









CHEMICAL CONSTITUENTS OF MALAYSIAN AQUILARIA CRASSNA PIERRE (GAHARU)

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FACULTY OF SCIENCES AND MATHEMATICS UNIVERSITI PENDIDIKAN SULTAN IDRIS

2012





















ii

DECLARATION

I hereby declare that the work in this dissertation is my own except for the quotations and summaries which have duly acknowledged.

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SYUKUR KE HADRAT ILAHI





















PREFACE

This thesis reported my work which has carried out in the Department of Chemistry and Biology, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, Malaysia under the supervision of Dr. Saripah Salbiah Binti Syed Abdul Azziz. Some parts of my work described in this thesis have been reported in the following publications:

- 1. Norzafneza M. A., Saripah S. S. A. A., Hasimah A., (2009) Kolokium Kebangsaan Pasca Siswazah Sains dan Matematik 2009, Biological Activities of Leaves from *Aquilaria crassna* (Gaharu), Universiti Pendidikan Sultan Idris, 21 Disember 2009 (Oral presentation).
- 2. Norzafneza M. A., Saripah S. S. A. A., Hasimah A., Mohd Aspollah H. M. S., Mat Ropi M., Abdul Hamid A. H., (2010) 12th Medicinal and Aromatic Plant Seminar, Chemical Constituents of Leaves from *Aquilaria crassna* (Gaharu) and Biological Activities, FRIM, 3-4 August 2010 (Poster presentation).
- 3. Hasimah A., Norzafneza M. A., Saripah S. S. A. A., Ramli I., Faridahhanim M. J., Mohd Aspollah M. S., (2011) UMT 11th International Annual Symposium on Sustainability Science and Management (UMTAS), Biological Activities of Leaf and Bark from *Aquilaria crassna* Pierre (Gaharu), UMT, 11-13 July 2011 (Poster presentation).
 - 4. Norzafneza M. A., Saripah Salbiah S. A. A., Hasimah A., Mohd Aspollah M. S., Humera N. (2012) Chemical study of *Aquilaria crassna* Pierre. Journal Chemistry of Natural Compound (In press, Ref. No. 18.12)





















ABSTRACT

Studies of Aquilaria crassna Pierre from the leaves and barks had been divided into four parts; phytochemical screening, essential oils, chemical constituents and antimicrobial activities. Samples were extracted using three solvents; hexane, dichloromethane and methanol. All six extracts; three leaves and three barks had underwent phytochemical screening to detect the presence of secondary metabolites. Fresh samples of leaves and barks went through hydrodistillation process using Dean-Stark apparatus to obtain essential oil. Isolation of chemical compounds had been done using several chromatography techniques such as column chromatography (CC), preparative thin layer chromatography (PTLC) and monitoring with thin layer chromatography (TLC). Totally, seven pure compounds successively isolated. Four pure compounds isolated from leaves; 5-hydroxy-7,4'-dimethoxyflavone (29) and epifriedelanol (30) from hexane extract while squalene (31) and 5-hydroxy-7,4'dimethoxyflavone (29) from dichloromethane extract. Further extraction on barks pure compounds; sec-nonacosyl ester (32),5-hydroxy-7,4'dimethoxyflavone (29) and bis (2-ethylhexyl) phthalate (33). Finally, crude extracts had been screened for antimicrobial activities towards four bacteria. Results show that Staphylococcus aureus was the most susceptible bacteria to all six extracts. Whilst, the least susceptible strain was Pseudomonas aeruginosa.



























KANDUNGAN KIMIA DALAM AQUILARIA CRASSNA PIERRE (GAHARU) MALAYSIA

ABSTRAK

Kajian terhadap Aquilaria crassna Pierre daripada daun dan batang terbahagi kepada empat bahagian; saringan fitokimia, minyak pati, komponen kimia dan aktiviti Sampel diekstrak menggunakan tiga jenis pelarut; heksana, diklorometana dan metanol. Kesemua enam ekstrak; tiga ekstrak daun dan tiga ekstrak batang telah menjalani proses penyaringan fitokimia untuk mengesan kehadiran metabolit sekunder. Sampel segar juga telah melalui proses penyulingan hidro penggunaan peralatan Dean-Stark bagi menghasilkan minyak pati. Proses pemencilan sebatian kimia menggunakan teknik kromatografi seperti kromatografi turus (KT), plat kromatografi lapisan nipis (PKLN) dan seterusnya dipantau dengan kromatografi lapisan nipis (KLN). Sejumlah tujuh sebatian kimia telah berjaya dipisahkan. Empat sebatian tulen diperolehi daripada daun; 5-hidroksi-7,4'dimetoksiflavon (29) dan epifriedelanol (30) daripada ekstrak heksana manakala 5-hidroksi-7.4'-dimetoksiflavon (29) daripada (31) dan dikloromethana. Pengekstrakkan bahagian batang pula menghasilkan tiga sebatian tulen; sek-nonakosil ester (32), 5-hidroksi-7,4'-dimetoksiflavon (29) dan phthalatebis-2 (etilheksil) (33). Akhir sckali, ekstrak mentah disaring untuk aktiviti antimikrob ke atas empat bakteria. Keputusan menunjukkan Staphylococcus aureus adalah bakteria yang paling mudah direncat oleh kesemua ekstrak. Manakala, bakteria yang paling kurang direncat adalah Pseudomonas aeruginosa.





















