HIGH INTENSITY ULTRASOUND-ASSISTED EXTRACTION OF MEDICINAL BLUE DYE FRO AS

(PENGEKSTRAKKAN ULTI SITI TINGGI PEWARNA BIRU BERUB CURCAS)

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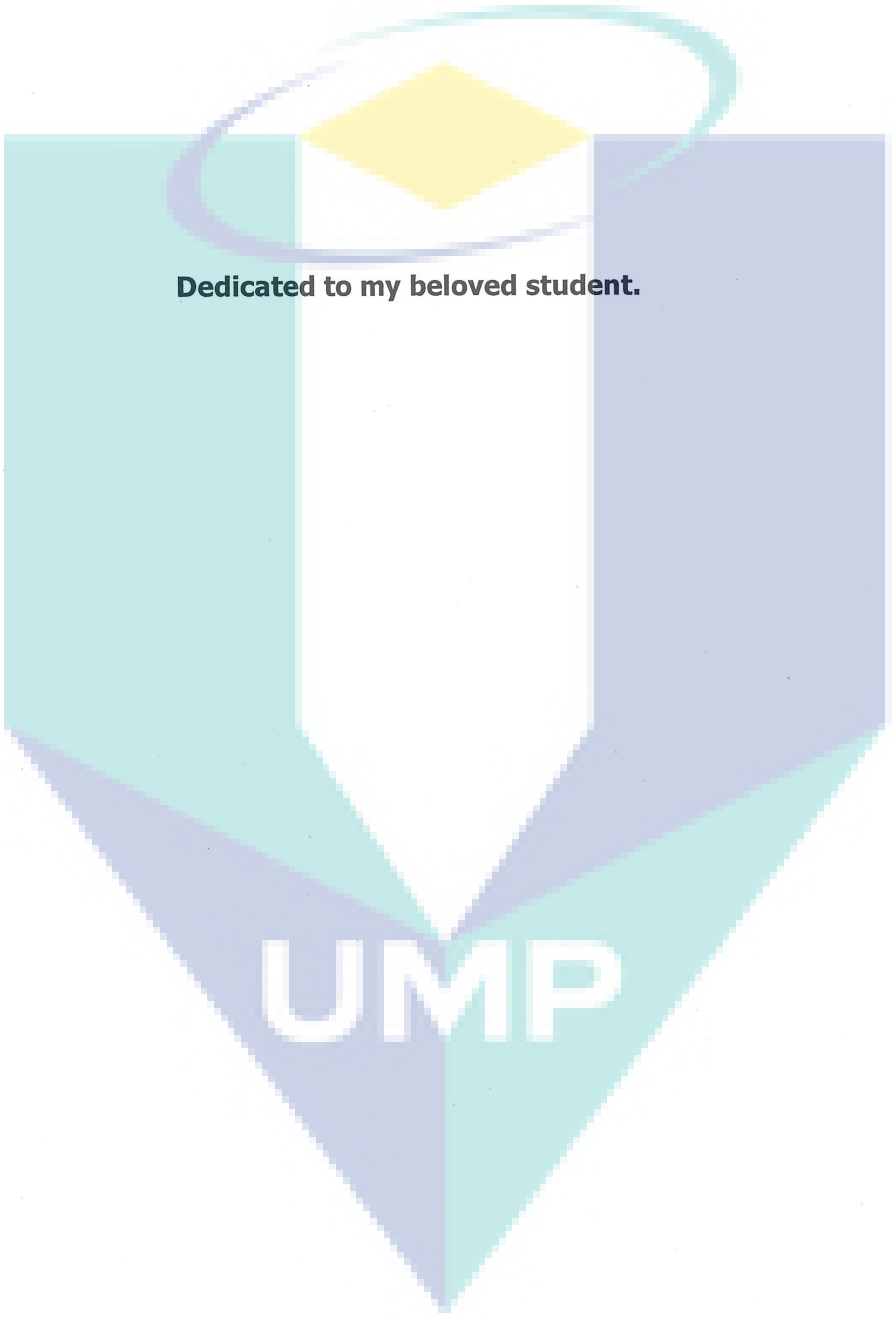
DEDICATION



Dedicated to my beloved student.

ABSTRACT

Extraction and product recovery are the most crucial steps in evaluation of valuable active compounds from various plant parts. In this study, extraction of gallic acid from *Jatropha curcas* stem bark was investigated using different techniques of extraction. The aims of this study were to optimize the extraction parameters as well as to do mathematical modeling of the extraction process. The work can be divided in five main stages namely as sample preparations, extraction studies, extraction analysis, parameters optimization and modeling. In sample preparations, the bark was stripped from the branches, cut into small pieces and dried in an oven. The samples were then grounded and sieved to obtain sample size of approximately 1 mm. Four extraction techniques were employed such as shake flask extraction, Soxhlet extraction, ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE). Temperature, solvent composition, time and power for UAE and MAE were the extraction parameters used in the extraction studies. The extracts were further undergone analysis process. Total phenolic content (TPC) determined the amount of phenolic compounds in the extracts. Quantification of gallic acid in the extracts was done using high performance liquid chromatography (HPLC). In optimization part, response surface methodology (RSM) was employed to optimize the extraction parameters. UAE gave the best results among all the extraction techniques. The optimal conditions for UAE were: employing 50% ethanol as solvent, time 30 min, temperature 40°C and power 76.68 Watt. The extraction efficiency increased in the following order: shake flask < Soxhlet< MAE< UAE.

ABSTRAK

Pengekstrakkan dan produk pemulihan adalah langkah yang amat penting dalam menilai kompaun aktif dari pelbagai bahagian tumbuhan. Dalam kajian ini, pengekstrakkan asid gallic daripada kulit kayu pokok *Jatropha curcas* telah dikaji dengan menggunakan pelbagai teknik pengekstrakkan yang berlainan. Tujuan kajian ini adalah untuk mengoptimumkan parameter pengekstrakkan serta melakukan pemodelan matematik untuk proses pengekstrakkan ini. Kajian ini telah dibahagikan kepada lima bahagian iaitupenyediaan sampel, pengekstrakkan, analisis pengekstrakkan, mengoptimumkan parameter dan pemodelan matematik. Dalam penyediaan sampel, kulit kayu tersebut telah dikupas dari batangnya, dipotong kecil dan dikeringkan di dalam ketuhar. Sampel itu kemudiantelah dikisar dan ditapis untuk mendapatkan saiz sampel *kira-kira 1 mm. Empat teknik pengekstrakkan telah digunakan seperti* pengekstrakkan kaedah goncang kelalang, pengekstrakkan Soxhlet, pengekstrakkan ultrasonic-berbantu (UAE) dan pengekstrakkan gelombang mikro-berbantu (MAE). Suhu, komposisi pelarut, masa dan kuasa untuk UAE dan MAE adalah parameter yang telah digunakan dalam kajian ini. Ekstrak yang dipeolehi kemudian dianalisis. Jumlah kandungan fenolik (TPC) menentukan amaun fenolik di dalm ekstrak. Kuantifikasi asid gallic di dalam ekstrak ditentukan dengan menggunakan kromatografi cecair berprestasi tinggi (HPLC). Dalam bahagian pengoptimunan, metodologi tindak bals permukaan (RSM) telah digunakan untuk mengoptimumkan parameter pengekstrakkan. Pengekstrakkan ultrasonik-berbantu (UAE) telah memberi keputusan yang baik daripada semua teknik pengekstrakkan. Keadaan optimum bagi pengekstrakkan ultrasonik-berbantu (UAE) adalah: 50% komposisi pelarut etanol, masa 30 min, suhu 40°C dan kuasa 76.68 Watt. Kecekapan pengekstrakkan bertambah mengikut susunan berikut: kaedah goncang kelalang< Soxhlet< MAE< UAE.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

It is well known that plants provide a wide range of the complex mixture of bioactive compounds such as lipids, phytochemical, pharmaceutics, flavors, fragrances and pigments. Adverse usage of plant extracts had gained a lot of interest in the food, pharmaceutical, agriculture and cosmetics industries. Extraction is the first key step to obtain such valuable bioactive compounds from plants for further commercialization. The extraction techniques can be divided into two groups which are classical or conventional and modern extraction techniques. Selection of an appropriate extraction technique lies on the amount of recovery, costs and efficiency of the process.

