the risk of thermal degradation of the molecules tested is eliminated (Bondet et al., 1997). The antioxidant activity of the extracts may attributed to the reduction of free radicals, chelation of metal ions, or a combination there of presence of

phytoconstituents. As the extraction temperature was increased, these mangiferin compounds would be degraded and resulted in the loss of antioxidant capacity. Mueller-Harvey (2001) reported that some mangiferin compounds decomposed rapidly under high temperature and thus caused a reduction in the antioxidant capacity of plant sample.



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(a) (b)

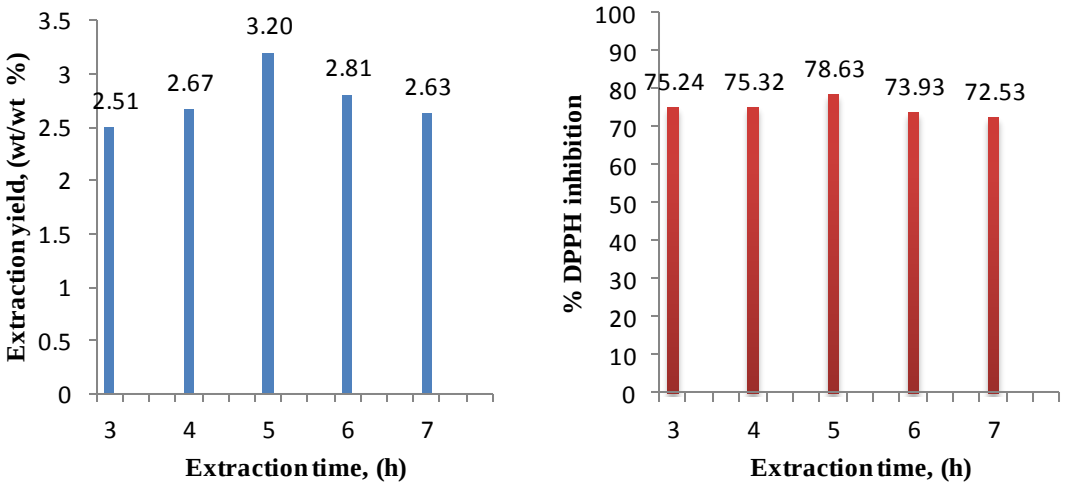
Figure 4.2: Effect of different extraction temperature on (a) mangiferin yield and (b) antioxidant activity. This solid to solvent ratio and time were fixed at 60 g/L and 5 h, respectively.

4.3 The Effect of Time

Figure 4.3 show the effect of extraction time on the mangiferin yield of Mahkota Dewa. The extraction yield was increased from 2.51 wt/wt % to 3.20 wt/wt % with extraction time increases from 3 h to 5 h. then, the extraction yield reduced to 2.63 wt/wt % at extraction time of 7 h. A longer extraction time would give the bark and solvent better equilibrium and also mass transfer. However the longer extraction also may cause decreasing of extraction yield because of degradation of mangiferin when it was exposed to the high temperature for an extended period of time (Kim et al., 2010), an equilibrium between the sample components still bound to the matrix and the water phase in which the components are already solubilized might be reached. This

phenomenon could be explained by Fick's second law of diffusion revealing that final equilibrium will be attained between the solution concentrations in the solid matrix and solvent after a particular duration (Pinelo et al., 2006). If this becomes the case, the efficiency of the extraction procedure will not increase beyond this point and the degradation of some compounds could occur more easily. According to Zhang et al. (2007) the effective parameter in subcritical water extraction process is extraction time.

The increased of DPPH inhibition from 75.24 % to 78.63 % at 3 h to 5 h. Then the reading was reduced to 72.53 % at 7 h. The higher of DPPH inhibition which is 78.63 % at 5 h was showed highest of antioxidant activity because the smallest of % remaining DPPH in the sample. This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Norziah et al., 2015). The activity of natural antioxidants often decreases during their isolation and purification due to their decomposition (Bandoniene and Murkovic, 2002). Extraction time would lead to exposure of more oxygen and thus increase the chances for occurrence of oxidation on mangiferin compounds (Naczk and Shahidi, 2004).



