SOLID-LIQUID EXTRACTION OF HYDROLYSABLE TANNIN (GALLIC ACID) FROM STEM BARK OF *JATROPHA CURCAS* USING VARIOUS TYPE OF EXTRACTION

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Solid-Liquid Extraction of Hydrolysable Tannin (Galic Acid) from Stem Bark of *Jatropha Curcas* Using Various Type of Extraction

AMIRAH BINTI IZAM

A thesis submitted in fulfillment of the requirements
for the award of the degree of
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STATEMENT OF AWARD FOR DEGREE

Thesis submitted in fulfillment of the requirements for the award of the degree of Master of Engineering in Chemical

SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Engineering in Chemical.

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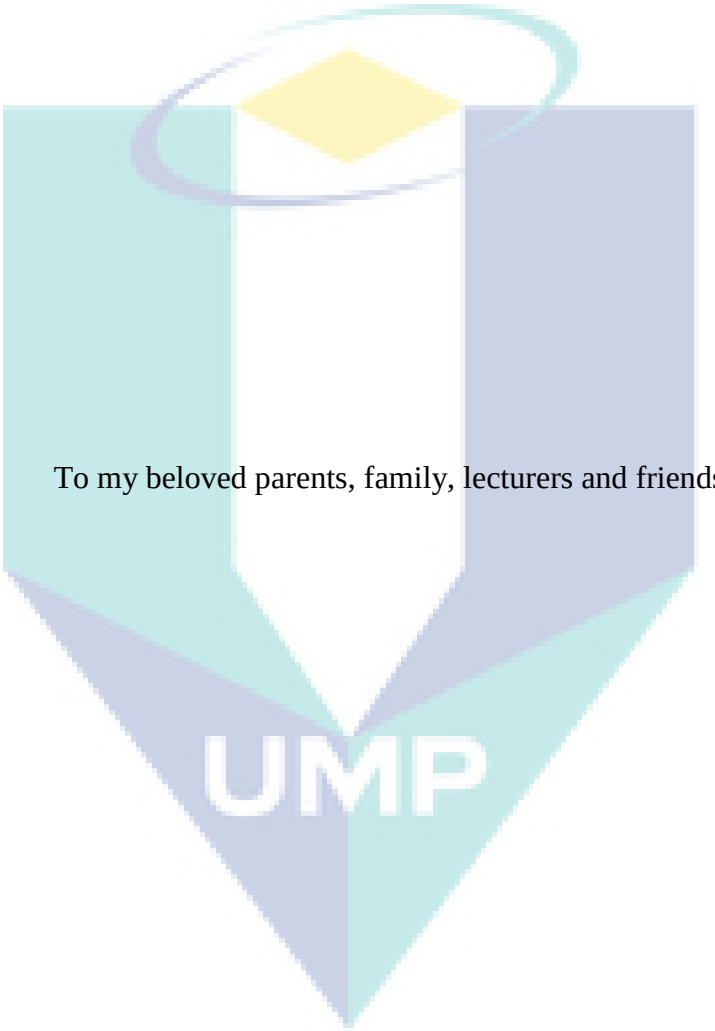

To my beloved parents, family, lecturers and friends

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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. The aims of this study were to study the extraction parameters using four different extraction techniques, to estimate the kinetic studies of gallic acid, to optimize the ultrasonic-assisted extraction (UAE) parameters and to compare the efficiency of extraction techniques. Two conventional extraction techniques were employed namely shake flask extraction and Soxhlet extraction. Ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE) were the modern extraction techniques utilized. The effect of solvent composition, extraction time, extraction temperature and power for UAE and MAE were the extraction parameters used in the extraction studies. For the shake flask extraction and Soxhlet extraction, two parameters namely as effect of solvent composition and extraction time were evaluated. The extracts were further undergone analysis process. Quantification of gallic acid in the extracts was done using high-performance liquid chromatography (HPLC). In general, all the extraction techniques were capable of isolating gallic acid from the bark, but the recovery obtained using modern techniques was higher than the conventional techniques. It was found that all the parameters studied had given significant effect towards the yield of gallic acid. Kinetic studies were done to estimate the washing coefficient and the slow extraction coefficient. In optimization part, the parameters of UAE (solvent composition, extraction temperature and extraction time) were optimized using Box-Behnken Design (BBD). The optimal conditions were as follows: solvent composition of 49.97%, extraction temperature of 35.7^{o}C and extraction time of 50.71 min. Under these conditions, the experimental yield of gallic acid was 21.6253 ± 0.0528% mg gallic acid/ 100g bark, which was agreed close to the predicted value 21.6367 mg gallic acid/ 100g bark. The efficiency of the extraction techniques was in the following order: shake flask extraction < Soxhlet < UAE < MAE.

ABSTRAK

Pengekstrakan dan pemulihan produk adalah langkah-langkah yang paling penting dalam penilaian sebatian aktif dari pelbagai bahagian tumbuhan yang berharga. Dalam kajian ini, pengekstrakan asid gallic dari kulit kayu batang Jarak Jatropha telah disiasat. Tujuan kajian ini adalah untuk mengkaji parameter pengekstrakan yang menggunakan empat teknik pengekstrakan yang berbeza, untuk menganggarkan kajian kinetik asid gallic, untuk mengoptimumkan pengekstrakan ultrasonik (UAE) parameter dan untuk membandingkan kecekapan teknik pengekstrakan. Dua teknik pengekstrakan konvensional telah digunakan seperti pengekstrakan kelalang goncang dan pengekstrak Soxhlet. Pengekstrakan ultrasonik (UAE) dan pengekstrakan gelombang mikro (MAE) adalah teknik pengekstrakan moden yang juga digunakan dalam kajian ini. Kesan komposisi pelarut, masa pengekstrakan, suhu pengekstrakan dan kuasa untuk UAE dan MAE adalah parameter pengekstrakan yang digunakan dalam kajian pengekstrakan. Untuk pengekstrakan kelalang goncang dan pengekstrak Soxhlet, dua parameter iaitu sebagai kesan komposisi pelarut dan masa pengekstrakan dinilai. Ekstrak terus menjalani proses analisis. Kuantifikasi daripada asid gallic dalam ekstrak dilakukan menggunakan kromatografi cecair berprestasi tinggi (HPLC). Secara umum, semuanya teknik pengekstrakan mampu mengasingkan asid gallic daripada kulit batang, tetapi pemulihan yang diperolehi dengan menggunakan teknik moden adalah lebih tinggi berbanding teknik konvensional. Ia didapati bahawa semua parameter yang dikaji telah memberi kesan yang ketara ke arah hasil asid gallic. Kinetik kajian telah dilakukan untuk menganggar pekali basuh dan pekali perahan perlahan. Membasuh bahan ekstraktif dari permukaan fenomena zarah tumbuhan yang berlaku sebelum ia mencapai keseimbangan dan kemudian proses pengekstrakan perlahan yang berlaku sehingga ia mencapai keseimbangan. Di bahagian pengoptimuman, parameter UAE (komposisi pelarut, suhu pengekstrakan dan masa pengekstrakan) yang telah dioptimumkan menggunakan Box-Behnken Rekabentuk (BBD). Keadaan optimum adalah seperti berikut: komposisi pelarut 49.97%, suhu pengekstrakan 35.70^{o}C dan masa pengekstrakan 50.71 min. Dalam keadaan ini, hasil eksperimen asid Gallic adalah 21.6253 ± 0.0528% asid gallic mg / 100g kulit kayu, yang telah dipersetujui hampir kepada nilai diramalkan 21.6367 mg asid gallic / kulit 100g. Kecekapan pengekstrakkan mengikut susunan berikut: kaedah goncang kelalang < Soxhlet < UAE < MAE.

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LIST OF ABBREVIATIONS

^{o}C	degree Celcius
ANOVA	Analysis of Variance
BBD	Box-Behnken Design
cm	Centimeter
cP	Centipoises
GHz	Giga Hertz
g	Gram
g/ml	gram per mili litre
HPLC	High-Performance Liquid Chromatography
i.d	internal diameter
kHz	kilo Hertz
L	Litre
m	Meter
MAE	Microwave-Assisted Extraction
mg/ml	mili gram per mili litre
mg	mili gram
MHz	Mega Hertz
min	Minute
ml	mili litre
ml/min	mili litre per minute
mm	mili meter
mm Hg	mili meter mercury
mN/cm	mili Newton per centimeter
nm	Nanometer
rpm	Rotation per minute
RSM	Response Surface Methodology
SFE	Super Critical Extraction
UAE	Ultrasonic-Assisted Extraction

UV	Ultra Violet
v/v	volume per volume

LIST OF NOMENCLATURE

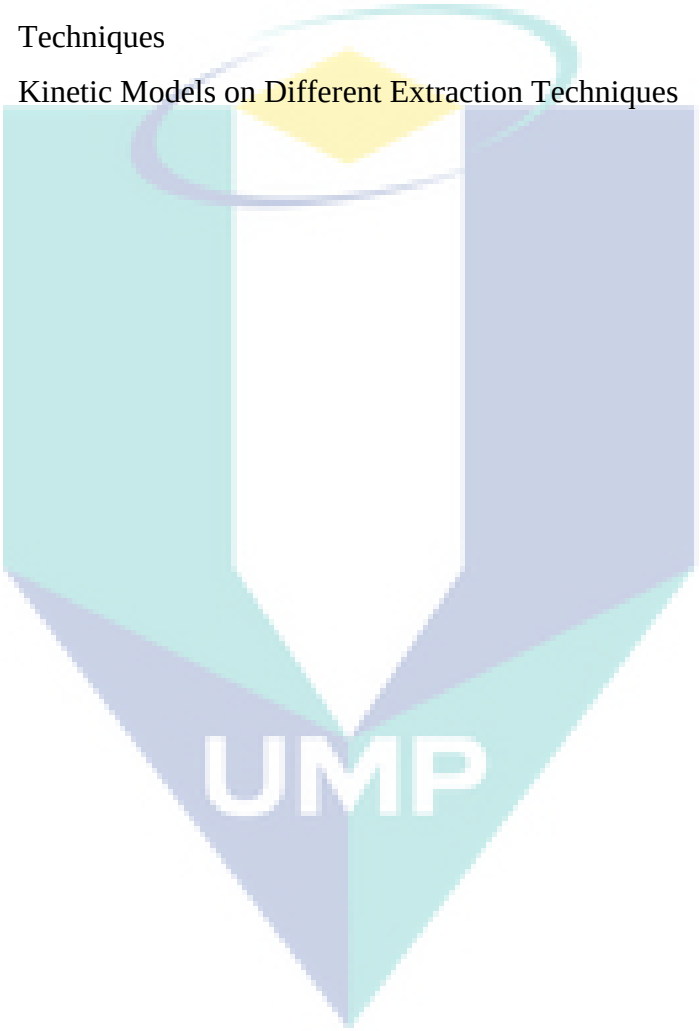
A	outer surface of the particles
b	washing coefficient according to the film theory
b’	washing coefficient of unsteady diffusion model
b’’	washing coefficient of the empirical model of Ponomaryov
c	concentration of extractable substances during extraction
$\bar{c}$	mean concentration of extractible substances in the particle
c_o	concentration of extractive substances in a particle at the beginning
c_∞	concentration of extractive substances on the surface of the particles
C_o	number of central points
c_s	concentration of saturated solution
C.V.	Coefficient of Variance
D_{ef}	effective diffusivity coefficient
h	radius for a particle geometry
k	number of factors
k	slow extraction coefficient according to film theory and unsteady diffusion model
k’	specific rate of slow extraction according to the empirical model of Ponomaryov
N	Number of experiments
P_m	Polarity index of solvent mixture
P_1	Polarity indices of solvent 1
P_2	Polarity indices of solvent 2
q	content of extractable substances in plant material
q_o	content of extractable substances present in plant material
R^2	determination of coefficient
R^2_{adj}	adjusted determination of coefficient
t	Time
V	Volume of particles
x_i	variables

W	Watt

Greek Symbols

α	constant values depend on the particle shape
β	constant values depend on the particle shape
β_o	constant term
β_i	coefficient of linear parameter/ first-order term
β_{ij}	coefficient of second-order interaction terms
μ	Micron
ε	residual associated to the experiments
ε'	dielectric constant
ε'_i	dielectric constant of *i*th solvent
ε'_m	dielectric constant of solvent mixture
ε''	dielectric loss
Φ_i	volume fraction
Φ_1	volume fraction of solvent 1
Φ_2	volume fraction of solvent 2
tan δ	dissipation factor
%	Percent
% EtOH	percent ethanol

LIST OF APEENDICES



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. The aims of this study were to study the extraction parameters using four different extraction techniques, to estimate the kinetic studies of gallic acid, to optimize the ultrasonic-assisted extraction (UAE) parameters and to compare the efficiency of extraction techniques. Two conventional extraction techniques were employed such as shake flask extraction and Soxhlet extraction. Ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE) were the modern extraction techniques utilized. The effect of solvent composition, extraction time, extraction temperature and power for UAE and MAE were the extraction parameters used in the extraction studies. For the shake flask extraction and Soxhlet extraction, two parameters namely as effect of solvent composition and extraction time were evaluated. The extracts were further undergone analysis process. Quantification of gallic acid in the extracts was done using high-performance liquid chromatography (HPLC). In general, all the extraction techniques were capable of isolating gallic acid from the bark, but the recovery obtained using modern techniques was higher than the conventional techniques. It was found that all the parameters studied had given significant effect towards the yield of gallic acid. Kinetic studies were done to estimate the washing coefficient and the slow extraction coefficient. Washing of extractive substances from the surface of plant particles phenomena is happening before it reaches equilibrium and then slow extraction process is happening until it reaches equilibrium. In optimization part, the parameters of UAE (solvent composition, extraction temperature and extraction time) were optimized using Box-Behnken Design (BBD). The optimal conditions were as follows: solvent composition of 49.97%, extraction temperature of 35.7oC and extraction time of 50.71 min. Under these conditions, the experimental yield of gallic acid was 21.6253 ± 0.0528% mg gallic acid/ 100g bark, which was agreed close to the predicted value 21.6367 mg gallic acid/ 100g bark. The efficiency of the extraction techniques increased in the following order: shake flask extraction > Soxhlet > UAE > MAE.

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^{o}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 has 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 is used on different 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 fertilizers, as insecticides/pesticides, as an energy source and many more. There is one potential aspect of this plant that gained interest from the researchers. This plant has the potential to have medicinal uses where it had been practiced traditionally by different communities of the world. 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 by the growers. This will promote better growth and reduce competition among the trees. But less had known that the branches can contribute to be a valuable product. Currently, the growers could not achieve the optimum benefits from the plant because the markets of different products from this plant have not been properly explored or quantified. As a result, the growers do not have ample information about the potential and economics of this plant to exploit it.

To my knowledge, there are no research had been conducted on extraction of gallic acid using various types of extraction process. This could be an opportunity to explore on different extraction process that provides better performance on the gallic acid extraction. 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 shake flask extraction 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-assisted extraction (UAE) and microwave-assisted extraction (MAE) 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 between the conventional and modern extraction techniques applied were done to identify which techniques give the comprehensive results of good isolation of gallic acid. Furthermore, an estimation of washing coefficient and slow extraction coefficient was obtained from kinetic study model of unsteady diffusion through plant material, the film theory and the empirical equation of Ponomaryov. Lastly, optimization of ultrasonic-assisted extraction (UAE) was done to attain the optimum condition of ultrasonic parameter.

1.3 OBJECTIVES OF STUDY

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

1. To extract the gallic acid from the stem bark of *Jatropha curcas* using conventional extraction techniques and modern extraction techniques.

2. To study on the several effects that can influence gallic acid extraction performance followed by RSM optimization using selected extractor.

3. To compare the gallic acid extraction performance of conventional extraction techniques with modern extraction techniques.

4. To model the kinetic of the extraction process at selected conditions.

1.4 SCOPES OF STUDY

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

1. Two methods of conventional extraction techniques (shake flask extraction and Soxhlet extraction) and two methods of modern extraction techniques (ultrasonic-assisted extraction and microwave-assisted extraction) were used in this study.

2. The effect of solvent composition, extraction time, extraction temperature and extraction power were studied and optimization was done using Design Expert 7.1.6 software by applying the Response surface methodology (RSM) method.

3. The extraction techniques employed were compared based on the parameters studied.

4. Three kinetic models were used namely as kinetic study model of unsteady diffusion through plant material, the film theory and the empirical equation of Ponomaryov.

1.5 SIGNIFICANT OF STUDY

The significant of doing this research study are as below.

1. To investigate the efficiency of different extraction methods 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 optimize 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 for 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 five 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 focused.

Chapter 2 discussed mainly about *Jatropha curcas*, phenolic compounds and extraction techniques. This chapter gives information on this plant and the chemical composition that contain in different parts of this plant. In addition, in this chapter discussed on the various uses of the plant to cure many diseases and illnesses. In addition, this chapter also introduced phenolic compound in general and specifically discussed on gallic acid. The used of gallic acid in many fields and the advantages of this compound can offer were discussed. The information on the extraction techniques and kinetic models equation are presented in this chapter. The general principle of extraction is introduced in order to understand the concept of extraction. This chapter also summarized on the principles, mechanisms, advantages and disadvantages of each extraction technique used in this study. In addition, the explanations of kinetic model equation were discussed in detail.

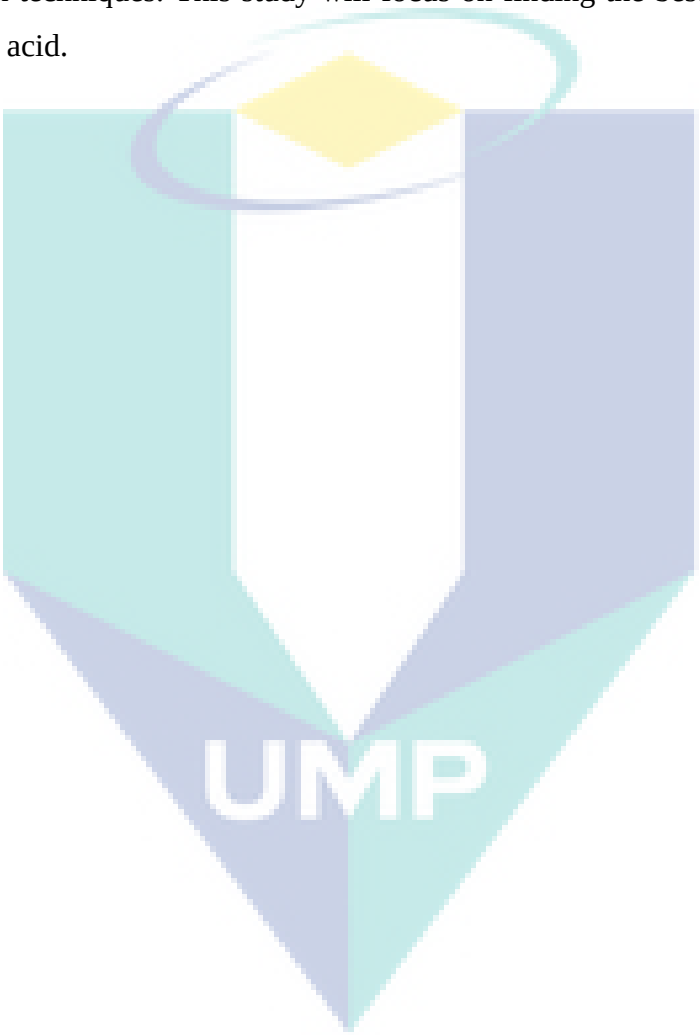
Materials and methods of experiment used in order to achieve the objectives of study are presented in Chapter 3. This chapter explains on the four stages that had been used to complete the experiments. These include the sample preparations, experimental studies using different extraction techniques, analysis of the extracts and lastly optimization of the studied parameters.

The main findings of this study are discussed in Chapter 4. The discussion covered the results for all the extraction techniques, optimization of the extraction techniques and the mathematical modeling for estimation of solid-liquid mass transfer.

Lastly, in Chapter 5 is the conclusion of the findings and 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.



CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

This chapter provides the information about *Jatropha curcas*, phenolic compounds and extraction techniques.

2.2 *JATROPHA CURCAS*

Jatropha curcas (Linnaeus) belongs to the Euphorbiaseae family. Carl von Linne, a botanist, had classified this plant in 1753 and gave the botanical name '*Jatropha curcas*' from the Greek word "jatros" which means doctor and "trophe" which means nutrition/food (Kumar and Sharma, 2008). *Jatropha curcas* is also known as physic nut or purging nut, is a small tree or large shrub that can reach a height of three to five meters but in favorable conditions its height can reach until 8 to 10 meters (Kumar and Sharma, 2008). This tree is well adapted to arid and semi-arid conditions. *Jatropha curcas* can grow almost anywhere except waterlogged lands, even on gravelly, sandy and saline soils. It can thrive on the poorest stony soil and can even grow in the crevices rocks. This plant can stand long period of drought by shedding most of its leaves to minimize transpiration loss and it requires extremely low water uptake. It also can grow on well-drained soils with good aeration and is well adapted to marginal soils with low nutrient content (Openshaw, 2000). *Jatropha curcas* is found in the tropics and sub-tropics areas such as Central and South America, Africa, India and South-East Asia (Schmook & Seralta-Peraza, 1997; Gübitz et al., 1999; Martínez-Herrera et al., 2006). From the Caribbean, where this species had been used by

the Mayas (Schmook & Serralta-Peraza, 1997), *Jatropha* curcas was probably distributed by the Portuguese seafarers via the Cape Verde Islands and Guinea Bissau to other countries in Africa and Asia (Heller, 1996).

Jatropha curcas has been used for different aspects of life. The tree itself has been used for erosion control, fire wood, as a hedge plant and for plant protection and as a commercial crop. Various parts of the plant have medicinal uses. Its bark contains tannin also yields a dark-blue dye. The flowers attract bees and thus the plant has a honey production potential. The leaves have been used for rearing silkworm, dyeing also as anti-inflammatory. Latex can be used as wound healing, pesticidal, mollusk control properties. The seeds have been used for insecticide and food. While the seed oil can produce soap, fuel, lubricant and as medicine. Seed cake or press cake has been potential as a fertilizer or biogas production (Staubmann et al., 1997; Gubitz et al., 1999). The roots contain yellow oil with strong anti-helminthic properties and can be used as to treat the snake bites. Even today, *Jatropha curcas* plant is mainly cultivated for the production of oil as fuel substitution.

2.2.1 Chemical Composition Isolated from Different Parts and Various Medicinal Uses

All the parts of *Jatropha curcas* have been used as traditional medicine and for veterinary purposes for a long time (Duke, 1985). It is known that these medicinal used had to do with the chemical compositions that are contained in different parts of this plant. The chemicals isolated from different parts of this plant had been identified by past research studies. Some of these chemicals compounds can be used in various industrial applications.

2.2.1.1 Leaves

The compounds that have been isolated from *Jatropha curcas* leaves include the flavonoid apigenin and its glycosides vitexin and isovitexin, the sterols stigmasterol, β-D-sitosterol and its β-D-glucoside (Chhabra et al., 1990). In addition, *Jatropha curcas* leaves

were reported to contain steroid sapogenins, alkaloids, the triterpenae alcohol, 1-triacontanol and a dimer of a tripene alcohol (Neuwinger, 1994). Staubmann et al. (1999) had isolated a complex of 5-hydroxypyrrolidin-2-one and pyrimidine-2, 4-dione by extraction with ethyl acetate.

In the island of Tonga, in Oceania, the leaves of *Jatropha curcas* have been used in folk medicine to treat vaginal bleeding (Singh et al., 1984). Rural communities of Churu district in the Thar Desert, India used the juice from leaves to cure diseases such as dysentery and colic (Parveen et al., 2007). The leaves were also applied to the breast to promote lactation (Parveen et al., 2007). In Southeast Asia and in some regions of Africa, the leaves are used as purgative, while on the Philippines and Cambodia the leaves are applied to wounds (Staubmann et al., 1999). In Cape Verde and Cameron, a decoction is used internally and externally against fever. In Cameron, the leaves are also in use as a remedy against rheumatism and in Nigeria against jaundice (Staubmann et al., 1999). Thomas (1989) investigations turned out that the leaves are also active against lymphocytic leukemia. An ethanolic extracts of the defatted leaves and twigs of *Jatropha curcas* have shown confirmed activity both in vivo and in vitro against P-388 lymphocytic leukemia (Hufford and Oguntimein, 1978). Fagbenro-Beyioku (1998) investigated and reported the anti-parasitic activity of the sap and crushed leaves of *Jatropha curcas*. In Mali, the leaves are known as a treatment for malaria (Henning, 1997). The leaves are utilized extensively in West Africa ethnomedical practice in different forms to cure various ailments like fever, mouth infections, jaundice, guinea worm sores and joint rheumatism (Irvine, 1961; Oliver-Bever, 1986).

2.2.1.2 Stem Bark, Branches, Twigs

Phytochemical screening of *Jatropha curcas* stems bark extracts revealed the presence of secondary metabolites such as saponins, steroids, tannins, glycosides, alkaloids, flavonoids also yields dark-blue dye (Igbinosa et al., 2009). These compounds are recognized to be biologically active that can aid the antimicrobial activities of *Jatropha*

curcas. These secondary metabolites exert antimicrobial activity through different mechanisms (Igbinosa et al., 2009). Shimada (2006) investigated that tannins have been found to form irreversible complexes with proline rich protein resulting in the inhibition of cell protein synthesis. Tannins reacted with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues (Parekh and Chanda, 2007). Herbs that have tannins as their main components are astringent in nature and good for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). As a result from these observations, it will support the use of *Jatropha curcas* in herbal remedies. The biological activities of tannins had been observed to have anticancer activity and can be used in cancer prevention. These suggest that *Jatropha curcas* has the potential as a source of important bioactive molecules for the treatment and prevention of cancer (Li et al., 2003). The presence of tannins in *Jatropha curcas* stem bark supports the traditional medicinal use of this plant in the treatment of different ailments through scientific investigations.

Alkaloid is another secondary metabolite compound observed in the stem bark extract of *Jatropha curcas*. One of the most common biological properties of alkaloids is their toxicity against the cells of foreign organisms. These activities have been widely studied by researchers for their potential use in the elimination and reduction of human cancer cell lines (Nobori et al., 1994). Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans. This, therefore, has led to the development of powerful painkiller medications (Kam and Liew, 2002).

The inhibitory effect of saponins on inflamed cells had been revealed by Just et al. (1998). Saponin was also found in *Jatropha curcas* stem bark extracts and have supported the usefulness of this plant in managing inflammation.

Steroidal compounds present in *Jatropha curcas* stem bark extracts are in large interest due to their relationship with various anabolic hormones, including sex hormones (Okwu, 2001). Quinlan et al. (2000) had worked on steroidal extracts from some medicinal

plants, which exhibited antibacterial activities on some bacterial isolates. Neumann et al. (2004) also confirmed the antiviral property of steroids through his study.

Flavonoid is another secondary metabolic compound found in *Jatropha curcas* stem bark extracts. It exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek et al., 2002). Hence, the presence of these compounds in *Jatropha* curcas supports the antimicrobial activities observed.

Rural communities of Churu district in the Thar Desert, India used the twigs of this plant as tooth brushes to strengthen the gum and to cure gum boils (Parveen et al., 2007). The extraction of *Jatropha curcas* branches using water as solvent inhibited strongly the HIV-induced cytopathic effects with cytotoxicity (Matsuse et al., 1999).

It can be concluded that *Jatropha curcas* stem bark could be a potential source of active antimicrobial agents. The detailed assessment of its in vivo potencies and toxicological profile is still ongoing. This plant can be a candidate for bio-prospecting for antibiotic and antifungal drugs (Igbinosa et al., 2009).

2.2.1.3 Latex

Folklore uses of *Jatropha curcas* latex are to cure toothache, as a mouth rinse to treat bleeding gums, as a hemostatic, wound dressing and many others (Fazwishni and Kristiani, 2007). The latex contains tannins, saponin, wax and resin (Perry et al., 1980; Watt et al., 1962). A proteolytic enzyme, curcain, can be obtained from the latex by alcohol and acetone precipitation (Nath and Dutta, 1991) and has been reported to have wound healing activity in mice (Nath and Dutta, 1997; Villegas et al., 1997). A novel cyclic octapeptide named curcacycline A was isolated from the latex, which inhibits the classical pathway of human complement and proliferation of human T-cells (Van de Berg et al., 1995). A new cyclic nonapeptide named curcacycline B was observed by Catherine et al.