The interest in the investigation of bioactive compounds, especially phenolic compounds from plants have greatly increased in recent years. Phenolic compounds are considered as secondary metabolites that are synthesized by plants (Harborne, 1982; Pridham, 1960). These compounds response when in stress conditions such as infection, wounding, UV radiation and many more (Beckman, 2000; Nicholson & Hammerschmidt, 1992). Simple phenols, phenolic acids, coumarins, flavonoids, tannins, lignans and lignins are included as phenolic compounds.

1.2 PROBLEM STATEMENT

Jatropha curcas is a multipurpose plant with many potential applications to be explored. In the present, this plant is gaining a lot of importance for the production of biodiesel as potential fuel substitution. Jatropha plant has been found to be used on various aspects in different communities in the world. The exploitation of this plant for various applications has been explored. The potential applications of *Jatropha curcas* can be as an oil crop, industrial uses, for enrichment of soil, medicinal uses, as food, as green manure and fertilizers, as

insecticides/pesticides, as an energy source and many more. There is one potential that gains interest from the researchers and also had been practiced traditionally by different communities of the world which is the medicinal uses of this plant. All parts of Jatropha have been used in traditional medicine and for veterinary purposes for a long time (Dalziel, 1955; Duke, 1985; Duke, 1988). Researches had conducted a lot of studies on medicinal value on different parts of this plant such as the latex, leaves, stem bark, roots and seed. Some of the ethnomedicinal uses of *Jatropha curcas* have received support from the results of scientific investigations in recent times. It has been reported that the bark of *Jatropha curcas* is rich in tannins but there is less study conducted on the contribution of this part in medicinal purposes. Recent study conducted by Igbinosa et al. 2009 revealed the presence of many secondary metabolites, including tannins that could be potential medicinal values.

Jatropha trees can live up to 50 years and can reach a height of 5 m like all perennial plants. It displays vigorous growth and continues growing towards maturity. A good sivicultural practice requires that the hedges are trimmed and pruned periodically. This will promote better growth and reduce competition among the tress. The branches or woods may be just as useful as the fruits, thus converting the wastes into something beneficial may be the desired goal.

This present study is introducing several extraction techniques that commonly used for solid-liquid extraction. Isolation of gallic acid from the stem bark of *Jatropha curcas* was done using conventional and modern extraction techniques. Conventional extraction techniques such as maceration and Soxhlet extraction efficiency depends on the type of solvent applied for the isolation and extraction time (Babic et al., 1998; Sporring et al., 2005). In the case of modern extraction techniques such as ultrasonic extraction and microwave extraction efficiency depends not only on the type of solvent used and extraction time, but also on many different parameters characteristics for every technique used (Pallaroni, 2003; Shen & Shao, 2005). The aim of this study is to investigate on the efficiency of these extraction techniques on the isolation of gallic acid from the stem bark. Comparisons were done to identify which techniques give the comprehensive results of good isolation, less time-consuming and effective.

1.3 OBJECTIVES OF STUDY

For this research study, there are three main objectives to be investigated as below.

1. To optimize the effect of the solvent composition, extraction time, extraction temperature and power on percentage recovery of gallic acid.
2. To compare the conventional extraction techniques with modern extraction techniques for the extraction of gallic acid.
3. To develop a mathematical model for estimation of the solid-liquid mass transfer coefficient of gallic acid.

1.4 SCOPES OF STUDY

To accomplish the objectives of this study, the scopes of studies are mainly as below.

1. Optimization of extraction parameters (solvent composition, extraction time, extraction temperature and power) was done using Design Expert 7.0 software by applying the Response Surface Methodology (RSM) method.
2. Two methods of conventional extraction techniques (maceration and Soxhlet extraction) and two methods of modern extraction techniques (ultrasonic-assisted extraction and microwave-assisted extraction) were used in this study. These extraction techniques were compared in terms of efficiency of extraction and also product recovery after the extraction process.
3. Estimation of the solid-liquid mass transfer coefficient was done by developing of the mathematical model.

1.5 SIGNIFICANT OF STUDY

The significant of doing this research study are as below.

1. To investigate the efficiency of different extraction method used in this research on the yield of gallic acid and comparison were made to determine the best extraction method.
2. To do a preliminary study on the parameters that can influence extraction efficiency and optimized the extraction parameters to obtain the best parameter that gives a better yield of gallic acid.
3. To achieve the optimum economic benefits from the plant by turning waste to wealth that gives the opportunity on research study to produce a marketable product.
4. To open up opportunities on the research study that is related to the medicinal value of this plant in order to exploit it commercially in the pharmaceutical interest.
5. To educate and provide adequate information to the growers of *Jatropha curcas* plant on the actual potential and economic benefits from the plant especially on its various uses.

1.6 THESIS OUTLINE

This thesis was organized by seven chapters beginning with Chapter 1. In Chapter 1, the background of study provides general information about this study. This chapter also listed the objectives and scopes of study to be focuses on.

Chapter 2 discussed mainly about *Jatropha curcas*. This chapter gives the information on this plant and the chemical composition that contain in different parts of this plant. In addition, this chapter also discussed on the various uses of this plant to cure many diseases and illness.

In Chapter 3, introduced phenolic compound in general and specifically discussed on gallic acid. The used of gallic acid in many fields and also the advantages of this compound can offer were discussed.

The information on the extraction techniques is presented in Chapter 4. The general principle of extraction is introduced in order to understand the concept of extraction. This chapter summarized on the principles, mechanisms, advantages and disadvantages of each extraction technique used in this study.

Materials and methods of experiments used in order to achieve the objectives of study are presented in Chapter 5. This chapter explains on the four stages that had been used to complete the experiments. These include the sample preparations, experimental studies using different extraction techniques, analysis of the extracts and lastly optimization of the studied parameters.

The main findings of this study are discussed in Chapter 6. The discussion covered the results for all the extraction techniques, optimization of the extraction techniques and also the mathematical modeling for estimation of solid-liquid mass transfer.

Lastly, in Chapter 7 is the conclusion of the findings, and also some recommendation are made to improve this research study.

1.7 SUMMARY

Investigation on the medicinal properties of *Jatropha curcas* has not been widely discovered although this plant offers many medicinal benefits. Separation of valuable targeted compound such as gallic acid from the stem bark of this plant can be done using many extraction techniques. This study will focus on finding the best extraction technique to extract gallic acid and to develop mathematical modeling on the extraction technique.

CHAPTER 2

TECHNICAL PAPER 1

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Microwave-assisted extraction of tannin from Jatropha curcas stem bark

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Abstract- A microwave-assisted extraction (MAE) method is presented for the extraction of tannin from stem bark of a medicinally important plant, *Jatropha curcas*. The effects of temperature, time, solvent composition and power were determined. The extraction yield of the extract was compared to Soxhlet extraction method. The percent yield of tannin was found to be increase with increasing time, temperature and power, and was highly dependent on the ethanol composition. From the points of extraction time and temperature, MAE was more effective than the Soxhlet extraction method. The most effective extraction was achieved with extraction of 50%v/v ethanol, extraction time of 2 min, microwave power of 320 W and at temperature of 40°C.

Keywords-Microwave-assisted extraction; Jatropha curcas; stem bark; tannin; Soxhlet extraction

I. INTRODUCTION

Jatropha curcas, a plant which is cultivated in Central and South America, Southeast Asia, India and Africa [1-3], has recently gain a great deal of interest by scientist due to the pharmaceutical values this plant offers. Kumar and Sharma [4] have published a review of *Jatropha curcas* research, which summarizes the multipurpose use of this plant including its medicinal uses. The stem bark of Jatropha plant contains active compounds such as tannin, which posses various therapeutic properties [5]. These include anti-microbial, anti-inflammation, anti-cancer activities as well as anti-septic [5-6]. This makes the compound potentially useful in several medical applications. However, there is limited study in extraction of tannin from this plant using ultrasonic. Tannins are found in leaves, fruits, bark and wood of most trees. Tannin is considered to be polyphenolic metabolites of plants with a molecular weight larger than 500 and with the ability to precipitate gelatin and other proteins from solution [7].

