



UNIVERSITI PUTRA MALAYSIA

***ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF MALAYSIAN
PHYLLANTHUS AMARUS THONN & SCHUM, PHYLLANTHUS NIRURI
LINN, AND PHYLLANTHUS URINARIA LINN***

TAN POH HWA

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LINN, AND *PHYLLANTHUS URINARIA* LINN**

By

TAN POH HWA

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirements for the Degree of Master of Science**

May 2012

***Dedicated to my loving parents, Tang Wang and Lang Kim and
brothers, Leet Hooi and Leet Fuh, and sister, Poh Fong and my
beloved husband, Chee Keong***



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment
of the requirement of the degree of Master of Science

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Chairman : Associate Prof Cheah Yoke Kqueen, PhD

Faculty: Medicine and Health Sciences

Natural products have long been consumed because of its convincing abilities in treating health related diseases. With the progression of science and technology, the use of natural products for medical applications is getting more popular and is being recognized. One of the most prominent traditional plant in Malaysia in which its medicinal values has yet to be discovered is *Phyllanthus* species. It originates from the family of Euphorbiaceae. This family comprises over 700 species in the world of flora and is well distributed mostly in tropical and subtropical countries.

We often face the restriction to obtain health benefited medicinal plants continuously from wild. And, the bioactive activities from plants may vary from different location because of different biotic growth environment. Beside that, there is limited research study on health related properties about this plant in Malaysia especially the comparison among three different species. Thus, this study was conducted to determine the antioxidant and antibacterial activities of three *Phyllanthus* species and

to identify possible polyphenols that are present in these plants. All plants were cultured at the same sheltered net house and growing condition. The supply of plants sources were continuous and standardized to minimize variation of active ingredient content which could occur as a result of environmental abiotic and biotic stress.

The aerial section of *Phyllanthus amarus*, *Phyllanthus niruri* and *Phyllanthus urinaria* was extracted with methanol and water. Water extract antioxidant activity and total phenolic content decreased in the order of *Phyllanthus niruri* > *Phyllanthus amarus* > *Phyllanthus urinaria*. However, methanol extract showed that this activity decreased in the order of *Phyllanthus niruri* > *Phyllanthus urinaria* > *Phyllanthus amarus*. From a positive correlation of $0.954 < r^2 < 1.000$ between antioxidant activity and total phenolic content, methanol extract showed higher total phenolic content and antioxidant activity as compared with water extract. ANOVA test indicated a significant difference of antioxidant activity among the species in water and methanol extract.

Water extract and methanol extract at the concentration of 100 mg/mL and 50 mg/mL of the three plant samples tested against both gram positive and gram negative bacterial. Both extract inhibited gram positive bacterial only at the range of inhibition zone of 1.15 to 1.70 cm. Lowest minimum inhibition concentration value for water extract and methanol extract were < 2.5 mg/mL and < 0.625 mg/mL. Value for minimum bactericidal concentration of water and methanol extracts were > 2.5 mg/mL and > 0.625 mg/mL.

Polyphenol compounds, gallic acid, digalloyglucopyranoside, corilagin and geraniin were identified from water and methanol extract. Gallic acid was found in water extract of all three species only. Geraniin was found in both water and methanol extract of all three species as major compounds and highest concentration. Corilagin and digalloyglucopyranoside were only identified in water and methanol extract of *Phyllanthus niruri*. Overall, water extract yielded more types of phytochemicals.

The study concluded that methanol extract showed higher and wider inhibition potential than water extract. *Phyllanthus niruri* possess the most potent protective and antioxidant activity among the species. *Listeria monocytogenes* and *Staphylococcus aureus* were the most sensitive strains.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

POTENSI ANTIOKSIDAN DAN ANTIBAKTERIA *PHYLLANTHUS AMARUS* THONN & SCHUM, *PHYLLANTHUS NIRURI* LINN, AND *PHYLLANTHUS URINARIA* LINN DARI MALAYSIA

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Produk semulajadi telah lama digunakan dalam aktiviti hidupan seharian kita. Ini adalah disebabkan keberkesanan produk semulajadi dalam mengubati penyakit and menyihatkan badan. Kemajuan sains dan teknologi membantu meningkatkan penggunaan produk semulajadi dalam bidang perubatan. Salah satu tumbuhan tradisi yang sangat berpotensi di Malaysia ialah *Phyllanthus* spesies. Tumbuhan ini berasal dari famili Euphorbiaceae dan terdiri daripada 700 species. Ia bertaburan luas di kawasan tropika dan subtropika di seluruh dunia.

