EXTRACTION OF MANGROVE COMPONENT FOR ISOLATION OF TRITERPENOID SAPONINS VIA ULTRASONIC EXTRACTION METHOD

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A dissertation submitted in partial fulfillment of the Requirements for the award of the degree of Bachelor of Engineering (Chemical Engineering)

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ABSTRACT

Ultrasound-assisted extraction was evaluated as a simpler and more effective alternative to conventional method for isolation of actives compound in plants. Ultrasonic extraction has been widely used for getting bioactive substances from different parts of a number of plants. The ultrasonic enhancement of extraction is attributed to disruption of cell walls, particle size reduction and enhanced mass transfer of the cell content via cavitations bubble collapse. Direct evidences are given, confirming that enhanced hydration process and plant material fragmentations are the primary benefits of sonification. In this paper, a method of ultrasonic-assisted extraction was used to extract total triterpenoid saponins from *Ceriops Decandra sp*, which have been reported to have several medicinal properties and uses. The extracts were directly determined by colorimetric method without any further treatment. Compared with thermal extraction or soxhlet extraction, ultrasonic extraction only need 15 min to give the highest yield of triterpenoid saponins at 0.9972%, while the other extraction methods need several hours or even more than 3 h and give lower yield. Several factors affecting the ultrasonic extraction rate were also discussed, such as extraction time, temperature and ratio of solvent to material. Optimal conditions of ultrasonic extraction can be concluded as follows: 30 min at 70˚C, the ratio of solvent to material is 25 ml/g by using 95% ethanol as the solvent.

ABSTRAK

Ekstrak secara ultrasonik merupakan kaedah yang paling mudah dan efektif serta kaedah yang berkesan untuk mendapatkan komponen-kompenen aktif dalam tumbuh-tumbuhan. Kaedah ini juga digunakan secara meluas bagi mendapatkan zat-zat bioaktif dari pelbagai bahagian tumbuhan. Peningkatan process ekstrak ultrasonik adalah disebabkan gangguan pada dinding sel, pengurangan saiz zarah dan perpindahan jisim yang disempurnakan oleh kandungan sel melalui pergeseran gelembung kavitasi. Ini terbukti bahawa proses penghidratan serta fragmentasi akan meningkat melalui proses ultrasonik. Dalam tesis ini, kaedah ekstrak secara ultrasonik telah digunakan untuk mengestrak triterpenoid saponins dari tumbuhan *Ceriops decandra sp* yang dikatakan mujarab dan boleh dijadikan ubat. Eksrak ini terus dikaji melalui kaedah kalorimetri tanpa melalui kaedah yang lain. Process ekstrak secara ultrasonik ini dibandingkan dengan ekstrak melalui soxhlet dan hasilnya menunjukkan bahawa ekstrak secara ultrasonic hanya memerlukan 15 minit untuk terus mendapatkan hasil triterpenoid saponins sedangkan kaedah ekstrak dengan soxhlet memerlukan masa yang lebih lama dan memberikan hasil yang rendah. Di samping itu, factor-faktor yang memberikan kesan kepada kadar ekstrak ultrasonik juga dibincangkan seperti masa, nisbah antara larutan dan bahan serta suhu. Keadaan optimum ekstraksi ultrasonik dapat disimpulkan sebagai berikut: 30 minit pada 70 ˚ C, nisbah pelarut untuk bahan adalah 25 ml / g dengan menggunakan etanol 95% sebagai pelarut.

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Abstract

Ultrasound-assisted extraction was evaluated as a simpler and more effective alternative to conventional method for isolation of actives compound in plants. Ultrasonic extraction has been widely used for getting bioactive substances from different parts of a number of plants. The ultrasonic enhancement of extraction is attributed to disruption of cell walls, particle size reduction and enhanced mass transfer of the cell content via cavitations bubble collapse. Direct evidences are given , confirming that an enhanced hydration process and plant material fragmentation are the primary benefits of sonification. In this paper, a method of ultrasonic-assisted extraction was used to extract total triterpenoid saponins from *Ceriops Decandra sp*, which have been reported to have several medicinal properties and uses. The extracts were directly determined by colorimetric method without any further treatment. Compared with thermal extraction or soxhlet extraction, ultrasonic extraction only need 15 min to give the highest yield of triterpenoid saponins at 0.9972%, while the other extraction methods need several hours or even more than 3 h and give lower yield. Several factors affecting the ultrasonic extraction rate were also discussed, such as extraction time, temperature and ratio of solvent to material. Optimal conditions of ultrasonic extraction can be concluded as follows: 30 min at 70˚C, the ratio of solvent to material is 25 ml/g by using 95% ethanol as the solvent.

