A COMPARISON BETWEEN THE MODERN HYDRO DISTILLATION TECHNIQUE AND MICROWAVE EXTRACTOR IN EXTRACTING LIGNANS FROM PHYLLANTHUS NIRURI

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ABSTRACT

Phyllanthus Niruri (Pokok Dukung Anak) can produce essential oil that is important in medicine and also important in developing our agriculture sector nowadays. To obtain Phyllanthus Niruri essential oil, entrepreneur and researcher nowadays use various extraction methods. So the objective of this study is to obtain lignin, a major component of essential oil from Phyllanthus Niruri plant source by comparing two different extraction methods which are modern hydro distillation technique and microwave extractor. The extraction process was conducted at three different temperatures which were 30°C, 80^0C and 100^0C. The power for the microwave extractor is set at 1000 W for all the temperatures. The raw materials were cut into small pieces and ethanol was used as extraction solvent in both methods. The weight for each run was fixed at 250g of samples. After the extraction process was completed, the samples were processed in rotary evaporator and water bath in order to separate the solvents from the oil. The weight of oil was recorded and calculations were performed to compare the efficiency yield. Finally all the samples were analyzed using Gas Chromatography Mass Spectrometer (GC-MS). The results showed that of all the six samples, lignin was detected only at temperature 30°C by using hydro distillation as the extraction method. This indicates that lignin is a highly volatile oil and the most suitable method to extract ligin is by hydro distillation method.

ABSTRAK

Phyllanthus Niruri (Pokok Dukung Anak), boleh menghasilkan minyak pati yang penting sebagai ubat dan juga penting dalam memajukan sektor pertanian pada zaman ini. Untuk memperoleh minyak pati Phyllanthus Niruri, usahawan dan penyelidik sedang membuat kajian dengan mengunakan pelbagai kaedah pengekstrakan. Objektif kajian ini ialah untuk mendapatkan lignin, iaitu komponen utama minyak dari sumber Phyllanthus Niruri iaitu dengan membuat perbandingkan antara dua kaedah pengekstrakan yang berbeza, iaitu teknik penyulingan hidro moden dan gelombang mikropengekstrak. Proses pengekstrakan ini telah dijalankan pada tiga suhu yang berlainan, iaitu 30 °C, 80°C dan 100 ° C. Kuasa untuk pengekstrak gelombang telah mikro ditetapkan pada 1000W untuk semua suhu. Bahan mentah dipotong dalam kepingan kecil, dan etanol telah digunakan sebagai pengekstrak pelarut dalam kedua-dua kaedah. Sampel untuk setiap eksperimen telah ditetapkan pada 250g. Selepas proses pengekstrakan telah dijalankan, sampel telah diproses dalam penyejat berputar dan rendaman air supaya pelarut dapat dipisahkan daripada minyak. Berat minyak yang diperolehi telah direkodkan dan pengiraan telah dibuat untuk membandingkan kecekapan hasil yang diperolehi. Akhirnya semua sampel telah dianalisis dengan menggunakan GasChromatography Mass Spectrometer (GC-MS). Keputusan menunjukkan, daripada semua ke-enam sampel, lignin dikesan hanya pada suhu 30°C dengan menggunakan penyulingan hidro sebagai kaedah pengekstrakan. Ini menunjukkan yang lignin merupakan minyak yang mudah meruap dan kaedah yang paling sesuai untuk menekstrak lignin adalah melalui cara penyulingan hidro.

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P	-	Pressure
m	-	Mass
^{o}C	-	Degree Celsius
kg	-	Kilogram
K	-	Degree Kelvin
mL	-	Mililiter

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CHAPTER 1

INTRODUCTION

1.0 Introduction

Extraction is phenomenon that can be defined as the process of separating desired components from a material. There are many types of extraction such as solid-liquid extraction and liquid-liquid. There are also a lot of extraction methods. Some of them are steam distillation, hydro distillation, solvent extraction, carbon dioxide extraction and cold pressing. The main objective in the extraction process is use to extract the essential oil. The main resources of essential oils are plants. In this research, the extraction will be conduct by using hydro distillation technique to extract essential oil from *Phyllanthus Niruri* plant.