(1997) can enhance the rotamase activity of cyclophilin B. In Mexico the latex is used for fungal infections in mouth, wasp and bee stings and digestive troubles in children (Watt and Breyer-Brandwijk, 1962; Schmook & Serralta-Peraza, 1997). In tropical Africa and Southeast Asia, the latex is said to effectively treat scabies, eczema and ringworm. Furthermore, it is used as a mouth rinse to treat bleeding gums and to sooth a baby's inflamed tongue. In The Philippines and Indonesia, a little latex on absorbent cotton is used to cure a toothache (Perry et al., 1980; Watt et al., 1962; Burkill 1935; Heyne, 1987; Suwondo, 1993).

One of the reported traditional used of *Jatropha curcas* latex is as hemostatic or styptic: for example, when the latex is applied directly to cuts and bleeding wounds, the bleeding will soon stops (Dalziel, 1955; Watt and Breyer-Brandwijk, 1962; Neuwinger, 1996). These observations indicate the presence of procoagulant activity in this plant. Osaniyi and Onajobi (2003) were interested in investigating coagulant activity of the latex as this has great medical potentials. Investigation of the coagulant activity of the latex of *Jatropha curcas* showed that whole latex significantly reduced the clotting time of human blood. They also discovered that diluted latex, however, exhibits anticoagulant activity. This indicates that *Jatropha curcas* latex possesses both procoagulant and anticoagulant activities (Osaniyi and Onajabi, 2003). Fazwishni and Kristiani (2007) conducted a mutagenicity test to evaluate the mutagenicity of the latex by Ames method, and the result showed that *Jatropha curcas* latex produces no mutagenicity activity. The latex can be used as anti-inflammation by massaging the latex to the traumatic area (De Feo, 1989).

Jatropha curcas latex also exhibited good antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes, Candida albicans and Trichophyton sp. (Oyi et al., 2007). The antimicrobial activities of the latex could be due to the presence of secondary metabolite such as tannins, flavonoids and saponins which have been confirmed to be present in the latex (Levens et al., 1979). Tannins coagulate the cell wall proteins resulting in bactericidal activity in high concentrations. Saponins are surface-active agents where they alter the

permeability of the cell wall thus facilitating the entry of toxic materials or leakages of vital constituents from the cell. Flavonoids are phenolic compound in nature; they act as cytoplasmic poisons where they inhibit the activity of enzymes (Iwu et al., 1990; Pathak et al., 1991).

2.2.1.4 Fruits and Seeds

The seeds of *Jatropha curcas* have a good potential as a fuel substitution. However, the seeds in general, are toxic to human and animals. Curcin is a toxic protein isolated from the seeds also contains a high concentration of phorbol esters (Adolf et al., 1984; Makkar et al., 1997). Two new esterases (JEA and JEB) and a lipase (JL) were isolated from the seeds (Staubmann et al., 1999). Esterases have been isolated from mammalian tissue as well as from microorganisms and plants. Because of the simple availability, especially microbial esterases are of interest for the application in industrial processes. They are widely used for the resolution of racemic mixtures of compounds, in order to produce pure enantiomers (Staubmann et al., 1999). Lipases constitute a significant portion of enzymes that have been investigated for use in organic synthesis (Hills et al., 1990). Lipases have potential use in lipid modification to produce synthetic lipids for various industrial applications (Macrae, 1983; Svensson et al., 1992).

In Egypt, the seed is used for the treatment of arthritis, gout and jaundice (Khafagy et al., 1977). The seed of this plant has also been used traditionally for the treatment of many ailments, including burns, convulsions, fever and inflammation (Osoniyi and Onajobi, 2003). The seed oil can be applied to treat eczema and skin diseases and to sooth rheumatic pain (Heller, 1996). The oil is also used externally for the treatment of sciatica, dropsy and paralysis (Mujumdar et al., 2000). The 36% linoleic acid (C18:2) content in *Jatropha curcas* kernel oil is the possible interest for skin care (Kumar and Sharma, 2008). The oil has a strong purgative action also is widely used for skin diseases and to sooth pain such as that caused by rheumatism. The seed oil is also reported can be a remedy against

syphilis. In South Sudan, the seeds as well as the fruits are used as a contraceptive or as abortifacient (List and Horhammer, 1979).

2.2.1.5 Roots

Jatropha curcas roots are used for treating eczema, scabies, ringworm and gonorrhea where these diseases are caused by fungi and bacterial infections (Aiyelaagbe et al., 2007). Phytochemical analysis of the extracts revealed the presence of many secondary metabolites, including steroids, alkaloids and saponins. Chen et al. (1988) had isolated diterpenoids jatropholone A and B and a new diterpenoid named jatropholol from the roots of *Jatropha curcas*. Previous work (Dekker et al., 1986; Aiyelaagbe et al., 2000; Aiyelaagbe, 2001) has shown that many Jatropha species possess antimicrobial activity. Aiyelaagbe et al. (2007) had investigated an in vitro antimicrobial activity against different microorganisms responsible for various infections, especially sexual transmitted diseases. The results displayed potent antimicrobial activity against the target organisms giving MIC as low as 0.75 μg/ml. It was confirmed the potency of this plant in treating infections, including sexually transmitted infection. The roots contain yellow oil with strong anti helminthic properties (Sirisomboon et al., 2007). The roots are also reported being used as an antidote for snake bites. During an ethnobotanical survey of Raignad and Ratnagiri districts of Konkan area, a part of the West coast of India, use of *Jatropha curcas* roots to control dysentery and diarrhea was recorded. In order to control these symptoms, a dose of two tablespoons of root suspension with butter milk is recommended, once or twice a day depending on the severity of the symptoms (Mujumdar et al., 2000). It is reported that the root is triturated with a little asafetida and given with butter milk in treating dyspepsia and diarrhea (Desai, 1975; Dymock et al., 1976; Nadkarni, 1976). Based on ethnobotanical practice the root extracts of this plant were investigated by Mujamdar et al. (2000) for pharmacognistic studies and evaluation of anti-diarrheal activity in albino mice. The proposed mechanism of action was through a combination of inhibition of elevated prostaglandin biosynthesis and reduction in propulsive movements of the small intestine (Mujamdar et al., 2000).

The roots of this plant are applied locally in paste form after crushing for the treatment of inflammation by Bhil tribes from Rajasthan area in India on an empirical basis (Joshi, 1995). Considering this information, Mujamdar and Visar (2004) evaluated for local and systematic anti-inflammatory activity using various animal models in the present investigation. Anti-inflammatory activity of topical application of *Jatropha curcas* root powder in paste form in TPA-induced ear inflammation was confirmed in albino mice and the successive solvent extraction of these roots was carried out by ether and methanol. The resultant anti-inflammatory activity might be due to the effects on several mediators and arachidonic acid metabolism involving cyclo-oxygenase pathway resulting in prostaglandin formation, anti-proliferative activity leading to reduction in granular tissue formation and leukocyte migration from the vessels (Mujamdar and Visar, 2004). The roots can also be used in decoction as a mouthwash for bleeding gums and toothache.

2.3 PHENOLIC COMPOUND

Phenolic compounds are compounds that have one or more hydroxyl groups attached directly to an aromatic ring. The entire group is based upon phenol structure. The aromatic ring is of course referring to benzene. Figure 2.1 illustrated the chemical structure of phenol.

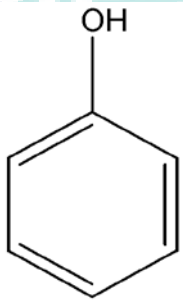


Figure 2.1: Phenol Chemical Structure

Phenolic compounds cover a very large and diverse group of chemical compounds. Classification of these compounds into groups based on the number of carbons in the

molecule had been done by Harborne and Simmonds (1964). Table 2.1 summarized the classification of phenolic compounds.

Table 2.1: Classification of Phenolic Compounds

Structure	**Class**
C_6	Simple phenolics
C_6 - C_1	Phenolic acids and related compounds
C_6 - C_2	Acetophenones and phenylacetyl acids
C_6 - C_3	Cinnamic acid, cinnamyl aldehydes, cinnamyl alcohols
C_{15}	Chalcones, aurones, dihydrochalcones
C_{15}	Flavans
C_{15}	Flavones
C_{15}	Flavonones
C_{15}	Flavononols
C_{15}	Anthocyanidines
C_{15}	Anthocyanins
C_{30}	Biflavonyls
C_6-C_1-C_6, C_6-C_2-C_6	Benzophenons, xanthones, stilbenes
C_6, C_{10}, C_{14}	Quinones
C_{18}	Betacyanins
Lignans, neolignans	Dimmers or oligomers
Lignin	Polymers
Tannins	Oligomers or polymers
Phlobaphenes	Polymers

Source: Vermerris and Nicholson, 2008

2.3.1 Plant Phenolics Occurrence

Phenolic compounds are synthesized by plants during normal development of plant tissues (Harborne, 1982; Pridham, 1960) and in response to stress conditions such as wounding, UV radiations, infections, and other stress (Beckman, 2000; Nicholson et al., 1992). In plant, phenolics may function as phytoalexins, anti-feedant, antioxidant, attractants for pollinators, contributors to the plant pigmentation, and protective against UV radiations (Shahidi and Naczk, 2004). In foods, phenolics may contribute to the bitterness, color, flavor, astringency, odour, and oxidative stability of the products (Shahidi and Naczk, 2004).

Phenolics are not distributed equally in plants at the tissue, cellular and sub-cellular levels. Soluble phenolics are compartmentalized within the plant cell vacuoles, while insoluble phenolics are the component of the cell walls (Beckman, 2000). The outer layers of plant cells contain higher levels of phenolics than those located in their inner parts (Bengoechea et al., 1997; Perez-Ilzarbe et al., 1991; Fernandez de Simon et al., 1992).

Cell walls of the plant comprise of lignins and hydroxycinnamic acids that are linked to various components (Wallace, 1994; Musel et al., 1997; Baucher et al., 1998). These compounds aiding the mechanical strength of cell walls play a regulatory role in plant growth and morphogenesis as well as in the cell response to stress and pathogen (Wallace, 1994; Baucher et al., 1998; Kamisaka et al., 1990; Lewis and Yamamoto, 1990; Scalbert, 1993). Ferulic and p-coumaric acids may be esterified to form pectins and arabinoxylans or cross-linked together to the cell wall polysaccharides in the form of dimmers (Brett et al., 1999). The role of these cross-links may involve in cell-cell adhesion (Ng et al., 1998), serve as a site for the formation of lignin (Grabber et al., 2000) and contribute to the thermal stability of plant food texture (Waldron et al., 1997).

2.3.2 Tannins

Tannins are found abundantly in many different plant species, particularly in leaves, fruits, barks and woods. Tannins are water-soluble compounds that have a molecular weight between 500 and 3000. Tannins have the ability to act as an anti-nutrient compound of plant origin because they can precipitate proteins, decrease the utilization of vitamins and minerals and inhibit the digestive enzyme (Karamac et al., 2007). In addition, it has been considered as a health promoting component in plant-derived foods and beverages. Research done by Cos et al. (2004) and Awika et al. (2006) had shown the ability of tannins to have anticarcinogenic, antimutagenic potential and antimicrobial properties. Several studies have reported on the antioxidant and anti-radical activity of tannins (Amarowicz et al., 2004; Alasalvar et al., 2006; Amarowicz, 2007). It can also exert physiological effects, such as reduce blood pressure, accelerate blood clotting, produce liver necrosis, decrease the serum lipid level and modulate immuno-responses (Zivkovic et al., 2009).

Tannins can be classified into two groups, which are condensed tannins and hydrolyzable tannins. Condensed tannins, which also referred to as proanthocyanidins are oligomeric or polymeric flavonoids consisting of flavan-3-ol (catechin) units. Hydrolyzable tannins are esters of gallic acid or its other derivatives with a polyol which is usually glucose.

2.3.3 Gallic Acid

Gallic acid is also known as 3,4,5-trihydroxybenzoic acid. The chemical formula for gallic acid is $C_6H_2(OH)_3COOH$. This compound is found in almost all plants. Gallic acid can occur as a free molecule in plants alone or as part of tannins. The chemical structure of gallic acid is shown in Figure 2.2.

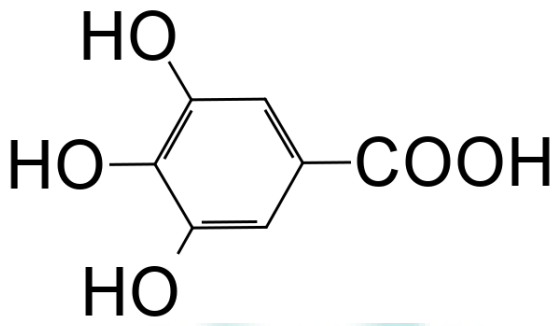


Figure 2.2: Chemical Structure of Gallic Acid

Gallic acid is an important chemical used in the food, agriculture and pharmaceuticals industries (Lu et al., 2008). It becomes an interest to these industries as it was found to have many significant biological activities. Gallic acid has antifungal and antiviral properties, which can protect the plant from fungus. Like all the other phenolic compounds, gallic acid is also a powerful antioxidant. It neutralizes free radicals and help to protect human cell against oxidative damage. Quite a few studies have shown that gallic acid shows cytotoxicity against cancer cells, without damaging the healthy cells. Gallic acid has astringent properties that can be used to treat hemorrhage, especially in chronic condition such as stomach ulcers. It can be used as gargle to cure inflamed mucous membrane or bleeding gums. In the form of ointments that contain gallic acid is used to treat psoriasis and external hemorrhoids. Gallic acid is also used to treat albuminuria and diabetes.

2.3.4 Extraction of Phenolic Compounds

Extraction process is the main step in the recovery and isolation of phenolic compounds from various plant-based materials. The aim of extraction using solvent is to remove all phenolic compounds from the plant material by transferring them into the liquid phase, which is the solvent. Phenolic compounds are often most soluble in a solvent that is less polar than water. Extraction efficiency of plant-based materials relies on their chemical nature, proper solvent selection, elevated temperature, sample particle size and mechanical agitation to maximize phenolic compound recovery. Some of them are prone to degradation

due to light and oxidation during sample preparation and extraction. It is recommended that all the samples are being analyzed within one day of extraction. The most frequently used solvents for extraction of phenolic compounds are methanol, ethanol, acetone, ethyl acetate, and their aqueous solvents (Antolovich et al., 2000).

There are various extraction techniques that can be employed to extract phenolic compounds. The common extraction techniques used nowadays are ultrasound-assisted extraction, microwave-assisted extraction, Soxhlet extraction, supercritical fluid extraction, and many more. Therefore, the extraction method employed should be carefully selected in order to obtain full recovery of targeted phenolic compounds.

2.4 GENERAL PRINCIPLE OF EXTRACTION

Extraction allows isolation of the analytes from plant material using various solvent, as a rule, in order to increase the polarity of the extracting agent (Nyiredy, 2004). When the plant material and the solvent are in intimate contact, the solutes can diffuse from the plant material into the solvent. This will result in the separation of the components originally in the plant material. However, the rate of diffusion may be comparatively slow because the cell walls of the plant material provide another resistance to diffusion. To overcome this, grinding of the plant material into sufficiently small sizes will increase the number of cells directly exposed to the solvent. Figure 2.3 shows the schematic diagram for the extraction process in solid-liquid.

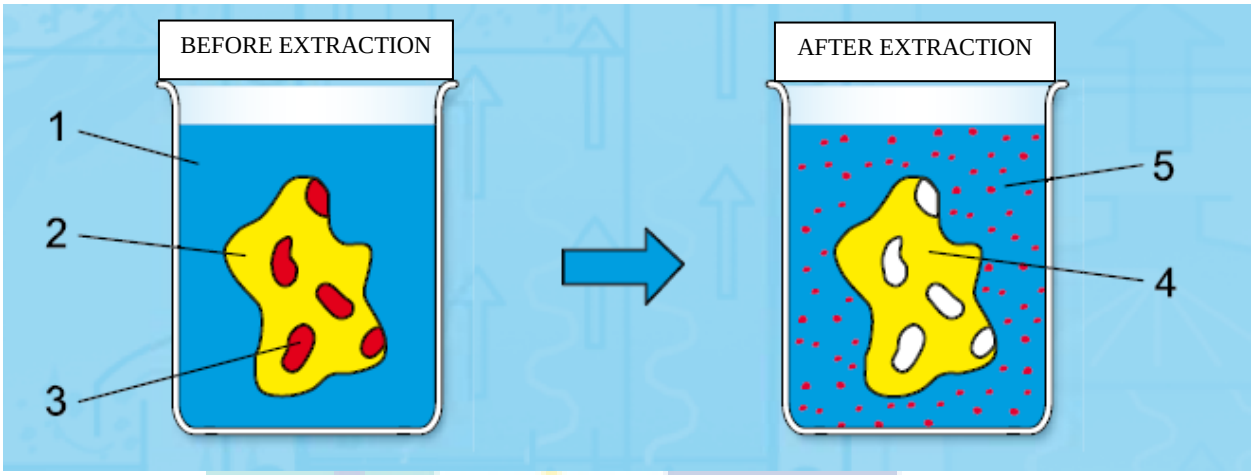


Figure 2.3: Schematic Diagram for the Extraction Process in Solid Liquid. 1. Solvent, 2. Extraction Material (Solid Phase), 3. Solute Components, 4. Depleted Solid Carrier Phase, 5. Solvent with Solute Components.

2.4.1 Solvent Choices

The solvent choices for the extraction process of compounds from plants depend on the nature and the type of compound to be extracted. There are many factors to be considered in order to choose the suitable extraction solvents. The factors are as follows:

- Polarity
- Boiling point – this should be low in order to facilitate removal of the solvent from the product
- Latent heat vaporization
- Reactivity – the solvent should not react chemically with the extract, nor should it readily decompose
- Safety in use – the solvent should, if possible, be non-flammable and should not present a toxicity hazard to technician or consumer; its disposal should not endanger the environmental
- The solvent should be available in substantial quantities
- Costs

- Suitability for re-use

The most common solvent used for the extraction of phenolic compounds from plant material are methanol, ethanol, water, ethyl acetate, acetone and their aqueous solvents (Antolovich et al., 2000). For this study, ethanol and water were chosen for the extraction process. This choice is due to the safety issues, costs and availability of the solvent to be purchased.

2.4.2 Shake Flask Extraction

Shaking flask extraction is one of the conventional extraction techniques that had been used for a long time. Mainly, the samples are soaked in the solvent in a conical flask and cover-up to avoid spilling. An orbital shaker with speed and time controller is used to facilitate shaking.

The main advantage of this technique is the shaking that promotes constant agitation and uniform contact of sample and solvent. Furthermore, it is a simple technique to be used. However, this extraction technique efficiency depends mainly on the type of solvent applied for the isolation. In addition, this technique consumes a lot of time and solvent (Babić et al., 1998).

2.4.3 Soxhlet Extraction

Soxhlet extraction is a general and well-established technique that has been used for a long time. The technique is used for the isolation and enrichment of extracts that have low volatility, and thermal stability targeted compound. The Soxhlet extractor enables the solids to be extracted with fresh warm solvent. It can increase extraction rate as the solid sample is in contact with fresh warm solvent.

The Soxhlet extraction system is shown in Figure 2.4. The solid sample is placed in a cellulose or ceramic thimble and placed in the thimble-holder. The thimble-holder is

connected to a flask containing extraction solvent at the bottom, and a condenser is connected above the thimble-holder. The extraction solvent is heated to reflux. The solvent vapor travels up a siphon and through the condenser, where it condensed and drips onto the solid sample in the thimble-holder. Once the solvent reaches overflow level, the thimble-holder is emptied by a siphon, with the solvent running back down to the flask carrying extracted solutes into the bulk solvent. In the flask, solute is separated from solvent using distillation. Solute is left inside the flask as the solvent vapors back into the thimble-holder via a siphon for the next cycle of extraction. This cycle is repeated until complete extraction is achieved.

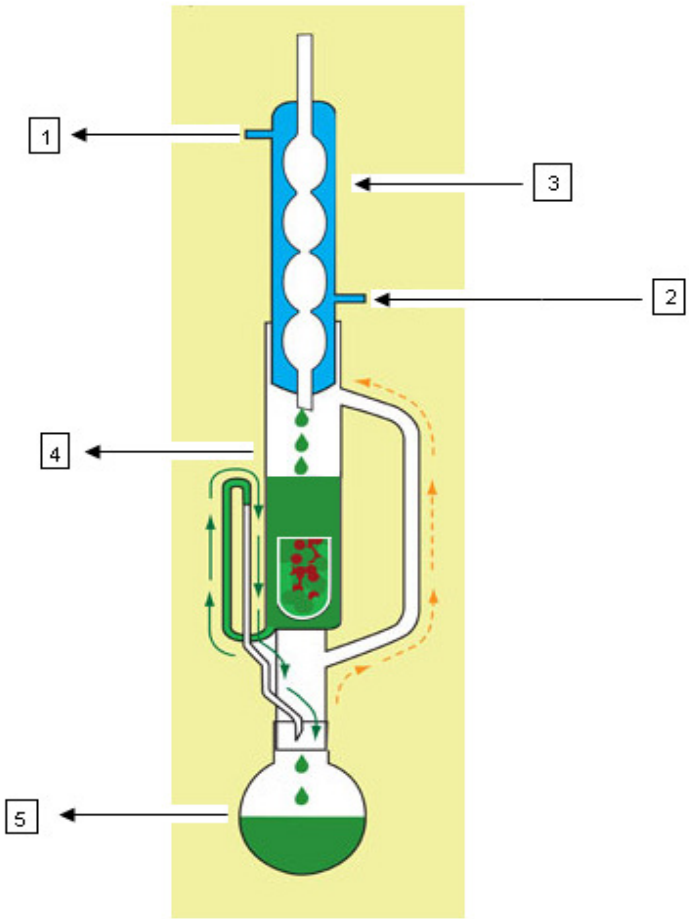


Figure 2.4: Soxhlet Extraction System. 1. Water Outlet, 2. Water Inlet, 3. Condenser, 4. Thimble-Holder, 5. Round Bottom Flask

Soxhlet extraction is commonly used and had been applied for the extraction of lipids and polycyclic aromatic hydrocarbons in natural products (e.g. coffee, soybean and coconut oil, mushrooms, fruits and vegetables) (Hofler et al., 1995; Lao et al., 1996; Prange et al., 1999). In addition, Lin and his co-workers (1999) also used Soxhlet extractor in the investigation of anti-inflammatory and antimicrobial activities of nine African plants.

Some advantages had been displayed by using Soxhlet extractor. The sample is repeatedly brought into contact with the fresh portions of the solvent, thereby helping to displace the transfer equilibrium. The temperature of the system remains relatively high since the heat applied to the distillation flask reaches the extraction cavity to some extent. There will be no filtration is required after the extraction step. Furthermore, the Soxhlet method is very simple and cheap (Luque de Castro and Garcia-Ayuso, 1998).

The main disadvantages of Soxhlet extractor are long extraction time and large consumption of solvents, cooling water and electricity. In addition, agitation cannot be provided in the Soxhlet device to accelerate the process. The large amount of solvent used requires an evaporation or concentration procedure. There will be a possibility of thermal decomposition of the target compounds cannot be ignored as the extraction usually occurs at the boiling point of solvent for a long time.

2.4.4 Ultrasonic-Assisted Extraction (UAE)

The term ‘ultrasonic’ represents sound waves having a wave frequency above approximately 20 kHz to 1 GHz. It usually generated by a transducer which converts mechanical or electrical energy into high-frequency vibrations (Sun et al., 2008). Sound waves are mechanical vibrations in solid, liquid or gas. Unlike electromagnetic waves, sound waves must travel in the matter and involved in expansion and compression cycles travelling through the medium. Expansion pulls molecules apart, whereas compression pushes the molecules together. The expansion cycle produces negative pressure in the liquid medium. When the negative pressure exceeds the tensile strength of the liquid,

bubbles or cavities are formed in the liquid. Once the bubbles are formed, it will absorb the energy from the sound waves and grow during the expansion cycles and recompress during compression cycles. The process by which the vapor bubbles form, grow and undergo implosive collapse is known as ‘cavitation’. Close to a solid boundary, the cavity will collapse and produces high–speed jets of liquid. The impact of the high-speed jets on the solid surface is very strong. This will result in serious damage to impact zones and can produce newly exposed, highly reactive surfaces (Luque-Garcia and Luque de Castro, 2003). Figure 2.5 shows the expansion and compression cycles produce by ultrasound.

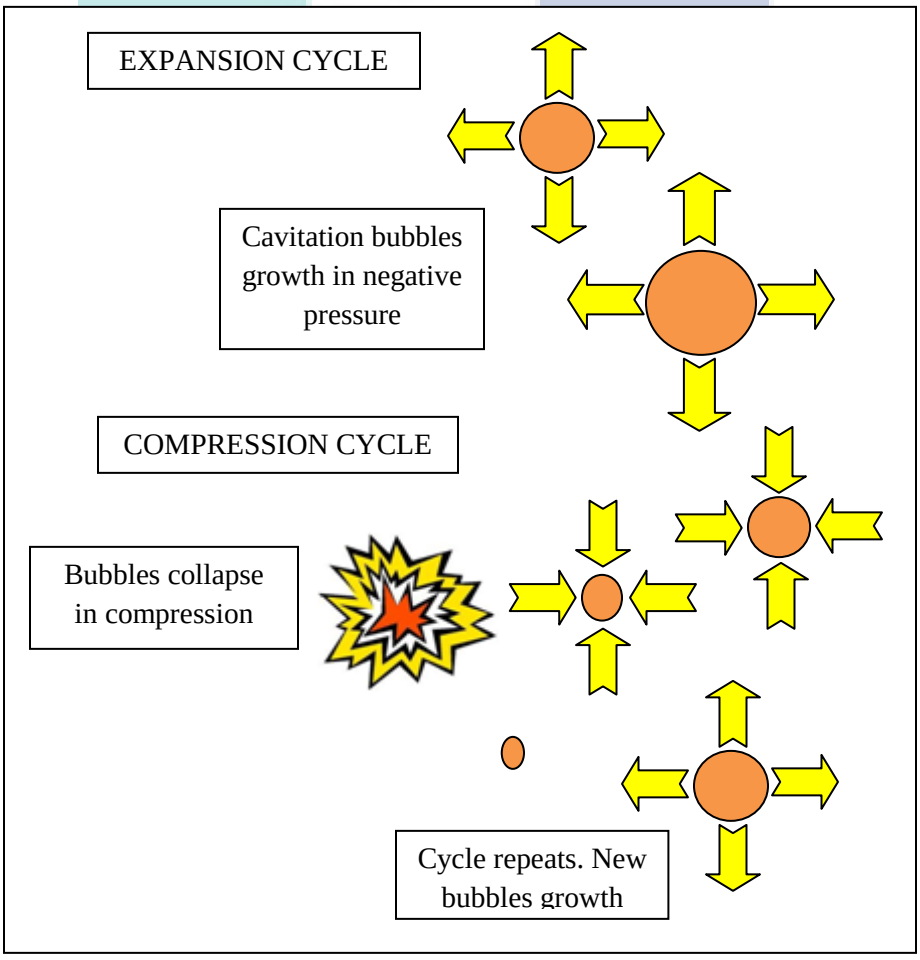


Figure 2.5: Expansion and Compression Cycles Produce by Ultrasound

The use of ultrasound in the extraction of analytes from different types of plant materials has been widely used. The mechanical effects of ultrasound induce a greater penetration of the solvent into cellular materials and improve mass transfer. Introducing ultrasound in the extraction process can disrupt cell walls and thus, facilitating the release of its contents. Therefore, efficient cell disruption and effective mass transfer are cited as two major factors leading to the effective extraction with ultrasonic power (Mason et al., 1996).

Practical issues for ultrasonic-assisted extraction should be taken into account in order to obtain efficient and effective extraction process. First of all, is the plant characteristic such as moisture content and size particles have significant effects towards the extraction process. Furthermore, other factors, including ultrasonic frequency, temperature, pressure, sonication time and solvent used for extraction may also contribute on the efficiency of the extraction process as well (Wang and Weller, 2006).