Microwaves are electromagnetic radiations with a frequency from 0.3 to 300 GHz [8]. The enhancement of product recovery by microwave is generally attributed to its heating effect, which causes dipoles rotation of the solvent in the microwave field. The highly localized temperature of the solvent can cause selective migration of the target compound from plant material to the solvent at a more rapid rate, thus increase in solubility. The polarity of solvent is very important in microwave extraction, because solvents with high dielectric constant absorb more microwave energy. Polar solvents are usually believed to be better than non-polar solvents [8]. Solvent heating by microwave can cause the molecules of polar solvent to not align themselves quickly enough to the high frequency electric field of microwave. This inconsistency causes the solvent molecules to dissipate the absorb energy in the form of heat [9]. Considering the dissipation factor, the higher this factor, the faster the heat will be distributed through the extraction matrix and the faster the heat will be transferred to the solvent [10]. The purpose of the present study was to examine the extraction process of tannin with microwave-assisted extraction method and to compare with the effects with Soxhlet extraction method. Various experimental conditions on the extraction yield were also studied (extraction time, temperature, power and solvent composition). Quantitative analysis of the extracts was also investigated.

II. MATERIALS AND METHODS

A. Preparation of plant material

Jatropha Curcas branches were collected from a plantation in Selangor, Malaysia. The branches were cleaned by hand to remove foreign materials. Then the bark was stripped from the branches and cut into small pieces prior to drying. An oven with a temperature of 60°C was used to dry the bark until constant weight was achieved. The dried bark was then grounded before it was sieved. A granulometric apparatus was used to obtain a homogenous particle size of 1.0mm. Separation of the grounded sample was carried out with a sieve shaker (Fritsch) including with various granulometric size sieves. The samples were kept in a seal plastic bag and store at room temperature.

B. Chemicals and reagents

Ethanol (with purity of 95% v/v) and hydrochloric acid were purchased from R&M Chemicals Ltd. Gallic acid, vanillin and catechin were purchased from Sigma-Aldrich, USA. Methanol and acetonitrile were HPLC grade was purchased from Merck, USA.

C. Soxhlet extraction

A Soxhlet apparatus was employed in which 10 g of sample was place into a thimble with 300 ml of 50% ethanol contained in a 500 ml round-bottom flask. Extraction was carried out for up to 8 h. The extract was then filtered with filter paper (Whatman no. 1, USA) and the filtrate was concentrated under vacuum at 50°C using a rotary evaporator.

D. Microwave-assisted extraction (MAE)

MAE was carried out using domestic microwave (NN-S215WF, 2450MHz, Panasonic) with total capacity of 800W. It was equipped with one 1000 ml closed quartz vessel, a temperature sensor, a temperature controller and a condenser. Ten grams of grounded *Jatropha curcas* stem bark was placed in quartz extraction vessel and 300 ml of solvent was added. Extraction process was carried out under different MAE conditions. The ranges of parameters studied are listed in Table 1. Then each extract were filtered through filter paper (Whatman no.1, USA) and concentrated under vacuum at 50°C using a rotary evaporator.

TABLE 1. RANGES OF EXPERIMENTAL PARAMETERS

Parameters	Ranges
Temperature, °C	35, 40, 45, 50, 55
Time, min	1.5, 2, 3, 4, 5
Solvent composition, % EtOH	0, 20,50,70,95
Power, Watt	160, 320, 480, 640, 800

E. Yield determination

Yield of extraction was calculated using the following equation:

$$\text{Yield (\%)} = \frac{W_e}{W_t} \times 100 \quad (1)$$

Where W_e is the mass of tannin extracted from the sample (g) and W_t is the mass of sample used for extraction.

F. Measurement of total tannin

The concentration of tannin extracts were analyzed by measuring the absorbance at 500 nm using UV-Vis Spectrophotometer, following the spectroscopic method from Robert [11]. A calibration curve of catechin solutions in various concentrations were used as reference.

III. RESULTS AND DISCUSSION

A. Effect of solvent composition

Solvent extraction that was used in this experiment was 95% v/v ethanol. Fig.1 shows that the extraction of tannin was greatly influenced by ethanol composition. When the ethanol volume percentage in the solvent was lower than 50% v/v, the extraction was increased with the increased of ethanol concentration. Concentration of ethanol higher than 50% shows decreased of extraction yield. This could be due to the increase in swelling of plant material by water, which increased the contact surface area between the plant matrix and the solvent [13,14,15]. Another reasonable explanation could be due to the increased in polarity of ethanol-water mixture. Increase in water content increases the polarity of the solvent, thus enhance the solubility of tannin in the mixture. Furthermore, by addition of some amount of water, the mixture dielectric constants increase. This helps absorb microwave energy and therefore increase in extraction efficiency. It should be noted that even though addition of water into ethanol increase the mixture dielectric constants, the dissipation factor decreases. This means that although the solvent mixture can absorb more microwave energy than pure ethanol as a result of increased dielectric constant, the mixture would not be able to dissipate the heat as effectively. As found in this result, in a mixture with too high water content, i.e. 20% ethanolic solution, the extraction efficiency was low and unfavorable. So, 50% v/v of ethanol concentration was used in the following experiments.

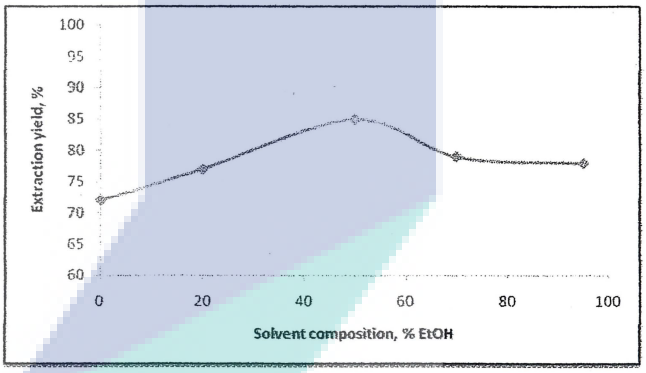

Fig. 1. Effects of solvent composition on the extraction yield

B. Effect of MAE duration time

Experiments were conducted to study the effects of time on extraction efficiency. The extraction was performed with 50% v/v ethanol at 40°C. The duration of microwave radiation was 1.5, 2, 3, 4 and 5 min respectively. Longer extraction time was not investigated because it may have negative effects resulting from degradation or conversion of the analytes. In Fig. 2 shows the effect of time on the extraction yield. The result indicates that the yield of extraction decreased as the time increase. If the time of extraction was more than 2 min, the extraction percentage decreased because tannin easily decomposed if they were

kept at high temperature for a long period of time. Therefore, 2 min was chosen to continue the experiment.