1.0 Introduction

Medicinal properties of mangrove trees provide a wide domain for medical uses, most yet to be explored. This is the basic idea towards the contribution to this study which is to find the magical healing properties of the tree. From the research towards these medicinal plants, it was believe that root, leaf and stem extracts of *Rhizophora* trees have inhibitory properties, affecting the growth of various human pathogenic organisms. A physician in Cali, Colombia, reported to cure throat cancer, with gargles of mangrove bark (Garcia-Barriga, 1975). Recently in Japan, Premanathan et al. (1999) reported that a polysaccharide extracted from the leaf of *Rhizophora apiculata* (designated as RAP) may inhibit AIDS virus in an early stage of its life cycle. Recently, Alarcon-Aguilara et al. (1998) reported that extracts of *Rhizophora mangle* had anti-diabetic and anti-hyperglycemic property.

Extraction is the first important step for the recovery and purification of active ingredients of plant materials. The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents and the use of heat and/or agitation to increase the solubility of materials and the rate of mass transfer. Usually, the traditional techniques require long extraction hours and have low efficiency. Moreover, many natural products are thermally unstable and may degrade during thermal extraction (Grigonis, Venskutonis, Sivik, Sandahl, & Eskilsson, 2005; Wu, Lin, & Chau, 2001). Recently there have been numerous reports on the application of high intensity or power ultrasound in the extraction of various phytochemicals, such as alkaloids, flavonoids, polysaccharides, proteins and essential oils, from various parts of plant and plant seeds.

The extraction of organic compounds from various plant materials can be significantly improved with the aid of intense ultrasound, achieving higher product yields at reduced processing time and solvent consumption. The mechanical effects of ultrasounds induce a greater penetration of solvent into cellular membranes walls, facilitating the release of contents of the cells and improve mass transfer (Kiel, 2007). In addition, ultrasonic extraction can be carried out at lower temperatures, avoiding thermal damage to extracts and loss of volatile components in boiling. It has been suggested that the improvement of solvent extraction from plant material by ultrasound is due mainly to the mechanical effects of acoustic cavitation, which enhances both solvent penetration into the plant material and the intracellular product release by disrupting the cell walls

The main objective of this research is to extract active compounds of mangrove leaves *Ceriops Decandra sp.* In this study the use of ultrasonic irradiation to assist extraction of triterpenoid saponins from *Ceriops Decandra sp.* is presented. It is compared with traditional extraction method and the effects of various experimental conditions on the extraction yield are also studied (irradiation time, temperature and extractant volume). The colorimetric method with vanillin–acetic acid system is used for the quantification of triterpenoid saponins, which has been reported to be a simple, quick and accurate method (Dong & Gu, 2001; Gao & Wang, 2001). Ethanol is a safe solvent providing both good yield and high concentration for triterpenoid saponins, so it has been chosen as the best solvent in this study.

2.0 Experimental

2.1 Materials

Dried *Ceriops Decandra sp* leaves used in this study were collected from riverbank of Tanjung Lumpur, Kuantan. All samples were sliced and ground into fine powder before extraction. Ethanol was used as a extraction solvent. Acetic acid, perchloric acid, vannilin and ethyl acetate were used for the colorimetric analysis.

2.2 Apparatus

Blender (Waring Lab Blender), Rotary evaporator (Buchi Rotavapor R-200), Vacuum pump (Rocker 300), Ultrasonic cleaning bath (Daihan, Japan), Soxhlet extractor (Favorit), Water bath (Model BS-21), Incubator (Infors AG CH-4103), Double beam UV/Vis spectrophotometer (Hitachi, U-1800 Spectrophotometer)

2.3 Extraction method

2.3.1 Ultrasonic extraction

Extractions were carried out in an ultrasonic bath that allows variation of amplitude and temperature. Working frequency was set at 60 kHz. Four grams of material were extracted with 100 ml 95% (v/v) ethanol in a conical flask (100 ml) and kept for sonication for 15 min at 60˚C temperature. After the extraction, the contents were filtered and evaporated to dryness. The procedure of ultrasonic extraction of material was repeated twice under the same conditions.

2.3.2 Soxhlet extraction

Traditional extractions were carried out by soxhlet extractor. 15 gram of material were extracted with 500 ml 95% (v/v) ethanol The extraction was kept for 1 h at 95 ˚C. After the extraction, the contents were filtered and evaporated to dryness. The procedure of soxhlet extraction of material was repeated twice in the same manner.

2.3.3 Experimental design

The aim of this study was to establish the effect of the variables (factors) involved in the ultrasonic extraction and to find the optimum values of those factors that give a maximum in the analytical response. It is usual that many of the operational variables can affect the extraction process, only a few of them are truly important or active. In situations with little knowledge whose variables are really active, it is advisable to carry out an orthogonal array experimental design to identify the most probable active factors and possible interactions.. Three different factors, namely extraction time, temperature and ratio of liquid- solid, were investigated with five levels.