Ultrasonic-assisted extraction certainly provides many benefits in the extraction process. Among them include an increase in extraction yield with faster kinetics, reducing the operating temperature allowing the extraction of thermolabile compounds, less solvent consumption, and it is easy to operate. Cavitation increases the polarity of the system, including extractants, analytes and matrix, which can increase in the extraction efficiency. In addition, the operating time is invariably shorter than using Soxhlet extractor.

However, there are also some disadvantages in using ultrasonic-assisted extraction. The extraction yields and kinetics may be linked to the nature of the plant materials. The need for separation of the extract from the sample after the extraction lengthens the overall duration of the process.

2.4.5 Microwave-Assisted Extraction (MAE)

Microwave is electromagnetic waves, which consist of electric field and magnetic field. These fields oscillate perpendicularly to each other in the frequency ranging from 0.3 to 300 GHz. Microwave can penetrate into certain materials and interact with polar molecules to produce heat. As a result, microwaves can heat a whole material and penetrates deep within simultaneously. Microwave-assisted extraction technique is based on the principle of heating up the water molecules (moisture content) in the plant materials, where it is known as the microwave heating target. When the moisture evaporates, plant cell will swell, which produce pressure on the cell wall (Wang and Weller, 2006). The pressure pushes the cell wall from inside, stretching it and ultimately rupturing it. This will facilitate the active compounds to be released from the ruptured cells into the surrounding solvent (Mandal et al., 2007). The heating of the microwave energy acted directly on the molecules by ionic conduction and dipole moment (Sparr Eskilsson and Bjorklund, 2000). Thus only selective and targeted materials can be heated based on their dielectric constant.

The success of microwave-assisted extraction depends on two parameters that define the dielectric properties of the solvent. The first is the dielectric constant, ε'_{δ}, that describes the polarizability of the molecule to an electric field. It measures the ability of the material to store the electromagnetic radiation. The higher the dielectric constant, the more energy is absorbed by molecules and the faster the solvent reaches its boiling point. The second parameter is the dielectric loss factor, ε''_{δ}, which measure the efficiency of the absorbed microwave energy to convert into heat inside a material when an electric field is applied. From these two parameters, another solvent dielectric property can be defined as dissipation factor, tan δ, as given by Eq. 2.1 (Mandal et al., 2007).

$$\tan\delta = \frac{\varepsilon''_{\sigma}}{\varepsilon'_{\sigma}} \tag{2.1}$$

This dissipation factor measures the ability of the solvent to absorb microwave energy in the form of heat.

Microwave-assisted extraction may be largely affected by a variety of factors, including power and frequency of the microwave, duration of microwave radiation, moisture content and particle size of plant materials, type and concentration of the solvent, ratio of solid to liquid, extraction temperature, pressure and number of cycle (Wang and Weller, 2006, Yang and Zhang in press). Out of these factors, the most important factors to be consider is the solvent for microwave-assisted extraction, which can affect the solubility of the target components and the absorption of microwave energy determine by its dissipation factor (Chen et al., 2008). Furthermore, the addition of some amount of water in the solvent (i.e the concentration of aqueous solution) significantly will influence the extraction yield (Hemwimon et al., 2007). In general, the solvent choice is important not only towards its affinity with the target compound but also its ability to absorb microwave energy.

Microwave-assisted extraction has been recognized as a potential alternation extraction technique for the extraction of components in plant materials. Microwave-assisted extraction generally shows evident advantages with shorter time of extraction, higher extraction yield, higher selectivity and better quality of the target compound (Chen et al., 2007). This extraction technique surpasses other modern techniques such as supercritical fluid extraction due to its process simplicity and low cost. The main advantage that microwave-assisted extraction has over ultrasonic-assisted extraction is reduced in extraction time.

Nevertheless, microwave irradiation accelerates the chemical reactions or changing some of the target compounds (Ghani et al., 2008; Zhao et al., 2006a; Zhao et al., 2006b; Zhou et al., 2006) and other operational conditions (e.g., high extraction pressure) that might results in the reduction of extraction yield. An additional filtration or centrifugation is necessary to remove the solid residue from extract. Moreover, extraction efficiency may

be reduced when either the target compounds or solvent are non polar or when the viscosity of the solvent is too high (Cravotto et al., 2008; Wang and Weller, 2006). Extraction using microwave is unsuitable for the extraction of thermolabile compounds (Huie, 2002).

2.4.6 The Kinetics of Solid-Liquid Extraction in Suspension

The classical extraction process of bioactive compounds from plant materials by a solvent is via diffusion of extractive substances through cell walls. This generally involves two main stages which are dissolution of the soluble constituents on or near surfaces of plant material (so called washing) and mass transfer of soluble compounds from the plant material into the solution by diffusion and osmotic process (so called slow extraction) (Coulson and Richardson, 1991). The latter stage usually is slower than the former one and is responsible for limiting the rate of extraction process.

The kinetics of extraction process of bioactive compounds from plant materials has often been modeled using the unsteady diffusion through plant material (Ponomaryov, 1976), the film theory (Stankovic et al., 1994; Milenovic et al., 2002), and the empirical equation of Ponomaryov (Ponomaryov, 1976; Pekic et al., 1988).

2.4.6.1 Kinetic Model Based on Film Theory

This model is describing the extractive mass transfer rates of substances from the particles in solution where some particles form a thin diffusion layer. This model was assumes as below.

- Volume and external surface of the particles does not change during the extraction
- Suspension of plant material is homogeneous and the ideal solution had been mixed
- Solubility of extractive substances in the solvent at a temperature which the extraction is performed is known

- Washing of the extractive substances from the destroyed plant cells at the outer surface of the particles take place
- Mass transfer resistance is concentrated in the diffusion layer around the particles which the thickness does not change
- Speed of the overall process (internal diffusion and diffusion of outer surface of the particle solution) is proportional to the effective diffusion coefficient (approximately equal to the coefficient of diffusion of extractive substances in the solvent), which does not change during the extraction.

Mass balance of extractive substances in the liquid phase leads to the next differential equation.

$$\frac{D_{ef}}{\delta}=(c_s-c)A=V\frac{dc}{dt} \tag{2.2}$$

Where c – the concentration of the solution during extraction, V – volume of particles, A – outer surface of the particles, c_s – concentration of saturated solution, δ - the thickness of liquid film diffusion, D_{ef} – effective diffusivity coefficient and t – time. From Eq 2.2, after the separation of the variables, integration form of this equation was as follows.

$$\int_{c_w}^{c}\frac{dc}{c_s-c}=\frac{D_{ef}A}{\delta V}\int_0^t dt \tag{2.3}$$

Where

$$-\ln\frac{c_s-c}{c_s-c_w}=\frac{D_{ef}A}{\delta V}t \tag{2.4}$$

If you introduce the change

$$b = \frac{c_w}{c_s} \tag{2.5}$$

$$k = \frac{D_{ef} A}{\delta V} \tag{2.6}$$

Where b is the washing coefficient and k is slow extraction coefficient. Simplification of Eq 2.6 is as below.

$$\left(1 - \frac{c}{c_s}\right) = (1 - b)e^{-kt} \tag{2.7}$$

According to Eq 2.7, when t → ∞ and c → c_s, the concentration of the solution during extraction approaches exponentially to become concentration of saturated solution. If the value of b = 0, there is no washing happen and if b = 1, means it has wash completely. It is reliable value when it is 0 < b <1. The ratio A/V is describing the specific surface of particles of plant material and its reciprocal value can be understood as the characteristic dimension of the particles. Washing coefficient and slow extraction coefficient can be calculated from the linearized form of Eq 2.8.

$$\ln\left(1 - \frac{c}{c_s}\right) = \ln(1 - b) - kt \tag{2.8}$$

2.4.6.2 Kinetic Model Based on Unsteady Diffusion in Plant Material

The extraction of raw material in solid-liquid extraction takes place by diffusion. Easy diffusion mechanism is not as simple as in gases and liquids. Usually the speed of mass transfer of extractive substances through solid material soaked in the solvent can be described by Fick's law. The differential form of this equation is as follows.

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \tag{2.9}$$

Where c is the concentration of extractive substances, t refers to time, x is the length in the direction of mass transfer and diffusion coefficient and D is extractive substances. The assumptions for Eq 2.9 are as follows.

- The particles of plant material are isotropic and equal in size
- Mixing the suspension was extensively
- Diffusion occurs only in one direction
- The coefficient of diffusion of extractive substances through particle is constant
- The concentration of extractive substances in a particle at the beginning (c_o) is uniform
- The concentration of extractive substances on the surface of particles (c_∞) is constant
- Mean concentrations of extractive substances in the particle ($\bar{c}$) changes with time, according to the change of spatial distribution of concentration of extractive substances, and
- At each point in the axis of the particles $\left(\frac{\partial C}{\partial c}\right)_{x=0} = 0$

Intensive mixing effect is introduced to speed up the process. The assumption that the concentration of extractive substances on the surface of the particles is constant means that there is no resistance to diffusion of extractive substances in the fluid around the particles or the solution concentration in the suspension is very large, so that the solution concentration does not change with time, or that the solution is continuously renewed with fresh solvent. In most cases, the resistance to diffusion within the particles is large enough so that the valid assumption of negligible resistance to diffusion in solution.

When the plant material is immersed in solvent, extractive substances will diffuse through the outer surface of the particles. If extraction is longer, the concentration of extractive substances in the particles will reduce the value of c_∞. The difference (c_o-c_∞) is a measure of the mass of extractive substances that can be extracted from the solid phase for an infinitely long time, or they can stop the extraction at some time, t. If the mean concentration of extractive substances in the particles, $\bar{c}$ is used, then the difference ($\bar{c}$ - c_∞) is a measure of the mass of extractive substances of the residual particles up to the given moment. The ratio of mass of extractive substances in the residual particles and mass of extractive substances will be extracted after infinite time depends on the dimensionless criteria, Dt/h^2.

$$\frac{\bar{c} - c_\infty}{c_o - c_\infty} = f\,\frac{Dt}{h^2} \tag{2.10}$$

Where: h - flat plate or cylinder or sphere radius for a particle geometry. The function f (Dt/h^2) can be found in the literature (Ponomaryov, 1976). For example, for a flat plate, whose two dimensions are much larger than the thickness, the Eq 2.10 has the following form.

$$\frac{\bar{c} - c_\infty}{c_0 - c_\infty} = \sum_{n=0}^{\infty} \frac{8}{\lambda(2n-1)^2}\left(e^{-\left(\frac{\pi^2(2n-1)}{4}\right)\left(\frac{Dt}{h^2}\right)} \right) \tag{2.11}$$

or

$$\left(\frac{\bar{c} - c_\infty}{c_o - c_\infty}\right) = \frac{8}{\lambda^2} e^{-\frac{\pi^2}{4}\frac{Dt}{h^2}} \tag{2.12}$$

Eq 2.12 is applicable for $\frac{\pi^2 Dt}{4h^2} > 1.2$

In the case of other simple geometric shapes (spheres or cylinders) a, similar procedure as above is used, the equations of general form is as below.

$$\frac{\bar{c} - c_\infty}{c_o - c_\infty} = \alpha e^{-\beta \frac{Dt}{h^2}} \tag{2.13}$$

Where α and β are constants whose values depend on the particle shape If it is assumed that c_∞ = 0, then the Eq 2.13 can be simplified to Eq 2.14

$$\frac{\bar{c}}{c_o} = \alpha e^{-\beta \frac{Dt}{h^2}} \tag{2.14}$$

If the concentration of c_o and $\bar{c}$ are replace with the corresponding masses of extraneous substances present in plant raw material at the beginning, q_o, and after some time of extraction, q, we get

$$\frac{q}{q_o} = \alpha e^{-\beta \frac{Dt}{h^2}} \tag{2.15}$$

If the constants of α and β replaced by rinsing coefficient b' and slow extraction k'

$$b' = 1 - \alpha \tag{2.16}$$

$$k' = \frac{\beta D}{h^2} \tag{2.17}$$

Substitute Eq 2.16 and Eq 2.17 in the Eq 2.15 it becomes

$$\frac{q}{q_o} = (1 - b')e^{-k't} \tag{2.18}$$

To calculate the parameters from the Eq 2.18, a linearized form is used as follows.

$$\ln \frac{q}{q_o} = \ln(1 - b') - k't \tag{2.19}$$

2.4.6.3 Empirical Equation of Ponomaryov

This model assumes that the slow extraction period is a linear relationship between the normalized mass of extracted substances $\frac{q_o - q}{q_o}$ and time, t (Ponomaryov, 1976).

$$1 - \frac{q}{q_o} = b'' + k''t \tag{2.20}$$

Where: b" - washing coefficient, k" - coefficient depending on the direction linearly. The washing coefficient is a measure of mass of extractive substances which are dissolved in a solvent, that is, $b''' = \left(\frac{q_o - q}{q_o} \right)_{t=0}$. Parameter k" is the dissolution rate of extractive substances in relation to their initial mass in slow extraction period, so it can be considered a slow specific speed extraction. Eq 2.20 is applied to the determination of the slow extraction coefficient and the coefficient of washing line.

2.5 SUMMARY

A lot of medicinal uses of *Jatropha curcas* plant parts had been investigated and studied by the researchers. This plant typically contains mixtures of different chemical compounds that may act individually, additively or in synergy to improve health. Numerous biologically active substances have been isolated and characterized from all parts of the *Jatropha curcas* plant. Their mechanisms of action have been studied in associates to a great number of applications of *Jatropha curcas* in traditional medicines. However, the full

potential of *Jatropha curcas* plant is far from being realized both technically and economically for several reasons. The role of *Jatropha curcas* in medicinal uses should be taken into consideration as it shows promising potential in the pharmaceutical field. Commercializing on the medicinal product derived from *Jatropha curcas* may turn out to be more profitable than using Jatropha as fuel substitution. All non-energy uses of *Jatropha curcas* should be tabulated to provide a wide range of options. The investigations of economics of making such products and markets for the products should be vigorously developed.

Consumption of plant foods containing dietary phenolics may significantly contribute to human health. This had been proven by numerous studies, which revealed the benefits of phenolic compounds. Gallic acid is one of the phenolic compounds that also exerts significant biological activities, such as antioxidant, anti-inflammatory, anti-fungal and carcinogenic properties. This compound had attracted considerable interest among the foods, pharmaceutical and agricultural industries. Extraction of phenolic compounds is influenced by many factors and careful considerations need to be taken in order to effectively extract the targeted phenolic compounds.

In search of the best extraction techniques, it is better to compare both conventional extraction techniques with modern extraction techniques. Better extraction techniques mean lower economic costs, which is often the primary task in the extraction of natural products. In addition, the concentration of targeted compound in the extracts was another important aim. It can be concluded that, the selection of the best extraction technique would depend on various factors.

Kinetic model based on the film theory, unsteady diffusion on plant material and empirical equation of Ponomaryov can describe the mechanism that happen during the extraction process. The washing coefficient and slow extraction coefficient can be calculated using these three equations.

CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter describes the materials, instruments and methods to accomplish the objectives and scopes of this study. There are four stages involved namely as sample preparations, extraction process, analysis of extract and optimization.

The first stage is the preparation of sample before experiment, second stage involves in experimental process, third stage is where the analysis of the extract using several methods while forth stage is optimization of the parameters used in this study. Details of the procedures on each stage are discussed below

3.2 MATERIAL SELECTION

Jatropha curcas branches were obtained courtesy of Malaysian Agriculture Research and Development Institute (MARDI) situated in Serdang, Selangor. The samples were chosen in this place because there is a *Jatropha curcas* plantation in this institute. The other reason was because of the availability of the samples in substantial quantities to carry out the experiments.

Several chemicals were used to conduct this study had been purchased. For extraction process 95% v/v ethanol purchased from R&M Chemicals was chosen as a

solvent. Chemicals for analysis of extracts used were methanol and acetonitrile (≥ 99.9% HPLC grade from Merck), while phosphoric acid (85% v/v from Fisher-Scientific) were used in separation of fraction components in extracts. Gallic acid (99% HPLC grade from Sigma-Aldrich) was purchased and used as standard chemical for HPLC analysis.

3.2.1 Sample Preparations

The branches that had been collected were cleaned by hand to remove foreign materials. Then, the branches were stripped from the branches using a knife and cut into small pieces prior to drying. An oven with a temperature of 60^{o}C was used to dry the bark until constant weight was achieved. The drying temperature is kept at 60^{o}C to avoid thermal degradation. The dried bark was then cool and grounded using domestic grinder (Panasonic, Malaysia) 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, GmbH) with various granulometric size sieves (0.4 μm – 2.0 mm) to obtain a homogeneous size of ±1.0 mm. The samples were then kept in a seal plastic bag and store at temperature 25^{o}C**.**

Figure 3.1: Sieve Shaker (Fritsch, GmbH)

3.3 EXTRACTION PROCEDURES

Four extraction techniques had been selected in this study namely as shake flask extraction, Soxhlet extraction, ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE). The extraction solvent chosen were water and ethanol. Ethanol was chosen because it is non-toxic food organic solvent, environmental friendly and available in substantial amount.

3.3.1 Shake Flask Extraction

Approximately 20 g of sample were weighed and transferred to a 500 ml conical flask. The sample was then extracted 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 an orbital shaker (Certomat, B. Braun) and shake at the speed of 200 rpm at an ambient temperature of 25^{o}C for selected studied time shown in Table 3.1. A shaker was used to have an efficient mixing of solvent and sample. The extract was then filtered with filter paper (Whatman No. 1, USA) and the filtrate was concentrated under vacuum at 50^{o}C using a rotary evaporator (Heidolph, USA). Table 3.1 presents the ranges of parameters investigated in this study.

Figure 3.2: Orbital Shaker (Certomat, B. Braun)

Table 3.1: Ranges of Experimental Parameters for Shake Flask Extraction

Parameters	**Ranges**
Solvent composition, % Ethanol	0, 20, 50, 70, 95
Time, min	90, 180, 270, 360, 450

3.3.2 Soxhlet Extraction

A Soxhlet apparatus was employed in which approximately 10 g of sample was placed into a thimble with 300 ml of extracting solvent contained in a 500 ml round-bottom flask. Extraction was carried out according to the studied time. The extract was then concentrated under vacuum at 50^{o}C using a rotary evaporator. All the rangers of parameters for this experiment are shown in Table 3.2.

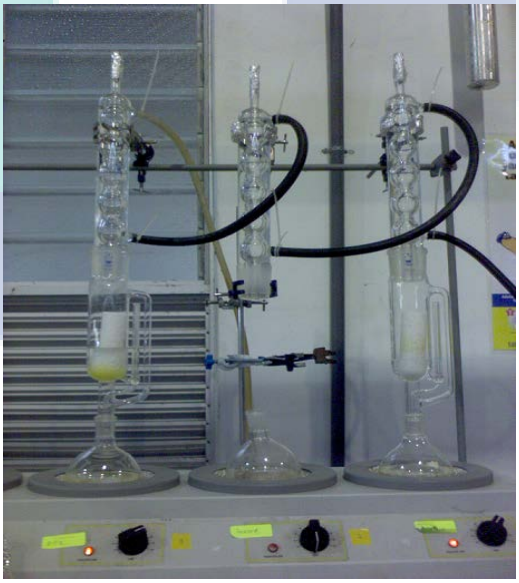


Figure 3.3: Soxhlet Extractor

Table 3.2: Ranges of Experimental Parameters for Soxhlet Extraction

Parameters	**Ranges**
Solvent composition, % Ethanol	0, 20, 50, 70, 95
Time, hours	2, 4, 6, 8, 10

3.3.3 Ultrasonic-Assisted Extraction (UAE)

An ultrasonic bath (model P1800D, Crest Ultrasonic, USA) with a frequency of 40 kHz and power of 230 Watt was used in this study. The extraction of gallic acid was performed by adding approximately 20 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, USA) and the filtrate was concentrated under vacuum at 50^{o}C using a rotary evaporator. Table 3.3 summarizes the ranges of parameters investigated in this study. All the experiments were performed in triplicate.

Figure 3.4: Water Bath Ultrasonic Extractor (Crest Ultrasonic, USA)

Table 3.3: Ranges of Experimental Parameters for Ultrasonic-Assisted Extraction

Parameters	**Ranges**
Solvent composition, % Ethanol	0, 20, 50, 70, 95
Time, min	10, 20, 30, 40, 50, 60
Temperature, ^{o}C	20, 25, 30, 35, 40, 45
Power, Watt	25.56, 51.12, 76.68, 102.24, 127.8, 204.48

3.3.4 Microwave-Assisted Extraction (MAE)

MAE was carried out using domestic microwave (model NN-S215WF, 2450 MHz, Panasonic, Malaysia) with total capacity of 800 Watt. 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 3.4. Then each extract were filtered through filter paper (Whatman no.1, USA) and concentrated under vacuum at 50^{o}C using a rotary evaporator.

Table 3.4: Ranges of Experimental Parameters for Microwave-Assisted Extraction

Parameters	**Ranges**
Solvent composition, % EtOH	0, 20, 50, 70, 95
Time, min	1.5, 2.0, 3.0, 4.0, 5.0
Temperature, ^{o}C	35, 40, 45, 50, 55
Power, Watt	160, 320, 480, 640, 800

3.4 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HPLC is a powerful instrument in analysis. It is a chromatographic system that uses separation technique to separate the mixture of compounds to be analyzed. These compounds are separated from one another by the column packing. There are various chemical and/or physical interaction involves during the separation between the compound molecules and the packing particles. The separated compounds are then detected at the end of the column by a detector that measures their amount. An output from the detector is called a 'liquid chromatogram' which represents a separated compound. For this experiment, gallic acid was separated from the mixture of compounds in the extract by using HPLC. The amount of gallic acid was then quantified.

Figure 3.5: High-Performance Liquid Chromatography (HPLC) (Agilent Technologies, USA)

3.4.1 Sample Preparations

Reference standard solutions for gallic acid were prepared first. Dilutions of gallic acid in water were prepared with concentration ranging from 66.7 µg/ml – 0.53 mg/ml. Two mobile phases which were 0.085% phosphoric acid in water and acetonitrile were used for HPLC detection. There were prepared in a 2 litre Schott bottle and filter it using

vacuum filter. All of the prepared standard solutions were ultrasonicated for 15 minutes and 30 minutes for the mobile phases to remove air bubbles and to ensure the solids were completely dissolved before analysis with HPLC. Prior to injection, all standard solutions and samples were filtered through 0.45 μm PTFE syringe filter into the 1.5 ml vials.

3.4.2 Detection and Identification

Identification of gallic acid was carried out using HPLC equipped with a UV-Visible detector and an auto-sampler (Agilent Technologies 1200 series, USA). The column used for the analysis was a reverse-phase Amide with 150 mm x 4.5 mm i.d. and 5 μm particle diameters (Supelco, Sigma-Aldrich, USA). The chromatographic separation was developed using a mobile phase of 0.085% phosphoric acid in water (solvent A) and acetonitrile (solvent B) at a flow rate of 1 ml/min. The injection volume was set at 10 μL, the detection in UV absorbance at 280 nm and temperature of 30^{o}C. For the reference standard solutions, they were injected at different concentration that had been prepared earlier. A linear regression analysis on the data of peak area versus concentration was carried out. Linear calibration with accuracy of 99.9% was obtained. Chromatographic peaks of samples were identified through comparison with the retention time of gallic acid standards. The content of gallic acid in the extracts was calculated based on the corresponding peak areas and the linear equation obtained from the prepared standard curve.

3.5 KINETIC MODEL

3.5.1 Kinetics of Gallic Acid from Different Extraction Techniques

The kinetic studies of gallic acid for each extraction techniques were estimate using the results of the effect of extraction time. The methods on doing so had been explained in Section 3.3.1, 3.3.2, 3.3.3 and 3.3.4.

3.6 OPTIMIZATION

Optimization refers to the improvement of the performance of a process, a system or a product in order to obtain maximum benefit from it. The term optimization has been commonly used in analytical chemistry to discover the conditions that produces the best possible response (Araujo and Brereton, 1996; Bezerra et al., 2008).

The parameters of UAE were used as the variables in optimizing the gallic acid extraction by using experimental design. An experimental design determined the effect of the independent variable on the dependent variable (response), which then relation between them was expressed through a regression model. In this section, software called Design Expert 7.1.6 (Stat-Ease Inc., Minneapolis, MN) was employed for the experimental design, data analysis and modeling of the experiment. The Box-Behnken design (BBD) of response surface methodology was used to obtain data that fits a second order polynomial in five replicates at the center of the design.

3.7 SUMMARY

The process to obtain gallic acid from the stem bark of *Jatropha curcas* has started from sample preparation and then followed by extraction process using four different extraction techniques namely as shake flask extraction, Soxhlet extraction, ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE). Different extraction techniques used had several factors that contribute to the efficiency of the extraction process that need to be studied.

The kinetics studies of gallic acid were developed using the results of the effect of extraction time for each extraction techniques. For the optimization, the factors that affected the extraction of gallic acid using UAE were determined to find out the optimum points in enhancing the amount of gallic acid. A Box-Behnken design (BBD) was

employed to determine the optimum values of the parameters that produced highest amount of gallic acid.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 INTRODUCTION

This chapter will discussed on the results obtained from each extraction techniques used with different extraction parameters, kinetic studies of gallic acid for each extraction techniques, optimization of UAE parameters, and also comparison of extraction techniques used.

4.2 SHAKE FLASK EXTRACTION

4.2.1 Effect of Solvent Composition

There are many kinds of solvents that can be used to extract phenolic compounds. The most common solvent used in the extraction of plant material is ethanol (Ong et al., 2000) because it is non-toxic and economical. Moreover, ethanol is an acceptable for practical use as it is in compliance with Good Manufacturing Practice (GMP). Throughout the experiments conducted, ethanol and water were the solvents used in the extraction process.

Extraction of gallic acid was done using solvent mixture of ethanol-water of 0%, 20%, 50%, 70% and 95% of ethanol. The yield of gallic acid is shown in Figure 4.1. The mixed solvent system had a better extraction performance. The result indicates that there

was an increased in the yield from 0% (1.605 mg gallic acid/ 100g bark) to 50% (5.521 mg gallic acid/ 100g bark) solvent composition, after which it began to decrease.

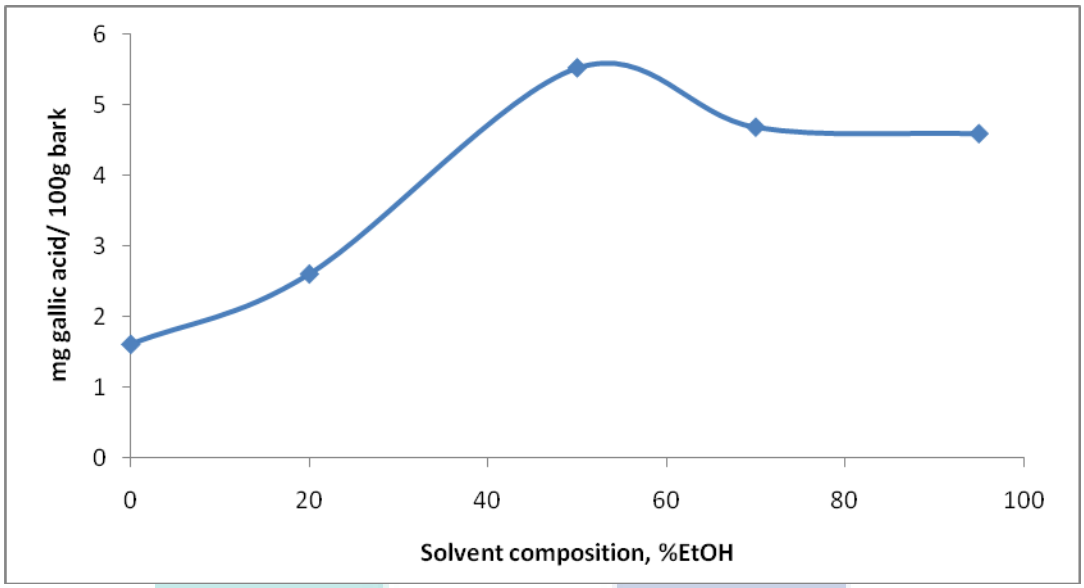


Figure 4.1: Effect of Solvent Composition on the Yield of Gallic Acid for Shake Flask Extraction (20 g Bark with 300 ml Solvent at 25^{o}C for 24 hrs)

It shows that solvent polarity plays an important role. The polarities of the different solvent composition are listed in Table 4.1. The values of polarity index of the solvent mixture can be calculated from the following equation (Lide, 1992).

$$P_m = \varphi_1 P_1 + \varphi_2 P_2 \quad (6.1)$$

The $\varphi_1$and φ_2 are the volume fractions of solvent 1 and 2, respectively, while P_1 and P_2 are the polarity indices of solvent 1 and 2, respectively.