(a) (b)

Figure 4.3: Effect of different extraction time on (a) mangiferin yield and (b) antioxidant activity. This solid to solvent ratio and extraction temperature were fixed at 60 g/L and 100 °C, respectively.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The subcritical water extraction of Mahkota Dewa was successful using pressure vessel reactor, Buchiglasuster. The extraction parameters that produce the highest of mangiferin yield are solid to solvent ratio at 60 g/L 87, extraction temperature at 100°C, and extraction time at 5 hours. A high solid to solvent ratio could promote an increasing concentration gradient, thus resulting in increasing of diffusion rate, which allows greater extraction of solid by solvent. While, for highest antioxidant activity were obtained at solid to solvent ratio of 50 g/L, extraction temperature at 125°C, and extraction time at 5 hours. This is because of free radical in low level of reactivity for producing toxic substance may prevent oxidation, through lowering the remaining DPPH in the sample.

5.2 Recommendation

Although this work has successfully extract the mangiferin from Mahkota Dewa further works should be done for improvement. Below are some recommendations for future work:

i. To include the analysis of other bioactive compounds in Mahkota Dewa which is polyphenol, flavonoid, and saponin by using High Performance Liquid Chromatography (HPLC) and Liquid Chromatography - Mass Spectrometer (LCMS).

ii. To produce dried solid form of Mahkota Dewa extract by using spray-drying process. This idea is essential as most of the food and alternative medicine acts as supplement to human health and also base of cosmetics are marketed in solid form.

iii. To conduct the measurement of antioxidant activity in Mahkota Dewa extract by using different method apart from DPPH assay method, such as oxygen radical absorbance capacity (ORAC), and total radical-trapping antioxidant parameter (TRAP).

iv. To conduct a cytotoxicity analysis to test the potential use of Mahkota Dewa extract as anticancer. It can be further explore as complementary and alternative medicine (CAM) as recently it gained popularity among cancer patients since the current treatment of cancer using commercial drugs is quite expensive.

REFERENCES

Ahmad Faried and others, 'Potential of Indonesian Herbal Medicine, Phaleria Macrocarpa (Scheff.) Boerl, for Targeting Multiple Malignancy Signaling Pathways: An Introductory Overview', *European Journal of Medicinal Plants*, 11.2 (2016), 1–17.

Al-Farsi, M. A. and Chang Y. L. 2007. Optimization of phenolics and dietary fibre extraction from date seeds. Food Chemistry 108(3): 977-985.

Ali RB, Atangwho IJ, Kaur N, Abraika OS, Ahmad M and Mahmud R, (2012). Bioassay-guided antidiabetic study of *Phaleria macrocarpa* fruit extract. *Molecules (*17), 4986–5002.

Amin Ilghami, Saeed Ghanbarzadeh and Hamed Hamishehkar, (2015). 'Optimization of the Ultrasonic-Assisted Extraction of Phenolic Compounds, Ferric Reducing Activity and Antioxidant Activity of the Beta Vulgaris Using Response Surface Methodology', *Pharmaceutical Sciences*, 21(1) 46–50.

Anggraini, T.,&Lewandowsky, P. (2015). The Exotic Plants of Indonesia : Mahkota Dewa (Phaleria macrocarpa), Sikaduduak (Melastoma malabathrium Linn) and Mengkudu (Morinda citrifolia) as Potent Antioxidant Sources, 5(2), 59-62.

Antolovich M, Prenzler P, Patsalides E, McDonald S, Robards K: Methods for testing antioxidant activity. The Analyst 2002, 127:183-198.

Arnao M. B. (2000). Some methodological problems in the determination of antioxidant activity using chromogen radicals:a practice case. Trends in Food Science & Tehnology , 11(11):419–421. doi: 10.1016/S0924-2244(01)00027-9

Asl, A.H.; Khajenoori, M., (2013). Subcritical water extraction. In Mass Transfer - Advances in Sustainable Energy and Environment Oriented Numerical Modeling (pp. 459–487).

Azmin, S. N. H. M., Manan, Z. A., Alwi, S. R. W., Chua, L. S., Mustaffa, A. A., & Yunus, N. A. (2016). Herbal Processing and Extraction Technologies. *Separation &Purification Reviews*, *45*(4), 305–320.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, *117*(4), 426–436.

Bandoniene, D. and Murkovic, M. (2002). On-Line HPLCDPPH screening method for evaluation of radical scavenging phenols extracted from apples (Malus domestica L.). Journal of Agricultural and Food Chemistry 50 (9): 2482–2487.