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2.4 Colorimetric method for quantification analysis

2.4.1. The standard curve

The determination of the total content of triterpenoid saponins from the plant was performed as described by Xiang, Tang, Chen, and Shi (2001). The standard curve which was used as the benchmark for the yield determination was obtained as follows. A mixed stock solution consisting of Oieanolic acid (604 g l_1) was prepared. The different volumes of the stock solution with 0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml were transferred into a 10 ml test tube, respectively. After the solvent was heated to evaporation in a water-bath, 0.2 ml new mixed 5% (w/v) vanillin–acetic acid solution and 1.2 ml perchloric acid were added, mixed and incubated at 70 _C for 15 min. The tubes were taken out and cooled in running water for 2 min. Then ethyl acetate was added in order to make the total volume being 5 ml. After being cooled to room temperature, with a blank solution as reference, the absorbance was scanned using a Double beam UV/Vis spectrophotometer in the range of 200–700 nm. Scanning results showed that the maximum adsorption was at 550 nm, so the absorbance A at Vis 550 nm was determined with a glass cell of 1 cm.

2.4.2. Determination of total triterpenoid saponins

The extracted compounds were dissolved in ethanol to a designed concentration and 0.2 ml extract solutions were added to a tube. The absorbency of the sample was determined by the colorimetric method as described in Section 2.4.1. The contents of triterpenoid saponins in *Ceriops Decandra sp* were determined by reading the values from the standard curve.

3.0 Result and discussion

3.1 Calibration curves and determination of total triterpenoid saponins

The standard curve was shown in Fig. 1
Regression gives the linear relationship:

$$C = 0.103A \qquad (R^2 = 0.9985) \qquad \text{Eq. 1}$$

where C (mg/ml) is the concentration of triterpenoid saponins of solution for colorimetric analysis, and A is the absorbance at Vis 550 nm. According to Eq. (1), the yield Y was calculated by

$$Y = 0.515 \times A \times \frac{v}{v_1} \times \frac{1}{m} \times 100\% \qquad \left(\%; \frac{m}{m}\right), \qquad \text{Eq. 2}$$

where V is the total volume of extraction solvent (ml), V1 is the analysis volume of extraction liquid (ml), m is the mass of ginseng sample (g), and A is the same as in Eq. (1).

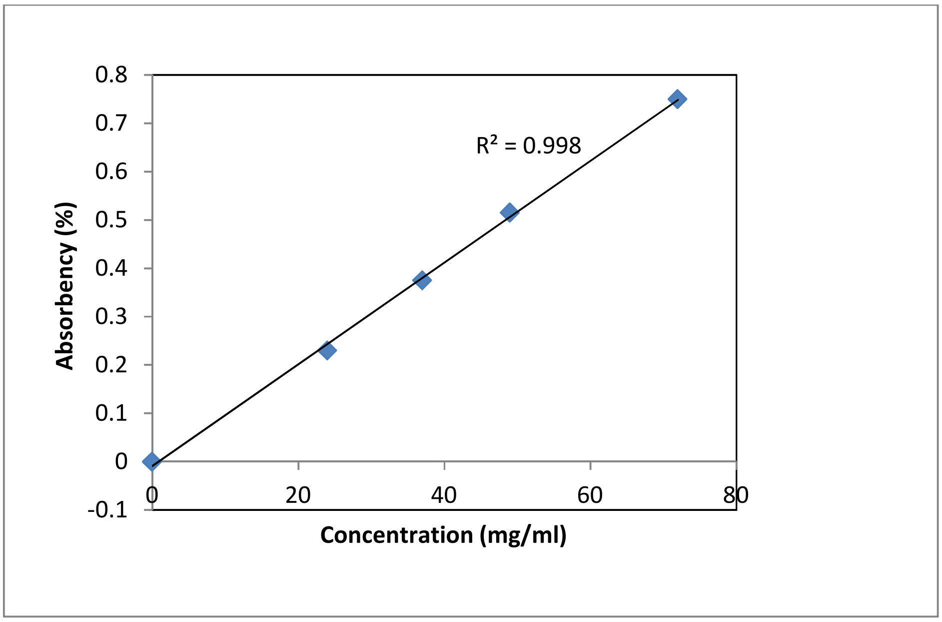

Figure 1: The Standard Curve of Oleanolic Acid.

3.2 Parameter manipulated for ultrasonic extraction

Ultrasonic recoveries may be influenced by the extraction time, temperature and ratio of liquid–solid. These effects were investigated. In this experiment, there are three parameters that being experimented which is duration of the extraction for both ultrasonic and soxhlet extraction, respectively, ratio of solvent to material (Ultrasonic only) and effect of temperature to the extraction process (Ultrasonic only).