According to Snyder (Brawick, 1997), polarity can be defined as the relative ability of a molecule to engage in strong interactions with other polar molecules. Therefore, polarity represents the ability of a molecule to enter into interactions of all kinds (Brawick,

1997). The addition of water to ethanol tremendously increases the extract yield. Spigno et al., (2007), found out that mixtures of alcohols and water had revealed to be more efficient in extracting phenolic compound compared to the mono-component solvent. Usually, addition small amount of water to organic solvent creates a more polar medium which can facilitate the extraction of phenolic compounds (Spigno et al., 2007). By increasing the proportion of water to ethanol, the polarity of the solvent system is able to extract phenolic substances (highest polarity, low polarity as well as moderate polarity substances) (Zhang et al., 2007). However, the amount of gallic acid decreased with the decreasing amount of water. This could be due to the relative polarity of solvent mixture and the decrease of effective swelling of plant materials. The values of viscosity, surface tension and vapor pressure of water and ethanol are presented in Table 4.2.

Table 4.1: Polarity Index of Different Solvent Composition

Solvent composition, % Ethanol	Solvent polarities index
0%, water	9.00
20%	8.24
50%	7.10
70%	6.34
95%	5.39
100%, ethanol	5.20

Table 4.2: Properties of Viscosity, Surface Tension and Vapor Pressure of Water and Ethanol

	Water	Ethanol
Viscosity, cP	0.89	1.20
Surface tension, mN/cm	72.80	23.70

Vapor pressure, mmHg	23.80	59.02

Addition of water certainly can lower the mixture viscosity, thus; mass transfer improves. The higher water content reduces the product recovery due to increase of the mixture polarity. It is not favorable for the extraction of gallic acid. Another explanation is that, gallic acid is less soluble in water rather than in ethanol (Daneshfar et al., 2008). The solvent mixture contains with a high amount of water is less effective for extract gallic acid. In addition, phenolic compounds are often more soluble in solvent less polar than water (Dac-Ok and Chang, 2002). From the result, 50% of solvent composition gave high amount of gallic acid.

4.2.2 Effect of Extraction Time

The effect of extraction time for shake flask extraction is shown in Figure 4.2. As expected the gallic acid amount was increased by increasing of the extraction time from 5 hours (2.743 mg gallic acid/ 100g bark) to 20 hours (4.1409 mg gallic acid/ 100 g bark). As observed in Figure 4.2, the highest value of gallic acid was at 20 hours and after that there was no significant increase of the amount. Even though this extraction process could give positive results on the yield, longer extraction time is not favorable. It was probably because longer time will increase cost and not practical to conduct in larger scale.

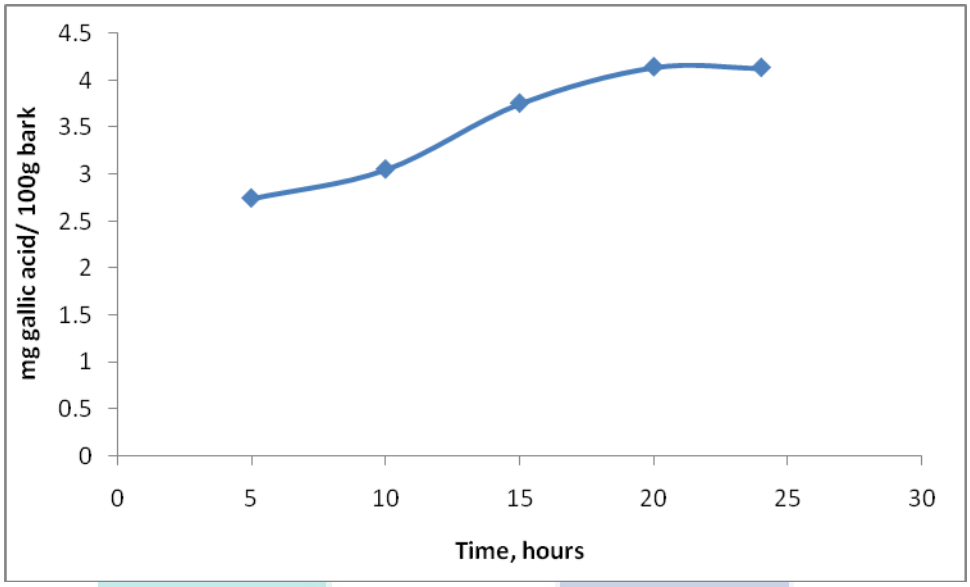


Figure 4.2: Effect of Extraction Time on the Yield of Galic Acid for Shake Flask Extraction (20 g Bark with 300 ml 50% Solvent Composition at 25^{o}C)

4.3 SOXHLET EXTRACTION

4.3.1 Effect of Solvent Composition

The effect of solvent composition on the yield of gallic acid was studied using standard Soxhlet extraction method. Different amount of the water-ethanol were considered as solvent for the extraction process. The solvent mixtures were prepared by adding water and ethanol to give the solvent composition of 0%, 20%, 50%, 70%, and 95% of ethanol. Figure 4.3 presents the yield of gallic acid extracted with different range of solvent composition. The results indicate that the amount of gallic acid increased with the increasing of ethanol concentration from 0% (2.698 mg gallic acid/ 100g bark) to 50% (9.1 mg gallic acid/ 100g bark), after which it began to decline.

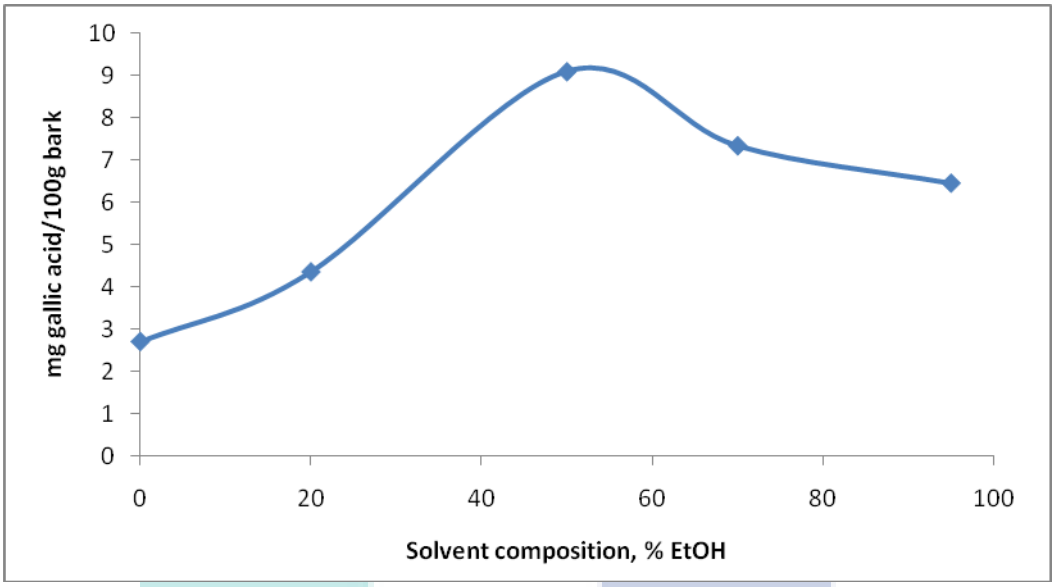


Figure 4.3: Effect of Solvent Composition on the Yield of Gallic Acid for Soxhlet Extraction (10 g Bark with 300 ml Solvent at Boiling Point for 8 hrs)

The same trend of result for Soxhlet extraction and shaking flask extraction was observed for this experiment. The difference between these two extraction processes is on the heating process that Soxhlet extraction needed. The same explanation for the result obtained can be used. It should be noted that Soxhlet extraction only works at the boiling points of the solvent. The surface tension and viscosity of the solvent is greatly reduced at a higher temperature. Hence, the solvent can reach the active sites in the matrix far more easily. As a conclusion, 50% of ethanol composition gave higher amount of gallic acid.

4.3.2 Effect of Extraction Time

Extraction time is also an important factor for the extraction of gallic acid. It is associated with the efficiency of extraction, final amount of gallic acid and the energy cost. The effects of extraction time on the yield of gallic acid was investigated and is shown in Figure 4.4. As a result, the yield of gallic acid increased very fast with the increasing amount of extraction time from 2 hours (2.975 mg gallic acid/ 100g bark) to 8 hours (9.965

mg gallic acid/ 100g bark). Over 8 hours, the yield almost had no significant changes. This could be probabbly because almost all the substance has been extracted out.

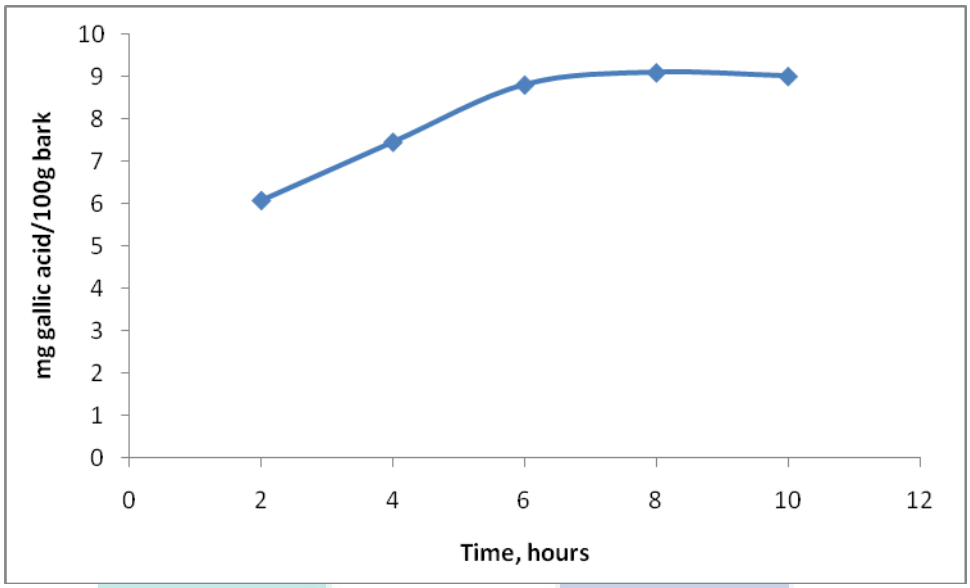


Figure 4.4: Effect of Extraction Time on the Yield of Gallic Acid for Soxhlet Extraction (10 g Bark with 300 ml 50% Solvent Composition at Boiling Point)

Soxhlet extraction took longer time to achieve high amount of gallic acid. Based on the practical and economical aspects, longer extraction time is not favorable because it will increase the cost. According to Mane et al. (2007), shorter extraction time was aimed to decrease tannin and its derivatives degradation. With this result, 8 hours of extraction time gave high amount of gallic acid.

4.4 ULTRASONIC-ASSISTED EXTRACTION (UAE)

4.4.1 Effect of Solvent Composition

Different mixtures of water-ethanol were considered as solvent for studying the effect of solvent composition. The solvent mixtures were prepared by mixing ethanol and water to make up the solvent composition of 0%, 20%, 50%, 70% and 95% of ethanol.

Figure 4.5 shows the results for the effect of solvent composition on the yield of gallic acid obtained.

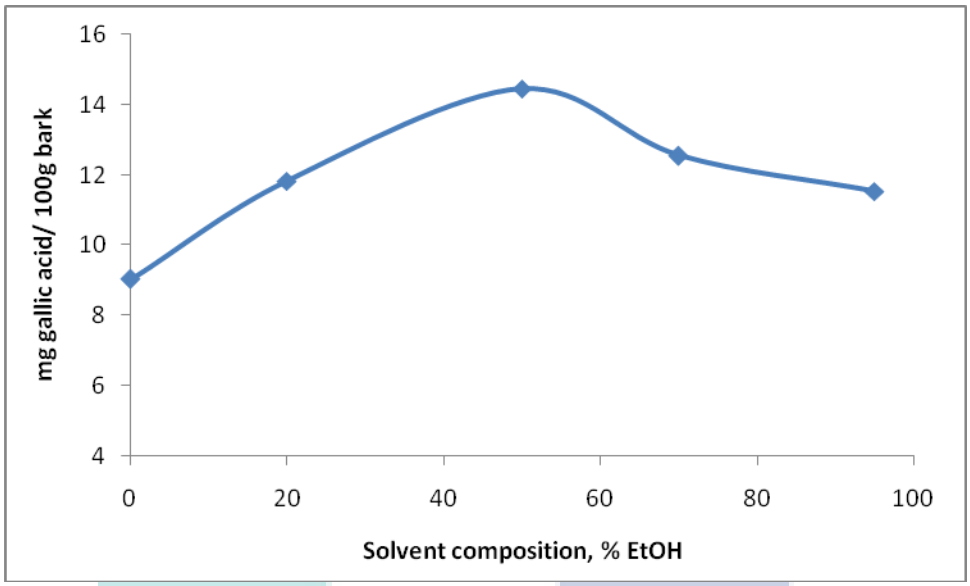


Figure 4.5: Effect of Solvent Composition on the Yield of Gallic Acid for UAE (20 g Bark with 150 ml Solvent at 27^{o}C for 30 min and 76.68 W of Ultrasonic Power)

As can be seen from figure above, the yield of gallic acid increases as the water concentration were decreased. The increment was observed up until 50% of solvent composition (14.45 mg gallic acid/ 100g bark) after which it starts to decrease. This observation could be due to solvent polarity, the ultrasonic cavitation effect and gallic acid chemical nature.

The enhancement of extraction by ultrasound is due to the phenomenon of cavitations produced in the solvent by the passage of an ultrasonic wave (Chen et al., 2007). As the ultrasound waves passes through the liquid, an expansion and compression cycles travelling in the liquid are involved. Expansion cycles pull the molecules apart, while compression cycles push the molecules together. The expansion cycles produced negative pressure in the liquid and create bubbles or cavities. This can happen when the negative

pressure exceeds the local tensile strength of the liquid. The bubbles will absorb energy from the ultrasound waves and grow during the expansion cycles and recompress during the compression cycles. The compression cycles lead the bubbles to collapse caused by the increase in pressure and temperature. This produces shock wave that passes through the solvent, enhancing the mass transfer within the plant materials.

The intensity of ultrasound cavitation in the solvent mixture was affected by the medium vapor pressure, viscosity and surface tension (Chen et al., 2007). In low vapor pressure liquid, ultrasonication produces few cavitational bubbles. However, ultrasonication in high vapor pressure liquid is not very effective. As more cavitational bubbles are created, they collapsed in less intensity due to a smaller internal and external pressure differential. For the influence of liquid viscosity, low liquid viscosity easily produces cavitational bubbles because the ultrasonic intensity applied could more easily exceed the molecular forces of the liquid. In addition, low viscosity liquid able to diffuse into the pores of the plant materials because the liquid has low density and high diffusivity (Djilani et al., 2006; Ou et al., 1997; Mason et al., 1996; Roldan-Gutierrez et al., 2008). Cavitational effects also affected by surface tension of the liquid. Lower energy needed to produce cavitation bubbles with liquid that has small surface tension.

The values of viscosity, surface tension and vapor pressure of water and ethanol are presented in Table 4.2. From Table 4.2, viscosity and vapor pressure of ethanol is higher than water, while the surface tension of water is larger than ethanol. Hence, for the mixture of water-ethanol solvent under ultrasonic irradiation, a non-linear behavior with an increase of the percent of pure solvent concentration is not uncommon.

Normally, the extraction efficiency will increase by varying the solvent from water to ethanol. From Figure 4.5, the product recovery decreases with decreasing the amount of water. This could be due to the relative polarity of solvent mixture and the decrease of effective swelling of plant materials. Addition of water certainly can lower the mixture viscosity, thus; mass transfer improves. At higher water contents, the product recovery

decreases due to increase of the mixture polarity, which is not favorable for the extraction of gallic acid. Another explanation is that, gallic acid is less soluble in water rather than in ethanol (Daneshfar et al., 2008). Solvent mixture with a high amount of water is less effective for extract gallic acid. In addition, phenolic compounds are often more soluble in solvent less polar than water (Dac-Ok and Chang, 2002). As a conclusion, 50% of ethanol composition gave higher amount of gallic acid.

4.4.2 Effect of Extraction Temperature

Temperature plays an important role during the extraction process. To study the effect of temperature on the yield of gallic acid, six temperatures were selected. The temperatures that had been chosen were 20, 25, 30, 35, 40 and 45^{o}C. This range of temperature was chosen because possible degradation of phenolic compounds takes place in increased temperature (Dac-Ok and Chang, 2002). During the ultrasonication process, the bath water temperature may raise caused by ultrasonic exposure. The temperature of bath water is controlled by the cooling system or ice. Figure 4.6 shows the result for the effect of temperature on the yield of gallic acid obtained.

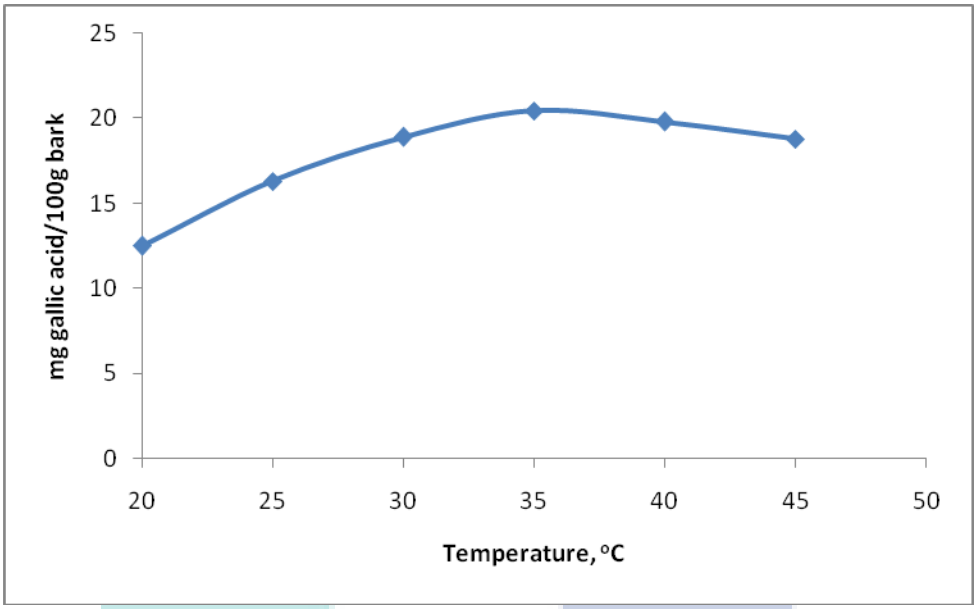


Figure 4.6: Effect of Extraction Temperature on the Yield of Gallic Acid for UAE (20 g bark with 150 ml 50% Solvent Composition for 30 min and 76.68 W Ultrasonic Power)

It was found that the increase in temperature from 20^{o}C (12.504 mg gallic acid/ 100g bark) to 35^{o}C (20.443 mg gallic acid/ 100g bark) enhanced the yield of gallic acid. The yield then decreasing as the temperature was raised from 35^{o}C (20.443 mg gallic acid/ 100g bark) to 40^{o}C (19.808 mg gallic acid/ 100g bark). Physical properties such as surface tension, density, viscosity, diffusivity, solubility and vapor pressure are affected when there is the treatment with temperature (Yang et al., 2008). This will directly affect the ultrasonic cavitation effect.

As the temperature rise, the solubility and diffusivity of gallic acid in the solvent will increase. The added thermal energy also helps to break the cell walls, allowing the analytes in the plant material to diffuse in the solvent (Pallaroni and von Holst, 2003). Furthermore, liquid viscosity and density decrease as temperature increase. The cavitational bubbles formed easily as the cohesive forces between the molecules decrease as a result of decrease in solvent viscosity. Hence, the tensile strength in the solvent was reduced (Toma

et al., 2001; Wu et al., 2001; Palma and Barraso, 2002). For the influence of vapor pressure, low vapor pressure is resulted from lower temperature and vice versa. The vapor pressure had great influence on the formation and the intensity of cavitational bubbles. At low vapor pressure, few caviatation bubbles were produced but it collapsed with greater force, resulting in enhancing cell tissues disruption during the extraction process. High vapor pressure produces more bubbles, but the bubbles were filled with vapor. Consequently, the implosion of the bubbles will be cushioned, and they collapsed with less intensity. This is called cushioning effect. Therefore, the cavitational effect would be less efficient. Formation and collapse of the cavitational bubbles is also affected by surface tension. High temperature will decrease the surface tension of the liquid. Thus, the bubbles are easily to form and collapse which may increase the mass transfer enhancement. From the result in Figure 6.6, amount of gallic acid that reaches the highest value was at the temperature of 35^{o}C.

4.4.3 Effect of Extraction Time

Plant materials can retain the analytes within the pores or other plan structures. It takes time to allow these compounds to be dissolved in the extraction solvent. Extraction time is one of the most important parameters during the extraction process. Thus, the extraction times that had been evaluated for this experiment were 10, 20, 30, 40, 50 and 60 min.

The effect of extraction time on the amount of gallic acid is shown in Figure 6.7. The amount of gallic acid increased for the first 50 min (16.875 mg gallic acid/ 100g bark), and after that it almost did not increase. This might be because most of the gallic acid had already been extracted for the first 50 min.

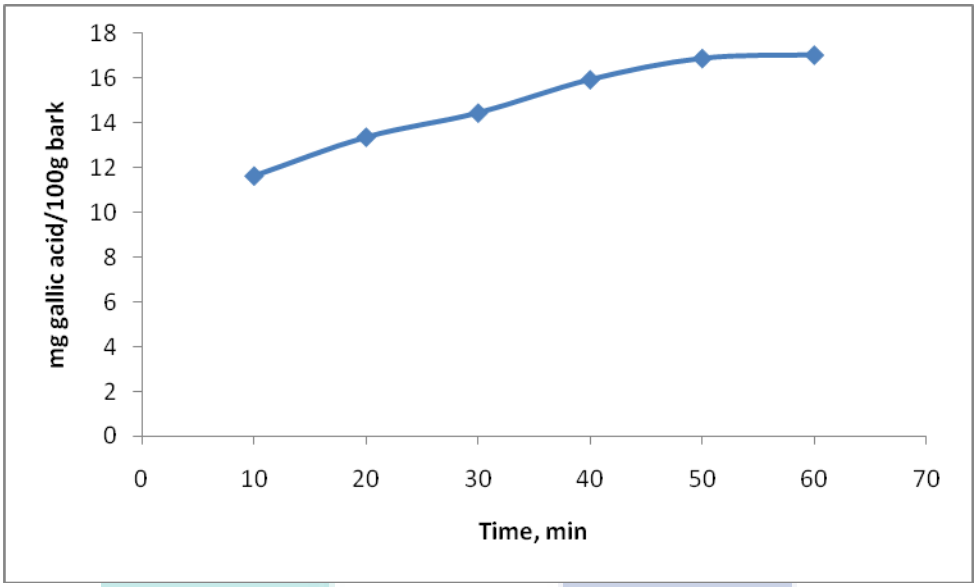


Figure 4.7: Effect of Extraction Time on the Yield of Gallic Acid for UAE (20 g Bark with 150 ml 50% Solvent Composition at 27^{o}C and 76.68 W of Ultrasonic Power)

The increment in the amount of gallic acid was due to the cavitational effect. The ultrasonic waves that produce cavitational bubbles collapsed near the tissue surfaces creating the micro-jet effect. This will cause tissues disruption and a good penetration of the extraction solvent into the plant material releasing the compound to the solvent. Therefore, a better mass transfer rate enhancing the extraction process. From this experiment, it was suggested that at 50 min of extraction time can give high amount of gallic acid.

4.4.4 Effect of Ultrasonic Power

The ultrasonic-assisted extraction experiments were conducted in an ultrasonic bath. This bath has a frequency of 40 kHz and 230 W of ultrasonic power rating on the scale of 0-9. The study of ultrasonic power was performed at the scale setting from 3 to 8. As the experiments were conducted, the bath water was regulated at constant desired temperature

to avoid the water bath temperature rise, caused by the ultrasonic exposure. The result for the effect of power is shown in Figure 4.8.

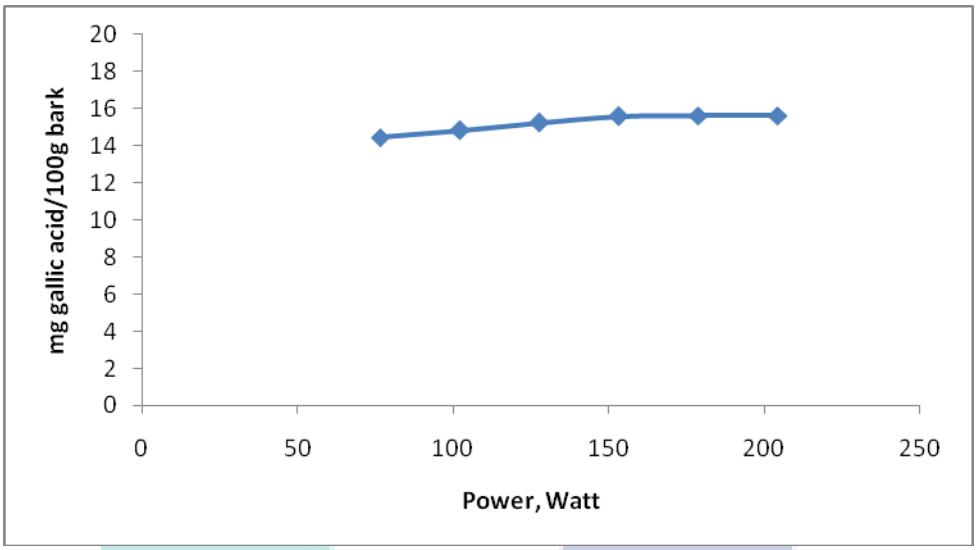


Figure 4.8: Effect of Ultrasonic Power on the Yield of Gallic Acid for UAE (20 g Bark with 150 ml 50% Solvent Composition at 27^{o}C for 30 min)

It can be seen that the amount of gallic acid was slightly increasing as the ultrasonic power increase. The reason for this is that as the ultrasonic amplitude increase, more cavitation bubbles are created and collapsed (Heminwol et al., 2006). It generates violent shock with high-speed jet effect which can disrupt the cell walls, allowing the penetration of solvent to the plant material. The release of compounds from the plant material into the solvent were significantly accelerated. Meanwhile, the mass transfer rate was also enhancing. However, the extraction efficiency was not favorable, probably because only a small fraction of ultrasonic power was transferred into the extraction solvent in this ultrasonic system. The rest of the energy was absorbed by the water bath or the sides of metal bath. For this reason, the ultrasonic power give insignificant effect towards the extraction process.

4.5 MICROWAVE-ASSISTED EXTRACTION (MAE)

4.5.1 Effect of Solvent Composition

The effect of solvent composition on the extraction yield was determined at five different solvent compositions (0%, 20%, 50%, 70% and 95% of ethanol). The rest of the parameters employed were extraction time of 3 min, extraction temperature of 40^{o}C, and microwave power of 480 W. From Figure 4.9 it can be observed that the yields of gallic acid were greatly influenced by the ethanol concentration. It was found that the yield of gallic acid gradually increased with the increasing of ethanol concentration up to 50% (25.3665 mg gallic acid/ 100g bark). When extracted with 70% and 95% of solvent composition, the extraction yields were decreased. From this result, it is clear that the addition of some quantity of water improved the extraction efficiency. The possible reason for increases in the efficiency could be because of increasing in swelling of plant materials by water, which increased the contact surface area between the plant material and solvent (Li et al., 2004; Rostagno et al., 2003).