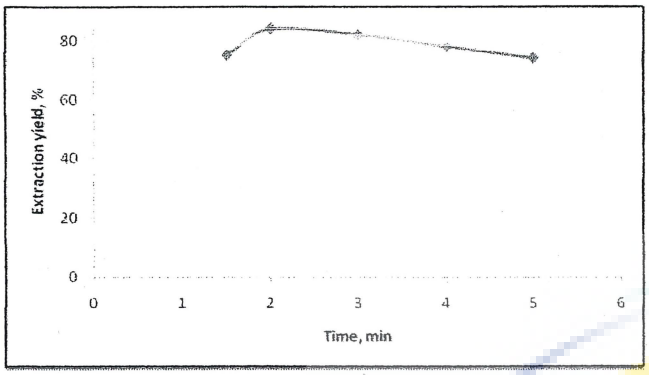

Fig. 2. Effect of time on the extraction yield

C. Effect of MAE power

Microwave irradiation energy disrupts hydrogen bonds, because of microwave-induced dipole rotation of molecules and migration of dissolved ions. Microwave irradiation energy can enhance the penetration of the solvent into the matrix and deliver efficiently to materials through molecular interaction with the electromagnetic field and offer a rapid transfer of energy to the solvent and matrix, allowing the dissolution of components to be extracted [16]. The effect of power on percent of yield is demonstrated in Fig 3. From this result, it can be noted that the use of microwave power can influenced the percent yield. When microwave power was lower than 320 W, the extraction increased with the increase in microwave power. When microwave power was more than 320 W the extraction leveled out might be because all the tannin in the stem bark had been extracted at lower microwave power.

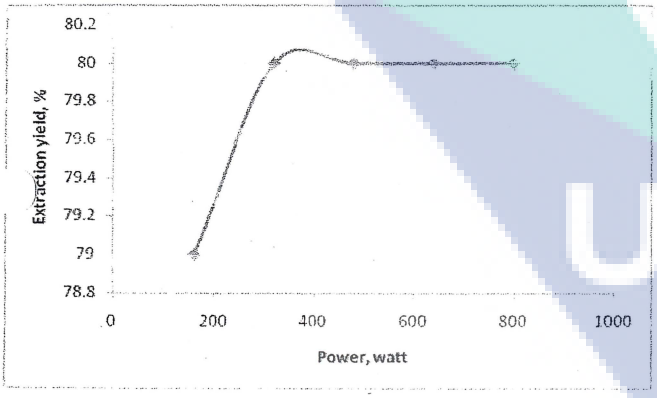

Fig. 3. Effect of power on the extraction yield

D. Effect of MAE temperature

Generally, the higher the extracting temperature is profitable for extraction due to increased in solubility. The elevated temperatures result in improved extraction efficiencies, since desorption of analytes from active sites in the matrix will increase. Additionally, increased in temperature will allow the solvent to have higher capacity to solubilize analytes, while surface tension and solvent viscosity decrease with temperature which will improve sample wetting and matrix penetration, respectively [17,18]. Fig. 2 showed the effect of different temperature on the extraction yield. The present results revealed that the extraction yield achieved highest percentage at the temperature of 40°C and decrease at higher temperature might be because tannin had been degraded.

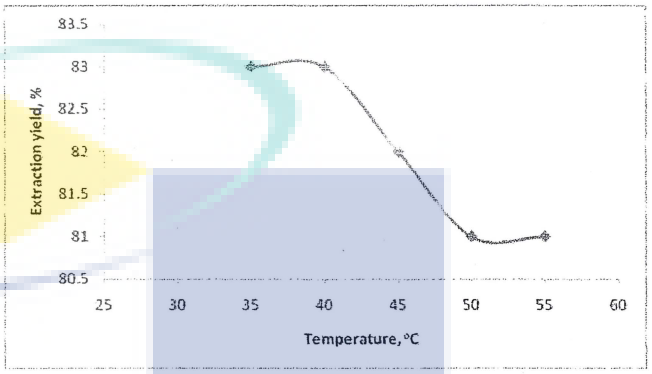

Fig. 4. Effect of temperature on the extraction yield

E. Comparison of MAE with Soxhlet extraction

The selection of extraction methods depends on the advantages and disadvantages of the processes, such as extraction yield, complexity, production cost, environmental friendliness and safety. Soxhlet extraction is the most common method of extraction. They are definitely user friendly but the drawbacks are that they used a large amount of solvent and long time of extraction needed. Considering the massive use of solvent and long extraction time, this extraction method is not favorable from a commercial prospective.

MAE has received increasing attention as an alternative method. It has been used for several reasons: (1) reduced extraction time (2) reduced solvent usage and (3) improved extraction yield. By considering the economical and practical aspects, MAE is a strong novel extraction technique.

The efficiency of extraction using Soxhlet and MAE was compared and shown in Table 2. From the results shows that at shorter time, MAE give higher extraction yield compared to Soxhlet extraction. The MAE can give the highest extraction selectivity.

TABLE 2 COMPARISONS OF PERCENTAGE YIEL AND EXTRACTION CONDITONS FOR DIFFERENT EXTRACTION METHODS

Extraction methods	Time	Temperature	Type of solvent	Yield (%)
Soxhlet	6 hrs	80°C	50% Ethanol	96
MAE	2 min	40°C	50% Ethanol	76

CONCLUSION

MAE gives the highest yields while requiring the shortest extraction times when compare with Soxhlet extraction. The main mechanism for enhanced recovery of tannin with MAE was the dipole rotation of the polar solvent in the microwave field, which was highly influenced by the dielectric constant and dissipation of the solvent. The appropriate condition of tannin extraction using MAE was extraction with 50% v/v ethanol, extraction time of 2 min, microwave power of 320W and at temperature of 40°C. These results demonstrate the potential of MAE to extraction tannin from stem bark of *Jatropha curcas*.

ACKNOWLEDGEMENT

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CHAPTER 3

TECHNICAL PAPER 2

STATUS: PENDING IN PUBLISHING

Comparison of extraction techniques on extraction of gallic acid from stem bark of *Jatropha curcas*

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ABSTRACT: In this paper, effectiveness of three different commonly applied extraction techniques for the determination of gallic acid in the stem bark of *Jatropha curcas* has been compared. The three different techniques that were used are namely as Soxhlet extraction, ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE). Quantification of gallic acid in the extracts was done using HPLC. In general, all extraction techniques were capable of extracting gallic acid from the stem bark, but the recovery obtained using modern extraction techniques was higher than using conventional extraction techniques. In particular, MAE extracts presented a higher amount of gallic acid than Soxhlet and UAE extracts. Therefore, MAE was a simple and rapid technique that was useful for extraction of *Jatropha curcas* stem bark. In contrast, Soxhlet extraction only results in slightly ruptured cell pores, which could explain its low amount of gallic acid produced.

Key words: Gallic acid, Soxhlet, UAE, MAE, HPLC

1. INTRODUCTION

Extraction and product recovery are the most imperative steps in evaluation of valuable biologically active compound from various plant parts. A desirable extraction technique should be simple, inexpensive, efficient, selective, environmentally friendly and compatible with various analytical techniques. However, limitation of the extraction process is usually due to the time constraint, labour intensive, lengthy operation techniques and highly cost of operations.

Classical extraction technique such as maceration and Soxhlet extraction are time consuming, require a huge amount of solvent and possible degradation of target compound due to overheating [1]. Modern extraction techniques, including microwave-assisted extraction (MAE) and ultrasonic-assisted extraction (UAE) are much faster and efficient.

Various parts of *Jatropha curcas* (Euphorbiaceae) had been used in many traditional medicines to cure many ailments. Gallic acid (3, 5, 7 - trihydroxybenzoic acid) and its derivatives are the natural products of hydrolysis of tannin [2]. Significant biological activities of gallic acid such as an antioxidant [3], anti-inflammatory [4], antifungal [5] and carcinogenic properties [2] have attracted considerable interest in pharmaceutical, food and agriculture industries [6].

The purpose of extracting phenolic compounds from plant materials is to release the compounds from the plant part where they are found by rupturing the plant tissues or by a diffusion process [7]. A high extraction efficiency that produces high extraction recovery is required in every extraction process. In order to increase extract recovery and preserve bioactivity, the extraction techniques need to be revised. Thus, the innovative extraction techniques such as UAE and MAE need to be explored and compared with the conventional extraction techniques.

The present study compares the ability of three extraction techniques namely as Soxhlet, UAE and MAE to extract gallic acid compound from the stem bark of *Jatropha curcas*. Four factors such as solvent composition, time, temperature and power had been studied for UAE and MAE. Each technique's performance was assessed by quantifying the amount of gallic acid using HPLC.

