Solid-Liquid Extraction of Hydrolysable Tannin (Gallic 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 Master of Engineering in Chemical

Faculty of Chemical Engineering and Natural Resources
UNIVERSITI MALAYSIA PAHANG

MARCH 2012
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°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. Kesaran 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 kesaran 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 diikaji telah memberi kesaran 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°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.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE PAGE</td>
<td>i</td>
</tr>
<tr>
<td>STATEMENT OF AWARD FOR DEGREE</td>
<td>ii</td>
</tr>
<tr>
<td>SUPERVISOR’S DECLARATION</td>
<td>iii</td>
</tr>
<tr>
<td>STUDENT’S DECLARATION</td>
<td>iv</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>vi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>viii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xviii</td>
</tr>
<tr>
<td>LIST OF NOMENCLATURES</td>
<td>xx</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>xxii</td>
</tr>
</tbody>
</table>

## CHAPTER 1 INTRODUCTION

1.1 Background of Study  
1.2 Problem Statement  
1.3 Objectives of Study  
1.4 Scopes of Study  
1.5 Significant of Study  
1.6 Thesis Outline  
1.7 Summary  

## CHAPTER 2 JATROPHA CURCAS

2.1 Introduction
2.2  *Jatropha curcas*

2.2.1 Chemical Composition Isolated from Different Parts and Various Medicinal Uses

2.2.1.1 Leaves
2.2.1.2 Stem Barks, Branches, Twigs
2.2.1.3 Latex
2.2.1.4 Fruits and Seeds
2.2.1.5 Root

2.3 Phenolic Compound

2.3.1 Plant Phenolics Occurrence
2.3.2 Tannins
2.3.3 Gallic Acid
2.3.4 Extraction of Phenolic Compounds

2.4 General Principle of Extraction

2.4.1 Solvent Choices
2.4.2 Shake Flask Extraction
2.4.3 Soxhlet Extraction
2.4.4 Ultrasonic-Assisted Extraction (UAE)
2.4.5 Microwave-Assisted Extraction (MAE)
2.4.6 The Kinetic of Solid-Liquid Extraction in Suspension

2.4.6.1 Kinetic Model Based on Film Theory
2.4.6.2 Kinetic Model Based on Unsteady Diffusion in Plant Material
2.4.6.3 Empirical Equations of Ponomaryov

2.5 Summary

CHAPTER 3 MATERIALS AND METHODS

3.1 Introduction
3.2 Material Selection
3.2.1 Sample Preparations 39

3.3 Extraction Procedures 40
3.3.1 Shake Flask Extraction 40
3.3.2 Soxhlet Extraction 41
3.3.3 Ultrasonic-Assisted Extraction (UAE) 42
3.3.4 Microwave-Assisted Extraction (MAE) 43

3.4 High-Performance Liquid Chromatography (HPLC) 44
3.4.1 Sample Preparations 44
3.4.2 Detection and Identification 45

3.5 Kinetic Model 45
3.5.1 Kinetics of Gallic Acid from Different Extraction Techniques 45

3.6 Optimization 46
3.7 Summary 46

CHAPTER 4 RESULTS AND DISCUSSION 48

4.1 Introduction 48
4.2 Shake Flask Extraction 48
4.2.1 Effect of Solvent Composition 48
4.2.2 Effect of Extraction Time 51
4.3 Soxhlet Extraction 52
4.3.1 Effect of Solvent Composition 52
4.3.2 Effect of Extraction Time 53
4.4 Ultrasonic-Assisted Extraction (UAE) 54
4.4.1 Effect of Solvent Composition 54
4.4.2 Effect of Extraction Temperature 57
4.4.3 Effect of Extraction Time 59
4.4.4 Effect of Ultrasonic Power 60
4.5 Microwave-Assisted Extraction (MAE) 62
4.5.1 Effect of Solvent Composition 62
4.5.2 Effect of Extraction Time 64
4.5.3 Effect of Microwave Power 66
4.5.4 Effect of Extraction Temperature 67

4.6 Kinetics Models of Extraction Techniques 69
4.6.1 Kinetic Study of Gallic Acid for Shake Flask Extraction 69
4.6.2 Kinetic Study of Gallic Acid for Soxhlet Extraction 72
4.6.3 Kinetic Study of Gallic Acid for UAE 75
4.6.4 Kinetic Study of Gallic Acid for MAE 78