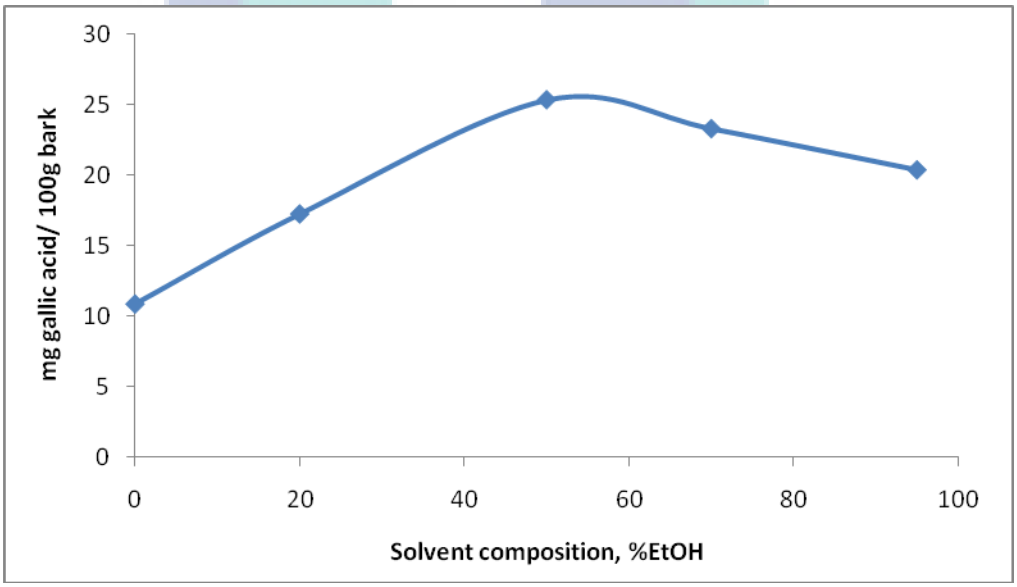


Figure 4.9: Effect of Solvent Composition on the Yield of Gallic Acid for MAE (10 g Bark with 300 ml Solvent at 40^{o}C for 3 min and 480 W Microwave Power)

Under the influence of microwave, the success of MAE depends on two parameters defining the dielectric properties of the solvent. The first parameter is dielectric constant, or relative permittivity, ε', that describes the polarizability of the molecule to an electric field. The second parameter is dielectric loss factor, ε'', that measures the efficiency of the absorbed microwave energy to convert into heat inside a material when an electric field is applied. From these two parameters, it defined another property called dissipation factor, δ, which is expressed mathematically by:

$$\tan \delta = \varepsilon'' / \varepsilon' \qquad (4.2)$$

The dissipation factor is a measure of the ability of the solvent to absorb microwave energy and dissipate that energy in the form of heat.

Another reasonable explanation is due to the different polarity of the solvent mixture used. As can be seen in Table 4.1, increase of water contact will decrease the polarity index of the mixture. Figure 4.9 suggested that at a certain degree of increase in the solvent polarity up to 50% of water could enhance the solubility of gallic acid in the mixture. In addition, the solvent mixture dielectric constant increases by the addition of some amount of water. This helps to absorb microwave energy, hence increasing the extraction efficiency. The dielectric constant of the solvent mixture, ε'_m, can generally be calculated from the following equation:

$$\varepsilon'^{1/3}_m = \sum(\varphi_i) \, \varepsilon'^{1/3}_i \qquad (4.3)$$

Where φ_i is the volume fraction of *i* solvent and ε'_i is the dielectric constant of *i*th solvent (Hao et al., 2002). The values of dielectric constants for various solvent compositions, as well as water and ethanol are presented in Table 4.3.

Table 4.3: Dielectric Constants of Water, Ethanol and Solvent Compositions

Solvent	Dielectric constant, ε'
Ethanol	24.30
Water (0%)	78.90
20% ethanol	64.50
50% ethanol	46.37
70% ethanol	36.40
95% ethanol	26.09

It should be noted that although the addition of water will increase the solvent mixture dielectric constant, the dissipation factor decreases. This means that although the solvent mixture can absorb high microwave energy compared to pure ethanol as a result of increased of dielectric constant, the solvent mixture would not be able to dissipate the heat effectively. As found in the experiment, solvent mixture with too high water content, i.e. 20% solvent composition, the extraction efficiency was low and unfavorable. As a result, 50% of solvent composition gave a better yield.

4.5.2 Effect of Extraction Time

As in other extraction techniques, time is another parameter for MAE that needs to be taken in account. Studies were carried out at different extraction time of 1.5 min, 2 min, 3 min, 4 min and 5 min. The rest of the parameters employed were solvent composition of 50%, extraction temperature of 40^{o}C and microwave power of 480 W. As confirmed in Figure 4.10, with increasing in extraction times from 1.5 min to 4 min, the yields of gallic acid increased rapidly and reached its maximum at 4 min (25.667 mg gallic acid/ 100g bark). Then, the extraction yields decreased with extension of extraction time. Generally,

the quality of analytes will increased with increasing in extraction time, although there is a risk of degradation may occur (Mandal et al., 2007).

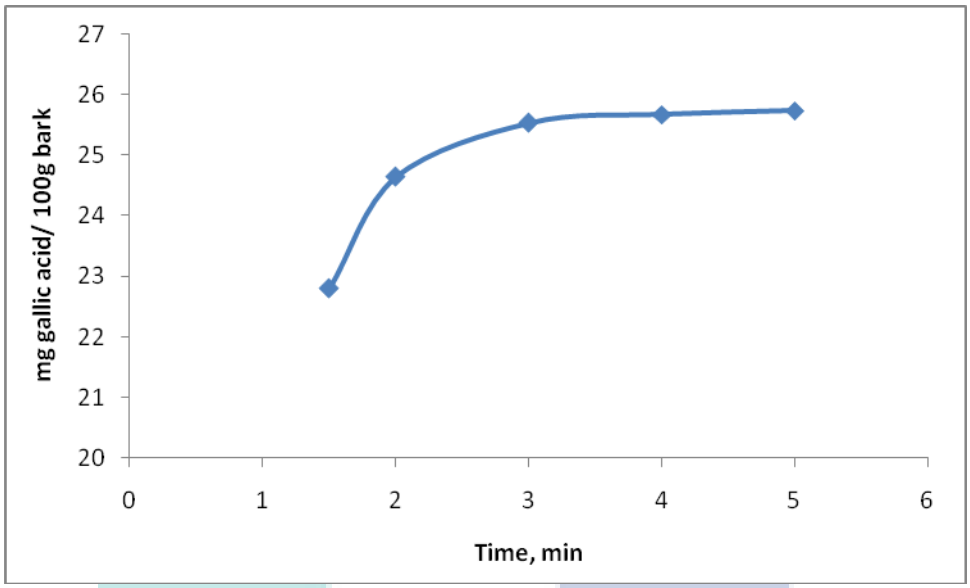


Figure 4.10: Effect of Extraction Time on the Yield of Gallic Acid for MAE (10 g Bark with 300 ml 50% Solvent Composition at 40^{o}C and 480 W Microwave Power)

MAE delivers fast recovery of gallic acid in such a short time. The effect of microwave heating is thought to be the theoretical basis of MAE (Romanik et al., 2007; Sticher, 2008). During the microwave-assisted extraction of the secondary metabolites from plant materials, microwave quickly delivers energy to the extractant and plant matrix. The energy is efficiently absorbed by some substances in the plant cells that cause heating up the water molecules (moisture content) of the plant cells. This is known as microwave heating target. As a result, the internal temperature of the plant cells increases drastically which may cause the liquid vaporization. The pressure on the cell walls is producing by plant swelling (Wang and Weller, 2006) that pushes the cell wall from the inside, stretching and ultimately rupturing it. This could facilitates leaching out of the active compounds from the ruptured cells into the extraction solvent (Mandal et al., 2007), thus allowing the effective extraction.

For MAE, oftenly 15 to 20 minutes is sufficient for the extraction process, but even in 40 seconds of extraction time can deliver excellent recovery too (Li et al., 2004; Wang et al., 2007). Over exposure to microwave radiation even at low temperature or low operating power was found to decrease the product recovery. This might be due to the loss of chemical structure of active compounds (Hao et al., 2002; Wang et al., 2009). Hence, 4 minutes of extraction time gave better yields of gallic acid.

4.5.3 Effect of Microwave Power

In order to evaluate the effect of microwave power on MAE, different microwave powers were controlled, e.g., 160 W, 320 W, 480 W, 640 W and 800 W. The rest of the parameters employed were solvent composition of 50%, extraction time of 4 min and extraction temperature of 40^{o}C. It can be seen in Figure 4.11 that high microwave power enhanced the yields of gallic acid when the power was lower than 480 W. However, when the power was higher than 480 W, the yield declined. The amount of gallic acid at this highest point was 25.704 mg gallic acid/ 100g bark.

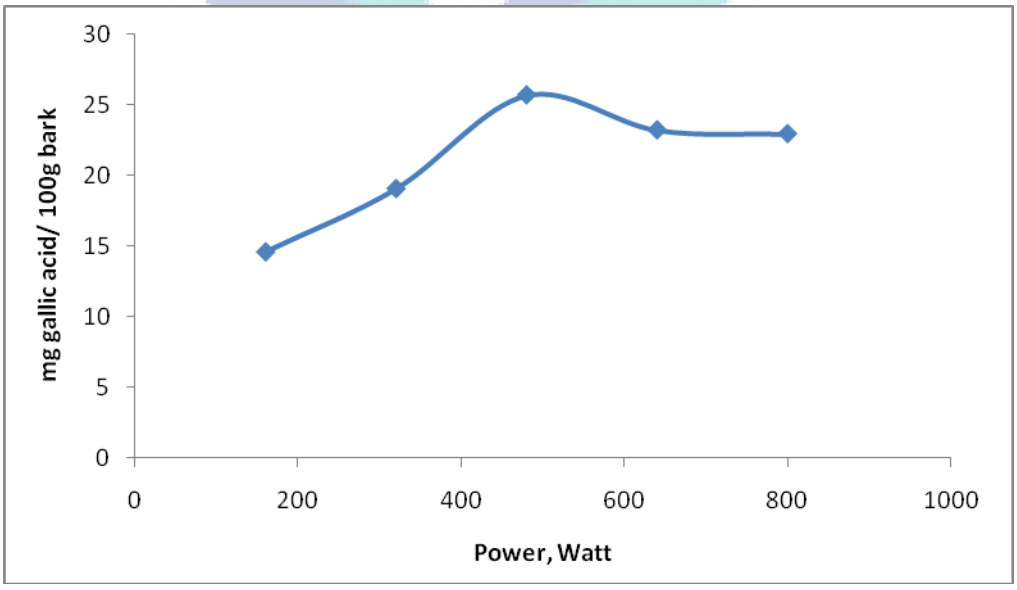


Figure 4.11: Effect of Microwave Power on the Yield of Gallic Acid for MAE (10 g Bark with 300 ml 50% Solvent Composition at 40^{o}C for 4 min)

Microwave power provides localized heating in the sample, and it acts as a driving force to destroy the plant material to release the active compounds and dissolve in the solvent. Increasing the microwave power will generally improve the extraction yield and result in a shorter time (Chan et al., 2011). Microwave power and extraction time are two factors that give much influence in the extraction process to a great extent. A wise approach may be better with a combination of low or moderate microwave power with longer extraction time (Mandal et al., 2007). At low microwave power, the cell wall will be ruptured gradually enables the selective MAE. High power can give superfluous energy to the solvent and plant material. It can drastically disturb the molecular interactions and become unorderly (Yan et al., 2010). Furthermore, high power with prolonged exposure always involves the risk of thermal sensible compounds. In general, the extraction yields increase proportionally with microwave up to limit before the increase becomes insignificant or decline (Mandal and Mandal, 2010; Xiao et al., 2008; Chemat et al., 2005; Kwon et al., 2003). Hence, 480 W was chosen as an appropriate microwave power.

4.5.4 Effect of Extraction Temperature

Temperature is an important factor to ensure efficient extraction. The influence of extraction temperature on the yields of gallic acid is shown in Figure 4.12. the rest of the parameters employed were solvent composition of 50%, extraction time of 4 min and microwave power of 480 W.

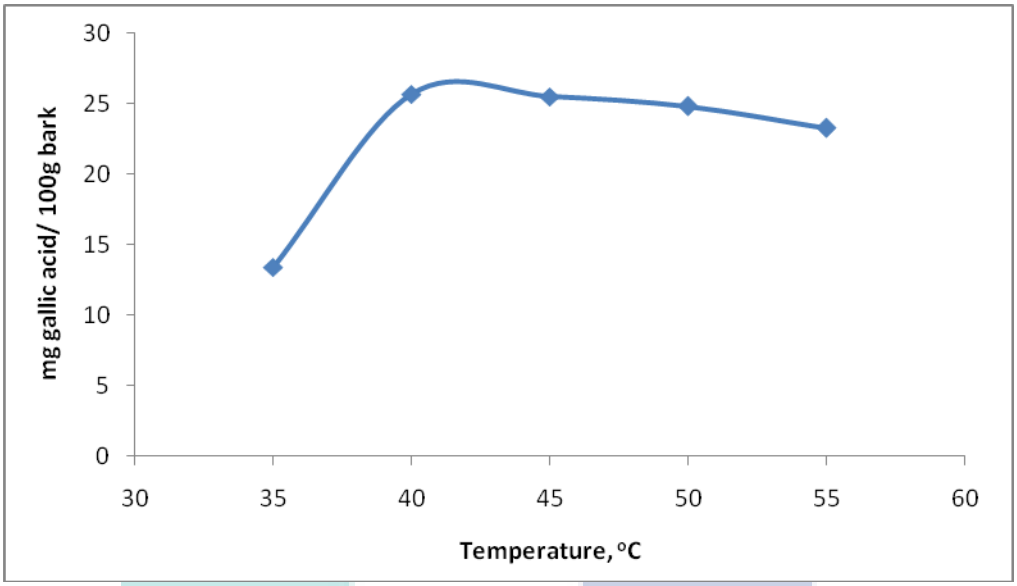


Figure 4.12: Effect of Extraction Temperature on the Yield of Gallic Acid for MAE (10 g Bark with 300 ml 50% Solvent Composition for 4 min and 480 W Microwave Power)

Theoretically, the temperature may well reach above the boiling point of the solvent when MAE is conducted in closed vessels. These high temperatures consequently improves the extraction efficiency, since the desorption of analytes from active sites in the matrix will increase. The present result revealed that the yields of gallic acid steadily increased with the increase of temperature until 40^{o}C (25.677 mg gallic acid/ 100g bark), and then decreases. This might be probably due to the increased diffusivity of solvent into the internal parts of the bark when the temperature increase. At high temperature, some physical properties will change such as solvent viscosity, diffusivity and surface tension. Solvent viscosity and surface tension will decrease with high temperature, while the diffusivity will increase. This improve sample wetting and matrix penetration; thus, the efficiency of extraction increased (Camel, 2000; Pan et al., 2000). Hence, extraction temperature of 40^{o}C gave better yield rather than other temperature.

4.6 KINETIC MODELS OF DIFFERENT EXTRACTION TECHNIQUES

4.6.1 Kinetic Study of Gallic Acid for Shake Flask Extraction

Figures 4.13 to 4.15 illustrate the linearized form of the corresponding basic kinetic equation given in Eqs. 2.8, 2.19 and 2.20. Parameters of kinetic models were calculated from the experimental data and the linear regression from each figures were used to obtain the washing coefficient and specific rate of slow extraction (the coefficient of linear correlation was higher than 0.92). The values of the kinetic parameters for shake flask extraction are listed in Table 4.4. The washing coefficients, b were greater than the slow extraction coefficients, k for film theory and unsteady diffusion. It can be seen in Figure 4.2 that the washing process took 20 hrs to reach the slow extraction process. At this point, the dissolution of soluble constituents on or near the surface of the bark took place. After 20 hrs, slow extraction happened whereby most of the soluble constituents had already been extracted. This process is slower which contributed in lower slow extraction coefficient. The maximum concentration of gallic acid was determined from Figure 4.2 which is at 1200 min. The shake flask extraction can successfully be described mathematically using the unsteady diffusion through plant material, the film theory and the empirical equation of Ponomaryov.

Table 4.4: Values of Kinetic Parameters for Shake Flask Extraction

Model	**Washing coefficient, b**	**Slow extraction coefficient, k (min^{-1})**
Film theory	0.01523	0.00248
Unsteady diffusion	0.01523	0.00248
Ponomaryov's equation	0.50952	0.00038

Figure 4.16 shows the comparison between the concentration of gallic acid from experiment and the theoretical concentration of gallic acid calculated from Eq 2.7. A good fitting between experimental and theoretical data was obtained.

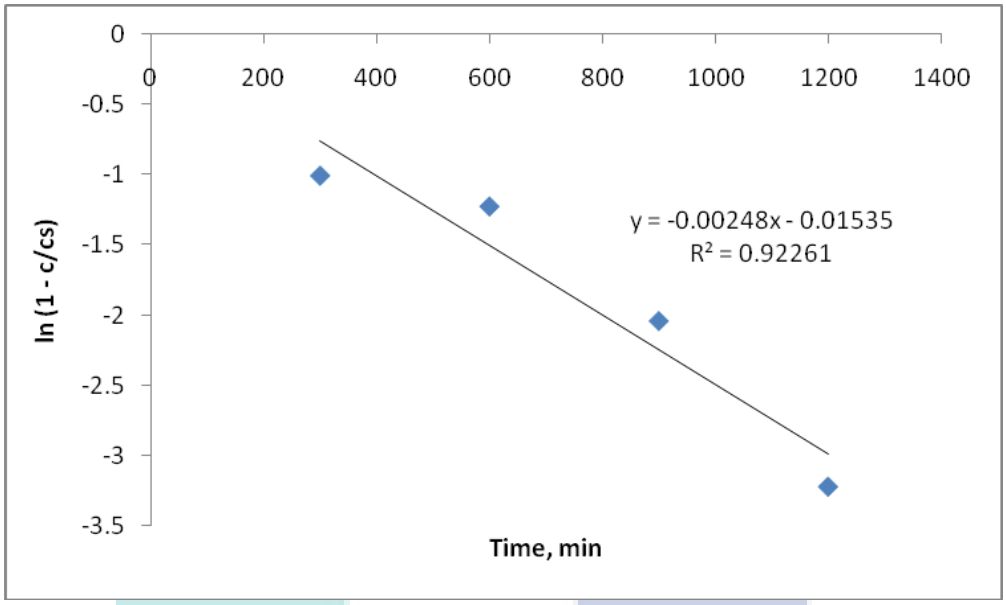


Figure 4.13: Linearized Form of Kinetic Equation Model Based on Film Theory for Shake Flask Extraction

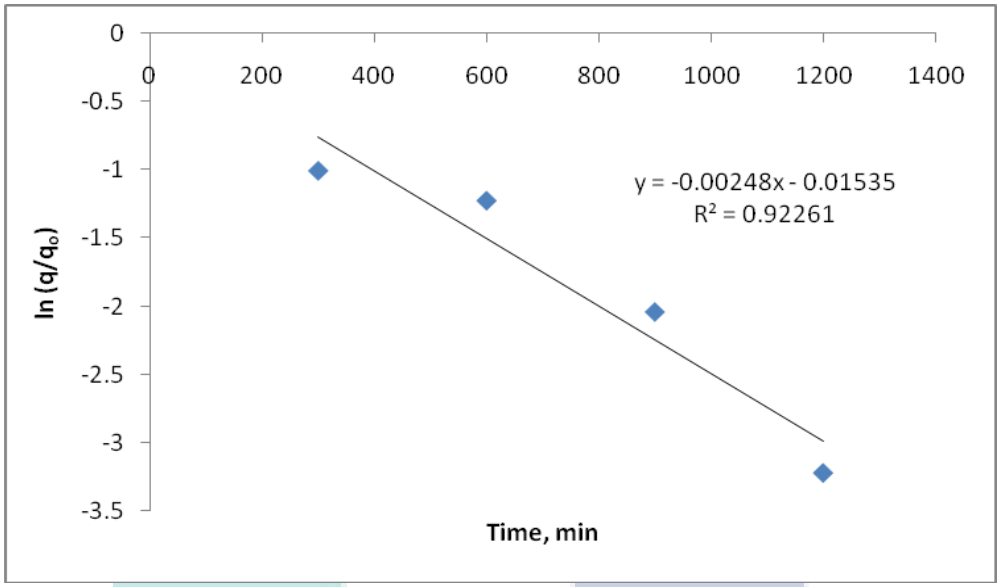


Figure 4.14: Linearized Form of Kinetic Equation Model Based on Unsteady Diffusion Through Plant Material for Shake Flask Extraction

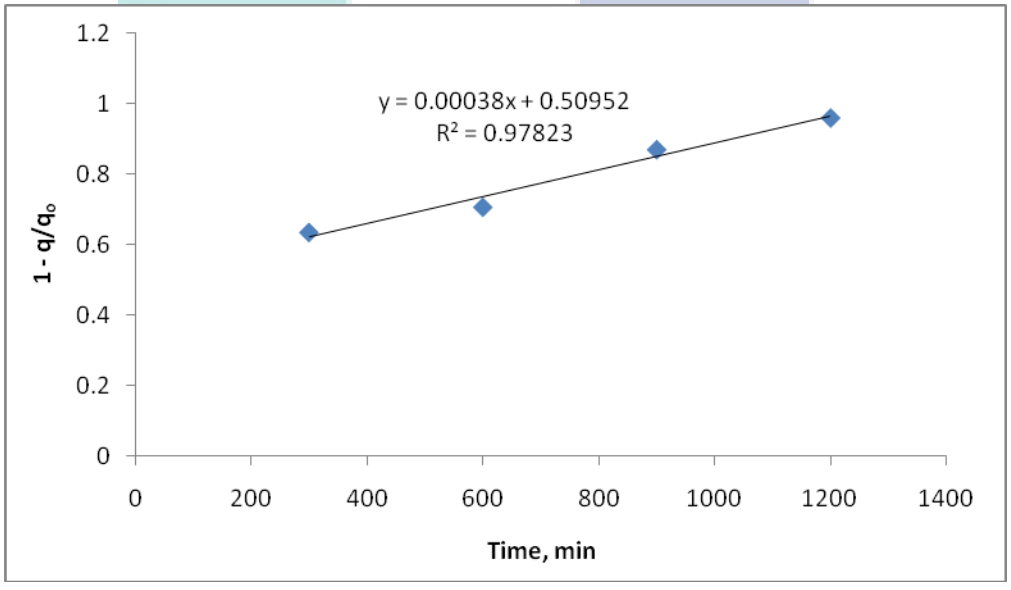


Figure 4.15: Linearized Form of Kinetic Equation Model Based on Ponomaryov's Empirical Equation for Shake Flask Extraction

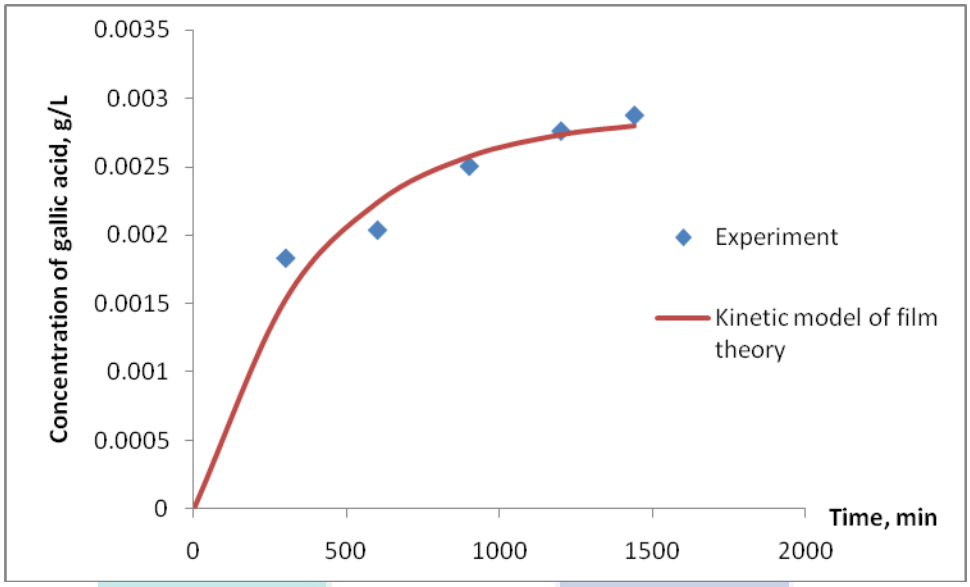


Figure 4.16: Comparison Between Experimental Concentration of Gallic Acid and Model Data for Shake Flask Extraction

4.6.2 Kinetic Study of Gallic Acid for Soxhlet Extraction

The linearized form of the coressponding kinetic equation are shown in Figures 4.17 to 4.19. Parameters of kinetic models were calculated from the experimental data and the linear regression from each figures were used to obtain washing coeeficient and specific rate of slow extraction (the coefficient of linear correlation was higher than 0.93). The values of the kinetic parameters for Soxhlet Extraction are listed in Table 4.5. The washing coefficients, b were lower than the slow extraction coefficients, k for film theory and unsteady diffusion. From Figure 4.4, the washing process took 8 hrs to reach slow extraction process. The low value of washing coefficient could be because the solvent and the bark particles was not in direct contact which might hinder the washing process. The mass transfer of soluble constituents from the bark into the solution by diffusion or osmotic process was faster during the slow extraction process. This might occurred when the bark particles were soaked by the solvent. The maximum concentration of gallic acid was determined from Figure 4.4 which was at 480 min. Figure 4.20 shows the comparison between the concentration of gallic acid from experiment and the theoritical concentration

of gallic acid calculated from Eq 2.7. A good fitting between the experimental data and the theoritical data was obtained.