2. MATERIALS AND METHODS

2.1 Preparation of plant materials

Jatropha curcas branches were collected from a plantation in Selangor, Malaysia. The branches were cleaned by hand to remove foreign materials. Then the bark was stripped from the branches and cut into small pieces prior to drying. An oven with a temperature of 60°C was used to dry the bark for 8 hours. The dried bark was then grounded before it was sieved. A granulometric apparatus was used to obtain a homogenous particle size. Separation of the grounded sample was carried out with a sieve shaker (Fritsch) with various granulometric size sieves to obtain a homogeneous size of 1.0 mm. The samples were then kept in a seal plastic bag and store at room temperature.

2.2 Chemicals and reagents

Ethanol (with purity of 95% v/v) was purchased from R&M Chemicals Ltd. Acetonitrile and orthophosphoric acid both HPLC grades were purchased from Merck, USA. Gallic acid (HPLC grade) was purchased from Sigma-Aldrich, USA.

2.3 Soxhlet extraction

A Soxhlet apparatus was employed in which 10 g of sample was placed into a thimble with 300 ml of solvent composition (0, 20, 50, 70,95 % ethanol) in a 500 ml round-bottom flask. Extraction was carried out for up to 6 h. The extract was then filtered with filter paper (Whatman No. 1) and the filtrate was concentrated under vacuum at 50°C using a rotary evaporator.

2.4 Ultrasonic-assisted extraction (UAE)

An ultrasonic bath (40kHz, 230Watt) was used in this study. The extraction of gallic acid was performed by adding 10 g of sample with 150 ml of solvent in a 250 ml of conical flask. The flask was then partially immersed into the ultrasonic bath. The bottom of the flask was approximately 5 cm from the bottom of the bath. Water in the ultrasonic bath was circulated and regulated to avoid temperature rising caused by exposure of ultrasonic. Four different factors were investigated namely as solvent composition, time, temperature and ultrasonic power. The extract was then filtered with filter paper (Whatman No. 1) and the filtrate was concentrated under vacuum at 50°C using a rotary evaporator. Table 1 summarizes the ranges of parameters investigated in this study. All the experiments were performed in triplicate.

Table 1: Ranges of experimental factors for UAE

Factors	Ranges
Temperature, °C	35, 40, 45, 50, 55
Time, min	10, 20, 30, 40, 50
Solvent composition, % EtOH	0, 20, 50, 70, 95
Power, Watt	25.56, 51.12, 76.68, 102.24, 127.8

2.5 Microwave-assisted extraction (MAE)

MAE was carried out using domestic microwave (NN-S215WF, 2450MHz, Panasonic) with total capacity of 800W. It was equipped with one 1000 ml closed quartz vessel, a temperature sensor, a temperature controller and a condenser. Ten grams of grounded *Jatropha curcas* stem bark was placed in quartz extraction vessel and 300 ml of solvent was added. Extraction process was carried out under different MAE conditions. The same factors as in UAE had also been studied in MAE. The ranges of parameters studied are listed in Table 2. Then each extract were filtered through filter paper (Whatman no.1, USA) and concentrated under vacuum at 50°C using a rotary evaporator.

Table 2: Ranges of experimental factors for MAE

Factors	Ranges
Temperature, °C	35, 40, 45, 50, 55
Time, min	1.5, 2, 3, 4, 5
Solvent composition, % EtOH	0, 20,50,70,95
Power, Watt	160, 320, 480, 640, 800

2.6 HPLC analysis of gallic acid

Gallic acid was determined by high-performance liquid chromatography (HPLC). HPLC analysis was performed on an Agilent 1100 liquid chromatography system (Agilent Technologies, USA), equipped with vacuum degasser, four single solvent delivery pumps, a thermostated column compartment, a 20µL sample loop manual injector and a diode-array detector. Samples were separated using

Supelco Ascentis RP-Amide column (15 cm x 4.5 mm i.d., 5µm particles size) at a temperature of 30°C. The mobile phase delivered at a flow rate of 1.0 ml/min was a mixture of 85:15 (v/v) 0f 0.085% aqueous orthophosphoric acid and acetonitrile over 11 min. The diode-array detector was performed at 280 nm. The peak detected was identified by comparing their retention time with the standard. The concentration was calculated based on the calibration curve.

3. RESULTS AND DISCUSSION

3.1 Effect of solvent composition

The effect of solvent compositions on the amount of gallic acid for three different extraction methods was shown in Fig 1. All three extraction methods demonstrated that the improvement of extraction efficiency was observed with the addition of some amount of water. The amount of gallic acid increased as the solvent composition increased up until 50%. Usually, by varying the solvent polarity from water to ethanol, the extraction yield will increase. At the same time, product recovery will decrease with decreasing water percentage. This could be due to the relative polarity and the decrease in effective swelling of plant materials [8]. Alcoholic solvents have been commonly used as solvent to extract polyphenols from natural sources where they will give higher yield of total extract, even though they are not highly selective for phenols. Mixtures of alcohol and water have revealed to be more efficient in extracting phenolic constituents then compared to mono-component solvent system [9]. Addition of water to organic solvents usually creates a more polar medium which facilitates the extractions of polyphenols [9].

Higher amount of gallic acid was observed when some outer forces were introduced. The outer forces involved were ultrasonic wave and electromagnetic radiation for UAE and MAE respectively. In the case of UAE, a phenomenon called ultrasonic cavitation was produced in the solvent by the passage of an ultrasonic wave [10]. The intensity of ultrasonic cavitations in the solvent mixture was affected by the surface tension, viscosity and medium vapor pressure [10]. In the presence of water, the intensity of ultrasonic cavitation in the solvent mixture was increased as the surface tension increased while the viscosity and vapor pressure decreased [11]. Water has a higher surface tension than ethanol, which needs higher energy to produce cavitation bubbles. Ultrasonication in low vapor pressure produces few cavitation bubbles that collapsed at a high intensity produces a shock wave that passes through the solvent enhancing mass transfer within the plant materials. Furthermore, solvent with lower viscosity has low density and high diffusivity, which can easily diffuse into the pores of the plant materials [12-15].

For MAE, by addition of water, the mixture dielectric constant increase. This could help absorb microwave energy, thus increasing extraction efficiency. Although the addition of water increases the dielectric constant, the dissipation factor will decrease. This means that although the solvent mixture could absorb high microwave energy as a result in increased dielectric constant, the mixture could not dissipate the heat effectively [16]. As found in this investigation, solvent mixture with higher water content the extraction was low and not favorable.

In all the three methods used it shows the same pattern of results where 50% of ethanol concentration give the highest amount of gallic acid extracted. However, using MAE produced almost triple amount of gallic acid compared to Soxhlet and UAE. In this case, clearly 50% solvent composition was chosen to be the effective solvent composition and used to continue the study.

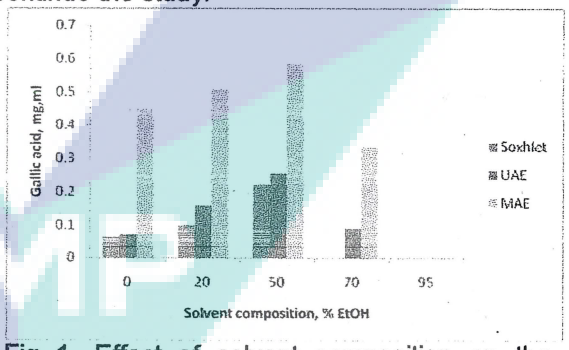

Fig 1. Effect of solvent composition on the amount of gallic acid for Soxhlet, UAE and MAE

3.2 Effect of time

The time of extraction was evaluated in UAE and MAE techniques. The techniques used for the isolation of gallic acid had a different effective extraction time. A comparison of the extraction time for the investigated techniques is shown in Fig 2 and Fig 3. The results showed

that an increased in extraction time will increase the amount of gallic acid for MAE and UAE. MAE can reach the highest amount of gallic acid in 2 min while UAE needed 40 min. To some extend of time, the amount of gallic acid decreased when the time increased. This effect is related to the overexposure to the radiation or thermal degradation. Although, both techniques disrupt the plant cells, MAE extraction was more effective because it uses cell's internal water as the conductor medium for microwave. The extraction time is the shortest in case of MAE compared to UAE and Soxhlet. Soxhlet extraction is the most time-consuming technique where it needed 6 h of extraction time. From the results obtained, MAE is a rapid technique on the recovery of gallic acid.