Bimakr, M., Rahman, R. A., Taip, F. S., Ganjloo, A., Salleh, L. M., Selamat, J., Zaidul, I. S. M. (2011). Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (Mentha spicata L.) leaves. *Food and Bioproducts Processing*, *89*(1), 67–72.

Bjoërklund, E., & Nilsson, T. (2000). Pressurised liquid extraction of persistent\norganic pollutants in environmental analysis. *Trends in Analytical Chemistry*, *19*(7), 434–445.

Bondet V, Brand-Williams W, Berset C. (1997). Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. Lebensmitt Wissenschaft Technologie Food Sci Technol. 1997;30:609–615. doi: 10.1006/fstl.1997.0240.

Cardenas-toro, F.P.; Alcazar-alay, S.C.; Forster-carneiro, T.; Meireles, M.A.A., (2014). Obtaining oligo- and monosaccharides from agroindustrial and agricultural residues using hydrothermal treatments, Food Public Health, 4(3): 123–139.

Chong SC, Dollah MA, Chong PP and Maha A, (2011). *Phaleria macrocarpa* (Scheff.) Boerl fruit aqueous extract enhances LDL receptor and PCSK9 expression *in vivo* and *in vitro*. *J Ethnopharmacol (*137), 817–827.

Easmin, M. S., Sarker, M. Z. I., Ferdosh, S., Shamsudin, S. H., Yunus, K. Bin, Uddin, M. S., … Khalil, H. P. S. A. (2015). Bioactive compounds and advanced processing technology: Phaleria macrocarpa (sheff.) Boerl, a review. *Journal of Chemical Technology and Biotechnology*, *90*(6), 981–991.

Eikani, M.H., Golmohammad, F., & Rowshanzamir, S. (2007). Subcritical water extraction of essential oils from coriander seeds (Coriandrum sativum L.). Journal of Food Engineering, 80(2), 735-740.

Ghoreishi, S. M., & Shahrestani, R. G. (2009). Subcritical water extraction of mannitol from olive leaves. *Journal of Food Engineering*, *93*(4), 474–481.

Gong, Y.; Zhang, X.; He, L.; Yan, Q.; Yuan, F.; Gao, Y., (2013). Optimization of subcritical water extraction parameters of antioxidant polyphenols from sea buckthorn (Hippophaë rhamnoides L.) seed residue, J. Food Sci. Tech., 2 (1): 1–9.

Halliwell. B., Aeschbach. R., Loliger. J and Aruoma. O. I. (1995). The characterization of antioxidants. Food Chem. Toxicol. 33, 601.

Hanus-fajerska, E., & Wiszniewska, A. (2012). Effectiveness of Daphne L . (Thymelaeaceae) In Vitro Propagation , Rooting Of Microshoots, *65*(1).

Harmanto, N., 2003. Conquering Disease in Unison with Mahkota Dewa, *Phalerai macrocarpa*. 1st Edn, P.T. Mahkota Dewa, Jakarta, Indonesia, pp:14.

Health, N. J. (2011). Antioxidant compound shows promising radioprotective properties, (March), 2–3.

Hendra R, Ahmad S, Oskoueian E, Sukari Aand ShukorMY, (2011). Antioxidant, anti-inflammatory and cytotoxicity of *Phaleria macrocarpa* (Boerl.) Scheff fruit. *BMC Complement Alt Med* (11), 1472–6882.

Hendra R, Ahmad S, Sukari A, Shukor MY and Oskoueian E, (2011). Flavonoid analyses and antimicrobial activity of various parts of *Phaleria macrocarpa* (Scheff.) Boerl fruit. *Int J Mol Sci* (12), 3422–3431.

Herrero, M., Mendiola, J. A., & Ib, E. (2013). Subcritical water extraction of bioactive components from algae. http://doi.org/10.1533/9780857098689.3.534.

Herrero, M., Ibanez, E., Senora NS, J and Cifuentes, A. (2004), Pressurized liquid extracts from *Spirulina platensis* microalga: Determination of their antioxidant activity and preliminary analysis by micellar electrokinetic chromatography, *J. Chromatogr. A* , 1047 , 195–203.

Ibanez, E., Herrero, M., Mendiola, J.A., Castro-Puyana, M., (2012). Extraction and characterization of bioactive compounds with health benefits from marine resources: macro and micro algae, cyanobacteria, and invertebrates. In: Hayes, M. (Ed.), Marine Bioactive Compounds: Sources, Characterization and Applications.Springer, pp. 55-98.