Table 4.5: Values of Kinetic Parameters for Soxhlet Extraction

Model	Washing coefficient, b	Slow extraction coefficient, k (min^{-1})
Film theory	0.00144	0.00764
Unsteady diffusion	0.00144	0.00764
Ponomaryov's equation	0.659106	0.00087

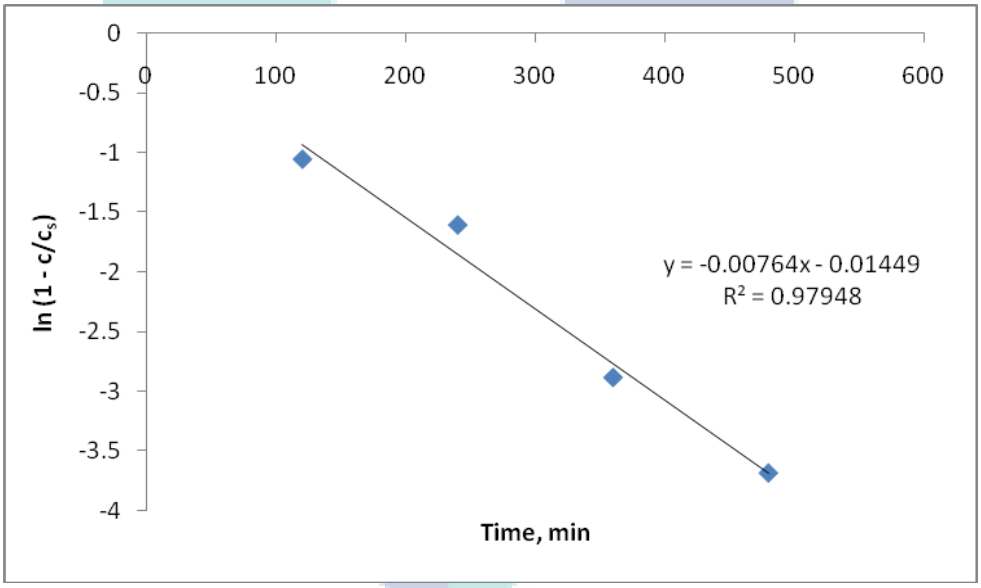


Figure 4.17: Linearized Form of Kinetic Equation Model Based on Film Theory for Soxhlet Extraction

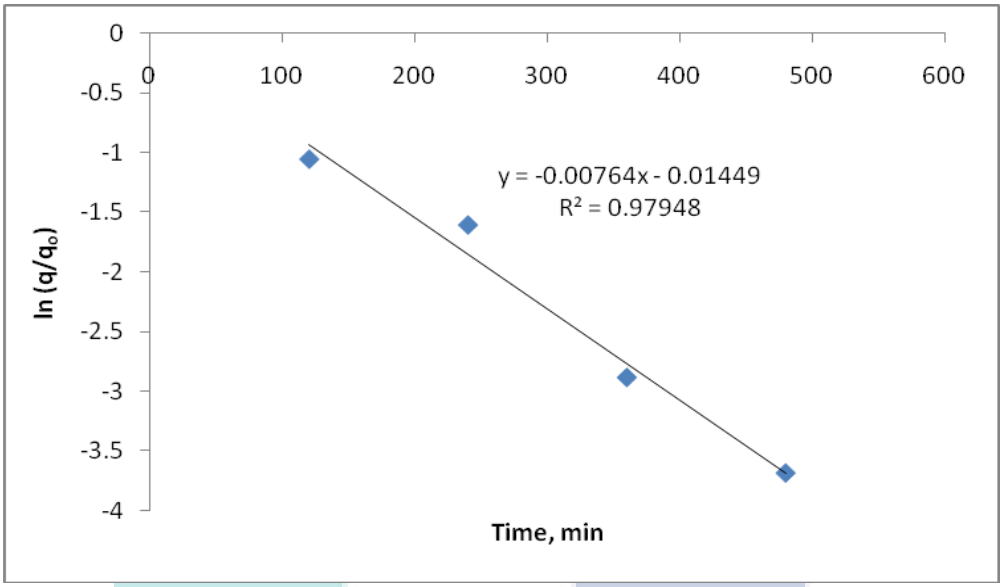


Figure 4.18: Linearized Form of Kinetic Equation Model Based on Unsteady Diffusion Through Plant Material for Soxhlet Extraction

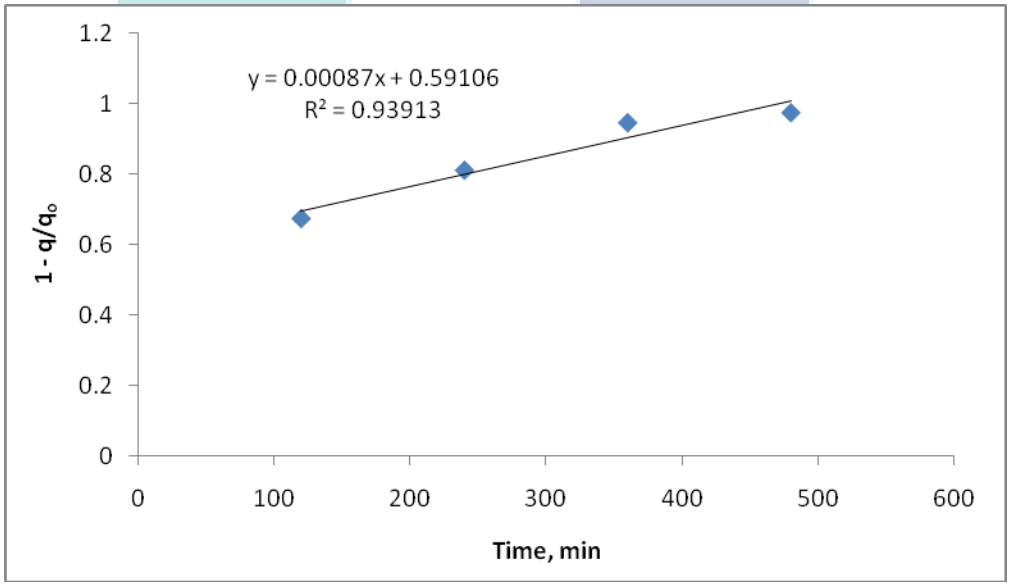


Figure 4.19: Linearized Form of Kinetic Equation Model Based on Ponomaryov's Empirical Equation for Soxhlet Extraction

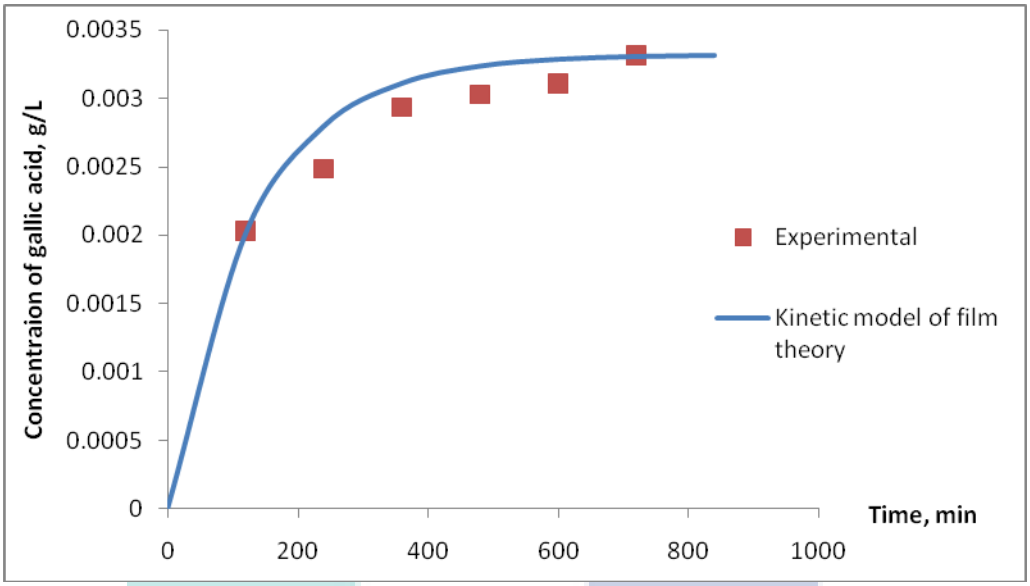


Figure 4.20: Comparison Between Experimental Concentration of Gallic Acid and Model Data for Soxhlet Extraction

4.6.3 Kinetic Study of Gallic Acid for UAE

The linearized form of the coressponding kinetic equation are shown in Figures 4.21 to 4.23. Parameters of kinetic models were calculated from the experimental data and the linear regression from each figures were used to obtain washing coeeficient and specific rate of slow extraction (the coefficient of linear correlation was higher than 0.91). The values of the kinetic parameters for UAE are lisred in Table 4.6. The washing coefficeints, b were higher than the slow extraction coefficients, k for both film theory and unsteady diffusion. The washing process took 50 min to happen until it reached the slow extraction process as shown in Figure 4.7. The value of washing coefficients were higher because of the ultrasonic effect that help the washing process to be faster. The value of slow extraction coefficients were lower could be because that almost all the soluble constituents had diffused out into the solution for the first 50 min. Figure 4.24 shows the comparison between the concentration of gallic acid from experiment and the theoritical concentration of gallic acid calculated from Eq 2.7. A good fitting between the experimental data and the theoritical data was obtained.

Table 4.6: Values of Kinetic Parameters for UAE

Model	Washing coefficient, b	Slow extraction coefficient, k (min^{-1})
Film theory	0.1707	0.06711
Unsteady diffusion	0.1706	0.0.6712
Ponomaryov's equation	0.6124	0.00759

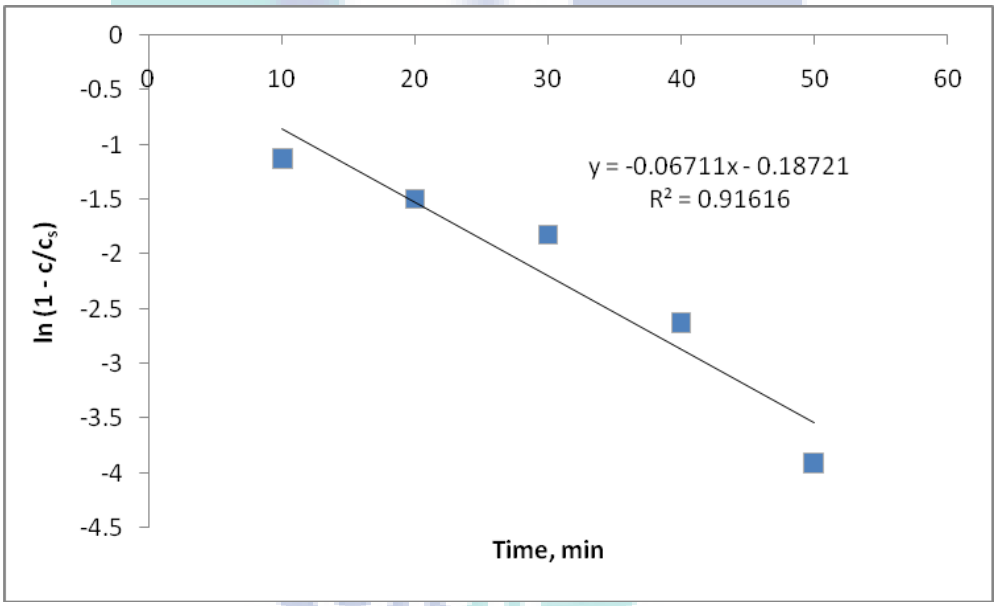


Figure 4.21: Linearized Form of Kinetic Equation Model Based on Film Theory for UAE

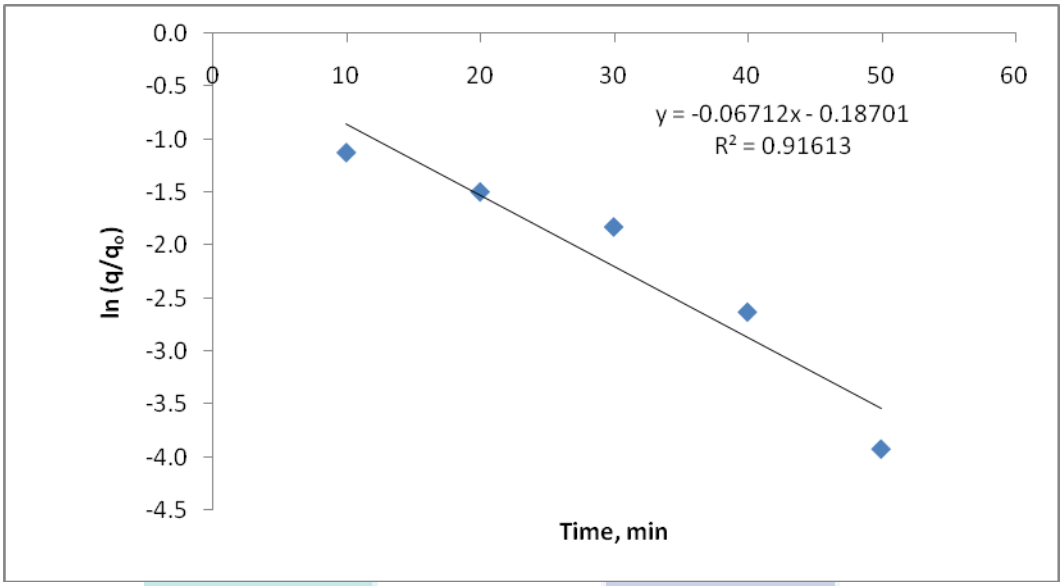


Figure 4.22: Linearized Form of Kinetic Equation Model Based on Unsteady Diffusion Through Plant Material for UAE

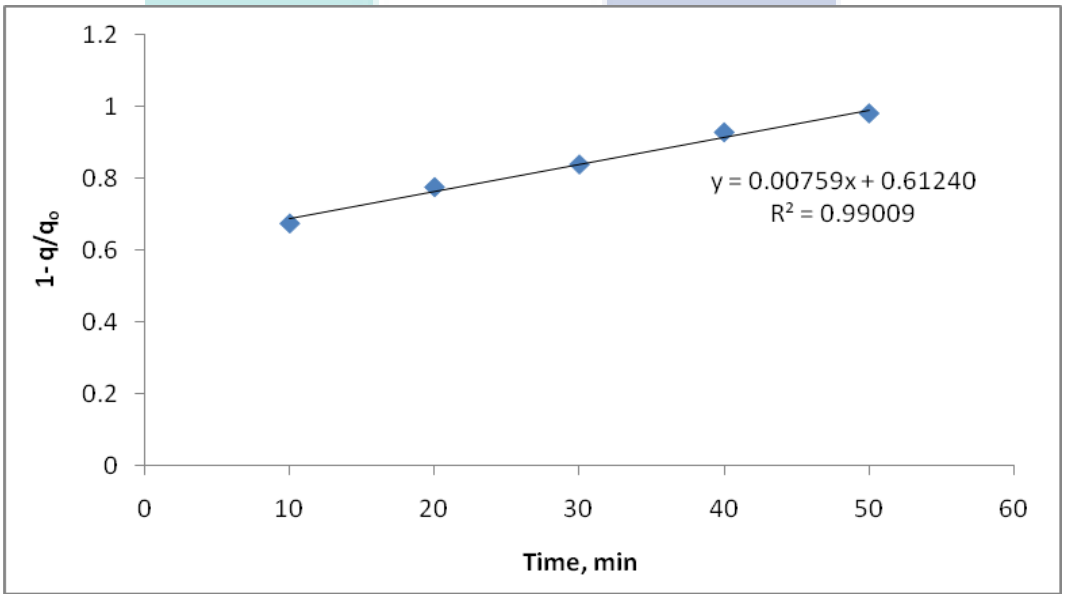


Figure 4.23: Linearized Form of Kinetic Equation Model Based on Ponomaryov's Equation for UAE

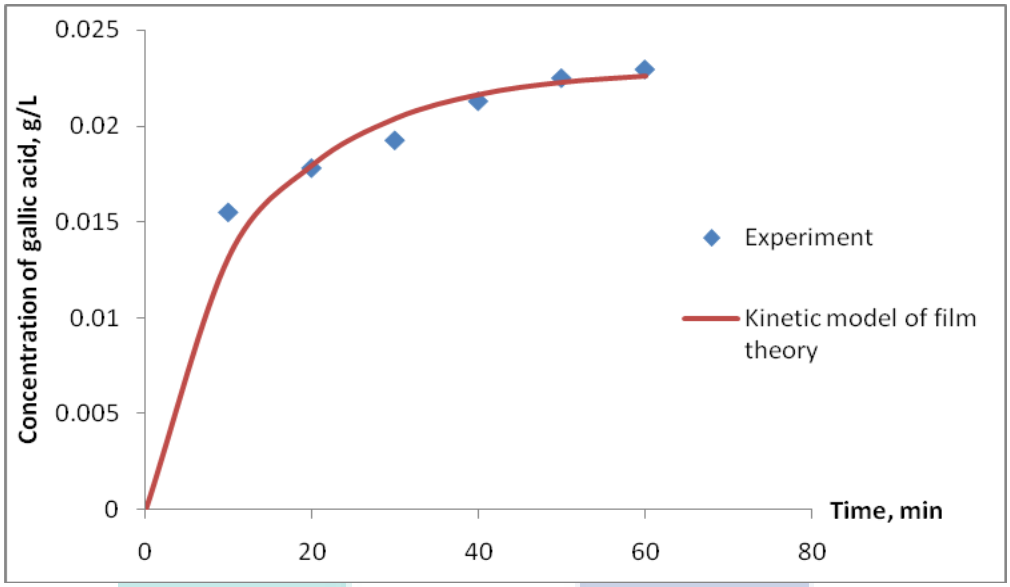


Figure 4.24: Comparison Between Experimental Concentration of Gallic Acid and Model Data for UAE

4.6.4 Kinetic Study of Gallic Acid for MAE

The linearized form of the coressponding kinetic equation are shown in Figures 4.25 to 4.27. Parameters of kinetic models were calculated from the experimental data and the linear regression from each figures were used to obtain washing coeeficient and specific rate of slow extraction (the coefficient of linear correlation was higher than 0.8). The values of the kinetic parameters for MAE are listed in Table 4.7. The washing coefficients, b were lower than the slow extraction coefficients, k for both film theory and unsteady diffusion. As shown in Figure 4.10 the washing process took 3 min which might not be enough time for dissolution. But during the slow extraction process, the mass transfer of the soluble constituents from the bark into the solution was faster due to the microwave effect. The maximum concentration of gallic acid was determined from Figure 4.10 which was at 4 min. Figure 4.28 shows the comparison between the concentration of gallic acid from experiment and the theoritical concentration of gallic acid calculated from Eq 2.7.

Table 4.7: Values of Kinetic Parameters for MAE

Model	Washing coefficient, b	Slow extraction coefficient, k (min^{-1})
Film theory	0.05023	1.51359
Unsteady diffusion	0.05023	1.51359
Ponomaryov's equation	0.85184	0.04044

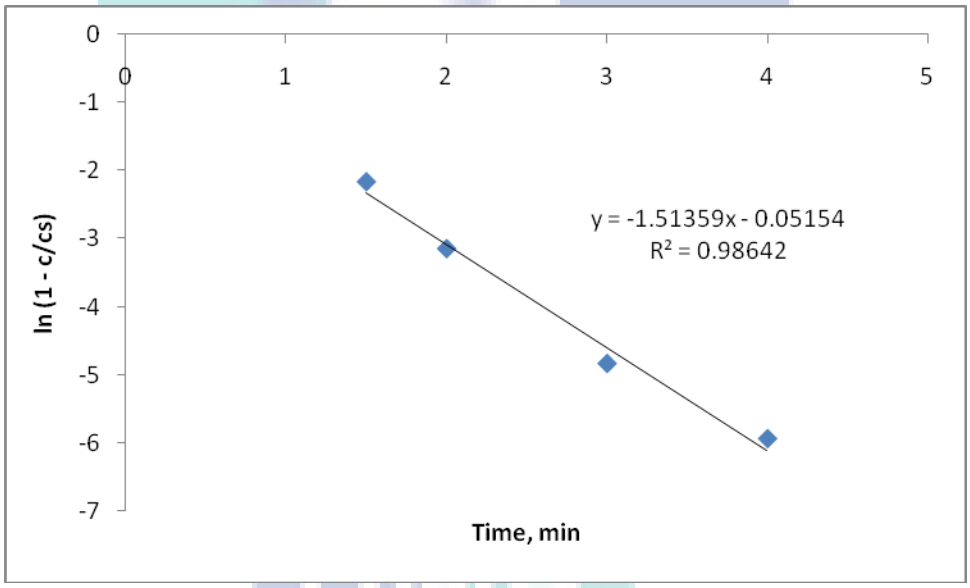


Figure 4.25: Linearized Form of Kinetic Equation Model Based on Film Theory for MAE

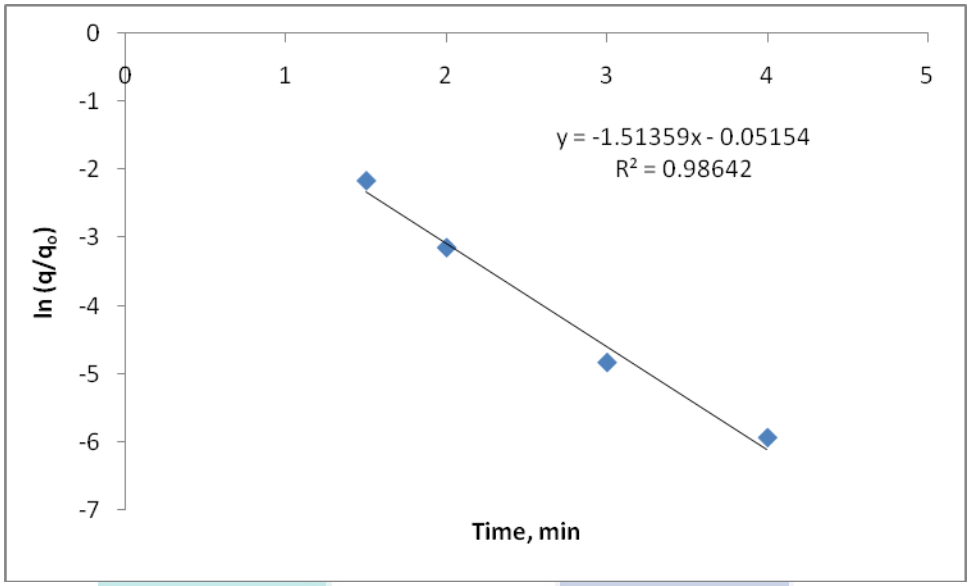


Figure 4.26: Linearized Form of Kinetic Equation Model Based on Unsteady Diffusion Through Plant Material for MAE

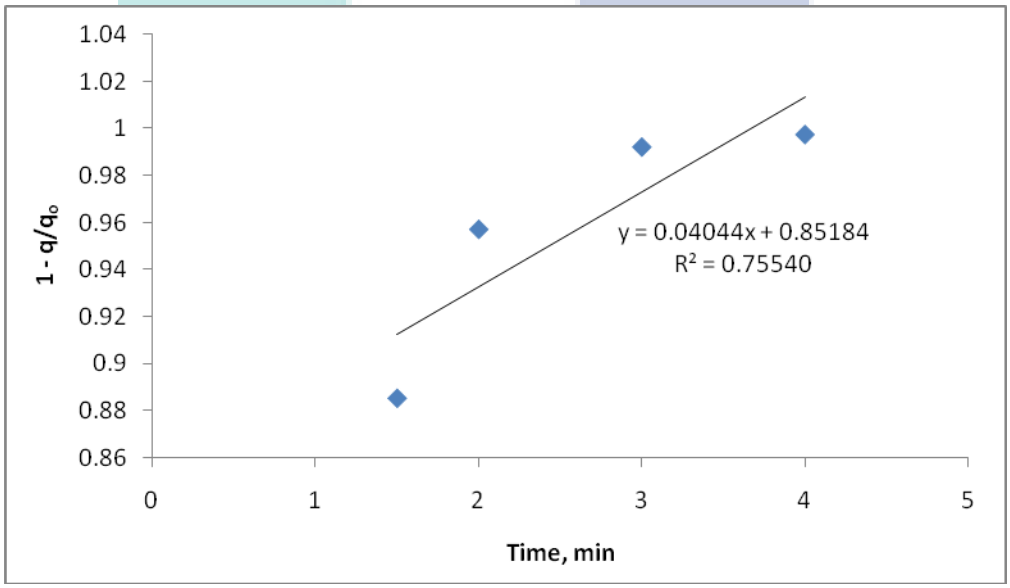


Figure 4.27: Linearized Form of Kinetic Equation Model Based on Ponomaryov's Equation for MAE

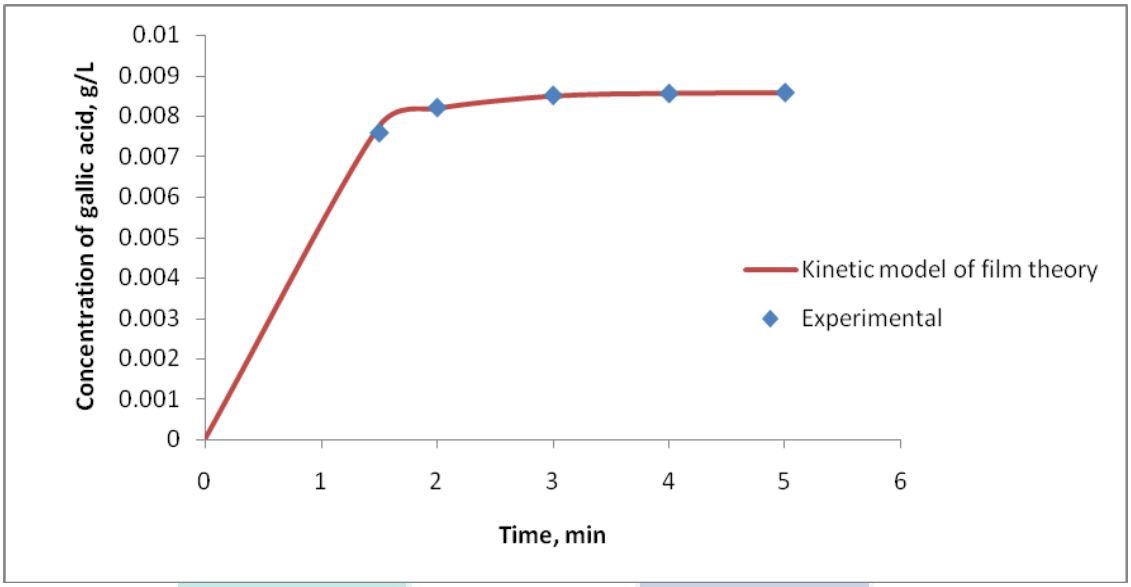


Figure 4.28: Comparison Between Experimental Concentration of Gallic Acid and Model Data for MAE

4.7 OPTIMZATION OF ULTRASONIC-ASSISTED EXTRACTION PARAMETERS

In this work, it was shown that MAE gave better results compare to UAE. From the results in Figures 4.11 and 4.12, due to the effect of microwave power and extraction temperature the amount of gallic acid was decreased. This could be because of the overexposure to the radiation and thermal degradation which was not favored. By using ultrasonic the chances on gallic acid degradation was lower. For this reason, the optimization of UAE was done.

4.7.1 Determination of Levels for Independent Variables

The three levels of the solvent composition variable were determined according to the results of a series of experiments carried out for 0, 20, 30, 50, 70 and 80% at the extraction temperature of 27^{o}C for 30 min. When the solvent composition was varied from 0% to 50%, a remarkable increase in the amount of gallic acid was observed. Beyond that

solvent composition, decreasing the amount of gallic acid was obtained. Therefore, 30%, 50% and 70% solvent composition were choosing for the coded solvent composition variable levels at -1, 0 and +1, respectively.

The effect of extraction temperature was investigated at 20, 25, 30, 35, 40 and 45^{o}C for 30 min and 50% of solvent composition. An increase in the amount of gallic acid was observed at extraction temperature ranging from 20^{o}C to 35^{o}C and no significant increase of the amount of gallic acid after that. The three design levels selected for the temperature variable were 30^{o}C, 35^{o}C and 40^{o}C.

The effect of extraction time on the amount of gallic acid was examined at 10, 20, 30, 40, 50 and 60 min at 27^{o}C and 50% solvent composition. The amount of gallic acid shows great improvement as the extraction time was increased. Therefore, 40 min, 50 min and 60 min were selected as three variable levels for extraction time.

4.7.2 Response Surface Optimization of Ultrasonic-Assisted Extraction Conditions

A three variable, three levels Box-Behnken factorial design (BBD) was applied to determine the best combination of extraction variables for the yield of gallic acid. Box-Behnken design (BBD), one of the response surface methodology (RSM), were chosen because it has only three levels and fewer experiments are needed. This design is more efficient and easy to arrange and interpret experiments compare to other design. In addition, this design had been used widely by the researchers (Ferreira et al., 2007). Three extraction variables that were considered were X_1 (solvent composition), X_2 (temperature) and X_3 (time). The proper range of the three variables had been determined from the above discussion on the basis of single-factor experiments.

Table 4.8 listed the whole experimental design consisted of 17 experimental points including five replicates (treatment 13-17) at the centre of the design that were used to

estimate a pure error sum squares. The experimental data were fitted to a quadratic polynomial model and regression coefficients obtained as shown in the following equation.

$$Y = \beta_0 0 + \sum_{i=0}^{4} \beta_i X_i 0 + \sum_{i=0}^{4} \beta_{ii} X_i^2 0 + \sum_{i=0}^{4} \sum_{j=0}^{4} \beta_{ij} X_i X_j \quad (4.4)$$

Where Y is the measured response variable, β_o, β_i, β_{ii}, β_{ij} are the regression coefficients of variables for intercept, linear, quadratic and interaction terms, respectively. The terms X_i is the coded levels of independent variables. While the terms X_iX_j and X_i^2 represent the interaction and quadratic terms, respectively. The quality of the fit of the polynomial model equation was expressed by the coefficient of determination R^2, and the significance of the regression coefficient was checked by F-value and p-value.

