ULTRASONIC-ASSISTED EXTRACTION (UAE) OF TANNINS FROM STEM BARK OF *JATROPHA CURCAS*

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ABSTRACT

Jatropha curcas, is gaining lot of importance in medicinal uses. The bark of *Jatropha Curcas* presents a good source of functional compounds, such as polyphenols. Ultrasound-assisted Extraction is evaluated as a simpler and more effective to conventional extraction for isolation of tannins from *Jatropha Curcas* stem bark. The purposes of this study are to extract tannins from stem bark of of *Jatropha Curcas* and also to determine the optimum condition to extract the tannins. The effects of solvent ratio (ethanol:water), temperature, extraction time, and amplitude of sonication were studied. Ethanol was used as solvent in this experiment. Extraction was done by using Ultrasounic-assisted extraction, which potentially enhances extraction of polyphenolics. Purification of tannins using rotary evaporator need to be done in order to obtained pure extract. Total phenolic content can be determined spectrophotometrically using Folin–Ciocalteau method. To obtain the amount of Gallic acid in extract, HPLC was used. In this experiment, the analysis revealed that the optimized conditions were when the particle was 1.0 mm in size, a temperature of 30°C, an amplitude of 40%, an extraction time of 30 minutes and 1:0 (v/v) ethanol:water ratio. For the conclusion, extraction of tannins using Ultrasound-assisted Extraction was achieved and the optimum condition to extract tannins from *Jatropha Curcas* stem bark was obtained.

ABSTRAK

Pokok jarak pagar, mempunyai banyak kepentingan dalam perubatan. Kulit pokok jarak pagar terdapat sumber berfungsi yang sangat baik, seperti polifenol. Pengekstrakan Gelombang Bunyi Bantu dinilai sebagai lebih sederhana dan lebih efektif untuk pengekstrakan konvensional untuk isolasi tanin dari kulit batang Jarak Pagar. Tujuan dari penelitian ini adalah untuk mengekstrak tanin dari kulit batang Jarak Pagar dan juga untuk menentukan keadaan optimum untuk mengekstrak tanin. Pengaruh nisbah pelarut (etanol: air), suhu, waktu pengekstrakan, dan amplitud sonikasi dipelajari. Etanol digunakan sebagai pelarut dalam percubaan ini. Pengekstrakan dilakukan dengan menggunakan Pengekstrakan Gelombang Bunyi Bantu, yang berpotensi meningkatkan pengekstrakan polifenol. Pengaslian tanin menggunakan Pengwapan secara pusingan perlu dilakukan dalam rangka untuk mendapatkan ekstrak yang asli. Jumlah fenolik kandungan dapat ditentukan secara spektrofotometri menggunakan kaedah Folin-Ciocalteau. Untuk mendapatkan jumlah asid Gaul dalam ekstrak, HPLC digunakan. Dalam kajian ini, analisis mendedahkan bahawa keadaan optimum adalah ketika zarah adalah pada 1.0 mm dalam saiz, dengan suhu 30 ^{0} C, amplitud 40%, waktu pengekstrakan selama 30 minit dan dengan nisbah pelarut 1:00 (v / v) etanol: air. Sebagai kesimpulan, pengekstrakan tannin menggunakan Pengekstrakan Gelombang Bunyi Bantu dan keadaan optimum untuk mengekstrak tannin dari pokok jarak pagar diperolehi.

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LIST OF ABBREVIATIONS AND SYMBOLS

Bhd.	-	Berhad
GAE	-	Gallic acid equivalent
HPLC	-	High-performance Liquid Chromatography
I.D	-	Inside Diameter
PVDF	-	Polyvinyledene Diflouride
PWE	-	Pressurized water extraction
TPC	-	Total phenol content
UAE	-	Ultrasonic-assisted Extraction
US	-	United State
SE	-	Soxhlet extraction
SFE	-	Supercritical fluid extraction
mg	-	miligram
mm	-	milimeter
mL	-	mililiter
min	-	minute
nm	-	nanometer
µg	-	Micron gram
v/v	-	Volume ratio
sp.	-	Species
s	-	Second
^{o}C	-	Degree celcius

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Jatropha curcas (Linnaeus) is a multipurpose bush/small tree belonging to the family of *Euphorbiaceae*. It is a plant with many attributes, multiple uses and considerable potential. The plant can be used to prevent and/or control erosion, to reclaim land, grown as a live fence, especially to contain or exclude farm animals and be planted as a commercial crop. It is a native of tropical America, but now thrives in many parts of the tropics and sub-tropics in Africa/Asia. It has few pests and diseases and will grow under a wide range of rainfall regimes from 200 to over 1500 mm per annum. In low rainfall areas and in prolonged rainless periods, the plant sheds its leaves as a counter to drought (Openshaw, 1999).