3.2.1 Effect of time of ultrasonic irradiation.

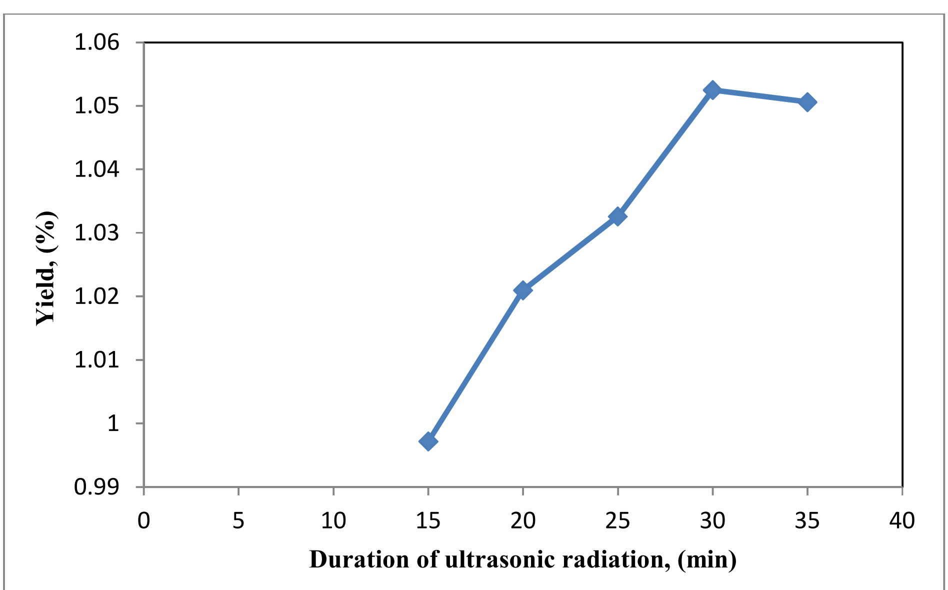

Figure 2: Graph of yield of triterpenoids saponins, (%) Vs Duration of ultrasonic irradiation, (min)

4 gram dried *Ceriops Decandra sp* leaves were extracted with 100 ml of 95% (v/v) ethanol at 60˚C and at working frequency 60 kHz . The duration of ultrasonic irradiation was 15, 20, 25, 30 and 35 min, respectively. Longer extraction time was not investigated because longer extraction time may not have further effects or have negative effects resulting from degradation or conversion of the analytes. Fig. shows the effect of ultrasonic time on the yield of triterpenoid saponins. The results indicated that the yield of triterpenoid saponins increased with the increase of ultrasonic time in the beginning of extraction. The yield could reach its maximum 1.0525% in 30 min during the ultrasonic process. If the ultrasonic time was more than 30 min, the extraction percentage of triterpenoid saponins decreased with the increase of ultrasonic time because triterpenoid saponins easily decomposed if they were kept at high temperature for a long period of time. This was also observed in the extraction of aromatic amines from leather, where the recovery of some amines decreases with increasing extraction time (Eskilsson & Bjo¨rklund, 2000). Therefore, 30 min were chosen as the optimal time for ultrasonic with the highest yield.