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LIST OF ABBREVIATIONS

ATP Attached proton test

broad br

CCcolumn chromatography

deuterated chloroform CDC₁₃

methyl group CH_3

chloroform CHCl₃

centimeter cm

H-H correlation spectroscopy **COSY**

Distortionless Enhancement by Polarization Transfer **DEPT**

doublet d

gs-4506832 pustaka.upsi.edu.mgram Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah

hydrochloric acid **HCI**

HMBC Heteronuclear Multiple Bond Coherence

Heteronuclear Multiple Quantum Coherence **HMQC**

Hertz Hz

Infrared IR

coupling constant (Hz) J

kilogram kg

potassium hydroxide **KOH**

meter m

multiplet m

methanol MeOH

milligram mg





















mega Hertz MHz

milliliter mL

millimeter mm

melting point m.p

Mass Spectrum MS

mass/charge m/z

Nuclear Magnetic Resonance **NMR**

methoxyl group OCH₃

hydroxyl group ОН

Preparative Thin Layer Chromatography **PTLC**

parts per million ppm



singlet

species sp

deuterated tetrahydrofuran THF-d

Thin Layer Chromatography TLC

tetramethylsilane **TMS**

Ultraviolet UV

micro liter μL





















CHAPTER 1

INTRODUCTION







Malaysia is one of the mega biodiversity countries that have more than 500,000 plant species which wildly grown in rainforests and also villages (Latiff, 2000). Malaysia owns a wide range of plant kingdom due to the location placed strategically in the equator where the balance of hot and wet climate.

Plants have been used as spices in culinary, remedies for diseases and many more. In Malaysia, there are about 12,000 plants out of which 1300 species have medical potential (Burkill, 1935). Many plant species have been used for traditional remedies even though their biological principle has not been discovered yet.



















Today, provided all the facilities and technologies, scientists are able to investigate and study the active compounds in selected plants organs, which can be useful in pharmaceutical and related industries. It is a current trend that people are going back to nature for healthier life style. They have turned back to previous method of medication and believe that natural product like herbs are safer than the synthetic substances (Abas, 2001). Normally, the whole plant or certain part of the plants are used for the purpose of treatment. The reason people used the herbs are as follows:

- a) Due to the culture and beliefs from the earlier generation.
- b) Due to economics, in which the herbs are cheaper than conventional medicines.
- c) Natural products are safer and do not contain hazardous chemical while some modern medicine have side effects.

Moreover, the scientists and researchers play important roles in exploring valuable herbs for the use of the next generation so that not only the herbs can be conserved but the traditional knowledge will also be preserved.

Eurycoma longifolia Jack or well known as Tongkat Ali had been explored widely due to its anti-malarial, antipyretic, anti-ulcer, anxiolytic, anticancer and aphrodisiac properties (Bhat et al.,2010). Nowadays, Aquilaria also had been explored well as this genus has been used traditionally as sedatives, analgesic and digestion medicine (Toru et al., 2005).









1.2 Genus Aquilaria

Genus Aquilaria belongs to the family Thymelaeceae. It is a fast growing evergreen tree that normally grown to 18 up to 40 m height with 40 cm diameter. The plants from this genus grow well in natural forest with few meters above sea level until about 1,000 meter. It is best grown on around 500 meter above sea level. Aquilaria can also survive in a wide range of soil including poor sandy soil. This genus usually occur particularly in the rain forest of Indonesia, Thailand, Cambodia, Laos, Vietnam, Malaysia, Northern India, Philippines Borneo and New Guinea.

There are 25 species in the genus *Aquilaria*. However, only 16 species were reported to produce gaharu (Barden, 2002). Table 1.1 shows the distribution of *Aquilaria* species in all over the world.