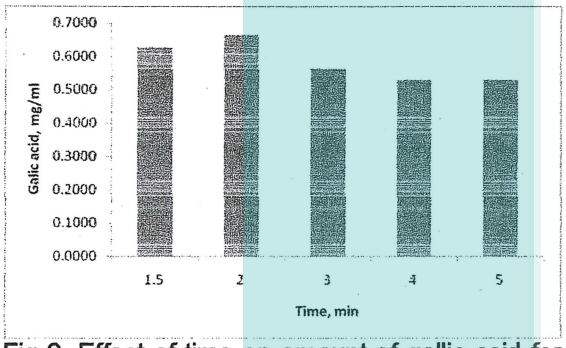

Fig 2. Effect of time on amount of gallic acid for MAE

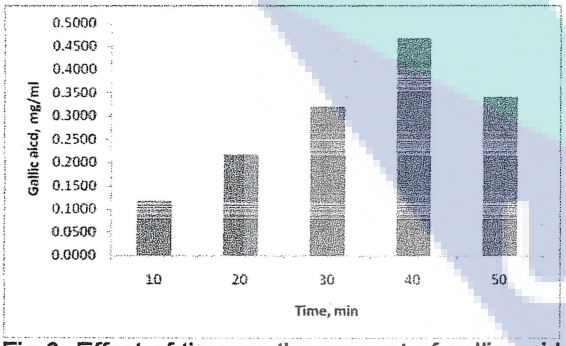

Fig 3. Effect of time on the amount of gallic acid for UAE

3.3 Effect of temperature

The effect of temperature on the amount of gallic acid is shown in Fig 4. It was found that the temperature from 35 to 55°C enhanced the product recovery. The amount of gallic acid will be decreased when the temperature was raised. Temperature affects many physicals properties, including viscosity, diffusivity, solubility and surface tension [17]. Additionally, increased in temperature will allow the solvent to have a higher capacity to solubilize analytes, while surface tension and solvent viscosity decrease with temperature, which will improve sample wetting and matrix penetration, respectively [18,19]. However, beyond certain temperature, phenolic compound can be denatured [9].

In all three extraction methods, extraction of gallic acid is taking place either in the range of studied temperature or at boiling point of solvent applied for the extraction. Higher recoveries were obtained using MAE than using Soxhlet extraction or UAE. There was no amount of gallic acid for MAE at the temperature of 35°C might be because the temperature is too low for the microwave heating to heat up the water molecules of the plant cells. For UAE, the highest amount of gallic acid that was produced was at the temperature of 35°C while MAE was at 40°C.

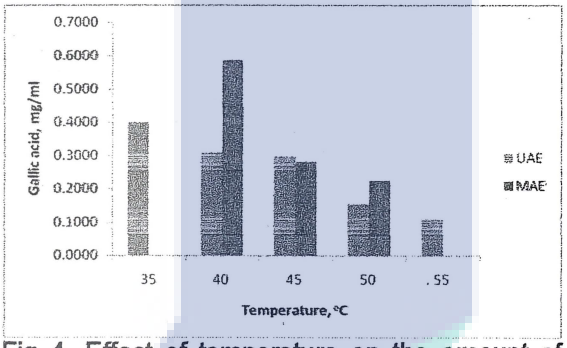

Fig 4. Effect of temperature on the amount of gallic acid for UAE and MAE

3.4 Effect of power

The power for both UAE and MAE were evaluated and is shown in Fig 5 and Fig 6. From the results obtained, it was clear enough that there was an effect towards the amount of gallic acid when the power of these two equipments was studied.

It can be seen that there are improvements in the extracts obtained as ultrasonic output power increases from 25.56 to 102.24 W. An explanation for this is that the larger the amplitude of ultrasound wave travel through the mass medium, the more the cavitation bubbles were created and collapse [16]. The violent shock wave and high speed jet might be generated, disrupting the cell walls. Solvent can penetrate into the cell and released the components from the cells into solvent. Meanwhile, the mass transfer rate was

significantly enhanced. A decreased in the amount of gallic acid was obtained might be due to that the compound had been degraded caused by excessive energy dissipation in the form of heat when high ultrasonic power was used. High amount of gallic acid was obtained when 102.24 W of ultrasonic power was used.

Microwave irradiation energy disrupts hydrogen bonds, because of microwave-induced dipole rotation of molecules and migration of dissolved ions. Microwave irradiation energy can enhance the penetration of the solvent into the matrix and deliver efficiently to materials through molecular interaction with the electromagnetic field and offer a rapid transfer of energy to the solvent and matrix, allowing the dissolution of components to be extracted [20]. From this result, it can be noted that the use of microwave power can influence the percent yield. When microwave power was lower than 320 W, the extraction increased with the increase in microwave power. When microwave power was more than 320 W the extraction leveled out might be because all the tannin in the stem bark had been extracted at lower microwave power.

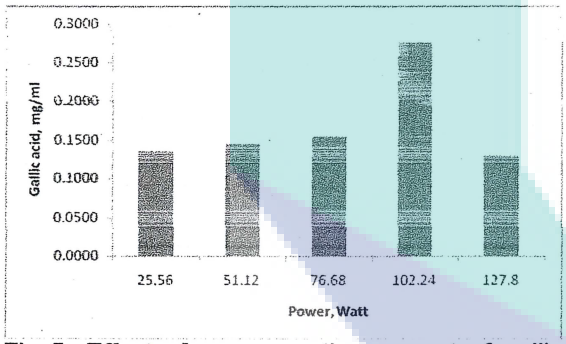

Fig 5. Effect of power on the amount of gallic acid for UAE

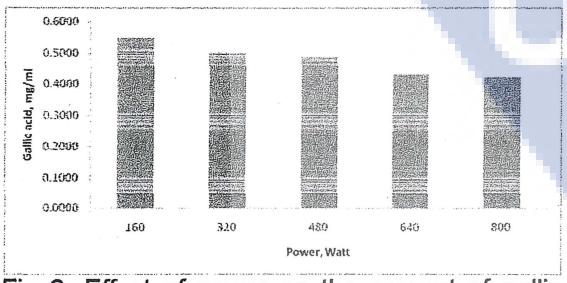

Fig 6. Effect pf power on the amount of gallic acid for MAE

3.5 Comparison of extraction methods

The selection of extraction methods depends on the advantages and disadvantages of the processes, such as extraction yield, complexity, production cost, environmental friendliness and safety. Soxhlet extraction is the most common method of extraction. They are definitely user friendly but the drawbacks are that they used a large amount of solvent and long time of extraction needed. Considering the massive use of solvent and long extraction time, this extraction method is not favorable to a commercial perspective.

UAE and MAE has received increasing attention as an alternative method. It has been used for several reasons: (1) reduced extraction time (2) reduced solvent usage and (3) improved extraction yield. By considering the economic and practical aspects, MAE is a strong novel extraction technique.

The efficiency of extraction using Soxhlet, UAE and MAE was compared and shown in Table 3. The findings demonstrate that UAE and MAE are promising extraction techniques that offer improved efficiency. From the comparison shows that at shorter time, MAE gave higher extraction yield compared to Soxhlet and UAE. The MAE can give the highest extraction selectivity for that extraction of gallic acid.