3.2.2 Effect of ratio of solvent to material

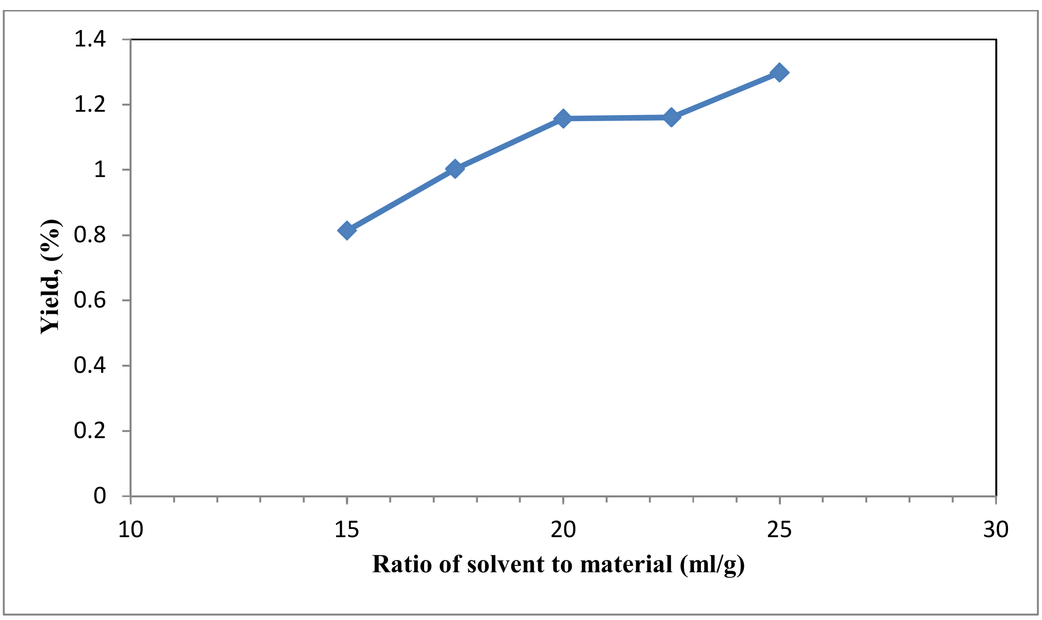

Figure 3: Graph of yield of triterpenoids saponins vs ratio of solvent to material

The solvent volume must be sufficient to ensure that the entire sample is immersed, especially when having a matrix that will swell during the extraction process. Generally in conventional extraction techniques a higher volume of solvent will increase the recovery, but in MAE a higher solvent volume may give lower recoveries (Eskilsson & Bjo¨rklund, 2000). 4 grams of *Ceriops Decandra Sp. leaves* were extracted at 60 ^{o}C for 20 min and at working frequency 60 kHz , with the different volumes of 95% (v/v) ethanol (60, 70, 80, 90, 100 ml, respectively). It can be seen in Fig. 4.3 that the yield of triterpenoids increased with the increase of ratio of solvent to material. From the graph, yield of triterpenoids saponins increase with the increase of volume of solvent. By increasing the volume of solvent, the ratio of solvent to material also increase resulting in increase of surface area and contact between the solid and liquid particles. Thus, the solute quickly diffuses from solid phase to the solvent and will increase the yield of extraction. From the result, the highest yield of triterpenoid saponins was 25 and consider as the optimal ratio of solvent to material for the ultrasonic process.

3.2.3 Effect of Temperature, ˚C

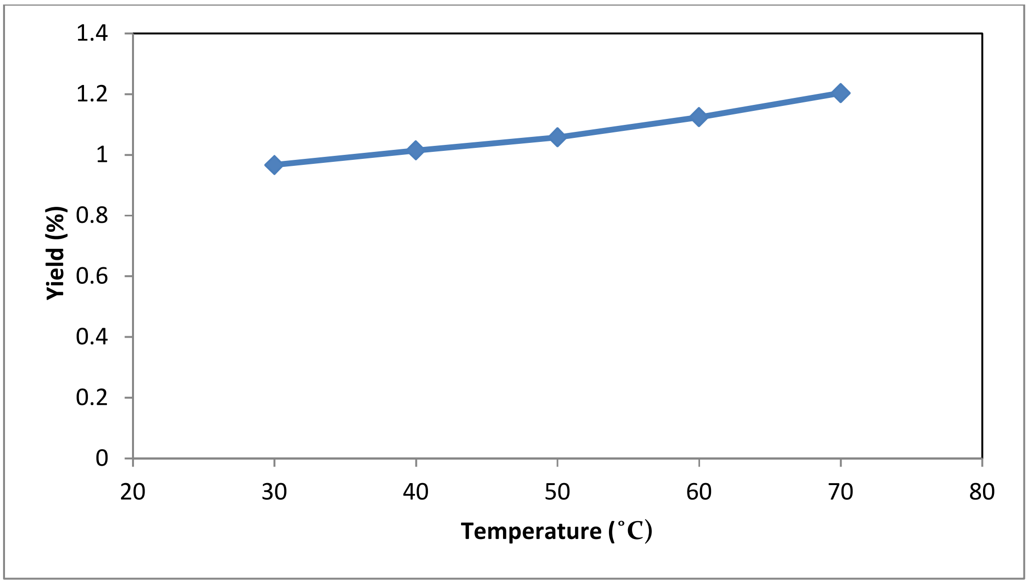

Figure 3: Graph of yield of triterpenoids saponins vs temperature

Experiments were conducted to study the effect of temperature on extraction efficiency. The extraction was performed with 95% ethanol for 20 min at five different temperatures of 30, 40, 50, 60, and 70˚C respectively. In theory, when ultrasonic is conducted in closed vessels, the temperature may well reach above the boiling point of the solvent. These elevated temperatures result in improved extraction efficiencies, since desorption of analytes from active sites in the matrix will increase. Additionally, solvents have higher capacity to solubilize analytes at higher temperatures, while surface tension and solvent viscosity decrease with temperature, which will improve sample wetting and matrix penetration, respectively. Fig. 4.4 showed the effect of different temperatures on extraction percentage of triterpenoids saponins. The present results revealed that the highest yield of triterpenoids was obtained with the value of 1.2033 %, when the sample was extracted with 95% ethanol at 70 ^{o}C. The yield of the compounds steadily increased with the increase of temperature until 70 ^{o}C, probably due to increased diffusivity of solvent into the internal parts of the matrix under elevated temperatures. Nevertheless, simultaneously with increased triterpenoids extractability, increased amount of matrix components would be co-isolated at higher temperature. Therefore, 70 ^{o}C should be optimal temperature for the ultrasonic process.