1.1 Essential Oil

Essential oils, also known as Volatile oils, are the odorous principles found in various plant parts. It can be found in the bark of the plant, the flower of the plant or even in the leaves of the plant. Essential oils are oils that are found in bags inside plants cell. These oils can be free from the leaves and extracted using a few of methods like hydro distillation. The oil is also known as volatile oils, ethereal oils, or essential oils because of their properties easily evaporated at ambient temperature.

Essential oils are usually colorless, particularly when fresh. Nevertheless with age essential oil may oxidize and resinify, which resulting the colour becomes darker. Therefore, essential oil need to be stored in a cool, dry place, tightly stoppered and

preferably full in amber glass containers. These essential oils have many uses. Most essential oils are produced for the perfume industry and minute amounts are used for flavour prepackaged foods. Besides that some essential oils that are produced from plants have high medical values in them like *Phyllanthus Niruri* plant. The *Phyllanthus Niruri* plant are used in beverages in Malaysia to improve health and for the treatment of kidney, bladder inflammation, gout and diabetes. (Wangner, 1982)

1.2 Phyllanthus Niruri.

Phyllanthus Niruri (Pokok Dokong Anak) is a medicinal herb found mainly throughout rainforests of the Amazon and other tropical areas throughout the world, including the Bahamas, southern India, China and also South East Asia. The *Phyllanthus* genus contains over 600 species of shrubs, trees, and annual or biennial herbs distributed throughout the tropical and subtropical regions of both hemispheres. It is believed to have antihepatotoxic, antilithic, analgesic, hypotensive, antispasmodic, antiviral, antibacterial, diuretic, antimutagenic, and hypoglycemic activities. It is used as a remedy for arteriosclerosis (capillary and circulatory disorders), kidney stones and nephritis. (Anon, 2001).

It is trusted for many centuries in treating ailments of the kidney, bladder stone, urinary tract infection, liver and bladder problems, diabetes, cold, flu, tuberculosis, and other viral infections; liver diseases and disorders including anemia, jaundice and liver cancer; and for bacterial infections such as cystitis, prostatitis, venereal diseases. It is also used to reduce cholesterol and blood pressure. Pokok dokong anak has a mild diuretic action, useful for flushing the kidneys and urinary tract. It also relieves spasms of the smooth muscle in the walls of the internal organs, making it valuable for gallbladder problems. (Hegnauer, 1966).

Researchers have found it to be mildly antiseptic as well. This plant has also various other names such as Chanca piedra, quebra pedra, stone-breaker, arranca-pedras, punarnava, amli, bhonya, bhoomi amalaki, bhui-amla, bhui amla, bhuianvalah, bhuimy-amali, bhuin-amla, bhumyamalaki, cane peas senna, carry-me-seed, creole senna, daun marisan, derriere-dos, deye do, erva-pombinha, elrageig, elrigeg, evatbimi, gale-wind grass, graine en bas fievre, hurricane weed, jar-amla, jar amla, kizha nelli,

malva-pedra, mapatan,para-parai mi, paraparai mi, pei, phyllanto, pombinha, quinine weed, sacha foster, cane senna, creole senna, shka-nin-du, viernes santo, ya-taibai, yaa tai bai, yah-tai-bai, yerba de san Pablo.

1.3 Problem Statement

In modern times, essential oils are used in the manufacture of high quality perfumes, as additives in many common products, and in the healing practice of aromatherapy. In this research, Hydro Distillation Unit and microwave extractor was used to get the *Phyllanthus Niruri* essential oil. In order to obtain a series of high quality extraction from *Phyllanthus Niruri*, a comparison between these two methods were applied.

Nowadays, in Malaysia, essential oil and oleoresin is gaining popularity as a herbal medication as it gave a lot of benefit to overcome the disease. It is because our people start to realize the important of healthy awareness. Hence, *Phyllanthus Niruri* essential oil and oleoresin has a clear commercial value. Therefore the identification of the constituents of *Phyllanthus Niruri* has been carried out extensively over the last 10 years. The Government wants to nurture the Malaysian herbal industry to be a leading international player. The local herbal industry is growing at an annual rate of between 15% and 20% and has a market value estimated at RM2 billions. (Malaysian Industrial Development Authority, 2005).