Table 3: Comparison of extraction techniques on the amount of gallic acid

Extraction method	Time	Temperature	% EtOH	Power	Gallic acid, mg/ml
Soxhlet	6 h	80°C	50	NA	0.2232
UAE	40 min	35°C	50	102.24 W	0.4005
MAE	2 min	40°C	50	160W	0.5477

4. CONCLUSION

Gallic acid degraded easily when they were exposed at high temperature, long period of time, high power for extraction and also affected by solvent composition. The amount of gallic acid was low when extracted using Soxhlet extraction technique. The effects of solvent composition, time, temperature and power had been investigated using UAE and MAE techniques. Comparisons of extraction techniques had been done towards the amount of gallic acid. MAE was the most efficient method where it provides high extraction efficiency in short time. Therefore, MAE is an alternative extraction technique for fast

extraction of gallic acid from the stem bark of *Jatropha curcas*.

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CHAPTER 4

TECHNICAL PAPER 3

STATUS: PENDING IN PUBLISHING

Ultrasonic-assisted extraction of Gallic acid from *Jatropha curcas* stem bark

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Abstract
Ultrasonic-assisted extraction was used to increase the extraction efficiency of gallic acid from the stem bark of Jatropha curcas. The effects of some extraction parameters such as solvent composition, extraction time, extraction temperature and ultrasonic power were evaluated, and the results have been compared to maceration and Soxhlet extraction. Quantification of gallic acid in the extract was done using HPLC. It was found that ultrasonic-assisted extraction requires a shorter time of extraction and a reduced solvent consumption. The optimum conditions of ultrasonic-assisted extraction were with extraction time of 40 minutes, extraction temperature of 35°C, solvent composition of 50% and ultrasonic power of 153.36 W. Under the optimum extraction conditions, the amount of gallic acid can reach twice as much as compared to Soxhlet extraction. From this research work, it can be concluded that ultrasonic-assisted extraction was faster and economical than the Soxhlet extraction in the extraction of gallic acid. The ultrasonic-assisted extraction was not only more efficient but also convenient for the recovery and purification of the active compounds from plant materials.

Keywords: *Jatropha curcas*, ultrasonic-assisted extraction, Soxhlet extraction, gallic acid, HPLC

1. Introduction

Jatropha curcas (Euphorbiaceae), a plant which is cultivated in Central and South America, Southeast Asia, India and Africa [1-3], has recently gained a great deal of interest by scientists due to the pharmaceutical values this plant offer. Kumar and Sharma [4] have published a review of Jatropha curcas research, which summarizes the multipurpose use of this plant, including its medicinal uses. Various parts of Jatropha curcas had been used in many traditional medicines to cure many ailments. The stem bark of Jatropha plant contains secondary metabolite active compounds, which have various therapeutic properties [5]. Gallic acid (3, 5, 7 - trihydroxybenzoic acid) and its derivatives are the natural products of hydrolysis of tannin [6]. Significant biological activities of gallic acid such as an antioxidant [7], anti-inflammatory [8], antifungal [9] and carcinogenic properties [6] have attracted considerable interest in pharmaceutical, food and agriculture industries [10].

The enhancement of product recovery using ultrasound is attributed to a phenomenon called cavitation produced in the solvent by the passage of an ultrasonic wave [11-12]. Cavitation bubbles are produced and compressed during the application of ultrasound, allowing higher penetration of the solvent into the plant materials and releasing intracellular product by disrupting the cell walls. Ultrasound has been shown to aid extraction in a number of plant materials by significantly reducing extraction time and increasing maximum extraction yield, respectively [13-14]. The extraction of gallic acid from stem bark of Jatropha curcas using ultrasonic has never been reported. The purpose of the present study was to investigate the extraction process of gallic acid with the ultrasonic method and to compare with the effects with maceration and Soxhlet extraction method. Various experimental conditions on the extraction yield were also studied (extraction time, temperature, amplitude and solvent composition). Quantitative analysis of the extracts was finally investigated using HPLC.

2. Materials and methods

2.1 Preparation of plant material

Jatropha curcas branches were collected from a plantation in Selangor, Malaysia. The branches were cleaned by hand to remove foreign materials. Then the bark was stripped from the branches and cut into small pieces prior to drying. An oven with a temperature of 60°C was used to dry the bark for 8 hours. The dried bark was then grounded before it was sieved. A granulometric apparatus was used to obtain a homogenous particle size. Separation of the

grounded sample was carried out with a sieve shaker (Fritsch) with various granulometric size sieves to obtain a homogeneous size of 1.0 mm. The samples were then kept in a seal plastic bag and store at room temperature.

2.2 Chemicals and reagents

Ethanol (with purity of 95% v/v) was purchased from R&M Chemicals Ltd. Acetonitrile and orthophosphoric acid both HPLC grades were purchased from Merck, USA. Gallic acid (HPLC grade) was purchased from Sigma-Aldrich, USA.

2.3 Maceration

A 10 g of sample were weighed and transferred to a 250 ml conical flask. The sample was then mixed with 300 ml of extracting solvent. The upper part of the conical flask was covered with parafilm and later with aluminium foil at the top. The conical flask was placed on a shaker (Certomat, B. Braun) for 6 h at an ambient temperature and shake at the speed of 200rpm. A shaker was used to efficient mixing of solvent and sample. The extract was then filtered with filter paper (Whatman No. 1) and the filtrate was concentrated under vacuum at 50°C using a rotary evaporator.

2.4 Soxhlet extraction

A Soxhlet apparatus was employed in which 10 g of sample was placed into a thimble with 300 ml of 50% ethanol contained in a 500 ml round-bottom flask. Extraction was carried out for up to 6 h. The extract was then filtered with filter paper (Whatman No. 1) and the filtrate was concentrated under vacuum at 50°C using a rotary evaporator.

2.5 Ultrasonic-assisted extraction (UAE)

An ultrasonic bath (40kHz, 230Watt) was used in this study. The extraction of gallic acid was performed by adding 10 g of sample with 150 ml of solvent in a 250 ml of conical flask. The flask was then partially immersed into the ultrasonic bath. The bottom of the flask was approximately 5 cm from the bottom of the bath. Water in the ultrasonic bath was circulated and regulated to avoid temperature rising caused by exposure of ultrasonic. The extract was then filtered with filter paper (Whatman No. 1) and the filtrate was concentrated under vacuum at 50°C using a rotary evaporator. Table 1 summarizes the ranges of parameters investigated in this study. All the experiments were performed in triplicate.

Table 1. Ranges of experimental parameters

Parameters	Ranges
Temperature, °C	20, 25, 30, 35, 40
Time, min	10, 20, 30, 40, 50
Solvent composition, % EtOH	0, 20, 50, 70, 95
Power, Watt	25.56, 51.12, 76.68, 102.24, 127.8

2.6 HPLC analysis for gallic acid

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3. Results and discussion

3.1 Effect of solvent composition

The effect of solvent compositions on the amount of gallic acid for three different extraction methods was shown in Fig 1. All three extraction methods demonstrated that the improvement of extraction efficiency was observed with the addition of some amount of water. The amount of gallic acid increased as the solvent composition increased up until 50%. Usually, by varying the solvent polarity from water to ethanol, the extraction yield will increase. At the same time, product recovery will decrease with decreasing water percentage. This could be due to the relative polarity and the decrease in effective swelling of plant materials [15]. Alcoholic solvents have been commonly used as solvent to extract polyphenols from natural sources where they will give higher yield of total extract, even though they are not highly selective for phenols. Mixtures of alcohol and water have revealed to be

more efficient in extracting phenolic constituents then compared to mono-component solvent system [16]. Addition of water to organic solvents usually creates a more polar medium which facilitates the extractions of polyphenols [16].

Higher amount of gallic acid was observed when ultrasound was introduced. This could be due to the phenomena called ultrasonic cavitation produced in the solvent by the passage of an ultrasonic wave [17]. The ultrasonic wave could disrupt the cell walls. So larger contact between solvent and plant material was created, and more product recovery obtained. In the presence of water, the intensity of ultrasonic cavitation in the solvent mixture was increased as the surface tension increased while the viscosity and vapor pressure decreased [14]. In all the three methods used it shows the same pattern of results where 50% of ethanol concentration give the highest amount of gallic acid extracted. Thus, 50% solvent composition was chosen to be the effective solvent composition and used to continue the study.