4.7 Optimization of Ultrasonic-Assisted Extraction Parameters 81
4.7.1 Determination of Levels for Independent Variables 81
4.7.2 Response Surface Optimization of Ultrasonic-Assisted Extraction Conditions 82
4.7.3 Analysis of Response Surface 87

4.8 Comparison of Extraction Techniques on the Extraction of Gallic Acid 92
4.8.1 Comparison of Extraction Techniques on the Effect of Solvent Composition 93
4.8.2 Comparison of Extraction Techniques on the Effect of Extraction Time 95
4.8.3 Comparison of Extraction Techniques on the Effect of Extraction Temperature 96
4.8.4 Comparison of Extraction Techniques on the Effect of Extraction Power 97
4.8.5 Overall Comparison of Extraction Techniques on the Yield of Gallic Acid 97

4.9 Summary 98
## CHAPTER 5  CONCLUSIONS AND RECOMMENDATIONS

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Conclusion</td>
<td>100</td>
</tr>
<tr>
<td>5.1.1</td>
<td>Effect of Extraction Parameters on the Extraction of Gallic Acid</td>
<td>100</td>
</tr>
<tr>
<td>5.1.2</td>
<td>Comparison of Extraction Techniques</td>
<td>102</td>
</tr>
<tr>
<td>5.1.3</td>
<td>Kinetic Model of Different Extraction Techniques</td>
<td>102</td>
</tr>
<tr>
<td>5.1.4</td>
<td>Optimization of Ultrasonic-Assisted Extraction</td>
<td>103</td>
</tr>
<tr>
<td>5.2</td>
<td>Recommendations</td>
<td>103</td>
</tr>
</tbody>
</table>