Table 4.8: Box-Behnken Experimental Design with the Independent Variables

Run	Independent variable			Gallic acid (mg/100g bark)
	X_1 (solvent composition, % EtOH)	X_2 (extraction temperature, ^oC)	X_3 (extraction time, min)	
1	30 (-1)	30 (-1)	50 (0)	11.2045
2	70 (+1)	30 (-1)	50 (0)	10.9583
3	30 (-1)	40 (+1)	50 (0)	11.7114
4	70 (+1)	40 (+1)	50 (0)	11.5472
5	30 (-1)	35 (0)	40 (-1)	11.3116
6	70 (+1)	35 (0)	40 (-1)	11.4113
7	30 (-1)	35 (0)	60 (+)	11.4988
8	70 (+1)	35 (0)	60 (+1)	11.4996
9	50 (0)	30 (-1)	40 (-1)	19.1727
10	50 (0)	40 (+1)	40 (-1)	19.9999
11	50 (0)	30 (-1)	60 (+1)	19.8742
12	50 (0)	40 (+1)	60 (+1)	20.1467
13	50 (0)	35 (0)	50 (0)	25.1731
14	50 (0)	35 (0)	50 (0)	25.2192
15	50 (0)	35 (0)	50 (0)	25.1961
16	50 (0)	35 (0)	50 (0)	25.1908
17	50 (0)	35 (0)	50 (0)	25.1491

The regression coefficients of the intercept, linear, quadratic and interaction terms of the model were calculated using the least square technique and analysis of variance (ANOVA) is listed in Table 4.9. According to Liyana-Pathirana and Shahidi (2005), checking of the model adequacy is important as the exploration and optimization of a fitted response surface may produce poor or misleading results, unless the model shows a better fit.

Table 4.9: Analysis of Variance (ANOVA) for the Fitted Quadratic Polynomial Model of Extraction of Gallic Acid.

Parameter	Estimated coefficients	Standard error	DF	Sum of squares	F-value	Prob > F
Intercept				**Model**		
β_o	21.61	0.057	1	383.48	2579.92	< 0.0001
X_1	-0.039	0.045	1	0.012	0.73	0.4221
X_2	0.27	0.045	1	0.60	36.48	0.0005
X_3	0.14	0.045	1	0.16	9.56	0.0175
X_1^2	0.021	0.064	1	0.001681	0.10	0.7590
X_2^2	-0.025	0.064	1	0.002445	0.15	0.7118
X_3^2	-0.14	0.064	1	0.077	4.66	0.0678
X_1X_2	-9.31	0.063	1	365.16	22110.03	< 0.0001
X_1X_3	-0.94	0.063	1	3.76	227.46	< 0.0001
X_2X_3	-0.87	0.063	1	3.18	192.79	< 0.0001
Lack of fit			3	0.074	2.34	0.2148
Pure error			4	0.042		
R^2	0.9997			Adjusted R^2	0.9993	
C.V%	0.78					

According to Table 4.9, the F-test suggested that the model had a very high F-value (F-value = 2579.92) and a very low p-value ($p < 0.0001$) indicating this model is highly significant. The significance of the F-value depends on the degree of freedom (DF) in the model and is shown in the p-value column (95% confidence level). The p-value is a tool to check the significance of each coefficient and the interaction strength between each independent variable (Muralidhar et al., 2001). More significant of the corresponding variables will be if the F-value is greater and smaller p-value (Atkinson and Donev, 1992). Hence, the effects of having p-value less than 0.05 are significant (Cai et al., 2008; Qiao et al., 2009). It was evident that the two linear (X_2 and X_3) and three quadratic parameters (X_{12}, X_{22} and X_{32}) were significant model terms at level $p < 0.05$, whereas the other parameters were insignificant ($p > 0.05$). The results indicate that the effect of temperature was the major contributing factor to the yield of gallic acid.

The lack of fit measures the failure of the model to represent the data in the experimental domain at points which are not included in the regression (Zhong and Wang, 2010). The F-value and p-value of lack of fits in the ANOVA table were 2.34 and 0.2148, respectively. This shows that it was insignificant ($p > 0.05$) relative to the pure error and suggested that the model equation was enough for predicting the yield of gallic acid under any combination of values of the variables. The determination of coefficient (R^2) of the model was 0.9997, implicating that the model suitably represented the real relationship between the parameters chosen. The R^2_{Adj} (adjusted determination coefficient) is the correlation measure for testing the goodness-of-fit of the regression equation. The higher the value of R^2_{Adj}, the better degree of correlation between observed and predicted values (Ravikumar et al., 2006). The value of R^2_{Adj} was 0.9993, which reasonably close enough to 1. This suggested that the model was highly significant and indicated a high degree of correlation between the observed and predicted data. Coefficient of variation (C.V.) indicates the degree of precision with which the experiments are compared (Zhong and Wang, 2010). The value of the coefficient of variation (C.V.) was 0.78, which showed better precision and reliability of the experiments carried out. The small value of C.V. suggested that the model is reproducible (Wanasundara and Shahidi, 1996). Predicted

response Y for the yield of gallic acid could be expressed by the following second-order polynomial equation in terms of coded value.

$$Y = 21.61 - 0.039X_1 + 0.27X_2 + 0.14X_3 + 0.021X_1X_2 - 0.025X_1X_3 - 0.14X_2X_3 - 9.31X_1^2 - 0.94X_2^2 - 0.87X_3^2 \quad (4.5)$$

4.7.3 Analysis of Response Surface

The best way of expressing that relationship between the independent and dependant variables is to generate the three-dimensional response surface plots generated by the model for the yield of gallic acid. The 3D response surface is the graphical representations of the regression equation. It provides a method to visualize the relationship between responses and experimental levels of each variable and the type of interactions between two test variables. Two variables were depicted in one three-dimensional surface plots while the other variable was kept at level zero.

Figure 4.29 shows the effect of the solvent composition and extraction temperature on the yield of gallic acid at a fixed extraction time of 50 min. Gallic acid yield increased with increasing of the solvent composition and reached the highest value at 49.97%, after which it declined. At a definite extraction temperature, the gallic acid yields increased slightly with an increase of the solvent composition. It nearly reached a peak (35.70^{o}C) in the highest solvent composition.

The interaction between the solvent composition and extraction time is shown in Figure 4.30. The gallic acid yields increased with the solvent composition while the extraction time had slight increased. The maximum gallic acid recovery was obtained at 49.97% of solvent composition and extraction time of 50.71 min.

The change in gallic acid yield could be due to the solvent polarity which provides the most suitable extraction efficiency for gallic acid extract. Addition of the extra water in

ethanol tremendously increase the extract yield because it creates a more polar medium which can facilitates the phenolic compounds (Spigno et al., 2007).

It was shown that the interactions between the solvent composition, and other two extraction variables gave an impact on the yield of gallic acid significantly (Table 4.9, Figure 4.29 and 4.30), in spite of the solvent composition was the minor factor affecting the yield of gallic acid.

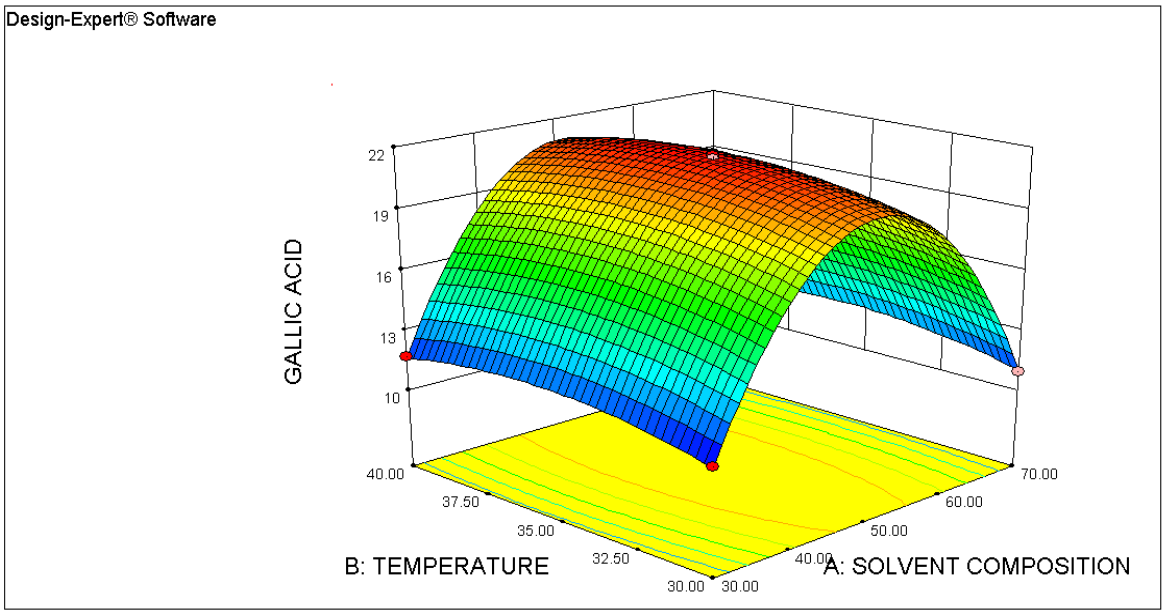


Figure 4.29: Response Surface Plot of Solvent Composition and Extraction Temperature on the Yield of Gallic Acid

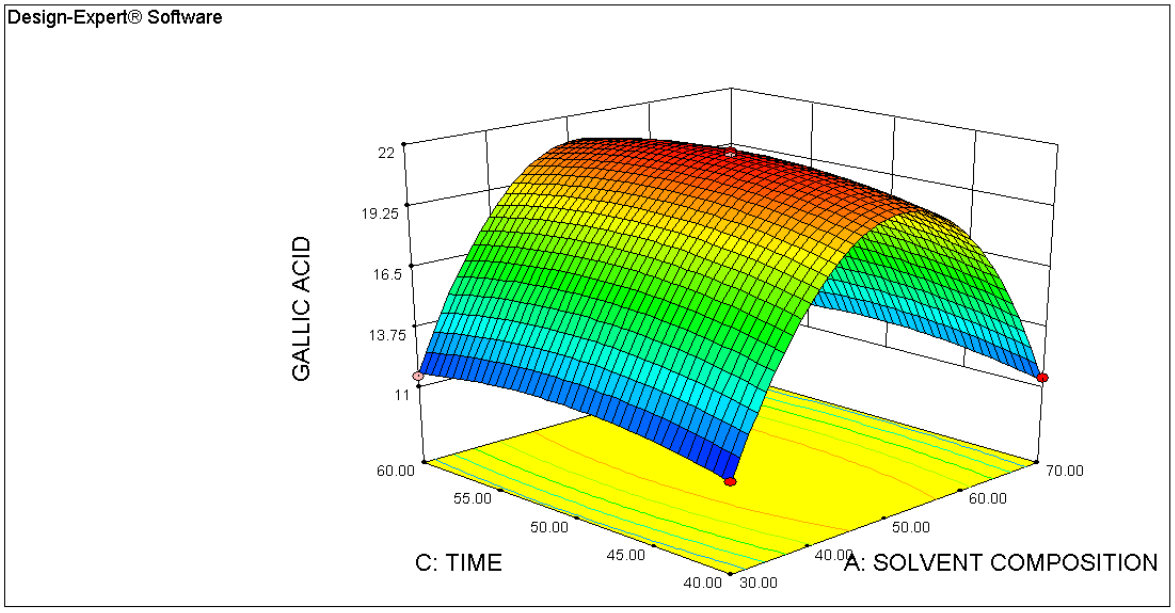


Figure 4.30: Response Surface Plot of Solvent Composition and Extraction Time on the Yield of Gallic Acid

Figure 4.31 showed the response surface plot at various extraction temperature and extraction time. The response curves demonstrated that the yield of gallic acid increased with extraction temperature from 30^{o}C to 35^{o}C. This is due to increase in temperature will accelerates mass transfer and improved extraction yield, while more than 35^{o}C appeared to be a disadvantage on the extracts. The extraction time also showed a positive impact on the yield of gallic acid. Increased in extraction from 40 min to 50 min resulted in increasing of the yield, and thereafter it decreased. The extraction time also showed a remarkable effect on the yield of gallic acid.

Figure 4.32 shows the normal probability of the studentized from the linear model. This plot is satisfactory and concludes the quadratic model is adequate to describe the gallic acid response surface of the gallic acid extraction

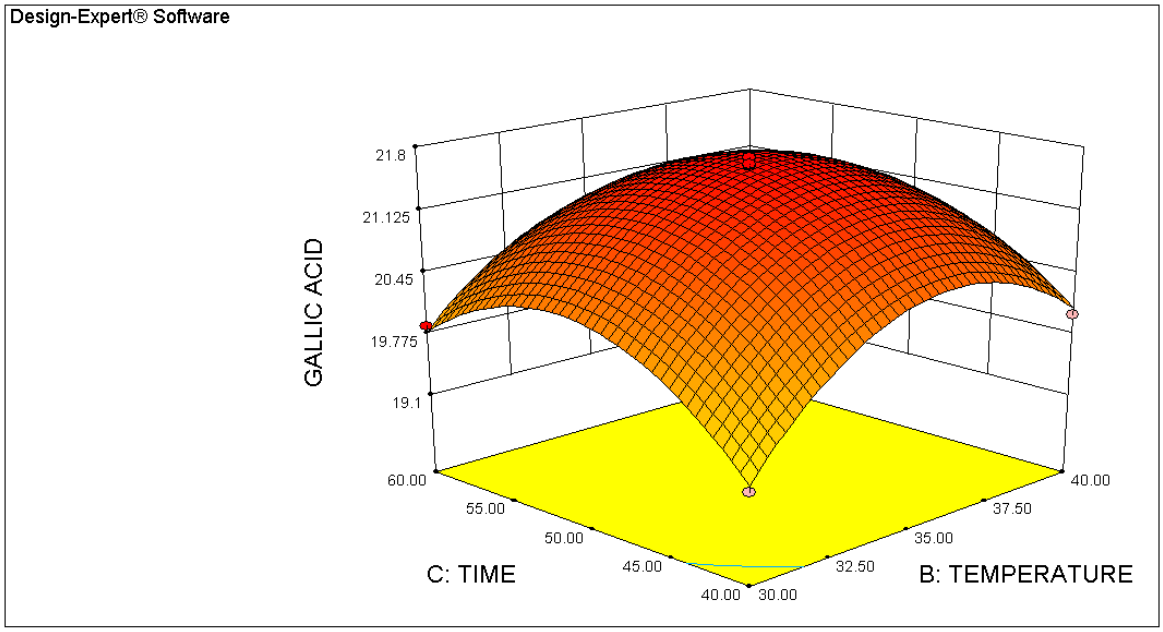


Figure 4.31: Response Surface Plot of Extraction Temperature and Extraction Time on the Yield of Gallic Acid

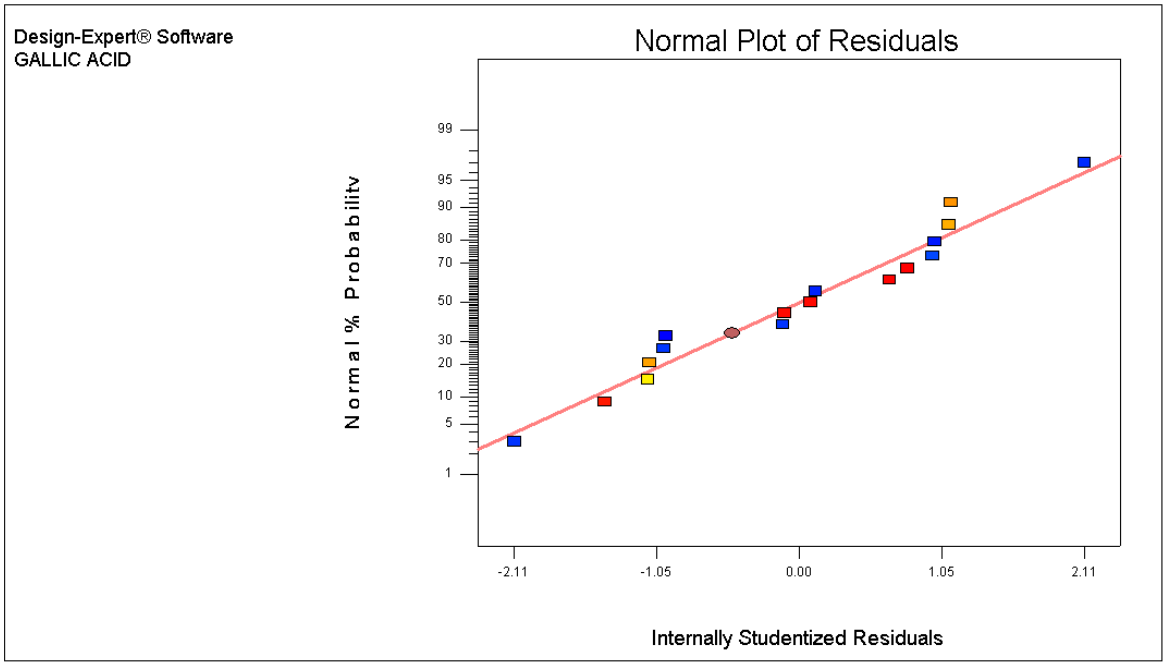


Figure 4.32: The Normal Probability Plot of Studentized Residual

According to Figure 4.29 - 4.31 and above single parameter study, it can be concluded that the optimal conditions of gallic acid from *Jatropha curcas* stem bark were solvent composition of 49.97%, extraction temperature of 35.70^{o}C and extraction time of 50.71 min. Among these three factors studied extraction temperature was the most significant factor to affect the extraction yield of gallic acid, followed by extraction time and solvent composition according to regression analysis (Table 4.9) and three-dimensional response surface plots.

The suitability of the model equation for predicting the optimum response values was tested by using the selected optimal conditions. The maximum predicted yield and experimental yield of gallic acid were given in Table 4.10. Additional experiments by using the predicted optimum conditions for gallic acid extraction were carried out: solvent composition of 49.97%, extraction temperature of 35.70^{o}C, extraction time of 50.71 min, and the model predicted a maximum response of 21.6367 mg gallic acid/100 g bark. To ensure the predicted result was not biased toward the practical value, rechecking of the experiment was performed using these modified optimum conditions: solvent composition of 50%, extraction temperature of 36^{o}C and extraction time of 51 min. A mean value of 21.6253 ± 0.0528% (N = 3) was gained, which was in agreement with the predicted value significantly ($p > 0.05$), obtained from real experiments, demonstrated the validation of the RSM model. The results of analysis confirmed that the response model was adequate for reflecting the expected optimization, and the model of Eq. 4.5 was satisfactory.

Table 4.10: Optimum Conditions and the Predicted and Experimental value of Response at the Optimum Conditions

	Solvent composition, % EtOH	**Extraction temperature, ^{o}C**	**Extraction time, min**	**Amount of gallic acid, mg gallic acid/ 100g bark**
Optimum conditions	49.97	35.70	50.71	21.6367 (predicted)
Modified conditions	50	36	51	21.6253 ± 0.0528% (actual)

4.8 COMPARISON OF EXTRACTION TECHNIQUES ON THE EXTRACTION OF GALLIC ACID

Extraction and product recovery are the most imperative steps in evaluation of valuable compounds from various plant parts. A desirable extraction technique should be simple, inexpensive, efficient, selective, environmental friendly and compatible with various analytical techniques. However, limitations of the extraction process are usually due to the time constraint, labor intensive, lengthy operation techniques and high cost of operation.

This present study compares the ability of four extraction techniques (shake flask extraction, Soxhlet extraction, UAE and MAE) to extract gallic acid from the stem bark of *Jatropha curcas*. Four parameters had been studied namely as solvent composition, extraction temperature, extraction time and power usage for UAE and MAE. However for the shake flask extraction and Soxhlet extraction, two parameters, which were solvent composition and extraction time were studied.

4.8.1 Comparison of Extraction Techniques on the Effect of Solvent Composition

The effect of solvent compositions on the yield of gallic acid for four different extraction methods is presented in Figure 4.33. In all four extraction techniques, it demonstrated that the improvement of extraction efficiency was observed with the addition of some amount of water. The yield of gallic acid increased as the solvent composition increased up to 50% and then declined. Higher amount of gallic acid was observed when some forces were introduced. The outer forces involved were ultrasonic wave and electromagnetic radiation for UAE and MAE, respectively.

In case of UAE a phenomenon called ultrasonic cavitation was produced in the solvent by the passage of an ultrasonic wave (Chen et al., 2007). The intensity of ultrasonic cavitations in the solvent mixture was affected by the surface tension, viscosity and medium vapor pressure (Chen et al., 2007). 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 (Rostagno et al., 2003). 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 (Djilani et al., 2006; Ou et al., 1997; Mason et al., 1996; Roldan-Gutierrez et al., 2008).

For MAE technique, the extraction mechanism is based on heating up the water molecules (moisture content) of the plant cells. As the heating process started, the moisture content will evaporates, and pressure on the cell walls is produced by plant swelling (Wang and Weller, 2006). The pressure build up pushes the cell walls from the inside, stretching and finally rupturing it. This could facilitate leaching out of compounds from the ruptured cells into the solvent (Mandal et al., 2007). Although, both UAE and MAE techniques

disrupt the plant cells, MAE was better than UAE since it uses the cell's internal water as conductor medium for microwave. In addition, adding some amount of water increase the dielectric constant of the solvent mixture. 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 (Hemwimol et al., 2006). In other words, solvent mixture with higher water content has low efficiency and not favorable.

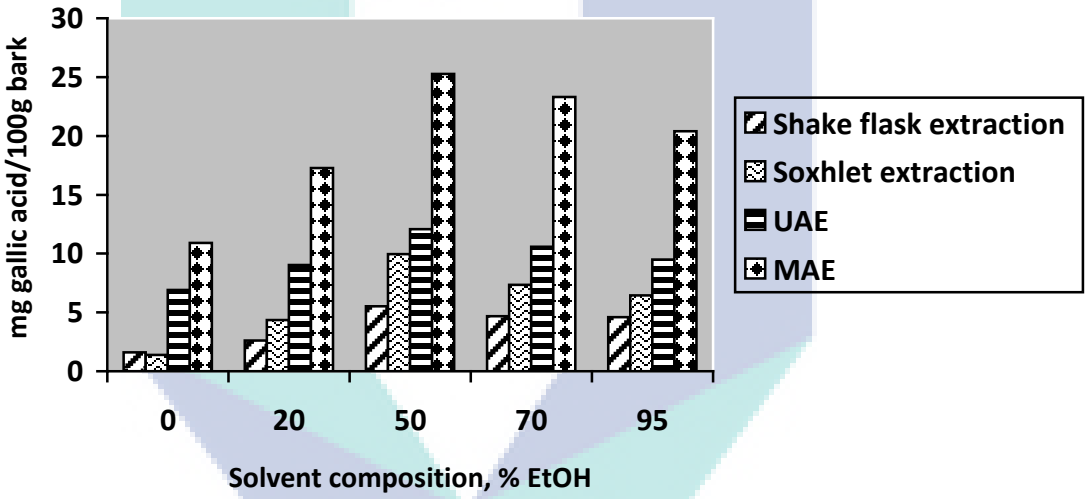


Figure 4.33: Comparison of the Extraction Techniques on the Effect of Solvent Composition

Shake flask extraction had the lowest extraction yield since this method only using stirring effect and rely on the diffusivity of the solvent into the plant material. Soxhlet extraction has a technique of heating up the solvent to its boiling point. The solvent vapor that evaporates was condensed and flooded the thimble-holder that contain with the bark. Once the solvent reaches the overflow level, the thimble-holder is emptied with the solvent running back down to the flask carrying the extracted compounds into the bulk solvent. Since the solvent is very hot, it might have an effect towards the thermal sensitive

compound like phenolic compound. The reason why the yield of gallic acid for Soxhlet extraction was higher than shake flask extraction might be because of the treatment using heat that could enhance the extraction process.

All the four extraction techniques used had shown the same pattern of results where 50% of solvent composition gave the highest amount of gallic acid extracted. This could be that 50% solvent composition provides a better solvent polarity to extract gallic acid from the stem bark. However, using MAE produced better yield compared to the other three extraction techniques.

4.8.2 Comparison of Extraction Techniques on the Effect of Extraction Time

The time of extraction had been evaluated within the scope of studies. The extraction 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 Figure 4.34.

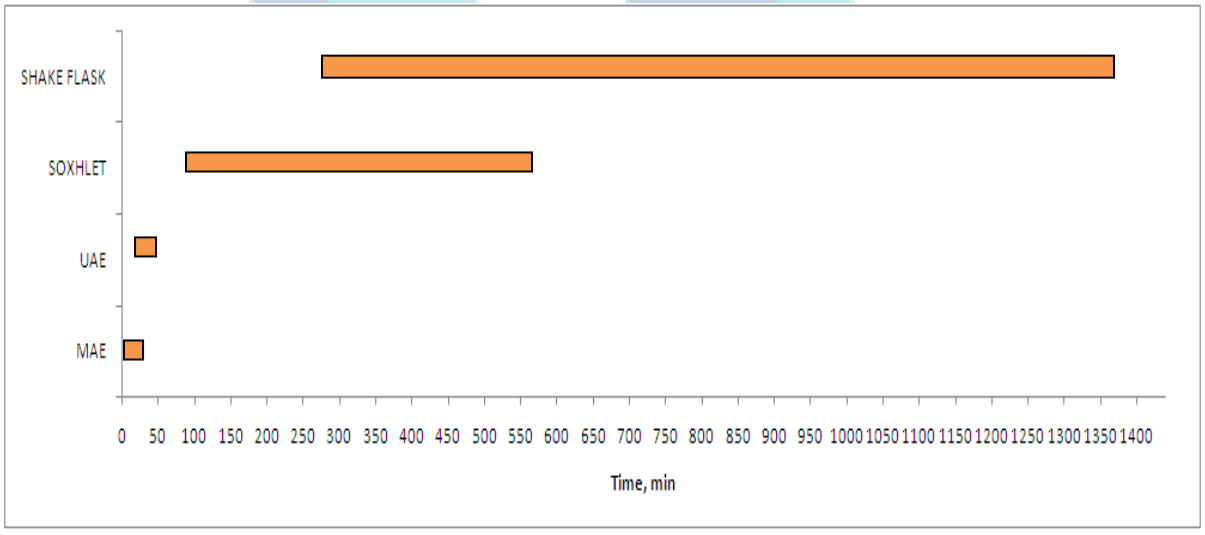


Figure 4.34: Comparison of Extraction Time for Shake Flask Extraction, Soxhlet Extraction, UAE and MAE

The results showed that extraction time is the shortest in the case of MAE comparable with the used of shake flask extraction, Soxhlet and UAE. Shortening the extraction time is important in the chemical analysis since sample preparation step is often obligatory and time consuming part of the analytical procedure. In the case of shake flask extraction, optimal extraction time is very long comparison with all the other techniques. Moreover, filtration and clean-up steps are nearly always essential, what cause that shake flask extraction is the most time consuming extraction technique.

4.8.3 Comparisons of Extraction Techniques on the Effect of Extraction Temperature

Temperature plays an important role too in the extraction process. The viscosity of the solvent and solubility of the target compound in the solvent used for extraction increased as the temperature rise. The added thermal energy helps to break the plant cells. However, in most techniques, isolation of gallic acid was done either at the ambient temperature or at the boiling point of the solvent, except for UAE and MAE, for which extraction temperature can be evaluated.

Figures 4.6 and 4.12 depicted the effect of extraction temperature on both UAE and MAE. The ranges of temperature studied for UAE and MAE were from 20^{o}C – 45^{o}C and 35^{o}C – 55^{o}C, respectively. The different ranges of temperatures were studied, while conducting UAE. MAE principle is based on heating up the water molecules in the plant cells, so higher range of temperature was studied. The highest recovery of gallic acid was using MAE at the temperature of 40^{o}C, while 35^{o}C for UAE gave the highest recovery. These observations show that, at a low temperature, it could give a good result for extraction of gallic acid. However, beyond a certain value of temperature might denature the phenolic compound (Spigno et al., 2007).

4.8.4 Comparison of Extraction Techniques on the Effect of Extraction Power

The extraction power was only evaluated for both UAE and MAE. The results are shown in Figures 4.8 and 4.11. The results in Figure 4.8 show that the ultrasonic power almost had no significant effect on the yield of gallic acid. This could be probably because of only small fraction of ultrasonic power was transferred into the extraction solvent in this ultrasonic system. The rest of the energy was absorbed by the water bath or the sides of metal bath.

Meanwhile, in MAE there was an increased of the yield when the microwave power was increased. Microwave power acts as a driving force to destroy the plant material to release the active compounds, and dissolve in the solvent. From these results, it can be noted that microwave power can influence the yield. The highest yield of gallic acid was obtained at the microwave power of 480 W.