Common names include Barbados Nut, Purging Nut, and Physic Nut. *J. curcas* is a poisonous, semi-evergreen shrub or small tree, reaching a height of 6 m (20 ft) (www.wikipedia.com). The genus name Jatropha derives from the Greek word *jatros* (doctor) and *trophe* (food), which implies medicinal uses (Kumar et al., 2006).

Table 1.1: Uses of *Jatropha Curcas*: (www.jatrophabiodiesel.com)

Whole Plant	Roots	Leaves	Latex	Seeds	Bark	Twig
Planted to prevent water erosion and for conservation	Used as ethno medicine	Used as ethno medicine	Resembles shellac	Source of oil (30-40%) suitable as fuel for diesel engine	Yields tannins (37%)	Used as medicine
Promosing live fence		Yield a dye used to give tan & brown	Used for making ink	Useful as illumitant, lubricant, in soap and candle making.		Used as Dataun (herbal tooth brush)
Useful as green manure		Useful as botanical	Used as ethno medicine	Used as medicine both internally and externally		Young one cooked and eaten

The most part of the *Jatropha Curcas* tree can be used as medicine. *Jatropha Curcas* is the sources of secondary metabolites of medicinal importance. The leaf, fruits, latex and bark of *Jatropha Curcas* contain glycosides, tannins, phytosterols, flavonoids, and steroidal sapogenins that exhibit wide ranging medicinal properties (www.jatrophabussiness.com). In this study, the bark of *Jatropha Curcas* is used to extract tannins.

The bark presents a good source of functional compounds, such as polyphenols. To produce the typical tannin effect, the hydrolyzable tannins react with proteins. Medicinally, this is important for treatment of inflamed or ulcerated tissues. They also contribute most of the astringent quality that is noted when drinking tannin-containing beverages.

Furthermore, this study is a preliminary research and later it will be a pioneer to do more research on this tannins extraction from bark of *Jatropha Curcas*.

Extraction process is the best method to separate the polyphenols from the bark. In the production of polyphenols, an organic solvent such as ethanol is used to extract polyphenols from bark of *Jatropha Curcas*. Selection of extraction conditions depends on the nature of the extraction process, the temperature, pH and residence time could have an effect on the yield and selectivity (www.cheresource.com). For this experiment, extraction process was conducted by using Ultrasound-assisted Extraction (UAE).

1.2 Problem Statements

A lot of studies had been conducted by using other parts of *Jatropha Curcas* tree such as seeds, fruits, stems, leaves, twigs and also latex. But studies on bark of *Jatropha Curcas* are limited. This is due to lack of exposure on the benefits of medicinal uses in the bark of *Jatropha curcas*.

Since know that the stem or branch is cut off 20 to 30 cm above the soil surface or from last pruning, which pruning could be done after the plant 6 weeks of age, the stem have been wasted without knowing that it have a lot of medicinal purposes. So this research could be turned the waste to wealth, without any cost in getting the branches.

This study was conducted to extract tannins to give more pleasant consume towards the consumers. Tannins have been proved can act as an alternative medicine to people. However, this is a preliminary research as there is not much evidence to prove the benefits of bark of *Jatropha Curcas* which contain tannin

1.3 Objectives

The purposes of this study were to extract tannins from bark of *Jatropha Curcas* using ultrasonic-assisted extraction and to determine the optimum condition for extraction of tannins from *Jatropha curcas* bark.

1.4 Scopes of Study

Raw material used in this study is bark of *Jatropha Curcas*. Preparation of the raw material before the process of extraction is needed to increase extraction yield.

In the extraction by using Ultrasound-assisted Extraction (UAE), ethanol as solvent is used to extract tannins. Ethanol is chose because it is easy to get from any chemical supplier companies in Malaysia and also, it is an organic solvent and suitable for consumers.