Kita sering menghadapi masalah mencari sumber tumbuhan yang berterusan dari alam semulajadi. Kandungan bahan bioaktif juga berbeza jika tumbuhan ditumbuhkan dari lokasi yang berlainan disebabkan keadaan pertumbuhan biotik yang berbeza. Selain itu, pengetahuan dan kajian mengenai efikasi tumbuhan adalah tidak mencukupi terutamanya mengenai tiga spesies tersebut. Dengan ini, kajian ini dijalankan untuk menentukan aktiviti antioksidan, antibakteria dan kandung jumlah

polifenol yang wujud dalam ekstrak. Tiga spesies ini ditumbuhkan dalam rumah hijau dan keadaan terkawal. Dengan ini, sumber tumbuhan ini boleh didapati dengan berterusan dan variasi kandungan bahan aktif dapat dikurangkan.

Bahagian atasan tumbuhan sahaja digunakan dalam proses ekstraksi dengan air dan methanol. Keputusan bagi aktiviti antioksidan dan jumlah kandungan fenolik dari ekstrak air mengikut kedudukan yang paling baik adalah *Phyllanthus niruri* > *Phyllanthus amarus* > *Phyllanthus urinaria*. Keberkesanan bagi ekstrak methanol pula ialah *Phyllanthus niruri* > *Phyllanthus urinaria* > *Phyllanthus amarus*. Korelasi ($0.954 < r^2 < 1.000$) yang baik ditunjukkan antara jumlah kandungan fenolik dan aktiviti antioksidan. Ekstrak methanol menunjukkan antioksidan yang paling berkesan dan jumlah kandungan phenolic yang lebih tinggi berbanding dengan ekstrak air.

Ekstrak air dan methanol pada kepekatan 100 mg/mL dan 50 mg/mL telah diuji keupayaan menghalang pertumbuhan bakteria gram positif dan gram negatif. Kedua-dua jenis ekstrak kasar hanya menunjukkan kesan antibakteria terhadap gram positif pada julat zon rencatan antara 1.15 cm ke 1.70 cm. Kepekatan < 2.5 mg/mL and < 0.625 mg/mL adalah nilai MIC terendah bagi ekstrak air dan ekstrak methanol terhadap bakteria gram positif yang dinilai. MBC yang ditunjukkan dari ekstrak air dan methanol adalah > 2.5 mg/mL dan > 0.625 mg/mL. Ekstrak methanol lebih berkesan dalam merencatkan pertumbuhan bakteria dan membunuh bakteria.

Bahan aktif polifenol, asid galik, digalloylglucopyranosida, korilagin dan geraniin telah diidentifikasikan dari kedua-dua jenis ekstrak. Asid galik hanya dapat dikesan

dari ekstrak air. Tetapi, geraniin wujud di kedua- dua ekstrak air dan methanol sebagai bahan aktif utama dan komposisi yang tinggi. Korilagin dan digalloyglucopyranosida hanya dijumpai dari kedua-dua jenis ekstrak *Phyllanthus niruri*. Secara keseluruhan, ekstrak air mengandung bahan aktif yang lebih bervariasi.

Kesimpulannya, ekstrak methanol menunjukkan kemampuan aktiviti antioksidan dan antibakteria terhadap bakteri gram positif lebih berkesan daripada ekstrak air. *Phyllanthus niruri* merupakan spesies yang paling berkesan dan berpotensi sebagai sumber baru agen antibakteria dan antioksidan. *Listeria monocytogenes* dan *Staphylococcus aureus* merupakan bakteri yang sangat sensitif terhadap ekstrak air and methanol.