It is appropriate to improve the current extraction method because of the energy and cost wasting. The current extraction method cannot give the highest purity and quality of *Phyllanthus Niruri* essential oil. It is hard to determine the exact amount of the solvent. So the aiming of this research is to get the maximum quality of essential oil by using the modern hydro distillation method and microwave extractor.

1.4 Research Objectives

The main objective of this study is to extract lignans from *Phyllanthus Niruri* essential by using microwave assisted solvent extractor and hydro distillation methods. The other objective is to investigate the performance of potential of hydro distillation method and microwave extractor in terms of the quantity and yield of oil extracted.

1.5 Research Questions

1. What are the prospects of oil extraction in future market?
2. What are the problems faced and how to overcome them while extracting oil.
3. Which method is the most convenient method to extract oil in terms of quantity and quality?

1.6 Scopes of the Research

To achieve the objective, scopes have been identified in this research. The scopes of this research are listed as below:-

- Prepare the *Phyllanthus Niruri* samples for the experiment. The samples are prepared so that it has fix amount of weight for both methods before the extraction procedures are carried out.

- The operating conditions are set at three different temperatures which are 30^0C, 80^0C and 100^0C.for both the methods. This indicates that for each method there will be three runs.

- To study the product analysis using GC-MS.

CHAPTER II

LITERATURE REVIEW

2.0 Introduction

In this chapter, the characteristics of *P.niruri* are studied thoroughly. Besides that, both the extraction methods, the extraction solvents and the essential oil separation and analyzing is discussed here.

2.1 Overview of *Phyllanthus Niruri*

Nowadays, the consumption of herbal based products are getting a wide spread acceptance among consumers because of the numerous beneficial therapeutic impacts they could give to our body and indirectly helps us sustaining a healthy condition. Although artificial and synthetic drugs are common in the market to combat a lot of chronic diseases, but these medications usually have negative effects to our body. Nowadays, people start to realize the differences between traditional and modern medication technique. Therefore, products from herbs, especially the Dukung Anak (*Phyllanthus Niruri)* are the right choice in treating certain kinds of ailments or diseases without introducing side effects to our body if consumed accordingly based on scientific findings and research. This is where our herbal products comes in which are confidently introduced to the public and backed by scientific research and findings from local and overseas scientist to prevent, reduce or to the extent in aiding the cure of certain types of chronic diseases or ailments.

According to Santos (1990), the scientific term of Phyllanthus Niruri have other synonyms such as *Phyllanthus carolinianus, Phyllanthus sellowianus, Phyllanthus fraternus, Phyllanthus kirganella, Phyllanthus lathyroides, Phyllanthus lonphali, and Nymphanthus niruri*. This herb is also known by its vernacular names such as Chanca piedra (Spain), quebra pedra (Portugal), stone-breaker (Britain), punarnava (India), bhoomi amalaki (Thailand), bhui-amla (Vietnam), cane peas senna (Cuba), daun marisan (Indonesia), derriere-dos (Philiphinnes), erva-pombinha (Colombia), elrageig (South Africa), quinine weed (USA), shka-nin-du (Japan), viernes santo (Argentina), ya-taibai, yaa tai bai (Cambodia) and in Malaysia this plant is known as Pokok Dukung Anak. This herb is spread from India, Indo- China and Thailand through Malaysia to tropical forest of Australia. Throughout Malaysia, it is abundantly be found but is apparently rare in Borneo, Sulawesi and the Moluccas. It is also now grown in South East Asia, Africa, Georgia and Cuba.

2.1.1 Characteristic of *Phyllanthus Niruri*

Phyllanthus niruri originated in India, usually occurring as a winter weed throughout the hotter parts. The Phyllanthus genus contains over 600 species of shrubs, trees and annual or biennial herbs distributed throughout the tropical and subtropical areas. *Phyllanthus niruri* is a herb of Euphorbiaceae family that grows upto 60 cm. Phyllanthus means "leaf and flower" because the flower, as well as the fruit, seem to become one with the leaf. The details of *P.niruri* are shown below.

Botanical aspects and geographical distributions

Kingdom	: Plantae
Division	: Magnoliophyta
Class	: Magnoliopsida
Order	: Euphorbiales
Family	: Euphorbiaceae
Genus	: *Phyllanthus*
Species	: *niruri*

Figure 2.1: *Phyllanthus niruri* L.; (A) whole plant (B) aerial part (C) leaves.