Parameters used to extract tannins, were time of extraction (10 – 50 min), amplitude (40 – 80%), temperature (20 - 60°C), and solvent ratio (ethanol: water) (0 – 100% of ethanol) and size of particles (>2.0mm, 2.0mm, 1.0mm, 0.5mm, and <0.5mm).

Analysis was done by using UV-Vis Spectrophotometer to determine the concentration of total phenol contents in extracts, while High-performance Liquid Chromatography was done to determine the amount of Gallic acid in extracts.

1.5 Rationale and Significance

The rationales of doing this study are:

1. To increase the extraction yield by monitoring parameters so that the pure extracts can be used effectively.
2. To do preliminary research on the extracted tannins using bark of *Jatropha Curcas*. This study is a pioneer toward further research and studies.

CHAPTER 2

LITERATURE REVIEW

2.1 The Bark of *Jatropha Curcas*

Jatropha Curcas stem bark extracts revealed the presence active biological of saponins, steroids, tannins, glycosides, alkaloids and flavonoids in phytochemical screening investigation and aid the antimicrobial activities of *Jatropha Curcas*. These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins have been found to form irreversible complexes with prolinerich protein (Shimada, 2006) resulting in the inhibition of cell protein synthesis.

Another secondary metabolite compound observed in the stem bark extract of *Jatropha Curcas* was alkaloid. Just et al. (1998) revealed the inhibitory effect of saponins on inflamed cells. Saponin was found to be present in *J. curcas* extracts and has supported the usefulness of this plant in managing inflammation. Steroidal compounds present in *J. curcas* extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones (Okwu, 2001). Quinlan et al. (2000) 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. Flavonoids, another constituent of *J. curcas* stem bark extracts exhibited a wide range of biological activities like

antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek et al., 2002).

Different parts of *J. curcas* contain the toxic alkaloids curcin and phorbal ester which prevent animals from feeding on it. Hence, the presence of these compounds in *Jatropha Curcas* corroborates the antimicrobial activities observed. It is concluded that *J. curcas* stem bark could be a potential source of active antimicrobial agents, and a detailed assessment of its *in vivo* potencies and toxicological profile is ongoing.

2.2 Tannins

Tannins are polyphenols that are obtained from various parts of different plants belonging to multiple species. Deriving it name from the technical word ‘tanning’ that meant converting animal hides to leather through chemical processes; tannin is basically used for this function. It is found in abundance in wood, fruit, fruit pod, leaves, roots, also in plant gall and particularly in the bark of oaks species and in sumac and myrobalan. Since earlier times, people obtained tannin for tanning from plants like wattle (*Acacia* sp.), oak (*Quercus* sp.), eucalyptus (*Eucalyptus* sp.), birch (*Betula* sp.), willow (*Salix caprea*), pine (*Pinus* sp.), quebracho (*Scinopsis balansae*) (www.herbs2000.com).

Tannins also called tannic acid, one of a group of pale-yellow to light brown amorphous substances in the form of powder, flakes, or a spongy mass, widely distributed in plants and used in tanning leather, dyeing fabric, making ink, and in various medical applications (www.britannica.com). Interestingly, tannins are found almost in all plants and in all climates all over the world. Although algae, fungi and mosses do not contain much tannin, the percentage of tannins present in the plants, however, varies. While they are present in significant proportions in some plants, many others have too little of them. Tannins are usually found in large quantities in

the bark of trees where they act as a barrier for micro-organisms like bacteria and fungi and protect the tree.

2.3 Medicinal Uses

All parts of *Jatropha* (seeds, leaves and bark) have been used in traditional medicine and for veterinary purposes for a long time (Dalziel, 1955; Duke, 1985 and Duke, 1988). Some compounds (Curcacycline A) with antitumor activities were reportedly found in this plant (Van den Berg et al., 1995).

In this study, the bark of *Jatropha Curcas* was used to extract medicinal tannins. Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). These observations therefore support the use of *J. curcas* in herbal cure remedies. Li and Wang (2003) reviewed the bio-logical activities of tannins and observed that tannins have anticancer activity and can be used in cancer prevention, thus suggesting that *Jatropha Curcas* has potential as a source of important bioactive molecules for the treatment and prevention of cancer. The presence of tannins in *Jatropha Curcas* supports the traditional medicinal use of this plant in the treatment of different ailments. Other than that, tannins can be as treatment of bleeding, including functional bleeding, hematochezia (blood in the stool), bleeding hemorrhoids, and topically for bleeding wounds and ulcerations. The tannins also can be as excessive discharge, such as enuresis and frequent urination, leucorrhea, hyperhidrosis (excessive sweating) and night sweating, involuntary seminal emission (Dharmananda, 2003).