3.2.4 Effect of time in Soxhlet Extraction

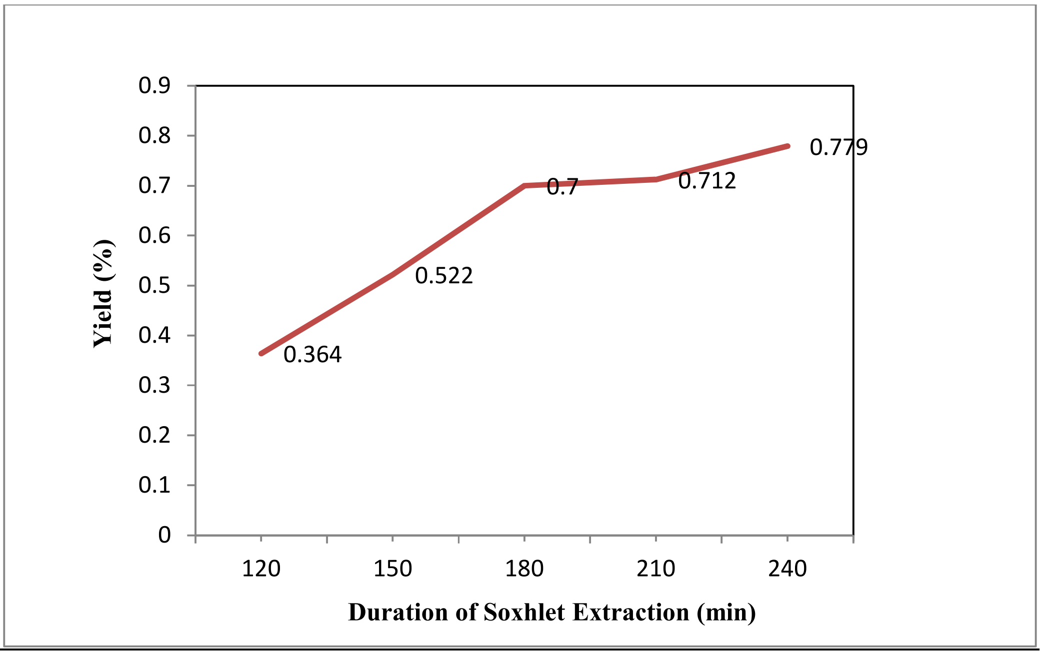

Figure 4: Graph of yield of triterpenoids saponins vs duration of soxhlet extraction

In Soxhlet extraction, 500 ml of ethanol solvent is used to extract 15 gram of dry *Ceriops Decandra Sp* leaves powder. Results generate the above graph. As indicate in the graph, yield of triterpenoids is increased with the increase of duration of Soxhlet extraction. This phenomena occur because when longer extraction time applied, solvent has been recycle back to the extraction chamber from the round bottom flask more repeatedly, causing it to extract more of the desired compound from the raw material. However, longer extraction time was not investigated because triterpenoids easily decomposed if they were kept at high temperature for a long period of time (Eskilsson & Bjo¨rklund, 2000)

3.3 Comparison between Ultrasonic assisted extraction with thermal extraction

The selection of an extraction method would mainly depend on the advantages and disadvantages of the processes, such as extraction yield, complexity, production cost, environmental friendliness and safety. In general, thermal extraction is the most frequently used extraction procedures. They are definitely very user friendly. The drawbacks of soxhlet extraction are the large amount of solvent and long extraction time needed. Considering the expensive solvent consumption and the long extraction period, these extraction methods are not favorable from a commercial perspective.

Meanwhile, Ultrasound-assisted extraction mainly depends on the ultrasonic effects of acoustic cavitations. The solid and liquid particles are vibrated and accelerated under ultrasonic action; as a result the solute quickly diffuses from solid phase to the solvent. In general, ultrasonic extraction is rapid and inexpensive. But the positions of the extraction vessels have to be carefully chosen and fixed during extraction, since generally the ultrasonic energy is not homogeneously distributed, which will induced the low precision of ultrasonic extraction, as already reported by Flotron et al. for the extraction of polycyclic aromatic hydrocarbons (PAH) from sewage sludge (Flotron et al., 2003).

So, from the comparison between soxhlet extraction methods and ultrasonic extraction, the ultrasonic assisted extraction method has the advantages based on gentle extraction conditions with relatively low extraction temperature, short time and minimal use of organic solvents.