The annual herb is 30-60 cm high, quite glabrous, stem often branched at the base. The leaves are numerous, sessile distichous often imbricating, elliptic oblong obtuse. Phyllanthus Niruri contains stipules and they are very acute.

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Figure 2.2: Leaves of *Phyllanthus Niruri*

The flowers of this plant are yellowish, very numerous, and axillaries. The male flowers are usually one to three in number while the female flowers are solitary in nature. It has capsules and it is about 2.5mm in diameter and the shape is depressed globes or smooth scarcely lobed.

Figure 2.3: The flowers of *Phyllanthus Niruri*

Phyllanthus Niruri fruits are small, matte-green grains with a rough surface and an oily white interior. They are roughly 1 cm (1/16 to 1/8 in) in diameter. The seeds are similar to onion seeds. The seeds have little bouquet, though when they are rubbed they give off an aroma reminiscent of oregano. It is also slightly bitter and peppery with a crunchy texture [12].

Figure 2.4: The fruits of *Phyllanthus Niruri*

2.1.2 Chemical Constituents of *Phyllanthus Niruri*

P. niruri has been the subject of much phytochemical studies since the mid-1960s. Different classes of organic compounds with various medical interest have been reported, the major being the lignans, tannins, polyphenols, alkaloids, flavonoids, terpenoids and steroids (Calixto *et al.*, 1998). The following chemical constituents have

been isolated from *P. niruri*. In scientific studies, *P. niruri* was found to exhibit antispasmodic, hypotensive, analgesic, antihepatotoxic, antihepatitis, antimutagenic, antiviral and antibacterial properties.

The seeds contain numerous esters of structurally unusual unsaturated fatty acids with terpene alcohols (7%); furthermore, traces of alkaloids are found which belong to two different types: isochinoline alkaloids are represented by nigellimin and nigellimin-*N*-oxide, and pyrazol alkaloids include nigellidin and nigellicin [14].

The therapeutic effects of *Phyllanthus Niruri* have been ascribed mainly to its phenylpropanoids, the most dominant constituent in the leaf which has been reported by Hollman & Katan (1999) to be effective in reducing oxidative stress by inhibiting the formation of lipid peroxidation products in biological systems. It could lead to some of the chronic diseases such as coronary heart disease and many more. This is proved by the research of Chung et al. (1999) and Venkatamuru et al. (1983) which among the different parts of plants studied, the leaves are reported to have the highest antioxidant properties whereas the highest fraction of phenols are obtained from root. It is the most active principle among the phytochemicals studied (Nakasugi & Komai (1998); Jung et al. (1999) & Pietta et al. (1998)). That is why the leaves of this herb is often used in traditional medicine compared to other parts of the plant because it contains much more active chemical components which contributes to the therapeutic benefits.

The specific polyphenol components that are dominant in the leaves of the Dukong Anak herb consists of four main polymethoxylated flavones, which are phyllanthin (PHL), estradiol (EST), 3'-hydroxy-5,6,7,4'-tetramethoxyflavone (TMF) and Gallic acid (GA), which is the major phenolic acid (Schut and Zwaving, 1993). Based on the studies by Akowuah et al. (2004), the GA component is the main polyphenol compound in the leaves of Dukong Anak, which is the most polar (water soluble) component compared to the three polymethoxylated flavones studied. The extract of the leaves of Dukong Anak using non- polar extracting solvents gave the highest activity of free radicals scavenging which is possibly due to the high concentration of caffeic acid derivatives, especially GA (Akowuah et al., 2005). Sumaryono et al. (1991) found that, the derivatives of caffeic acid, including GA was

reported to constitute 67% of total identified phenolics in aqueous methanol extract and about 94.6% in hot water extract. Therefore, by using the leaves of Dukong Anak in the form tablets and capsules which contain the extract of the herb is beneficial for health, especially when the active chemical components from the leaves are extracted using non - polar solvents.