Jintana, W.; Shuji, A., (2008). Extraction of functional substances from agricultural products or by-products by subcritical water treatment, Food Sci. Tech. Res., 14(4), 319–328.

J.Wu, L.Lin, F.Chau (2001). Ultrasonic-assisted extraction of giseng saporins from ginseng roots and cultured ginseng cells. Ultrasonic Sonochemistry 8.

Sealock, L. J., Elliott, D. C., Baker, E. G., & Butner, R. S. (1993). Chemical Processing in High-pressure Aqueous Environments . 1 . Historical Perspective and Continuing Developments, 1535–1541.

Kanter , M. (1998). Free radicals, exercise and antioxidant supplementation. Process Nutrition Society 57 : 9-13.

Khajenoori, M., Asl, A. H., & Eikani, M. H. (2015). Subcritical water extraction of essential oils from Trachyspermum ammiseeds, *5026* (10)

Kim, W. J., Veriansyah, B., Lee, Y. W., Kim, J., & Kim, J. D. (2010). Extraction of mangiferin from Mahkota Dewa (Phaleria macrocarpa) using subcritical water. *Journal of Industrial and Engineering Chemistry*, *16*(3), 425–430. http://doi.org/10.1016/j.jiec.2009.08.008.

King, J.W., Grabiel, R.D. & Wightman, J.D.(2003). Subcritical water extraction of anthocyanins from fruit berry substrates, In:*Proceedings of the 6th InternationalSymposium on Supercritical Fluids – Tome 1*. Versailles, France, 28-30 April, pp, 409 to 418.

Kurnia D, Akiyama K and Hayashi H, (2008). 29-Norcucurbitacin derivatives isolated from the Indonesian medicinal plant, *Phaleria macrocarpa* (Scheff.) Boerl. *Biosci Biotechnol Biochem (*72), 618–620.

Liang, X., & Fan, Q. (2013). Application of Sub-Critical Water Extraction in Pharmaceutical Industry. *Journal of Materials Science and Chemical Engineering*, *1*(October), 1–6.

Luong, D., Sephton, M. A., & Watson, J. S. (2015). Subcritical water extraction of organic matter from sedimentary rocks. *Analytica Chimica Acta*, *879*, 48–57.

Madhavi D.V, Despande S.S And Salunkhe D.K (1996), *Food antioxidants* . New York, Marcel Dekker.

Matkowski A, Kuś P, Góralska E, Woźniak D. (2013). Mangiferin - a bioactivexanthonoid, not only from mango and not just antioxidant. Mini Rev Med Chem. Mar;13(3):439-55.

Mueller-Harvey, I. (2001). Analysis of hydrolysable tannins. Animal Feed Science and Technology 91: 3-20.

Naczk, M. and Shahidi, F. (2004). Extraction and analysis of phenolics in food. Journal of Chormatography A 1054 (1-2): 95-111.

Norziah, M. H., Faezea, F. A., Bhat, R. and Ahmad, M. (2015). Effect of extraction solvents on antioxidant and antimicrobial properties of fenugreek seeds (Trigonella foenum-graecum L.) *22*(3), 1261–1271.

Nor Fariza I, Fadzureena J, Zunoliza A, Luqman Chuah A, Pin KY, et al. (2012) Anti-inflammatory Activity of the Major Compound from Methanol Extract of Phaleria macrocarpa leaves. J Appl Sci 12: 1195-1198.

Oshimi S, Zaima K, Matsuno Y, Hirasawa Y, Iizuka T and Studiawan H, (2008). Studies on the constituents from the fruits of *Phaleria macrocarpa*. *Jurnoul of Natural Medicines* (62), 207–210.

Parhizkar S, Yusoff MJ and DollahMA, (2013). Effect of *Phaleria macrocarpa* on sperm characteristics in adult rats. *Adv Pharmaceut Bull (*3), 345–352.

Pinelo, M., Sineiro, J. and Núñez, M. J. (2006). Mass transfer during continuous solid–liquid extraction of antioxidants from grape byproducts. Journal of Food Engineering 77(1): 57-63.

Prakash D, Upadhyay G, Singh B. N, Singh. H. B.(2007). Antioxidant and free radical-scavenging activities of seeds and agro-wastes of some varieties of soybean (Glycine Max). Food Chemistry;104: 783-790.