4 Conclusion

The efficiencies of triterpenoid saponins transferring into solvent from the dried *Ceriops Decandra Sp* by ultrasonic extraction and soxhlet extraction were compared. Ultrasonic was found to be the most efficient method for the extraction of triterpenoid saponins from *Ceriops Decandra Sp*, which was verified by the experimental results presented in this study. Compared with the soxhlet extraction, the ultrasonic method employed provides high extraction efficiency in short time and less solvent consumption. Therefore, ultrasonic is an alternative extraction technique for fast extraction of triterpenoid saponins from *Ceriops Decandra Sp*. By studying the effects of various factors on extraction, optimal conditions of ultrasonic extraction of triterpenoid saponins from *Ceriops Decandra Sp* could be concluded with the solvent of 95% ethanol, the ratio of solvent to material of 25 ml/g, duration of ultrasonic irradiation of 30 min, and the extraction temperature of 70˚C.

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CHAPTER 1

INTRODUCTION

1.0 Introduction

Medicinal properties of mangrove trees provide a wide domain for medical uses; most yet to be explored. This is the basic idea towards the contribution to this study which is to find the magical healing properties of the tree. From the research towards these medicinal plants, it was believe that root, leaf and stem extracts of *Rhizophora* trees have inhibitory properties, affecting the growth of various human pathogenic organisms. A physician in Cali, Colombia, reported to cure throat cancer, with gargles of mangrove bark (Garcia-Barriga, 1975). Recently in Japan, Premanathan et al. (1999) reported that a polysaccharide extracted from the leaf of *Rhizophora apiculata* (designated as RAP) may inhibit AIDS virus in an early stage of its life cycle. Recently, Alarcon-Aguilara et al. (1998) reported that extracts of *Rhizophora mangle* had anti-diabetic and anti-hyperglycemic property. Therefore, through the research, we are going to determine what are the active compounds contain in mangrove plant which are believed can cure diabetes.

1.1 Research Background and Problem Statement

Diabetes mellitus (DM) is a common metabolic disease characterized by elevated blood glucose levels, resulting from absent or inadequate pancreatic insulin secretion, with or without concurrent impairment of insulin action. This illness affects approximately 150 millions of people worldwide and its incidence rate is expected to double during the next 20 years (Cohen and Goedert, 2004). Epidemiological studies and clinical trials strongly support the notion that hyperglycemia is the main cause of complications related with coronary artery disease, cerebrovascular disease, renal failure, blindness, limb amputation, neurological complications and pre-mature death (Lopez-Candales, 2001). Recent studies suggest that postprandial hyperglycemia could induce the non-enzymatic glycosylation of various proteins, resulting in the development of chronic complications. Therefore, control of postprandial plasma glucose levels is critical in the early treatment of DM and in reducing chronic vascular complications (Shim et al., 2003).

The purpose of the research is to analyze the mangrove leaves through extraction process in order to determine active compounds that was believe can cure diabetes. Some studies on medicinal properties of mangroves state that root, leaf and stem extracts of mangrove trees have healing properties. The people living in coastal area have rich traditional knowledge and extensive practice of using mangrove for healing certain illness. Result from the finding of the cure of diabetes from mangroves could give positive impact on recent effort of finding alternative sources of medicine from locally available plant sources. But the main problem here is we need to know what is the active compounds involve and are they similar to the properties of medicine for diabetes nowadays.

1.2 Research objectives

- To extract active compounds of mangrove leaves
- To identify key compound in the extract
- To estimate total Triterpenoids in extract

1.3 Scope of research

The scope has been identified for this study in order to achieve the objective. The scopes of research are listed as below:

- To conduct extraction process via ultrasonic method
- To analyze the effect of temperature, time and solvent ratio towards the extraction process
- To determine the yield of the extract

1.4 Significance of the study

The rationales and significance of the study are:

- Creating a new development of diabetes medicine from medicinal plants
- Maximizing the production of extraction product via ultrasonic extraction
- Determining the chemical, biological and pharmacological properties of extract

CHAPTER 2

LITERATURE REVIEW

2.0 Medicinal properties of mangrove

The unique family of *Rhizophoraceae* includes the halophytic (salt tolerant) species of mangrove tree that are endemic to tropical coasts. The *Rhizophora* species are adapted to salt water. They possess a distinct property called 'vivipary' or 'live birth' where seeds germinate while still attached to the parent tree, avoiding the harsh salt-water environment. This unique property also enables the mature seedlings to settle in the immediate ecosystem viable for mangroves. Like many other species of higher plants *Rhizophora* trees release through their roots and leaves chemical compounds which prevent the growth of invasive plants and algae that may compete with the *Rhizophora* in its immediate ecosystem. This phenomenon is termed 'allelopathy' and is referred to any biochemical interaction among plants, including micro-organisms (Rice, 1974). Chemical compounds released by plants have wide medical applications. One issue of interest pertaining to the "allelopathic" characteristic of Rhizophora is the observation that certain substances secreted by the plants suppresses new tissue growth thus the prevention of invasive plants in its ecosystem. Research needs to be conducted to determine the suppressive inhibitory compounds because of the obvious implications for applications with out-of-control tumor growth and pathogens in humans.