Hydrolysable tannins are basically derived from simple phenolic acids like gallic acid or ellagic acid and when heated they give away pyrogallol. Pyrogallol is also known as hepatotoxic and has antiseptic as well as caustic properties. While condensed tannins are basically flavonoid dyes formed through bio-synthesis of flavins and catechins. Tannins can also be effective in curbing hemorrhages as well as restrict bare swellings. While tannins are proved haemostatics, they are also beneficial when applied on mucosal coating in mouth. Hence, herbs possessing tannins are widely used as mouthwashes, eyewashes, snuff and even as vaginal douches and also treat rectal disorders.

The other secondary metabolite compound observed in the stem bark extract of *Jatropha Curcas* was alkaloid. Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew, 2002).

2.4 Ultrasound-assisted Extraction (UAE)

Ultrasound is sound waves, which have frequencies higher than 20 kHz, are mechanical vibrations in a solid, liquid and gas. Unlike electromagnetic waves, sound waves must travel in a matter and they involve expansion and compression cycles during travel in the medium. Expansion pulls molecules apart and compression pushes them together. The expansion can create bubbles in a liquid and produce negative pressure. The bubbles form, grow and finally collapse (Wang & Weller, 2006).

Ultrasound-assisted extraction is an inexpensive, simple and efficient alternative to conventional extraction techniques. The main benefits of use of ultrasound in solid–liquid extraction include the increase of extraction yield and faster kinetics (Wu *et. al*, 2001). Ultrasound can also reduce the operating temperature allowing the extraction of thermolabile compounds. Compared with other novel

extraction techniques such as microwave-assisted extraction, the ultrasound apparatus is cheaper and its operation is easier (Wang & Weller, 2006). Furthermore, the ultrasound-assisted extraction, like Soxhlet extraction, can be used with any solvent for extracting a wide variety of natural compounds.

Two general designs of ultrasound-assisted extractors are ultrasonic baths or closed extractors fitted with an ultrasonic horn transducer. The mechanical effects of ultrasound induce a greater penetration of solvent into cellular materials and improve mass transfer. Ultrasound in extraction can also disrupt biological cell walls, facilitating the release of contents. Therefore, efficient cell disruption and effective mass transfer are cited as two major factors leading to the enhancement of extraction with ultrasonic power (Mason, Paniwnyk, & Lorimer, 1996). It is necessary to take into account plant characteristics such as moisture content and particle size, and solvent used for the extraction in order to obtain an efficient and effective ultrasound-assisted extraction. Furthermore, many factors govern the action of ultrasound including frequency, temperature, and sonication time.

Figure 2.1 Ultrasonic-assisted Extraction

CHAPTER 3

METHODOLOGY

3.1 Materials and Solvents

The raw material that used for this experiment was bark of *Jatropha Curcas* from a family of *Euphorbiaceae*. The raw material was obtained from a plantation in Serdang, Malaysia. Ethanol 95% v/v was purchased from R&M Chemicals and was used as a solvent in extraction of tannins from *Jatropha Curcas* Bark. Other chemical used were Folin-Ciocalteu Reagent (Fistcher Chemicals Bhd) used in Folin-Ciocalteu method, Acetonitrile and Ortho-phosphoric acid have been used as mobile phase in HPLC, and Gallic acid as the medium for preparation of Gallic acid stock solution.

3.2 Apparatus

Granulometric apparatus was used in sizing the sample, to get the homogenous particle size. In the extraction process, Ultrasound-assisted Extraction used to extract tannins from the barks. Purification of total phenol need to be done in order to obtained pure total phenol and rotary evaporator was the equipment responsible to remove the solvent from extracted. Uv-vis was used to do the chemical analysis which to get the concentration of extracted total phenol. While high performance liquid

chromatography (HPLC) was used in order to get the amount of tannins in extracted total phenol.