Prior RL, Wu X, Schaich K, J Agric Food Chem. (2005) May 18; 53(10):4290-302.

Raihan H. Mirza, Nan Chi, Chi Y. (2013). Therapeutic Potential of the Natural Product Mangiferin in Metabolic Syndrome. J. Nutr. Ther 2, 74-79.

Saufi A, Heimendahl CB, Alfermann AW and Fuss E, (2008). Stereochemistry of lignans in *Phaleria macrocarpa* (Scheff.) Boerl. *Z Naturforsch C (*63), 13–16.

Schaich KM (2006), Developing a rational basis for selection of antioxidant screening and testing methods, *Acta Hort*. , 709 , 79–94.

Shirsath S. R, Sonawane S. H. and Gogate P. R. (2012). Intensification of extraction of natural products using ultrasonic irradiations—A review of current status. Chem Eng Process Process Intensif. 53:10–23.

Shodikin A, (2009). Antimicrobial activity of etanol extract of Mahkota Dewa *(Phaleria macrocarpa)* fruits and leaves against pseudomonas aeruginosa by agar dilution and scanning electron microscopy. Microbiology, Surabaya: Faculty of Medicine, University of Airlangga.

Thiruvenkadam, S., Izhar, S., Yoshida, H., Danquah, M. K., & Harun, R. (2015). Process application of Subcritical Water Extraction (SWE) for algal bio-products and biofuels production. *Applied Energy*, *154*, 815–828. http://doi.org/10.1016/j.apenergy.2015.05.076.

Tunchaiyaphum, S.; Eshtiaghi, M.N.; Yoswathana, N., (2013). Extraction of bioactive compounds from mango peels using green technology, Int. J. Chem. Eng. Applications, 4(4): 194–198 (5 pages).

Wang, L., Weller, C.L., (2006). Recent advances in extraction of nutraceuticals from plants. Trends in Food Science & Technology 17 (6), 300-312.

Whaetley, G. W., & K. D. (2010). Patent WO2010034971A2 - Sub-critical water extraction of medicinal plants. Retrieved March 30, 2016, from http://www.google.com/patents/WO2010034971A2?cl=en#classifications.

Wiboonsirikul, J. et al. (2007) Properties of extracts from defat-ted rice bran by its subcritical water treatment. J. Agric. Food. Chem., 55, 8759-8765.

Wong SP, Leong LP, Koh JHW (2006). Antioxidant activities of aqueous extracts of selected plants. Food Chemistry; 99: 775-783.

Wu, J., Lin, L., & Chau, F. T. (2001). Ultrasound-assisted extraction of ginseng saponins from ginseng roots and cultured ginseng cells. *Ultrasonics Sonochemistry*, *8*(4), 347–352. http://doi.org/10.1016/S1350-4177(01)00066-9.

Yosie A, Effendy MAW, Sifzizul TMT and Habsah M, (2011). Antibacterial, radical-scavenging activities and cytotoxicity properties of *Phaleria macrocarpa* (Scheff.) Boerl leaves in HEPG2 cell lines. *Int J Pharm Sci Res (*2), 1700–1706.

Zhang, S. qin,Bi, H. min, & Liu, C. jiao. (2007). Extraction of bio-active components fromRhodiola sachalinesis under ultrahigh hydrostatic pressure. Separation and purificationTechnology, *57*(2), 277-282.

Zima, T.S., Fialova, L., Mestek, O., Janebova, M., Crkovska, J., Malbohan, I., Stıpek, S., Mikulıkova, L. & Popov, P. (2001). Oxidative stress, metabolism f ethanol and alcohol-related diseases, *Journal of. Biomedical. Science*, 8, 59-70.

APPENDIX

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The extract samples at different solid to solvent ratio.

The extract samples at different extraction temperature.

The extract samples at different extraction time

The sample of SWE were kept in schoot bottle of 1 L.

Sample in the vial was prepared for HPLC analysis

Kiloclave Laboratory Pressure was used as SWE

Agilent Technologies 1100 HPLC

Column : **C18 4.6 mm x 250 mm,**

Detector : **UV 280 nm**

Flow rate **: 1.0 mL/min**

Injection Volume **: 10 µL**

Mobile Phase **:**

- **0.1% Ortho phosphoric acid(69%)**
- **Methanol (31%)**