Healing properties are attributed to *Rhizophora* trees in popular/folk medicine. Root, leaf and stem extracts of *Rhizophora* trees have inhibitory properties, affecting the growth of various human pathogenic organisms. Among these are bacteria, fungi and viruses (Hernandez and Perez, 1978). A physician in Cali, Colombia, reported to cure throat cancer, with gargles of mangrove bark (Garcia-Barriga, 1975). Bark of red mangrove trees have been used in folk remedy for a wide array of diseases (Duke and Wain, 1981; Morton, 1981). Recently in Japan, Premanathan et al. (1999) reported that a polysaccharide extracted from the leaf of *Rhizophora apiculata* (designated as RAP) inhibited HIV-1 or HIV-2 or SIV strains in various cell cultures and assay systems. According to this report, the RAP extract blocked the expression of HIV-1 antigen in MT-4 cells and abolished the production of HIV-1 p24 antigen in peripheral blood mononuclear cells (PBMC); RAP also reduced the production of viral mRNA when added before virus adsorption. These results suggest that RAP may inhibit AIDS virus in an early stage of its life cycle. Recently, Alarcon-Aguilara et al. (1998) reported that extracts of *Rhizophora mangle* had anti-diabetic and anti-hyperglycemic property. In Fiji mangrove stalks are called 'Titi'. The Fijians traditionally prepare tea by extracting juice from the Titi, and drink the warm extract to cure various ailments. Titi is used routinely for symptoms associated with the common cold such as nasal congestion, bronchial congestion, runny nose, etc. It is also used for a variety of other ailments.

2.1 Diabetes mellitus

Diabetes mellitus often simply referred to as diabetes is a condition in which a person has a high blood sugar (glucose) level, either because the body doesn't produce enough insulin, or because body cells don't properly respond to the insulin that is produced. Insulin is a hormone produced in the pancreas which enables body cells to absorb glucose, to turn into energy. If the body cells do not absorb the glucose, the glucose accumulates in the blood (hyperglycemia), leading to vascular, nerve, and other complications.

There are many types of diabetes, the most common of which are:

- Type 1 diabetes
- Type 2 diabetes
- Gestational diabetes

- Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes.

All forms of diabetes have been treatable since insulin became medically available in 1921, and type 2 diabetes can be controlled with tablets, but it is chronic condition that usually cannot be cured. Pancreas transplants have been tried with limited success in type 1 DM; gastric bypass surgery has been successful in many with morbid obesity and type 2 DM; and gestational diabetes usually resolves after delivery. Diabetes without proper treatments can cause many complications. Acute complications include hypoglycemia, diabetic ketoacidosis, or nonketotic hyperosmolar coma. Serious long term complications include cardiovascular disease, chronic renal failure, retinal damage. Adequate treatment of diabetes is thus important, as well as blood pressure control and lifestyle factors such as smoking cesation and maintaining a healthy body weight.As of 2000 at least 171 million people worldwide suffer from diabetes, or 2.8% of the population. Type 2 diabetes is by far the most common, affecting 90 to 95% of the U.S. diabetes population.

2.1.1 Type 1 diabetes

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to insulin deficiency. This type of diabetes can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, where beta cell loss is a T-cell mediated autoimmune attack. There is no known preventive measure against type 1 diabetes, which causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults but was traditionally

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termed "juvenile diabetes" because it represents a majority of the diabetes cases in children.

2.1.2 Type 2 diabetes

Type 2 diabetes mellitus is characterized by insulin resistance which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus due to a known defect are classified separately. Type 2 diabetes is the most common type. In the early stage of type 2 diabetes, the predominant abnormality is reduced insulin sensitivity. At this stage hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver. As the disease progresses, the impairment of insulin secretion occurs, and therapeutic replacement of insulin may sometimes become necessary in certain patients.

2.1.3 Gestational diabetes

Gestational diabetes is a form of glucose intolerance diagnosed during pregnancy. Gestational diabetes occurs more frequently among African Americans, Hispanic/Latino Americans, and American Indians. It is also more common among obese women and women with a family history of diabetes. During pregnancy, gestational diabetes requires treatment to normalize maternal blood glucose levels to avoid complications in the infant. Immediately after pregnancy, 5 to 10 percent of women with gestational diabetes are found to have diabetes, usually type 2. Women who have had gestational diabetes have a 40 to 60 percent chance of developing diabetes in the next 5 to 10 years.