### REFERENCES

<table>
<thead>
<tr>
<th>APPENDICES</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>List of Achievements</td>
<td>119</td>
</tr>
<tr>
<td>B</td>
<td>Calculations</td>
<td>121</td>
</tr>
<tr>
<td>C</td>
<td>Effects of Extraction Parameters on Different Extraction Techniques</td>
<td>127</td>
</tr>
<tr>
<td>D</td>
<td>Kinetic Models of Different Extraction Techniques</td>
<td>139</td>
</tr>
<tr>
<td>TABLE NO.</td>
<td>TITLE</td>
<td>PAGE</td>
</tr>
<tr>
<td>----------</td>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>2.1</td>
<td>Classification of Phenolic Compounds</td>
<td>17</td>
</tr>
<tr>
<td>3.1</td>
<td>Ranges of Experimental Parameters for Shaking Flask Extraction</td>
<td>41</td>
</tr>
<tr>
<td>3.2</td>
<td>Ranges of Experimental Parameters for Soxhlet Extraction</td>
<td>42</td>
</tr>
<tr>
<td>3.3</td>
<td>Ranges of Experimental Parameters for Ultrasonic-Assisted Extraction</td>
<td>43</td>
</tr>
<tr>
<td>3.4</td>
<td>Ranges of Experimental Parameters for Microwave-Assisted Extraction</td>
<td>43</td>
</tr>
<tr>
<td>4.1</td>
<td>Polarity Index of Different Solvent Composition</td>
<td>50</td>
</tr>
<tr>
<td>4.2</td>
<td>Properties of Viscosity, Surface Tension and Vapor Pressure of Water and Ethanol</td>
<td>50</td>
</tr>
<tr>
<td>4.3</td>
<td>Dielectric Constant of Water, Ethanol and Solvent Compositions</td>
<td>64</td>
</tr>
<tr>
<td>4.4</td>
<td>Values of Kinetic Parameters for Shake Flask Extraction</td>
<td>69</td>
</tr>
<tr>
<td>4.5</td>
<td>Values of Kinetic Parameters for Soxhlet Extraction</td>
<td>73</td>
</tr>
<tr>
<td>4.6</td>
<td>Values of Kinetic Parameters for UAE</td>
<td>76</td>
</tr>
<tr>
<td>4.7</td>
<td>Values of Kinetic Parameters for MAE</td>
<td>79</td>
</tr>
<tr>
<td>4.8</td>
<td>Box-Behnken Experimental Design With the Independent Variables</td>
<td>84</td>
</tr>
<tr>
<td>4.9</td>
<td>Analysis of Variance (ANOVA) for the Fitted Quadratic Polynomial Model of Extraction of Gallic Acid</td>
<td>85</td>
</tr>
<tr>
<td>4.10</td>
<td>Optimum Conditions and the Predicted and Experimental value of Response at the Optimum Conditions</td>
<td>92</td>
</tr>
<tr>
<td>4.11</td>
<td>Comparison of Extraction Techniques on the Yield of Gallic Acid</td>
<td>98</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Phenol Chemical Structure</td>
<td>16</td>
</tr>
<tr>
<td>2.2</td>
<td>Chemical Structure of Gallic Acid</td>
<td>20</td>
</tr>
<tr>
<td>2.3</td>
<td>Schematic Diagram for the Extraction Process in Solid Liquid</td>
<td>22</td>
</tr>
<tr>
<td>2.4</td>
<td>Soxhlet Extraction System</td>
<td>24</td>
</tr>
<tr>
<td>2.5</td>
<td>Expansion and Compression Cycles Produces by Ultrasound</td>
<td>26</td>
</tr>
<tr>
<td>3.1</td>
<td>Sieve Shaker (Fritsch, USA)</td>
<td>39</td>
</tr>
<tr>
<td>3.2</td>
<td>Orbital Shaker (Certomat, B. Braun)</td>
<td>40</td>
</tr>
<tr>
<td>3.3</td>
<td>Soxhlet Extractor</td>
<td>41</td>
</tr>
<tr>
<td>3.4</td>
<td>Water Bath Ultrasonic Extractor (Crest Ultrasonic, USA)</td>
<td>42</td>
</tr>
<tr>
<td>3.5</td>
<td>High–Performance Liquid Chromatography (HPLC) (Agilent Technologies, USA)</td>
<td>44</td>
</tr>
<tr>
<td>4.1</td>
<td>Effect of Solvent Composition on the Yield of Gallic Acid for Shake Flask Extraction</td>
<td>49</td>
</tr>
<tr>
<td>4.2</td>
<td>Effect of Extraction Time on the Yield of Gallic Acid for Shake Flask Extraction</td>
<td>52</td>
</tr>
<tr>
<td>4.3</td>
<td>Effect of Solvent Composition on the Yield of Gallic Acid for Soxhlet Extraction</td>
<td>53</td>
</tr>
<tr>
<td>4.4</td>
<td>Effect of Extraction Time on the Yield of Gallic Acid for Soxhlet Extraction</td>
<td>54</td>
</tr>
<tr>
<td>4.5</td>
<td>Effect of Solvent Composiiton on the Yield of Gallic Acid for UAE</td>
<td>55</td>
</tr>
<tr>
<td>4.6</td>
<td>Effect of Extraction Temperature on the Yield of Gallic Acid for UAE</td>
<td>58</td>
</tr>
<tr>
<td>4.7</td>
<td>Effect of Extraction Time on the Yield of Gallic Acid for UAE</td>
<td>60</td>
</tr>
<tr>
<td>4.8</td>
<td>Effect of Ultrasonic Power on the Yield of Gallic Acid for</td>
<td>61</td>
</tr>
</tbody>
</table>
4.9 Effect of Solvent Composition on the Yield of Gallic Acid for MAE 62
4.10 Effect of Extraction Time on the Yield of Gallic Acid for MAE 65
4.11 Effect of Microwave Power on the Yield of Gallic Acid for MAE 66
4.12 Effect of Extraction Temperature on the Yield of Gallic Acid for MAE 68
4.13 Linearized Form of Kinetic Equation Model Based on Film Theory for Shake Flask Extraction 70
4.14 Linearized Form of Kinetic Equation Model Based on Unsteady Diffusion Through Plant Material for Shake Flask Extraction 71
4.15 Linearized Form of Kinetic Study Equation Model Based on Ponomaryov Empirical Equation for Shake Flask Extraction 71
4.16 Comparison Between Experimental Concentration of Gallic Acid and Model Data for Shake Flask Extraction 72
4.17 Linearized Form of Kinetic Equation Model Based on Film Theory for Soxhlet Extraction 73
4.18 Linearized Form of Kinetic Equation Model Based on Unsteady Diffusion Through Plant Material for Soxhlet Extraction 74
4.19 Linearized Form of Kinetic Study Equation Model Based on Ponomaryov Empirical Equation for Soxhlet Extraction 74
4.20 Comparison Between Experimental Concentration of Gallic Acid and Model Data for Soxhlet Extraction 75
4.21 Linearized Form of Kinetic Equation Model Based on Film Theory for UAE 76
4.22 Linearized Form of Kinetic Equation Model Based on Unsteady Diffusion Through Plant Material for UAE 77
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.23</td>
<td>Linearized Form of Kinetic Study Equation Model Based on Ponomaryov Empirical Equation for UAE</td>
</tr>
<tr>
<td>4.24</td>
<td>Comparison Between Experimental Concentration of Gallic Acid and Model Data for UAE</td>
</tr>
<tr>
<td>4.25</td>
<td>Linearized Form of Kinetic Equation Model Based on Film Theory for MAE</td>
</tr>
<tr>
<td>4.26</td>
<td>Linearized Form of Kinetic Equation Model Based on Unsteady Diffusion Through Plant Material for MAE</td>
</tr>
<tr>
<td>4.27</td>
<td>Linearized Form of Kinetic Study Equation Model Based on Ponomaryov Empirical Equation for MAE</td>
</tr>
<tr>
<td>4.28</td>
<td>Comparison Between Experimental Concentration of Gallic Acid and Model Data for MAE</td>
</tr>
<tr>
<td>4.29</td>
<td>Response Surface Plot of Solvent Composition and Extraction Temperature on the Yield of Gallic Acid</td>
</tr>
<tr>
<td>4.30</td>
<td>Response Surface Plot of Solvent Composition and Extraction Time on the Yield of Gallic Acid</td>
</tr>
<tr>
<td>4.31</td>
<td>Response Surface Plot of Extraction Temperature and Extraction Time on the Yield of Gallic Acid</td>
</tr>
<tr>
<td>4.32</td>
<td>The Normal Probability Plot of Studentized Residual</td>
</tr>
<tr>
<td>4.33</td>
<td>Comparison of Extraction Techniques on the Effect of Solvent Composition</td>
</tr>
<tr>
<td>4.34</td>
<td>Comparison of Extraction Time for Shake Flask Extraction, Soxhlet Extraction, UAE and MAE</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