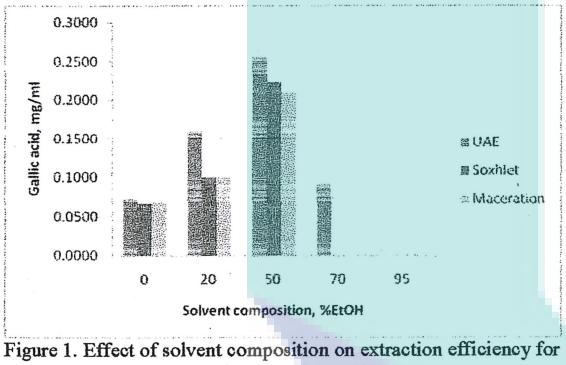

Figure 1. Effect of solvent composition on extraction efficiency for maceration, Soxhlet and UAE

3.2 *Effect of extraction time*

The effect of extraction time on the amount of gallic acid is shown in Fig. 2. The product recovery increased very fast for the first 40 min using UAE. However, the product recovery decreased when the sonication time was increased from 40 min to 50 min. This may be because most of the extracts had already been extracted during the first 40 min and that the power of ultrasound is able to degrade the target compounds for a long period of time. This effect is related to the ultrasound power towards the stability of compound and the medium [18]. This process indicates that the ultrasound is more effective in the first 40 min. Ultrasonic-assisted extraction significantly shortened the extraction time and improved extraction yield. Swelling and hydration of material could be accelerated by ultrasonic resulting in an enlargement in the pores of cell walls [19], leading to a better mass transfer of intracellular products into solvent. Rupture of cell walls due to micro jet effects cause an increase of penetration rate of solvent into tissue [20]. Therefore, ultrasonic-assisted extraction technique allowed target components to dissolve in the solvent in a higher speed thereby boosting yield with a relevant shorter time. Based on the results so far, higher extraction yield was obtained at 30 minutes of extraction time.

It compared to using maceration and Soxhlet, UAE was found to enhance product recovery much faster. This suggests that maceration and Soxhlet extraction are a time-consuming extraction method.

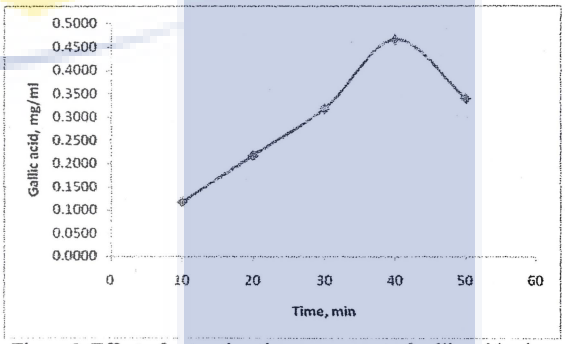

Figure 2. Effect of extraction time on amount of gallic acid using UAE

3.3 *Effect of temperature*

The effect of temperature on the amount of gallic acid is shown in Fig 3. It was found that the temperature from 20 to 35°C enhanced the product recovery. The amount of gallic acid had decreased when the temperature was raised from 35 to 40°C. Temperature affects many physicals properties, including viscosity, diffusivity, solubility and surface tension [21]. There are two factors that play the role when treatment at a high temperatures. First, the cavitation threshold is decreased so more cavitation bubbles can be produced. Secondly, the vapour pressure at the elevated temperatures will help to form vapour-filled bubbles and as a result, the implosion of those bubbles will be cushioned. Therefore, the cavitation effect would be less efficient. When there is an increase in temperature, it favors extraction. Thus, it enhances both solubility of the solute and the diffusion coefficients. However, beyond certain temperature, phenolic compound can be denatured [16].

In all three extraction methods, extraction of gallic acid is taking place either in ambient temperature or at boiling point of solvent applied for the extraction. Higher recoveries were obtained using UAE than using maceration and Soxhlet extraction.

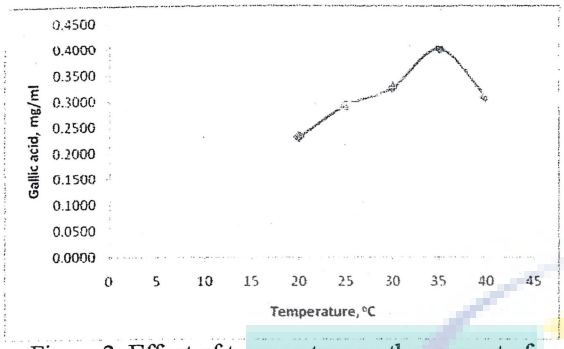

Figure 3. Effect of temperature on the amount of gallic acid using UAE

3.4 *Effect of ultrasound power*

The result for effect of ultrasound power on the amount of gallic acid is shown Fig 4. It can be seen that there are improvements in the extracts obtained as ultrasonic output power increases from 25.56 to 102.24 W. An explaination for this is that the larger the amplitude of ultrasound wave travel through the mass medium, the more the cavitation bubbles were created and collapse [22]. The violent shock wave and high speed jet might be generated, disrupting the cell walls. Solvent can penetrate into the cell and released the components from the cells into solvent. Meanwhile, the mass transfer rate was significantly enhanced. A decreased in the amount of gallic acid was obtained might be due to that the compound had been degraded caused by excessive energy dissipation in the form of heat when high ultrasonic power was used. High amount of gallic acid was obtained when 102.24 W of ultrasonic power was used.

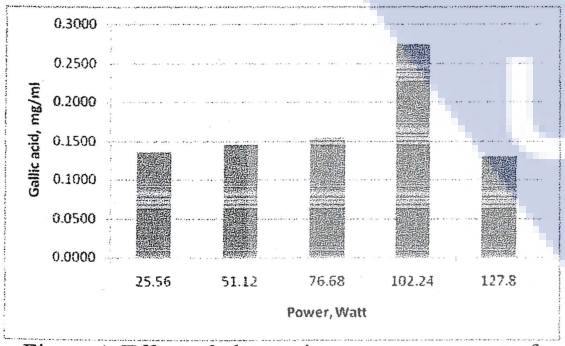

Figure 4. Effect of ultrasonic power on amount of gllic acid

3.5 *Comparison of ultrasonic-assisted extraction and conventional methods*

The comparisons of extraction efficiency of three extraction methods are summarized in Table 2. UAE gave significant higher value of gallic acid due to its cavitation phenomena. For extraction time, UAE was the fastest extraction method and just needs 40 min. Using conventional methods, it took long hours to achieve the same amount that UAE can produce. Clearly, by reducing the extraction time, ultrasonic can be a promising method that offers improved extraction efficiency.

Table 2. Comparison of different extraction methods on the amount of gallic acid

Extraction methods	Time	Temperature	Type of solvent	Gallic acid, mg/ml
Maceration	6 hrs	25°C	50% Ethanol	0.2094
Soxhlet	6 hrs	80°C	50% Ethanol	0.2232
UAE	40 min	40°C	50% Ethanol	0.2744

4. Conclusion

Three extraction methods for extraction of gallic acid from *Jatropha curcas* stem bark were investigated and compared. UAE was found to be faster and more effective comparing with other methods. The main mechanism for enhanced recovery of gallic acid with UAE was acoustic cavitation, a phenomena occurring in the liquid mediums under the influence of ultrasound. The ultrasound wave causes the bark tissues to disrupt, thus enhancing the mass transfer of the solute into the solvent. The amount of gallic acid increased with the increased in extraction time, temperature, ultrasonic power and was affected by the composition of solvent used.

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CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

As a conclusion, the research grant that is provided had helped this research study in terms of buying chemicals and instrument. From this research study, three technical papers had been accepted for publishing in three different journals. For this research study, there is some work that had already presented in conferences and will be presented in near future.

1.2 RECOMMENDATION

1. Research development is one of the crucial fields and need to be highly developed. It is good to provide potential research studies that have value added towards the industries and human society.

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