4.8.5 Overall Comparison of Extraction Techniques on the Yield of Gallic Acid.

The selection of extraction techniques depends on the advantages and disadvantages of processes, such as extraction yield, complexity, production cost, environmental friendliness and safety. Shake flask extraction and Soxhlet extraction are the most common techniques of extraction. They are definite 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, these extraction techniques are not favorable to a commercial perspective.

UAE and MAE had received increasing attention as the alternative extraction techniques. 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 shake flask extraction, Soxhlet, UAE, and MAE was compared and shown in Table 4.11. The findings demonstrate that UAE and MAE are promising extraction techniques that offer improved efficiency. From the comparison shows that at shorter time of extraction of MAE gave higher extraction yield than the shake flask extraction, Soxhlet, and UAE. The MAE can give the highest extraction selectivity for that extraction of gallic acid.

Table 4.11: Comparison of Extraction Techniques on the Yield of Gallic Acid

Extraction technique	% EtOH	Extraction time, min	Extraction temperature, ^{o}C	Power, W
Shake flask	50	1200	25	NA
Soxhlet	50	480	80	NA
UAE	50	50	35	153.36
MAE	50	3	40	480

4.9 SUMMARY

The studies on all four extraction techniques had revealed promising results for the extraction of gallic acid from the stem bark of *Jatropha curcas*. There were several factors that could affect the extraction efficiency for each extraction techniques. UAE and MAE had been alternative extraction techniques for the improvement of product recovery by many researchers. By comparing the effectiveness of all extraction techniques, MAE was found to give better results. It provides evident advantages with shorter time of extraction, higher extraction yield, and better quality of the target compound.

The kinetic of the extraction process of bioactive compounds from plant material has often been modeled using the unsteady diffusion through plant materials, the film theory and the empirical equation of Ponomaryov. The proposed models are based on two stages, which were washing, or dissolution of the bioactive compounds near the particle surface, and slow extraction or diffusion from the solid particles to the liquid extract.

In optimization part, the optimum parameters for UAE were obtained by employing Box-Behnken Design (BBD) using Design Expert software. The optimum values obtained were solvent composition of 49.97%, extraction temperature of 35.70^{o}C and extraction time of 50.71 min.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The principle objective of this research work was to investigate an extraction technique that can isolate gallic acid from the stem bark of *Jatropha curcas*. Comparisons between conventional and modern extraction techniques applied were done to identify which technique give the comprehensive results of good isolation of gallic acid. In addition, kinetic studies of gallic acid were estimate to obtain washing coefficient and slow extraction coefficient. Optimization of the UAE parameter was done to attain the optimum condition that provides higher yield of gallic acid.

This chapter presents the main results discussed in Chapter 6. Based on the results of this study, it can be concluded that:-

5.1.1 Effect of Extraction Parameters on the Extraction of Gallic Acid

According to the results of extraction parameters (solvent composition, extraction time, extraction temperature and power) on four different extraction techniques, the following conclusion can be made:-

i. The effect of solvent composition on the extraction yield was depending on the solvent polarity of the solvent composition. This study used the mixture of ethanol-water as extraction solvent to enhance the extraction process. By increasing the proportion of water to ethanol, the solvent polarity will increase and able to extract phenolic compound such as gallic acid. In all he cases of different extraction techniques, 50% of ethanol composition was found to give higher amount of gallic acid recovery. This was due to the suitability of the solvent polarity that could attract more gallic acid compound from the bark particles into the solvent system. Even though the solvent has higher water content and expected to extract more gallic acid, due to the increase of solvent polarity it is not favorable for the extraction of gallic acid. This was because gallic acid are often more soluble in the solvent that is less polar than water.

ii. The extraction techniques for the isolation of gallic acid had different effective extraction time. Clearly that in all extraction techniques used in this study showed that as the time of extraction prolong, the gallic acid recovery is improved. Shortening the extraction time is important in chemical analysis since the sample preparation and the steps after extraction process are often obligatory and time consuming part of analytical procedure. The results showed that extraction time was the shortest in MAE comparable to other extraction techniques. This could provide an advantage towards the extraction of gallic acid.

iii. Temperature plays a significant role during the extraction process. Physical properties such as surface tension, density, viscosity, diffusivity, solubility and vapor pressure are affected when there is treatment with temperature. As the temperature is increased, the viscosity of the matrix and solubility of gallic acid in the solvent system increases. The added thermal energy also helps to break the cell walls, allowing the target compounds in the plant material to diffuse in the solvent. It is important to take note that at elevated temperature phenolic compounds can denatured.

iv. The extraction power was only evaluated for UAE and MAE. The extraction of gallic acid was enhanced when the external forces of ultrasonic wave and electromagnetic radiation were introduced. These forces could destroy the plant material cell wallst o release the bioactive compounds and dissolved in the solvent. Increasing the power generally will improve the extraction yield. But this was not expected from UAE probably because of only small fraction of ultrasonic power was transferred into the extraction solvent in the ultrasonic system. The rest of the energy was absorbed by the water bath or the sides of metal bath. Meanwhile, MAE gave better results with an increase of the yield of gallic acid when the microwave power increased.

5.1.2 Comparison of Extraction Techniques

A desirable extraction technique should be simple, inexpensive, efficient, selective, environmentally friendly and compatible with various analytical techniques. For this study, two modern techniques (UAE and MAE) were compared to two conventional techniques (shake flask extraction and Soxhlet extraction). The efficiency of the extraction techniques is in the following order: shake flask extraction < Soxhlet < UAE < MAE.

5.1.3 Kinetic Model of Different Extraction Techniques

The mechanism of extraction process of bioactive compounds can be considered in two stages: first stage is the washing or dissolution of the compounds near the particle surface and second stage is the slow extraction of diffusion from solid particles to the liquid extracts. The kinetic of the extraction process of bioactive compounds from plant material has often been modeled using the unsteady diffusion through plant materials, the film theory and the empirical equation of Ponomaryov. The values of kinetic parameters depend on the applied kinetic model and operating conditions. Models on film theory and the unsteady diffusion through the plant materials are based on a simplified description of the diffusion of extractive substances from plant materials into the solution. Washing of extractive substances from the surface of plant particles phenomena is happening before it reaches equilibrium. Empirical

equation of Ponomaryov has no physical basis and is only a mathematical description of the amount of extractive substances changes in plant materials. The models based on unsteady diffusion through the plant materials and empirical equation of Ponomaryov only during slow extraction process.

5.1.4 Optimization of Ultrasonic-Assisted Extraction Parameters

Optimization of the UAE parameters was done using Box-Behnken Design (BBD). The parameters that were optimized were solvent composition, extraction temperature and extraction time. The results for these optimum conditions were 40.97% of solvent composition, 35.70^{o}C of extraction temperature and 50.71 min of extraction time.

5.2 RECOMMENDATIONS

Based on this study, some recommendations need to be taken into account in order to improve this research study.

1. Several other factors such as solid to liquid ratio, different type of solvents and particle size of the bark sample can also influence the extraction efficiency. Considering all these factors can give better performance of the extraction process.

2. Some additional experiments can be done to study on the mass transfer process of gallic acid during extraction process.

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APPENDIX A

LIST OF ACHIEVEMENTS

Publications

1. Amirah Izam and Reddy Prasad (2011). Ultrasonic assisted extraction of tannins form *Jatropha curcas* stem bark. Proceedings in International Conference of Chemical Innovation (ICCI 2011). Terengganu, Malaysia, May 23-24, 2011
2. Amirah Izam, D.M.Reddy Prasad, Zulkafli bin Hassan, Maksudur R Khan, Microwave-assisted extraction of tannin from the stem bark of the *Jatropha curcas*, CET-2011 International Conference, Shanghai, China, 814-817.
3. I. Amirah and D.M. Reddy Prasad. Ultrasonic-assisted extraction of gallic acid from *Jatropha curcas* stem bark. Proceedings in International Chemical and Environmental Engineering Conference (ICEEC 2011). Kuala Lumpur, Malaysia, December 21-23, 2011. Accepted for publication in Journal of Chemical & Environmental Engineering, ISSN 2078-0732
4. I. Amirah and D.M. Reddy Prasad. Comparison of extraction techniques on extraction of gallic acid from stem bark of *Jatropha curcas*. Proceedings in International International Conference of Chemical Engineering and Industrial Biotechnology (ICCEIB-SOMChe 2011). Pahang, Malaysia, November 28 – December 1, 2011. Accepted for publication in Journal of Applied Science.
5. Reddy Prasad, Amirah Izam, Maksudur R.Khan, MDH Beg and Rosli Mohd Yunus, Extraction of natural Compounds by Ultrasonic Assisted Extraction, World Academy of Science, Engineering and Technology 76, 789-793, 2011

6. Amirah Izam, D.M Reddy Prasad, M.D Maksudur Rahman Khan. Jatropha curcas: Plant of medicinal benefits. Accepted for publication in Journal of Medicinal Plant Research
7. M. Thorik Firdaus, Amirah Izam, Reddy Prasad, Rosli, Ultrasonic-assisted extraction of triterpenoid Saponins from mangrove leaves, The 13th Asia Pacific Confederation of Chemical Engineering Congress, 1-8. Taipei, Taiwan. October 5-8, 2010

Awards

1. **Silver Medal** in Pertandingan Rekacipta & Inovasi UMP 2010, 1st to 3rd October 2010, for the invention titled "Formulation of Medicated Herbal Toothpaste from the latex of Jatropha curcas (From Waste to Wealth)".
2. **Bronze Medal** in BioInno Awards, BIOMALAYSIA-2010, 1st to 4th November 2010, for the invention titled "Formulation of Medicated Herbal Toothpaste from the latex of Jatropha curcas (From Waste to Wealth)".

Patents

1. D.M. Reddy Prasad**,** Amirah Izam, Ummi Hajira Khairuddin, Rosli Bin Mohd Yunus, Medicated herbal toothpaste formulation from the latex of *Jatropha curcas.* PI 2010004919. 2010.
2. D.M. Reddy Prasad**,** Amirah Izam, Rosli Bin Mohd Yunus, A PHARMACEUTICAL COMPOSITION, Microwave Assisted Extraction of *Ceriops Decandra Sp.* Leaves for Hypoglycemic Active Compounds. PI2010005375, 2010.

APPENDIX B

CALCULATIONS

Calculation of Amount of Gallic Acid

$A = B \times V \times 10 \times 1000 \frac{mg}{g}$, for 10 g of sample

$A = B \times V \times \frac{100}{20} \times 1000 \frac{mg}{g}$, for 20 g of sample

Where:

A is the amount of gallic acid, mg gallic acid/ 100g bark

B is the amount of gallic acid obtained from HPLC, g/ml

V is the volume of concentrated extract, ml

For example calculation of determination of the amount of A, taking one of the data from Appendix C3

B = 0.000020757 g/ml

V = 13.0 ml

$A = 0.000020757 \times 13 \times 10 \times 1000$

= 2.69841 mg gallic acid/ 100g bark

The rest of the data was calculated from the equations above.

Calculation of Determination of Washing Coefficient

From the kinetic model based on film theory, unsteady diffusion in plant material and empirical equation of Ponomaryov in Equation 4.8, 4.19 and 4.20 the washing coefficients, b and slow extraction coefficient, k were calculated.

Film theory

The linearized film theory model is as below.

$$\ln\left(1-\frac{c}{c_s}\right)=\ln(1-b)-kt$$

Where:

c is the concentration of gallic acid during extraction obtained from HPLC, g/L

c_s is the saturated concentration of gallic acid in the solution, g/L

b is the washing coefficient

k is the slow extraction coefficient

t is time, min

To plot the above equation, a series of data for the concentration of gallic acid during extraction, c will be taken from the HPLC analysis. The saturated concentration of gallic acid, c_s in the solution was determined from graphs of effect of time for each extraction techniques. The graph of $\ln\left(1-\frac{c}{c_s}\right)$ with time was plotted to obtain the linear regression. From this linear regression, the washing coefficient, b and slow extraction coefficient, k can be calculated. For example of calculating the washing coefficient, b and slow extraction coefficient, k, the linear equation in Figure 6.13 was as follow.

$$y=-0.00248x-0.01535$$

To compare with the film theory model equation above,

$$\ln(1-b) = -0.01535$$
$$b = 1 - e^{-0.01535}$$
$$b = 0.01523$$

$k = 0.00248\ min^{-1}$

The rest of the data were also calculated the same as above

Unsteady diffusion

The linearized unsteady diffusion model is as below.

$$\ln\frac{q}{q_o} = \ln(1-b') - k't$$

Where:

q is the content of gallic acid during extraction process, g/g

q_o is the initial content of gallic acid, g/g

b'' is the washing coefficient

k'' is the slow extraction coefficient

t is time, min

To obtain the initial amount of gallic acid, q_o was from the maximum concentration of gallic acid at certain time determined from graphs of effect of time for each extraction techniques. For example taken the data from Apeendix C4 and Appendix D2 to calculate q_o is as follows.

$$q_o = \frac{C \times V}{m}$$

Where:

C is the maximum concentration of gallic acid at t = 600 min from Figure 6.4 and Appendix C4, g/L

V is the volume of solvent used for extraction, L

m is the mass of sample, g

$$C = 0.00006677 \frac{g}{ml} \times 14ml \times 1000 \frac{ml}{L} \times 300ml$$

$$C = 0.003111593 \frac{g}{L}$$

$$q_o = \frac{0.003111593 \times 0.3}{10}$$

$$q_o = 9.33478e^{-5} \frac{g}{g}$$

Then the content of gallic acid at certain time, q will be calculate from equation below

$$q = q_o - \left(\frac{CV}{m} \right)$$

$$q = 9.33478e^{-5} \frac{g}{g} - \left(\frac{0.002026665 \frac{g}{L} \times 0.3L}{10g} \right)$$

$$q = 3.2548e^{-5} \frac{g}{g}$$

The graph $\ln\frac{q}{q_o}$ was plotted against time to attain the value of washing coefficient, b' and slow extraction coefficient, k' from the linear regression equation. For example taken the linear equation from Figure 6.18

$$y = -0.00764x - 0.01449$$

To compare with the unsteady diffusion model equation above,

$$\ln(1-b') = -0.01449$$
$$b = 1 - e^{-0.01449}$$
$$b = 0.01439$$

k' = 0.00764 min^{-1}

The rest of the data were also calculated the same as shown.

Empirical equation of Ponomaryov

The equation of empirical equation of Ponomaryov is as follow.

$$1 - \frac{q}{q_o} = b'' + k''t$$

Where:

q is the content of gallic acid during extraction process, g/g

q_o is the initial content of gallic acid, g/g

b'' is the washing coefficient

k'' is the slow extraction coefficient

t is time, min

To calculate the value of q and q_o it was the same as shown above. The graph of $1-\frac{q}{q_o}$ with time to determine the washing coefficient, b'' and slow extraction coefficient, k''. For example taken the linear equation from Figure 6.19

$$y = 0.0087x + 0.59106$$

To compare with the empirical equation of Ponomaryov above,

b'' = 0.59106 and k'' = 0.0087 min^{-1}

Theoretical concentration of gallic acid

To calculate the theoretical concentration of gallic acid,c, the film theory model from Eq 4.7

$$\left(1-\frac{c}{c_s}\right) = (1-b)e^{-kt}$$

Since the values of c_s, t, b and k were known the equation above was applied to calculate the theoretical concentration of gallic acid, c.

APPENDIX C

EFFECTS OF EXTRACTION PARAMETERS ON DIFFERENT EXTRACTION TECHNIQUES

Table C1: Effect of Solvent Composition for Shake Flask Extraction

Mass, g	Solvent composiiton, % EtOH	Temperature, °C	Time, min	Particle size, mm	Amount of gallic acid from HPLC, g/ml	Volume concentrated, ml	Amount of gallic acid, mg gallic acid/100g bark
20	0	25	1440	2mm<d<1mm	0.000026757	12	1.60542
20	20	25	1440	2mm<d<1mm	0.000040024	13	2.60156
20	50	25	1440	2mm<d<1mm	0.000092015	12	5.5209
20	70	25	1440	2mm<d<1mm	0.000078021	12	4.68126
20	95	25	1440	2mm<d<1mm	0.000076483	12	4.58898

Table C2: Effect of Extraction Time for Shake Flask Extraction

Mass, g	Time, min	Temperature, °C	Solvent composition, %ETOH	Particle size, mm	Amount of gallic acid from HPLC, g/ml	Volume concentrated, ml	Amount of gallic acid, mg gallic acid/ 100g bark
20	300	25	50	2mm<d<1mm	0.000042195	13	2.742675
20	600	25	50	2mm<d<1mm	0.000046942	13	3.05123
20	900	25	50	2mm<d<1mm	0.000060059	12.5	3.7536875
20	1200	25	50	2mm<d<1mm	0.000069015	12	4.1409
20	1440	25	50	2mm<d<1mm	0.000071892	11.5	4.13379

Table C3: Effect of Solvent Composition for Soxhlet Extraction

Mass, g	Solvent composition, % EtOH	Temperature, °C	Time, min	Particle size, mm	Concentration of gallic acid from HPLC, g/ml	Volume concentrated, ml	Amount of gallic acid, mg gallic acid/100 g bark
10	0	BP	480	2mm<d<1mm	0.000020757	13	2.69841
10	20	BP	480	2mm<d<1mm	0.000030024	14.5	4.35348
10	50	BP	480	2mm<d<1mm	0.000065	14	9.1
10	70	BP	480	2mm<d<1mm	0.000052456	14	7.34384
10	95	BP	480	2mm<d<1mm	0.000047832	13.5	6.45732

Table C4: Effect of Extraction Time for Soxhlet Extraction

Mass, g	Time, min	Temperature, °C	Solvent composition, % EtOH	Particle size, mm	Concentration of gallic acid from HPLC, g/ml	Volume concentrated, ml	Amount of gallic acid, mg gallic acid/100 g bark
10	120	BP	50	2mm<d<1mm	0.000045037	13.5	6.079995
10	240	BP	50	2mm<d<1mm	0.00005525	13.5	7.45875
10	360	BP	50	2mm<d<1mm	0.000062942	14	8.81188
10	480	BP	50	2mm<d<1mm	0.000065	14	9.1
10	600	BP	50	2mm<d<1mm	0.00006677	14	9.3478

Table C5: Effect of Ultrasonic Power for Ultrasonic-Assisted Extraction (UAE)

Mass, g	Ultrasonic power, Watt	Temperature, °C	Time, min	Solvent composition, % EtOH	Particle size, mm	Amount of gallic acid from HPLC, g/ml	Volume concentrated, ml	Amount of gallic acid, mg gallic acid/100g bark
20	76.68	RT	30	50	2mm<d<1mm	0.000170000	17.0000	14.45
20	102.24	RT	30	50	2mm<d<1mm	0.000174500	17.0000	14.8325
20	127.8	RT	30	50	2mm<d<1mm	0.000179200	17.0000	15.232
20	153.36	RT	30	50	2mm<d<1mm	0.000183400	17.0000	15.589
20	178.92	RT	30	50	2mm<d<1mm	0.000183900	17.0000	15.6315
20	204.48	RT	30	50	2mm<d<1mm	0.000183700	17.0000	15.6145

Table C6: Effect of Extraction Time for Ultrasonic-Assisted Extraction (UAE)

Mass, g	Time, min	Temperature, ^{o}C	Ultrasonic power, Watt	Solvent composition, % EtOH	Particle size, mm	Amount of gallic acid from HPLC, g/ml	Volume concntrated, ml	Amount of gallic acid, mg gallic acid/100g bark
20	10	RT	76.68	50	2mm<d<1mm	0.000137000	17.0000	11.645
20	20	RT	76.68	50	2mm<d<1mm	0.000157300	17.0000	13.3705
20	30	RT	76.68	50	2mm<d<1mm	0.000170000	17.0000	14.45
20	40	RT	76.68	50	2mm<d<1mm	0.000179000	17.8000	15.931
20	50	RT	76.68	50	2mm<d<1mm	0.000187500	18.0000	16.875

Table C7: Effect of Extraction Temperature for Ultrasonic-Assisted Extraction (UAE)

Mass, g	Temperature, ^{o}C	Time, min	Ultrasonic power, Watt	Solvent composition, % EtOH	Particle size, mm	Amount of gallic acid from HPLC, g/ml	Volume concentrated, ml	Amount of gallic acid, mg gallic acid/100g bark
20	20	30	76.68	50	2mm<d<1mm	0.000156300	16.0000	12.504
20	25	30	76.68	50	2mm<d<1mm	0.000171800	19.0000	16.321
20	30	30	76.68	50	2mm<d<1mm	0.000236100	16.0000	18.888
20	35	30	76.68	50	2mm<d<1mm	0.000240500	17.0000	20.4425
20	40	30	76.68	50	2mm<d<1mm	0.000208500	19.0000	19.8075

Table C8: Effect of Solvent Composition for Ultrasonic-Assisted Extraction (UAE)

Mass, g	Solvent composition, % EtOH	Temperature, °C	Ultrasonic power, Watt	Time, min	Particle size, mm	Amount of gallic acid from HPLC, g/ml	Volume concentrated, ml	Amount of gallic acid, mg gallic acid/100g bark
20	0	RT	76.68	30	2mm<d<1mm	0.000106320	17.0000	9.0372
20	20	RT	76.68	30	2mm<d<1mm	0.000139200	17.0000	11.832
20	30	RT	76.68	30	2mm<d<1mm	0.000141500	17.5000	12.38125
20	50	RT	76.68	30	2mm<d<1mm	0.000170000	17.0000	14.45
20	70	RT	76.68	30	2mm<d<1mm	0.000147590	17.0000	12.54515

Table C9: Effect of Solvent Composition for Microwave-Assisted Extraction (MAE)

Mass, g	Solvent composition, % EtOH	Temperature, °C	Microwave power, Watt	Time, min	Particle size, mm	Amount of gallic acid from HPLC, g/ml	Volume concentrated, ml	Amount of gallic acid, mg gallic acid/ 100g bark
10	0	40	480	3	2mm<d<1mm	0.00009087	12.0000	10.9044
10	20	40	480	3	2mm<d<1mm	0.00013293	13.0000	17.2809
10	50	40	480	3	2mm<d<1mm	0.00018790	13.5000	25.3665
10	70	40	480	3	2mm<d<1mm	0.00017275	13.5000	23.32125
10	95	40	480	3	2mm<d<1mm	0.00015110	13.5000	20.3985

Table C10: Effect of Extraction Time for Microwave-Assisted Extraction (MAE)

Mass, g	Time, min	Temperature, °C	Microwave power, Watt	Solvent composition, % EtOH	Particle size, mm	Amount of gallic acid from HPLC, g/ml	Volume concentrated, ml	Amount of gallic acid, mg gallic acid/ 100g bark
10	1.5	40	480	50	2mm<d<1mm	0.00018996	12.0000	22.7946
10	2	40	480	50	2mm<d<1mm	0.00018955	13.0000	24.6415
10	3	40	480	50	2mm<d<1mm	0.00018919	13.5000	25.54065
10	4	40	480	50	2mm<d<1mm	0.00019020	13.5000	25.677
10	5	40	480	50	2mm<d<1mm	0.00019070	13.5000	25.7445

Table C11: Effect of Microwave Power for Microwave-Assisted Extraction

Mass, g	Microwave power, Watt	Temperature, °C	Time, min	Solvent composition, % EtOH	Particle size, mm	Amount of gallic acid from HPLC, g/ml	Volume concentrated, ml	Amount of gallic acid, mg gallic acid/100g bark
10	160	40	4	50	2mm<d<1mm	0.00011259	13.0000	14.63657
10	320	40	4	50	2mm<d<1mm	0.00013641	14.0000	19.09691
10	480	40	4	50	2mm<d<1mm	0.00019040	13.5000	25.704
10	640	40	4	50	2mm<d<1mm	0.00017210	13.5000	23.2328925
10	800	40	4	50	2mm<d<1mm	0.00017017	13.5000	22.972275

Table C12: Effect of Extraction Temperature for Microwave-Assisted Extraction (MAE)

Mass, g	Temperature, °C	Microwave power, Watt	Time, min	Solvent composition, % EtOH	Particle size, mm	Amount of gallic acid, g/ml	Volume concentrated, ml	Amount of gallic acid, mg gallic acid/100g bark
10	35	480	4	50	2mm<d<1mm	0.00010300	13.0000	13.39
10	40	480	4	50	2mm<d<1mm	0.00019020	13.5000	25.677
10	45	480	4	50	2mm<d<1mm	0.00018883	13.5000	25.49205
10	50	480	4	50	2mm<d<1mm	0.00018373	13.5000	24.80355
10	55	480	4	50	2mm<d<1mm	0.00017248	13.5000	23.2848

APPENDIX D

KINETIC MODELS OF DIFFERENT EXTRACTION TECHNIQUES

Table D1: Kinetic Models for Shake Flask Extraction

Time, min	Concentration of gallic acid from HPLC, g/ml	Volume concentrated, ml	Concentration of gallic acid in solution, g/L (experimental)	q	ln q/q_o	1 - q/q_o	ln 1-(c/c_s)	Theoritical concentration of gallic acid, g/L
0	0	0	0	0	0	0	0	0
300	0.000042195	13	0.00182845	1.5708E-05	-1.010140585	0.635832221	-1.0101406	0.001529943
600	0.000046942	13	0.002034153	1.2623E-05	-1.228826744	0.707364287	-1.2288267	0.002236173
900	0.000060059	12.5	0.002502458	5.5983E-06	-2.041871923	0.870214465	-2.0418719	0.00257178
1200	0.000069015	12	0.0027606	1.7262E-06	-3.218416908	0.959981639	-3.2184169	0.002731264

Table D2: Kinetic Models for Soxhlet Extraction

Time, min	Concentration of gallic acid from HPLC, g/ml	Volume concentrated, ml	Concentration of gallic acid in solution, g/L (experimental)	q	ln q/q_o	1 - q/q_o	ln (1 - c/c_s)	Theoritical concentration of gallic acid, g/L
0	0	0	0	0	0	0	0	0
120	0.000045037	13.5	0.002026665	3.2548E-05	-1.053620988	0.673407821	-1.0536212	0.002012685
240	0.00005525	13.5	0.00248625	1.876E-05	-1.604589368	0.811755085	-1.6045898	0.002798241
360	0.000062942	14	0.002937293	5.229E-06	-2.882112247	0.947531081	-2.8821141	0.003112303
480	0.000065	14	0.003033333	2.3478E-06	-3.682853583	0.976441666	-3.6828577	0.003237864

Table D3: Kinetic Models for UAE

Time, min	Concentration of gallic acid from HPLC, g/ml	Volume concentrated, ml	Concentration of gallic acid in solution, g/L (experimental)	q	ln q/q_o	$1-q/q_o$	$\ln(1-c/c_s)$	Theoritical concentration of gallic acid, g/L
0	0	0	0	0	0	0	0	0
10	0.000137	17	0.015526667	0.00005572	-1.128143	0.676366382	-1.1281185	0.013222641
20	0.0001573	17	0.017827333	3.8465E-05	-1.498734	0.776587094	-1.4986926	0.017980466
30	0.00017	17	0.019266667	0.00002767	-1.828134	0.839286751	-1.8280719	0.020412652
40	0.0001795	17.8	0.021300667	1.2415E-05	-2.629577	0.927891038	-2.629425	0.021655978
50	0.0001875	18	0.0225	3.42E-06	-3.918842	0.980135912	-3.9182595	0.022291563

Table D4: Kinetic Models of MAE

Time, min	Concentration of gallic acid from HPLC, g/ml	Volume concentrated, ml	Concentration of gallic acid in solution, g/L (experimental)	q	ln q/q_o	1 - q/q_o	ln (1 - c/c_s)	Theoritical concentration of gallic acid, g/L
0	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1.5	0.00018995	12.0000	0.007598	2.9505E-05	-2.166246365	0.885393	-2.16624636	0.00773859
2	0.00018955	13.0000	0.008213833	0.00001103	-3.150187272	0.9571559	-3.15018727	0.00818592
3	0.00018919	13.5000	0.00851355	2.0385E-06	-4.838591862	0.9920818	-4.83859186	0.00849437
4	0.00019020	13.5000	0.008559	6.75E-07	-5.943848693	0.99737808	-5.94384869	0.00856231