°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
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV</td>
<td>Ultra Violet</td>
</tr>
<tr>
<td>v/v</td>
<td>volume per volume</td>
</tr>
</tbody>
</table>
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
  mean concentration of extractible substances in the particle
c_o concentration of extractive substances in a particle at the beginning
  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
Watt

Greek Symbols

\( \alpha \) constant values depend on the particle shape
\( \beta \) constant values depend on the particle shape
\( \beta_0 \) constant term
\( \beta_i \) coefficient of linear parameter/ first-order term
\( \beta_{ij} \) coefficient of second-order interaction terms
\( \mu \) Micron
\( \varepsilon \) residual associated to the experiments
\( \varepsilon' \) dielectric constant
\( \varepsilon'_{i} \) dielectric constant of \( i \)th solvent
\( \varepsilon'_{m} \) dielectric constant of solvent mixture
\( \varepsilon'' \) dielectric loss
\( \Phi_i \) volume fraction
\( \Phi_1 \) volume fraction of solvent 1
\( \Phi_2 \) volume fraction of solvent 2
\( \tan \delta \) dissipation factor
\% Percent
\% EtOH percent ethanol
# LIST OF APPENDICES

<table>
<thead>
<tr>
<th>APPENDIX</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>List of Achievements</td>
<td>119</td>
</tr>
<tr>
<td>B</td>
<td>Calculations</td>
<td>121</td>
</tr>
<tr>
<td>C</td>
<td>Effect of Extraction Parameters on Different Extraction</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td>Techniques</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Kinetic Models on Different Extraction Techniques</td>
<td>139</td>
</tr>
</tbody>
</table>
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. Jatropa 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.