2.1.2.1 Lignans

Lignans isolated from *P. niruri* mostly belongs to two groups, the 1,4-diarylbutane and 1-aryltetralin though neolignans and lignans with other skeleton were also reported from this plant. The following lignans have been isolated from *P. niruri*:

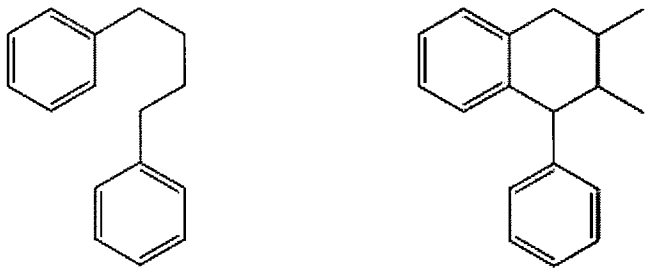

Figure 2.5:1,4-diarylbutane skeleton and 1-aryltetralin skeleton

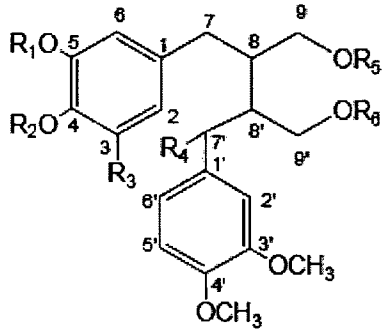

Figure 2.6: General structure of Lignans

phyllanthin R1 = R2 = CH3; R3 = R4 = H; R5 = R6 = CH3

niranthin R1 + R2 = CH2; R3 = CH3; R4 = H; R5 = R6= CH3

seco-isolariciresinol trimethyl ether R1 = R2 = CH3; R3 = R4 = R5 = H; R6 = CH3

hydroxyniranthin R1 + R2 = CH2; R3 = CH3; R4 = OH; R5 = R6= CH3

2,3-desmethoxyseco-isolintetralin	R1 + R2 = CH2; R3 = R4 = R5 = R6 = H
linnanthin	R1 = R2 = R3 = CH3; R4 = H; R5 = R6 = CH3
demethylenedioxyniranthin	R1 = R2 = H; R3 = CH3; R4 = H; R5 = R6 = CH3

2.1.2.2 Coumarins, Tannins and related Polyphenols

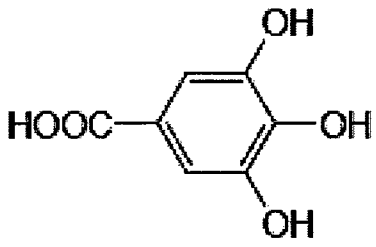

Figure 2.7: Ellagic Acid

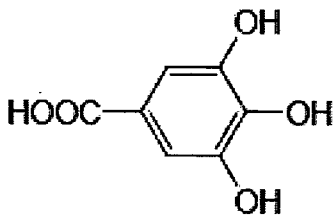

Figure 2.8: Gallic Acid

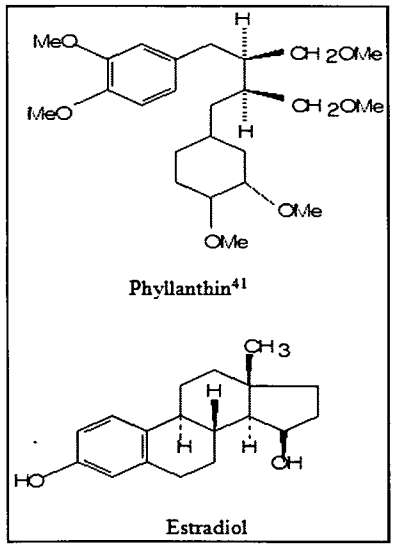

Figure 2.9: Molecules of Phyllanthin

(Ishimaru *et al.*, 1992)

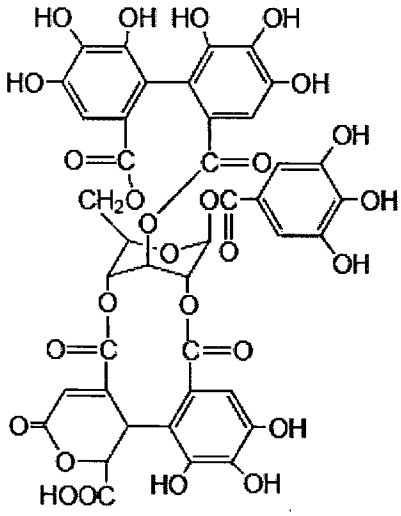

Figure 2.10: Geraniinic Acid