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I certify that a Thesis Examination Committee has met on 21st May 2012 to conduct the final examination of Tan Poh Hwa on her thesis entitled “Antioxidant and antibacterial potential of Malaysian type *Phyllanthus amarus*, *Phyllanthus niruri* and *Phyllanthus urinaria*” in accordance with the Universities and University Colleges Act 1971 and the Constitution of Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

TAN POH HWA

Date: 21st May 2012



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LIST OF ABBREVIATIONS

LCMSMS	Liquid Chromatography -Mass Spectrophotometer- Mass Spectrophotometer
MIC	Minimum inhibition concentration
MBC	Minimum bacteriacidal concentration
SPE	Solid phase extraction
TPC	Total phenolic content
DPPH	2,2-diphenyl-1-picryl-hydrazl
FRAP	Ferric reducing antioxidant potential
mg/mL	milligram per mililitre

CHAPTER 1

INTRODUCTION

Nature biodiversity is a precious asset for living organisms to live on from generation to generation. It has been created in such a way that consists of millions of flora and fauna to support the surviving chain of human and its uniqueness is yet to be fully explored. According to records, Oakes and Gahlin (2003) mentioned that the Egyptians were the first man who used drugs derived from plants which were later proven its medicinal efficiency from the modern studies. About 3000 to 6000 years ago, they have developed an effective pharmacological collection of numerous curing materials from natural resources (Halberstein, 2005). Plants were only consumed orally, applied topically and administered by fumigation or vapor inhalation without further processing. Herbal specialist need to be well versed in recognizing the medicinal plant and its medicinal value based on records because they played a major role in plants medicine application.

In the 18th century, herbal medicine instead of being killed off by medical science and pharmaceutical chemistry has made a comeback due to the mushrooming of science and technology. Herbal medicine has been found to have some impressive credentials, which cannot be substituted by modern medicine. People are turning back to Nature especially herbs again for answers about their medical ailments. It is because microbial infectious are giving a red alarm to human's health. Microbial infections are caused by microorganisms such as bacterial, virus or fungal. It responsible for a wide range of diseases from minor skin conditions like impetigo to more serious conditions such as meningitis, listeriosis. We depend on synthetic and

semi synthetic drugs to fight against the microbial infectious disease. But, these drugs are believed to bring the side effect to our health and causing the genetic mutation to the microbial strain which lead to antibiotic resistant microbial strain. A major problem associated with bacterial infection is the rise of methicillin resistant *S. aureus* and multidrug resistant strains (Venter *et al.*, 2004). It is important to look for alternatives that would not further increase drug resistance.

Malaysia's rainforest has been honorably listed as number 12 in the world oldest rainforest for nesting precious biodiversity (Star, 2006). Being part of the world's tropical rainforest is estimated to hold about 2000 species of medicinal plants and about one percent is being commercialized (Bernama, 2005). *Phyllanthus* spp. from the Euphorbiaceae family is popular among the traditional medicinal plant. *Phyllanthus niruri*, *Phyllanthus amarus* and *Phyllanthus urinaria* are the widely used species by traditional practitioners for its healing properties. They can be easily found growing wildly in wastelands and grasslands in tropical regions of the world. Their health related properties had been reported to have pharmacological effects such as antidiabetes (Hasenah *et al.*, 2006), antibacterial (Macrae *et al.*, 1988), antiviral activity (Bhattacharyya *et al.*, 2003), hepatoprotective agent (Rajesh and Parames, 2007) and anti-hepatitis B virus (Thyagarajan *et al.*, 1988). Thus, the health related properties of these species are getting more and more attention from scientists to be discovered as alternative novel drug.

Scientific study in Malaysia regarding the *Phyllanthus* species is still lacking even though this plant is widely distributed here and its health related properties has been reported by the scientist from oversea. The comparison study among the species is

insufficient and no specific area of study. Next, the herbs are collected from wild during study which caused the variation of results and inconsistency of findings. The bioactive compounds compositions vary according to the source of origin, soil, weather conditions, time of harvest and processing (Dweck, 2009). Therefore, it creates a difficulty for large scale plantation because the preservation of the active properties is unknown when planting in farm. It leads to a non-continuous supply of quality plants material. Therefore, the report from this study was undertaken to investigate potential antioxidant and antibacterial activity from *Phyllanthus amarus*, *Phyllanthus niruri* and *Phyllanthus urinaria*. Plants were grown in sheltered net house with same growing environment in order to obtain a continuous supply and minimize the variation of plants' quality.

1.1 Objectives of study

The study was undertaken for the following objectives:

1. To analyse water and methanol extracts of plants by using Liquid Chromatography-Mass Spectrophotometer-Mass Spectrophotometer (LCMSMS)
2. To determine the antioxidant potential from three *Phyllanthus* species by 2 different extraction methods.
3. To determine antibacterial potential from three *Phyllanthus* species by 2 different extraction methods

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