3.3 Standand Curve for UV-Vis Spectrophotometer Preparation

Standard curves are used to determine the concentration of substances. In order to prepare the standard curve for UV-Vis Spectrophotometer, there were a few first preparations before start the analysis. Folin-Ciocalteu reagent diluted with 10 times of water (1:10; Folin-Ciocalteu reagent:water), and sodium carbonate solution of 75 mg/mL were used in preparing the stock solution.

3.3.1 Preparation of Gallic Acid Stock Solution

Briefly, a calibration curve, using gallic acid with concentrations ranging from 8 to 80 µg/mL was prepared. In a 100 mL of volumetric flask, 0.5 gram of dry Gallic acid dissolved in 10 mL of ethanol, and dilute to volume 100 mL in water (0.005 g/mL). Gallic acid stock solutions in volumes ranging from 0.0016 to 0.016 mL were pipette out into test tubes. The final volume is made to 10 mL with ethanol in each test tube. Table 3.1 shows the preparation of Gallic acid stock solution.

The tubes then were kept five (5) minutes in water bath at temperature of 50°C and transferred to cold water. 2.5 mL of diluted Folin-Ciocalteu reagent was added to each test tube. After in ranging from 30 s to 8 min the Folin-Ciocalteu reagent was added, 2 mL of sodium carbonate was added. 0.02 mL was taken from different concentration resulting Gallic acid solution and negative control ethanol were mixed with 1.58 mL of diluted water in cuvette.

Analyze the stock solution by using UV-Vis Spectrophotometer to determine the concentration of stock solution. The absorbance was read at 760 nm and Gallic acid calibration curve was obtained by plotting the absorbance against concentration of Gallic acid, mg/mL.

Table 3.1 Preparation of Gallic acid Stock Solution

Gallic acid µg/mL	Gallic acid stock solution, mL	Ethanol, mL
0	0	1.0000
8	$1.6E10^{-3}$	0.9984
16	$3.2E10^{-3}$	0.9968
24	$4.8E10^{-3}$	0.9952
32	$6.4E10^{-3}$	0.9936
40	$8.0E10^{-3}$	0.9920
48	$9.6E10^{-3}$	0.9904
56	0.0112	0.9888
64	0.0128	0.9872
72	0.0144	0.9856
80	0.0160	0.9840

3.4 Preparation of Extracts

Briefly, upon the barks were obtained from the *Jatropha Curcas* tree, they were transported to the laboratory, strip the bark from the stem and cut it into small pieces. The barks then were dried in oven until the weight is constant (approximately 10 hours). After that, keep the bark in sealed clean plastic bags and label.

Figure 3.1 Strip the bark from the stem.

3.5 Sample Sizing

A granulometric apparatus was used to obtain a homogenous particle size and also to study the consequence of the granulometric size of bark to resulting extraction. Barks were ground and the separation of the obtain size was carried out with a sieve shaker Fritsch (Idar-Oberstein, Germany) including various granulometric sizes sieves (0.5mm – 2.0mm).

Figure 3.2 Sieve Shaker

3.6 Ultrasound-assisted extraction (UAE)

In Ultrasound-assisted extraction, 10 g of roots is mixed with 150 ml of ethanol as extracting solvent in a 250 ml of beaker. The beaker is immersed in ultrasound cleaning bath. The amplitude, time of extraction and frequency are set accordingly. Temperature of sample must be controlled manually by using water bath. Experiment is repeated by using different time of extraction, different solvents ratio, different temperature, and different amplitude of sonication. The solution is then filtered through a filter paper.

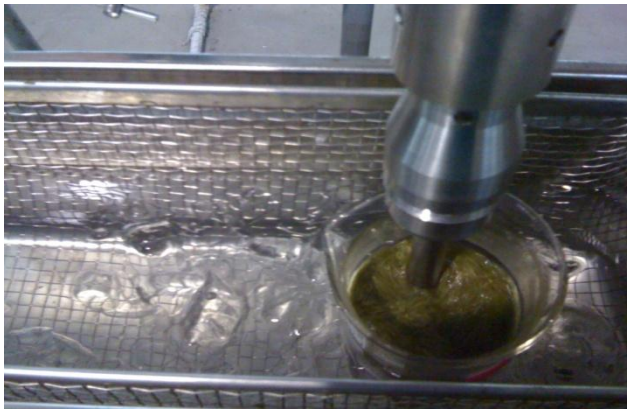

Figure 3.3 Sample extract in water bath by using UAE