Table 1.1

List of Genus *Aquilaria* and The Distributions

| Genus | Country |
|-------------------|---------------------------------|
| A. subintegra | Thailand |
| A. crassna | Malaysia, Thailand and Cambodia |
| A. malacensis | Malaysia, Thailand and India |
| A. apiculata | Philippines |
| A. baillonil | Thailand and Cambodia |
| A. baneomsis | Vietnam |
| A. beccarian | Indonesia |
| | |

(continue)

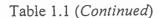












| 8 | A.brachyantha | Malaysia |
|----|----------------|------------------------|
| 9 | A. cuminggiana | Malaysia and Indonesia |
| 10 | A. filarial | China |
| 11 | A. grandiflora | China |
| 12 | A. hilata | Malaysia and Indonesia |
| 13 | A. khasiana | India |
| 14 | A. microcapa | Malaysia and Indonesia |
| 15 | A. rostrata | Malaysia |
| 16 | A. sinensis | China |

(gaharuonline.com, 2009)

The uniqueness of Aquilaria is that the matured tree start to produce gaharu at the age of 20 and will continue producing gaharu until 45 years old, depending on the injury methods by insect, physical cuts, bacterial infection or chemical stimulation. Gaharu or resin (agarwood, aloeswood, eaglewood) is resinous, fragrant and highly valuable heartwood produced by Aquilaria sp.

Aquilaria sp. is commonly known as gaharu in Malaysia. It is also called gaharu in Indonesia and Papua New Guinea. Other local names for Aquilaria such as Jin-koh (Japan), Chim-Hyuang (Korea), Ch'en Hsiang or Ch'en Xiang (China) and Oud in the Middle East. Although it is called differently according to different country but it still refers to species of Aquilaria.

The researchers started to realize the potential of the Aquilaria and explored this species due to its high price. The high quality of gaharu produce from Aquilaria









can reach as much as RM 10,000 - RM 100,000 per kilo depending on the grade. The global demand for gaharu is increasing, therefore could not meet the current need. This circumstance led to a variety of research efforts on gaharu, which include studies on the commercial plantings, artificial inoculations to stimulate the development of gaharu, grading system, chemical constituents, biological development, trading and economic aspects of gaharu, and differentiation of wild and cultivated Aquilaria trees.

1.3 Aquilaria crassna

A. crassna is a fast growing, large evergreen wood tree, which can grow over 15-30 m tall, sometimes can up to 40 m height with 1.5 - 2.5 m in diameter (Plate 1). The leaves usually 6.0 - 11.0 cm long with 2.0 - 4.0 cm broad with white umbellate flowers (Quan et al., 2003) (Plate 2).

The flowers are yellowish-green, produced in an umbel. Flowers are small, fragrant, fine-haired, pedicel up to 1.0 cm long, 4.0 mm long. The fruit is a woody capsule 3.0 - 3.5 cm long, 2.5 - 3.0 cm wide with 2 valves, each with 1 seed. The fruit capsule is hard or leathery when dry (Plate 3).

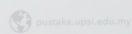


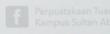
Plate 1: The Bark of A. crassna



Plate 2: The Leaves of A. crassna







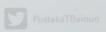




Plate 3: The Fruits of A. crassna

Its heartwood is black or sometimes brown in colour (Penpun et al., 2009). Prachakul (1989) reported that the aroma is due to the presence of sesquiterpene components in the heartwood.

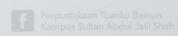
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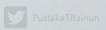
Even though the Aquilaria species has been explored for more than a decade ago, but only the resin from the heartwood of the Aquilaria has been investigated. To date, only a few studies have been done on leaves and barks of these plants.

On the other hands, the Aquilaria is known to have various applications in traditional medicine. Nevertheless, there is not much scientific evidence to support these claims. Thus, this study aimed to investigate the chemical compositions in the leaves and barks of Aquilaria, and to provide evidences for its folkloric uses. In addition, this research is worthwhile to embark the new source of active compounds for future benefits.





















1.5 Significance of the studies

This study would endow further information about phytochemical screening, essential oils, chemical constituents and antimicrobial activities of leaves and barks extracts of *A. crassna*, which would contribute to the public understanding about the plant. The finding might contribute an additional usage of the plant's organs that might have value to be commercialized, other than resin in the heartwood. It can be used in the discovery of new therapeutic agents and active compounds from this plant.

1.6 Objectives of the studies

- a) To conduct the phytochemical screening on leaves and barks
- b) To determine the presence of essential oil
- c) To extract, isolate and purify the chemical constituents
- d) To identify and elucidate the structure of the isolated compounds
- e) To run the antimicrobial activity of the crude extracts

1.7 Limitation of the studies

This study had been limited to one species which was A. crassna. The samples were collected from Kajang, Selangor on several field works in July 2008. The study focused only on the leaves and barks of the plant. Besides that, three solvents were chosen to extract the samples; hexane, dichloromethane and methanol. The extracts





















were tested on Pseudomonas aeruginosa (ATCC 10145), Bacillus spizizenii (ATCC 6633), Staphylococcus aureus (ATCC 1026) and Shigela flexneri (ATCC 12022) for antimicrobial test.



















